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(54) Title: SYSTEMS AND METHODS FOR PREDICTING THERAPEUTIC SENSITIVITY

(57) Abstract: Methods, systems, and software are provided for using organoid cultures, e.g., patient-derived tumor organoid cultures, to improve treatment predictions and outcomes.

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SYSTEMS AND METHODS FOR PREDICTING THERAPEUTIC SENSITIVITY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/924,621, filed on October 22, 2019, and U.S. Provisional Application No. 62/944,292, filed on December 5, 2019, which are expressly incorporated by reference in their entireties for all purposes.

TECHNICAL FIELD

[0002] The present disclosure relates generally to use of organoids for predicting patient sensitivity to therapeutic agents.

BACKGROUND

[0003] Precision oncology is the practice of tailoring cancer therapy to the unique genomic, epigenetic, and/or transcriptomic profile of an individual tumor. This is in contrast to conventional methods for treating a cancer patient based merely on the type of cancer the patient is afflicted with, e.g., treating all breast cancer patients with a first therapy and all lung cancer patients with a second therapy. Precision oncology was borne out of many observations that different patients diagnosed with the same type of cancer, e.g., breast cancer, responded very differently to common treatment regimes. Over time, researchers have identified genomic, epigenetic, and transcriptomic markers that facilitate some level of prediction as to how an individual cancer will respond to a particular treatment modality.

[0004] Therapy targeted to specific genomic alterations is already the standard of care in several tumor types (*e.g.*, as suggested in the National Comprehensive Cancer Network (NCCN) guidelines for melanoma, colorectal cancer, and non-small cell lung cancer). These few, well known mutations in the NCCN guidelines can be addressed with individual assays or small next generation sequencing (NGS) panels. However, for the largest number of patients to benefit from personalized oncology, molecular alterations that can be targeted with off-label drug indications, combination therapy, or tissue agnostic immunotherapy should be assessed. *See* Schwaederle *et al.* 2016 *JAMA Oncol.* 2, 1452-1459; Schwaederle *et al.* 2015 *J Clin Oncol.* 32, 3817-3825; and Wheler *et al.* 2016 *Cancer Res.* 76, 3690-3701. Large panel NGS assays also cast a wider net for clinical trial enrollment. *See* Coyne *et al.* 2017 *Curr. Probl. Cancer* 41, 182-193; and Markman 2017 *Oncology* 31, 158,168.

[0005] Genomic analysis of tumors is rapidly becoming routine clinical practice to provide tailored patient treatments and improve outcomes. *See* Fernandes *et al.* 2017 *Clinics* 72, 588–594. Indeed, recent studies indicate that clinical care is guided by NGS assay results for 30-40% of patients receiving such testing. *See* Hirshfield *et al.* 2016 *Oncologist* 21, 1315-1325; Groisberg *et al.* 2017 *Oncotarget* 8, 39254–39267; Ross *et al.* *JAMA Oncol.* 1, 40-49; and Ross *et al.* 2015 *Arch. Pathol. Lab Med.* 139, 642-649. There is growing evidence that patients who receive therapeutic advice guided by genetics have better outcomes. *See*, for example Wheler *et al.* who used matching scores (*e.g.*, scores based on the number of therapeutic associations and genomic aberrations per patient) to demonstrate that patients with higher matching scores have a greater frequency of stable disease, longer time to treatment failure, and greater overall survival (2016 *Cancer Res.* 76, 3690–3701). Such methods may be particularly useful for patients who have already failed multiple lines of therapy.

[0006] Targeted therapies have shown significant improvements in patient outcomes, especially in terms of progression-free survival. *See* Radovich *et al.* 2016 *Oncotarget* 7, 56491–56500. Further, recent evidence reported from the IMPACT trial, which involved genetic testing of advanced stage tumors from 3,743 patients and where approximately 19% of patients received matched targeted therapies based on their tumor biology, showed a response rate of 16.2% in patients with matched treatments versus 5.2% in patients with non-matched treatments. *See* Bankhead. “IMPACT Trial: Support for Targeted Cancer Tx Approaches.” *MedPageToday*. June 5, 2018. The IMPACT study further found that the three-year overall survival for patients given a molecularly matched therapy was more than twice that of non-matched patients (15% vs. 7%). *See Id.* and ASCO Post. “2018 ASCO: IMPACT Trial Matches Treatment to Genetic Changes in the Tumor to Improve Survival Across Multiple Cancer conditions.” *The ASCO POST*. June 6, 2018. Estimates of the proportion of patients for whom genetic testing changes the trajectory of their care vary widely, from approximately 10% to more than 50%. *See* Fernandes *et al.* 2017 *Clinics* 72, 588–594.

[0007] One example of a genomic trait that has been linked to the efficacy of particular therapies are mutations in the BRCA1, BRCA2, or PALB2 homologous recombination genes. A class of pharmacological inhibitors of Poly ADP ribose polymerase 1 (PARP1), known as PARP inhibitors (PARPi), have therapeutic efficacy for treating some cancers containing a mutation in the BRCA1, BRCA2, or PALB2 homologous recombination genes. PARP1 is an essential

enzyme in the error-prone microhomology-mediated end joining (MMEJ) DNA repair pathway. Sharma S. et al., Cell Death Dis. 6(3):e1697 (2015). In the absence of PARP1 activity, DNA replication forks stall when encountering a single-strand break. Fork stalling ultimately results in double-stranded chromosomal breaks that can be repaired by homologous recombination (HR) repair, which is much less error prone than the MMEJ pathway.

[0008] Unlike other DNA repair proteins, which are commonly deficient in cancer cells, PARP1 has been shown to be over-expressed in certain cancer types. It has been theorized that increased MMEJ DNA repair, relative to homologous repair, results in the accumulation of genomic mutations, which can lead to the development of cancer. However, the efficacy of PARP inhibitors is not completely understood. For instance, not all cancers with a BRCA1, BRCA2, or PALB2 mutation are sensitive to PARP inhibitors. Further, some cancers without a mutation in any homologous recombination protein are sensitive to PARP inhibitors.

SUMMARY

[0009] Given the above background, what is needed in the art are improved ways to identify which cancer patients will respond favorably to PARP inhibitors. The present disclosure addresses these and other needs by providing systems and methods for using organoid cultures to improve treatment predictions and outcomes. There is also a need for systems and methods for assessing the effectiveness of various drugs on one or more tumor organoid lines. Such systems or methods may be used to determine if a specific drug may be useful in killing cancer cells with specific genetic mutations or phenotypes.

[0010] Also provided herein is a high-throughput drug screening method and system more applicable for the unique characteristics of tumor organoids (TOs). The systems and methods provided herein are capable of measuring TO therapeutic response with high statistical confidence and exquisite inter-assay reproducibility. This approach can be utilized in research settings to elucidate heterogeneity of therapeutic responses within and among patients, and may be utilized in the clinical laboratory to potentially guide precision oncology treatments. In some embodiments, the platform couples high content fluorescent confocal imaging analysis with a robust statistical analytical approach to measure hundreds of discrete data points of TO viability from as few as 1×10^3 cells. This approach was validated through evaluating responses to

hundreds of small molecule inhibitors as well as a panel of chemotherapeutic agents in TO models derived from different patients.

[0011] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative embodiments of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different embodiments, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] Figure 1 illustrates a block diagram of an example of a computing device for using information derived from organoid-based assays to improve therapeutic outcomes, in accordance with some embodiments of the present disclosure.

[0013] Figure 2 illustrates an example of a distributed diagnostic environment for evaluating therapeutic regimes using information derived from tumor organoid studies, in accordance with some embodiments of the present disclosure.

[0014] Figures 3A, 3B, 3C, and 3D illustrate dose response curves for the treatment of four patient-derived tumor organoid cell lines with olaparib, in accordance with some embodiments of the present disclosure.

[0015] Figures 4A and 4B collectively show example image of high-content fluorescent confocal imaging analysis using the subject methods described herein. (Top panels) Brightfield (top left), Hoechst 33342 (top right), Caspase 3/7 (bottom left), and TO-PRO-3 (bottom right) staining is shown for vehicle control (Figure 4A) and staurosporine treated (Figure 4B) gastric cancer TOs. (Bottom panels) Overlays of the fluorescent channels and the result of image analysis are shown side by side. The outline of the larger objects in the right panels indicates the area of a given TO. Live and dead cells are shown in the image analysis panels. Scale bars represent 100 microns.

[0016] Figures 5A and 5B illustrate examples of dose-response curves determined from carbocyanine monomer nucleic acid staining (TO-PRO-3; Figure 5A) and caspase 3/7 staining (Caspase 3/7; Figure 5B), in accordance with some embodiments of the present disclosure.

[0017] Figure 6 shows a summary of a study, showing that a classifier trained using data sets obtained from the subject methods is capable of predicting morphological responses to pharmacological agents in a tumor organoid sample based on a brightfield image of the sample, in accordance with some embodiments of the present disclosure.

[0018] Figures 7A and 7B collectively illustrate a neural network-based model for predicting TO drug response. Figure 7A shows brightfield, real fluorescence, and generated fluorescence images for colon, lung, ovarian, and breast cancers. Figure 7B illustrates the correlation between the neural network predictions and fluorescent-based drug responses for the colon, lung, ovarian, and breast cancers.

[0019] Figure 8 illustrates AUC ROC curves between caspase and generated viabilities for the PARPi drug screen described in Example 5.

[0020] Figures 9A and 9B illustrate viability data for a tumor organoid having a low HRD score, a pathogenic BRCA1 mutation, no BRCA2 mutations and no BRCA1 or BRCA2 LOH. The x-axis label indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control).

[0021] Figures 10A and 10B illustrate viability data for a colorectal tumor organoid having a high HRD score, no BRCA1 or BRCA2 mutations and no BRCA1 or BRCA2 LOH. The x-axis label indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control).

[0022] Figures 11A and 11B illustrate viability data for a tumor organoid having a low HRD score, no BRCA1 or BRCA2 mutations and no BRCA1 or BRCA2 LOH. The x-axis label indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control).

[0023] Figures 12A and 12B illustrate viability data for a tumor organoid having a low HRD score, no BRCA1 or BRCA2 mutations and no BRCA1 or BRCA2 LOH. The x-axis label

indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control).

[0024] Figures 13A and 13B illustrate viability data for a tumor organoid having a high HRD score, no BRCA1 or BRCA2 mutations and no BRCA1 or BRCA2 LOH. The x-axis label indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control).

[0025] Figures 14A and 14B illustrate viability data for a tumor organoid having a low HRD score, no BRCA1 or BRCA2 mutations, BRCA1 LOH, and no BRCA2 LOH. The x-axis label indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control).

[0026] Figures 15A and 15B illustrate viability data for a tumor organoid having a high HRD score, no BRCA1 or BRCA2 mutations and no BRCA1 or BRCA2 LOH. The x-axis label indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control). Lower doses of PARPi were more effective for this organoid than the low HRD shown in the previous figures organoid.

[0027] Figures 16A and 16B illustrate viability data for a tumor organoid having a low HRD score, no BRCA1 or BRCA2 mutations and no BRCA1 or BRCA2 LOH. The x-axis label indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control).

[0028] Figures 17A and 17B illustrate viability data for a tumor organoid having a high HRD score, no BRCA1 or BRCA2 mutations, no BRCA1 LOH and having BRCA2 LOH. The x-axis label indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control).

[0029] Figures 18A and 18B illustrate viability data for a tumor organoid having a low HRD score, no BRCA1 or BRCA2 mutations and no BRCA1 or BRCA2 LOH. The x-axis label indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control).

[0030] Like reference numerals refer to corresponding parts throughout the several views of the drawings.

DETAILED DESCRIPTION

[0031] In some embodiments, the present disclosure provides systems and methods for using organoid cultures, e.g., patient-derived tumor organoid cultures, to improve treatment predictions and outcomes.

[0032] For instance, in one aspect, the disclosure provides methods and systems for performing methods of training a classifier to discriminate between two or more tumor sensitivities to a poly ADP ribose polymerase (PARP) inhibitor. In some embodiments, the method includes obtaining a data set comprising, for each respective tissue sample in a plurality of tissue samples, (i) a corresponding plurality of nucleic acid features of the tissue sample, and (ii) a corresponding indication of the sensitivity of a respective organoid cultured from one or more cells of the tissue sample to the PARP inhibitor. The method then includes training an untrained classifier against at least (i) the corresponding plurality of features and (ii) the corresponding indication of the sensitivity of the organoid to the PARP inhibitor, across the plurality of tissue samples, thereby obtaining a trained classifier that discriminates between the two or more tumor sensitivities to a poly ADP ribose polymerase (PARP) inhibitor.

[0033] In another aspect, the disclosure provides method and systems for performing methods of recommending therapy for treating a cancer in a subject. In some embodiments, the method includes obtaining a first test data set comprising a plurality of nucleic acid features of a tumor biopsy from the test subject. The method then includes evaluating the first test data set using a classifier trained to discriminate between two or more tumor sensitivities to a first poly ADP ribose polymerase (PARP) inhibitor. The classifier is trained against, for each respective training tissue sample in a plurality of training tissue samples, at least (i) the plurality of nucleic acid features obtained from the respective training tissue sample, and (ii) a corresponding indication of the sensitivity of a respective organoid cultured from one or more cells of the respective training tissue sample to the first PARP inhibitor. In some embodiments, the sensitivity of the training tissue sample is determined in a two-dimensional cell culture, a three-dimensional cell culture prepared in a matrix, a three-dimensional cell culture prepared in suspension culture, a cell culture suspension, or a xenograft model. A first tumor sensitivity in

the two or more tumor sensitivities to the first PARP inhibitor is associated with a first recommendation, in a plurality of recommendations, to treat the cancer in the subject with the first PARP inhibitor. A second tumor sensitivity in the two or more tumor sensitivities to the first PARP inhibitor is associated with a second recommendation, in the plurality of recommendations, not to treat the cancer in the subject with the first PARP inhibitor. In some embodiments, the method includes providing a respective recommendation, in the plurality of recommendations, for treating the cancer in the test subject based on the results of the evaluation.

[0034] In another aspect, the disclosure provides methods and systems for performing methods of treating, or assigning therapy for, a BRCA1-/BRCA2- cancer in a test subject. In some embodiments, the methods include determining a probability or likelihood that the cancer will be sensitive to a poly ADP ribose polymerase (PARP) inhibitor, and treating cancer in the subject. When the probability or likelihood that the cancer will be sensitive to the PARP inhibitor satisfies a sensitivity threshold, the method includes administering, or communicating, a recommended therapy comprising the PARP inhibitor to the test subject. When the probability or likelihood that the cancer will be sensitive to the PARP inhibitor does not satisfy a sensitivity threshold, the method includes administering, or communicating, a recommended therapy that does not include the PARP inhibitor to the test subject.

[0035] In another aspect, the disclosure provides methods and systems for performing methods of treating a cancer other than breast cancer or ovarian cancer in a test subject. In some embodiments, the methods include determining a probability or likelihood that the cancer will be sensitive to a poly ADP ribose polymerase (PARP) inhibitor, and treating cancer in the subject. When the probability or likelihood that the cancer will be sensitive to the PARP inhibitor satisfies a threshold likelihood, the method includes administering, or communicating, a recommended therapy comprising the PARP inhibitor to the test subject. When the probability or likelihood that the cancer will be sensitive to the PARP inhibitor does not satisfy a threshold likelihood, the method includes administering, or communicating, a recommended therapy that does not include the PARP inhibitor to the test subject.

[0036] In another aspect, the disclosure provides methods and systems for performing methods of providing a clinical report for a cancer patient to a physician. In some embodiments, the methods include obtaining a first test data set comprising features of a transcriptome from a

tumor biopsy from the cancer patient. In some embodiments, the methods include evaluating the first test data set using a classifier trained, e.g., as described herein, to discriminate between two or more tumor sensitivities to a first poly ADP ribose polymerase (PARP) inhibitor. The methods then include receiving a recommended therapy for the cancer patient from the classifier, and including the recommended therapy, or sending the recommended therapy to a third party for inclusion, in a clinical report for the cancer patient.

[0037] In another aspect, the disclosure provides methods and systems for performing methods of identifying an inhibitor of poly ADP ribose polymerase (PARP). In some embodiments, the methods include obtaining a tissue sample that is sensitive to a known PARP inhibitor, and culturing one or more organoids, each respective organoid in the one or more organoids from one or more cells of the tissue sample. The methods then include exposing the one or more of the cultured organoids to one or more concentrations of a candidate molecule, and measuring the fitness of cells in the one or more organoids following the exposure to the one or more concentrations of the candidate molecule. A reduced fitness of cells in the one or more organoids is indicative that the candidate molecule is a PARP inhibitor.

[0038] In another aspect, the disclosure provides methods and systems for performing methods of identifying a new use for a pharmaceutical compound of a first pharmaceutical class. In some embodiments, the methods include obtaining a plurality of tissue samples, wherein each respective tissue sample in the plurality of tissue samples is sensitive to a respective class of pharmaceutical agents in a plurality of classes of pharmaceutical agents that excludes the first pharmaceutical class. The methods then includes culturing, for each respective tissue sample in the plurality of tissue samples, one or more respective organoids from one or more cells of the respective tissue sample, thereby generating a plurality of organoid cultures. The methods then include exposing, for each organoid culture in the plurality of organoid cultures, the respective one or more organoids to one or more concentrations of the pharmaceutical agent, and measuring, for each organoid culture in the plurality of organoid cultures, the fitness of cells in the respective one or more organoids following the exposure to the pharmaceutical agent. Reduced fitness of the cells in the respective one or more organoids is indicative that the pharmaceutical molecule shares pharmacological properties with the class of pharmaceutical compounds in the plurality of classes of pharmaceutical classes to which the tissue sample corresponding to the respective one or more organoids is sensitive.

[0039] In another aspect, the disclosure provides methods and systems for performing methods of determining the eligibility of a cancer patient for a clinical trial of a candidate cancer pharmaceutical agent. In some embodiments, the methods include obtaining a tumor biopsy from the cancer patient, and culturing one or more tumor organoids from one or more cells of the tumor biopsy. The methods then include exposing the one or more tumor organoids to one or more concentrations of the candidate cancer pharmaceutical agent, and measuring the fitness of cells in the one or more tumor organoids following the exposure to the one or more concentrations of the candidate cancer pharmaceutical agent. The methods then include determining whether the cancer patient is eligible for the clinical trial based on at least the measured fitness of the cells in the one or more tumor organoids, wherein reduced fitness of the cells in the one or more tumor organoids is indicative that the cancer patient is eligible for the clinical trial.

[0040] In another aspect, the disclosure provides methods and systems for performing methods of determining the eligibility of a cancer patient for a clinical trial of a candidate cancer pharmaceutical agent. In some embodiments, the methods include obtaining a first test data set comprising a plurality of nucleic acid features of a tumor biopsy from the cancer patient. The methods then include evaluating the first test data set using a classifier trained to discriminate between two or more tumor sensitivities to the candidate cancer pharmaceutical agent. The classifier is trained against, for each respective training tissue sample in a plurality of training tissue samples, at least (i) the plurality of nucleic acid features obtained from the respective training tissue sample, and (ii) a corresponding indication of the sensitivity of a respective organoid cultured from one or more cells of the respective training tissue sample to the candidate cancer pharmaceutical agent. A first tumor sensitivity in the two or more tumor sensitivities to the candidate cancer pharmaceutical agent is associated with an indication that the cancer patient is eligible for the clinical trial. A second tumor sensitivity in the two or more tumor sensitivities to the candidate cancer pharmaceutical agent is associated with an indication that the cancer patient is eligible for the clinical trial. The methods then include determining whether the cancer patient is eligible for the clinical trial based on at least the evaluation.

[0041] In another aspect, the disclosure provides methods and systems for performing methods of identifying a resistance to a chemotherapeutic agent in a patient with cancer. In some embodiments, the methods include obtaining a first test data set comprising a plurality of

nucleic acid features of a tumor biopsy from the patient with cancer. The methods then include evaluating the first test data set using a classifier trained to discriminate between two or more tumor sensitivities to the chemotherapeutic agent. The classifier is trained against, for each respective training tissue sample in a plurality of training tissue samples, at least (i) the plurality of nucleic acid features obtained from the respective training tissue sample, and (ii) a corresponding indication of the sensitivity of a respective organoid cultured from one or more cells of the respective training tissue sample to the chemotherapeutic agent. A first tumor sensitivity in the two or more tumor sensitivities to the candidate cancer pharmaceutical agent is associated with an indication that the patient's cancer is resistant to the chemotherapeutic agent. A second tumor sensitivity in the two or more tumor sensitivities to the candidate cancer pharmaceutical agent is associated with an indication that the patient's cancer is not resistant to the chemotherapeutic agent. The methods then include providing a report indicating whether the patient's cancer is resistant to the chemotherapeutic agent based on at least the results of the evaluation.

[0042] In another aspect, the disclosure provides methods and systems for evaluating an effect of a cancer therapeutic agent. In some embodiments, the method includes providing a plurality of tumor organoids cultured in a tumor organoid culture medium, where the plurality of tumor organoids is divided into a plurality of tumor organoid subsets. The method then includes contacting each subset in the plurality of tumor organoid subsets with a cancer therapeutic agent. The method also includes contacting each subset in the plurality of tumor organoid subsets with one or more cell death detection agents and a total cell detection agent. The method then includes obtaining a tumor organoid profile for each subset in the plurality of subset. In some embodiments, the tumor organoid profile includes a cell viability value for every tumor organoid in the subset. The method then includes assessing the effect of the cancer therapeutic agent based on the tumor organoid profiles.

[0043] In another aspect, the disclosure provides methods and systems for assigning a treatment dosage of a cancer therapeutic agent for a subject in need thereof. In some embodiments, the method includes providing a plurality of tumor organoids cultured in a tumor organoid culture medium, where the tumor organoids are derived from a subject, and where the plurality of tumor organoids is divided into a plurality of tumor organoid subsets. The method then includes contacting each subset in the plurality of tumor organoid subsets with a different

dosage of a cancer therapeutic agent. The method also includes contacting each subset in the plurality of tumor organoid subsets with one or more cell death detection agents and a total cell detection agent. The method then includes obtaining a tumor organoid profile for each subset in the plurality of subset, where the tumor organoid profile includes a cell viability value for every tumor organoid in the subset. The method then includes determining a therapeutic agent dosage curve from the tumor organoid profiles. The method then includes assigning a treatment dosage of the cancer therapeutic agent to the subject based on the therapeutic agent dosage curve.

[0044] *Definitions.*

[0045] The terminology used in the present disclosure is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used in the description of the invention and the appended claims, the singular forms “a”, “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. It will also be understood that the term “and/or” as used herein refers to and encompasses any and all possible combinations of one or more of the associated listed items. It will be further understood that the terms “comprises” and/or “comprising,” when used in this specification, specify the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof. Furthermore, to the extent that the terms “including,” “includes,” “having,” “has,” “with,” or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term “comprising.”

[0046] As used herein, the term “if” may be construed to mean “when” or “upon” or “in response to determining” or “in response to detecting,” depending on the context. Similarly, the phrase “if it is determined” or “if [a stated condition or event] is detected” may be construed to mean “upon determining” or “in response to determining” or “upon detecting [the stated condition or event]” or “in response to detecting [the stated condition or event],” depending on the context.

[0047] It will also be understood that, although the terms first, second, *etc.* may be used herein to describe various elements, these elements should not be limited by these terms. These terms are only used to distinguish one element from another. For example, a first subject could

be termed a second subject, and, similarly, a second subject could be termed a first subject, without departing from the scope of the present disclosure. The first subject and the second subject are both subjects, but they are not the same subject. Furthermore, the terms “subject,” “user,” and “patient” are used interchangeably herein.

[0048] As used herein, the term “organoid” refers to an in vitro three-dimensional multicellular construct that is developed from stem cells (e.g., embryonic stem cells, induced pluripotent stem cells, and somatic stem cells or tissue derived progenitor cells) or cancer cells in a specific 3D organoid culture system. Organoids contain multiple cells types of the in vivo counter parts and organize similarly to the primary tissue. In some embodiments, the organoid culture system includes an organoid culture medium and an extracellular matrix or extracellular matrix substitute. An “organoid cell line” refers to a plurality of organoids that are derived and established from the same cell or cell population. A “tumor organoid” refers to an organoid derived from a tumor cell or population of tumor cells.

[0049] As used herein, the term “subject” refers to any living or non-living human. In some embodiments, a subject is a male or female of any stage (e.g., a man, a women or a child).

[0050] As used herein, the terms “control,” “control sample,” “reference,” “reference sample,” “normal,” and “normal sample” describe a sample from a subject that does not have a particular condition, or is otherwise healthy. In an example, a method as disclosed herein can be performed on a subject having a tumor, where the reference sample is a sample taken from a healthy tissue of the subject. A reference sample can be obtained from the subject, or from a database. The reference can be, e.g., a reference genome that is used to map sequence reads obtained from sequencing a sample from the subject. A reference genome can refer to a haploid or diploid genome to which sequence reads from the biological sample and a constitutional sample can be aligned and compared. An example of constitutional sample can be DNA of white blood cells obtained from the subject. For a haploid genome, there can be only one nucleotide at each locus. For a diploid genome, heterozygous loci can be identified; each heterozygous locus can have two alleles, where either allele can allow a match for alignment to the locus.

[0051] As used herein, the term “locus” refers to a position (e.g., a site) within a genome, e.g., on a particular chromosome. In some embodiments, a locus refers to a single nucleotide

position within a genome, i.e., on a particular chromosome. In some embodiments, a locus refers to a small group of nucleotide positions within a genome, *e.g.*, as defined by a mutation (*e.g.*, substitution, insertion, or deletion) of consecutive nucleotides within a cancer genome. Because normal mammalian cells have diploid genomes, a normal mammalian genome (*e.g.*, a human genome) will generally have two copies of every locus in the genome, or at least two copies of every locus located on the autosomal chromosomes, *e.g.*, one copy on the maternal autosomal chromosome and one copy on the paternal autosomal chromosome.

[0052] As used herein, the term “allele” refers to a particular sequence of one or more nucleotides at a chromosomal locus.

[0053] As used herein, the term “reference allele” refers to the sequence of one or more nucleotides at a chromosomal locus that is either the predominant allele represented at that chromosomal locus within the population of the species (*e.g.*, the “wild-type” sequence), or an allele that is predefined within a reference genome for the species.

[0054] As used herein, the term “variant allele” refers to a sequence of one or more nucleotides at a chromosomal locus that is either not the predominant allele represented at that chromosomal locus within the population of the species (*e.g.*, not the “wild-type” sequence), or not an allele that is predefined within a reference genome for the species.

[0055] As used herein, the term “single nucleotide variant” or “SNV” refers to a substitution of one nucleotide to a different nucleotide at a position (*e.g.*, site) of a nucleotide sequence, *e.g.*, a sequence read from an individual. A substitution from a first nucleobase X to a second nucleobase Y may be denoted as “X>Y.” For example, a cytosine to thymine SNV may be denoted as “C>T.”

[0056] As used herein, the term “mutation” or “variant” refers to a detectable change in the genetic material of one or more cells. In a particular example, one or more mutations can be found in, and can identify, cancer cells (*e.g.*, driver and passenger mutations). A mutation can be transmitted from apparent cell to a daughter cell. A person having skill in the art will appreciate that a genetic mutation (*e.g.*, a driver mutation) in a parent cell can induce additional, different mutations (*e.g.*, passenger mutations) in a daughter cell. A mutation generally occurs in a nucleic acid. In a particular example, a mutation can be a detectable change in one or more deoxyribonucleic acids or fragments thereof. A mutation generally refers to nucleotides that is

added, deleted, substituted for, inverted, or transposed to a new position in a nucleic acid. A mutation can be a spontaneous mutation or an experimentally induced mutation. A mutation in the sequence of a particular tissue is an example of a “tissue-specific allele.” For example, a tumor can have a mutation that results in an allele at a locus that does not occur in normal cells. Another example of a “tissue-specific allele” is a fetal-specific allele that occurs in the fetal tissue, but not the maternal tissue.

[0057] As used herein the term “cancer,” “cancerous tissue,” or “tumor” refers to an abnormal mass of tissue in which the growth of the mass surpasses and is not coordinated with the growth of normal tissue. A cancer or tumor can be defined as “benign” or “malignant” depending on the following characteristics: degree of cellular differentiation including morphology and functionality, rate of growth, local invasion and metastasis. A “benign” tumor can be well differentiated, have characteristically slower growth than a malignant tumor and remain localized to the site of origin. In addition, in some cases a benign tumor does not have the capacity to infiltrate, invade or metastasize to distant sites. A “malignant” tumor can be a poorly differentiated (anaplasia), have characteristically rapid growth accompanied by progressive infiltration, invasion, and destruction of the surrounding tissue. Furthermore, a malignant tumor can have the capacity to metastasize to distant sites. Accordingly, a cancer cell is a cell found within the abnormal mass of tissue whose growth is not coordinated with the growth of normal tissue. Accordingly, a “tumor sample” refers to a biological sample obtained or derived from a tumor of a subject, as described herein.

[0058] As used herein, the terms “sequencing,” “sequence determination,” and the like as used herein refers generally to any and all biochemical processes that may be used to determine the order of biological macromolecules such as nucleic acids or proteins. For example, sequencing data can include all or a portion of the nucleotide bases in a nucleic acid molecule such as an mRNA transcript or a genomic locus.

[0059] As used herein, the term “sequence reads” or “reads” refers to nucleotide sequences produced by any sequencing process described herein or known in the art. Reads can be generated from one end of nucleic acid fragments (“single-end reads”), and sometimes are generated from both ends of nucleic acids (e.g., paired-end reads, double-end reads). The length of the sequence read is often associated with the particular sequencing technology. High-

throughput methods, for example, provide sequence reads that can vary in size from tens to hundreds of base pairs (bp). In some embodiments, the sequence reads are of a mean, median or average length of about 15 bp to 900 bp long (e.g., about 20 bp, about 25 bp, about 30 bp, about 35 bp, about 40 bp, about 45 bp, about 50 bp, about 55 bp, about 60 bp, about 65 bp, about 70 bp, about 75 bp, about 80 bp, about 85 bp, about 90 bp, about 95 bp, about 100 bp, about 110 bp, about 120 bp, about 130, about 140 bp, about 150 bp, about 200 bp, about 250 bp, about 300 bp, about 350 bp, about 400 bp, about 450 bp, or about 500 bp). In some embodiments, the sequence reads are of a mean, median or average length of about 1000 bp, 2000 bp, 5000 bp, 10,000 bp, or 50,000 bp or more. Nanopore sequencing, for example, can provide sequence reads that can vary in size from tens to hundreds to thousands of base pairs. Illumina parallel sequencing can provide sequence reads that do not vary as much, for example, most of the sequence reads can be smaller than 200 bp. A sequence read (or sequencing read) can refer to sequence information corresponding to a nucleic acid molecule (e.g., a string of nucleotides). For example, a sequence read can correspond to a string of nucleotides (e.g., about 20 to about 150) from part of a nucleic acid fragment, can correspond to a string of nucleotides at one or both ends of a nucleic acid fragment, or can correspond to nucleotides of the entire nucleic acid fragment. A sequence read can be obtained in a variety of ways, e.g., using sequencing techniques or using probes, e.g., in hybridization arrays or capture probes, or amplification techniques, such as the polymerase chain reaction (PCR) or linear amplification using a single primer or isothermal amplification.

[0060] As used herein, the term “read segment” or “read” refers to any nucleotide sequences including sequence reads obtained from an individual and/or nucleotide sequences derived from the initial sequence read from a sample obtained from an individual. For example, a read segment can refer to an aligned sequence read, a collapsed sequence read, or a stitched read. Furthermore, a read segment can refer to an individual nucleotide base, such as a single nucleotide variant.

[0061] As used herein, the term, “reference exome” refers to any particular known, sequenced or characterized exome, whether partial or complete, of any tissue from any organism or pathogen that may be used to reference identified sequences from a subject. Example reference exomes used for human subjects as well as many other organisms are provided in the on-line genome browser hosted by the National Center for Biotechnology Information (“NCBI”).

[0062] As used herein, the term “reference genome” refers to any particular known, sequenced or characterized genome, whether partial or complete, of any organism or pathogen that may be used to reference identified sequences from a subject. Exemplary reference genomes used for human subjects as well as many other organisms are provided in the on-line genome browser hosted by the National Center for Biotechnology Information (“NCBI”) or the University of California, Santa Cruz (UCSC). A “genome” refers to the complete genetic information of an organism or pathogen, expressed in nucleic acid sequences. As used herein, a reference sequence or reference genome often is an assembled or partially assembled genomic sequence from an individual or multiple individuals. In some embodiments, a reference genome is an assembled or partially assembled genomic sequence from one or more human individuals. The reference genome can be viewed as a representative example of a species’ set of genes. In some embodiments, a reference genome comprises sequences assigned to chromosomes. Exemplary human reference genomes include but are not limited to NCBI build 34 (UCSC equivalent: hg16), NCBI build 35 (UCSC equivalent: hg17), NCBI build 36.1 (UCSC equivalent: hg18), GRCh37 (UCSC equivalent: hg19), and GRCh38 (UCSC equivalent: hg38).

[0063] As used herein, the term “assay” refers to a technique for determining a property of a substance, *e.g.*, a nucleic acid, a protein, a cell, a tissue, or an organ. An assay (*e.g.*, a first assay or a second assay) can comprise a technique for determining the copy number variation of nucleic acids in a sample, the methylation status of nucleic acids in a sample, the fragment size distribution of nucleic acids in a sample, the mutational status of nucleic acids in a sample, or the fragmentation pattern of nucleic acids in a sample. Any assay known to a person having ordinary skill in the art can be used to detect any of the properties of nucleic acids mentioned herein. Properties of a nucleic acids can include a sequence, genomic identity, copy number, methylation state at one or more nucleotide positions, size of the nucleic acid, presence or absence of a mutation in the nucleic acid at one or more nucleotide positions, and pattern of fragmentation of a nucleic acid (*e.g.*, the nucleotide position(s) at which a nucleic acid fragments). An assay or method can have a particular sensitivity and/or specificity, and their relative usefulness as a diagnostic tool can be measured using ROC-AUC statistics.

[0064] The term “classification” can refer to any number(s) or other characters(s) that are associated with a particular property of a sample. For example, in some embodiments, the term “classification” can refer to a type of cancer in a subject or sample, a stage of cancer in a subject

or sample, a prognosis for a cancer in a subject or sample, a tumor load in a subject, a presence of tumor metastasis in a subject, and the like. The classification can be binary (e.g., positive or negative) or have more levels of classification (e.g., a scale from 1 to 10 or 0 to 1). The terms “cutoff” and “threshold” can refer to predetermined numbers used in an operation. For example, a cutoff size can refer to a size above which fragments are excluded. A threshold value can be a value above or below which a particular classification applies. Either of these terms can be used in either of these contexts.

[0065] Several aspects are described below with reference to example applications for illustration. It should be understood that numerous specific details, relationships, and methods are set forth to provide a full understanding of the features described herein. One having ordinary skill in the relevant art, however, will readily recognize that the features described herein can be practiced without one or more of the specific details or with other methods. The features described herein are not limited by the illustrated ordering of acts or events, as some acts can occur in different orders and/or concurrently with other acts or events. Furthermore, not all illustrated acts or events are required to implement a methodology in accordance with the features described herein.

[0066] Reference will now be made in detail to embodiments, examples of which are illustrated in the accompanying drawings. In the following detailed description, numerous specific details are set forth in order to provide a thorough understanding of the present disclosure. However, it will be apparent to one of ordinary skill in the art that the present disclosure may be practiced without these specific details. In other instances, well-known methods, procedures, components, circuits, and networks have not been described in detail so as not to unnecessarily obscure aspects of the embodiments.

[0067] *Example System Embodiments.*

[0068] Now that an overview of some aspects of the present disclosure and some definitions used in the present disclosure have been provided, details of an exemplary system are now described in conjunction with Figure 1. Figure 1 is a block diagram illustrating a system 100 in accordance with some implementations. The device 100 in some implementations includes one or more processing units CPU(s) 102 (also referred to as processors), one or more network interfaces 104, a user interface 106, a non-persistent memory 111, a persistent memory 112, and

one or more communication buses 114 for interconnecting these components. The one or more communication buses 114 optionally include circuitry (sometimes called a chipset) that interconnects and controls communications between system components. The non-persistent memory 111 typically includes high-speed random access memory, such as DRAM, SRAM, DDR RAM, ROM, EEPROM, flash memory, whereas the persistent memory 112 typically includes CD-ROM, digital versatile disks (DVD) or other optical storage, magnetic cassettes, magnetic tape, magnetic disk storage or other magnetic storage devices, magnetic disk storage devices, optical disk storage devices, flash memory devices, or other non-volatile solid state storage devices. The persistent memory 112 optionally includes one or more storage devices remotely located from the CPU(s) 102. The persistent memory 112, and the non-volatile memory device(s) within the non-persistent memory 112, comprise non-transitory computer readable storage medium. In some implementations, the non-persistent memory 111 or alternatively the non-transitory computer readable storage medium stores the following programs, modules and data structures, or a subset thereof, sometimes in conjunction with the persistent memory 112:

- an optional operating system 116, which includes procedures for handling various basic system services and for performing hardware dependent tasks;
- an optional network communication module (or instructions) 118 for connecting the system 100 with other devices and/or a communication network;
- a test subject data store 120 for storing datasets containing biological information and/or personal characteristics about test subjects, including sequencing data 122, nucleic acid features 124, personal characteristics 126, and/or organoid sensitivities 128;
- an optional classifier training module 130 for training classifiers, e.g., to distinguish between disease states or predicted treatment outcomes, for example, based on information learned from studies of patient-based organoid cultures;
- an optional disease classification module 130 for classifying the cancer status of a subject based on test subject data, e.g., sequencing data 122, nucleic acid features 124, personal characteristics 126, and/or patient-derived organoid sensitivities 128 stored in test subject data store 120;
- an optional drug classification module 150 for classifying a utility of a therapeutic compound based on organoid-derived sensitivities to the therapeutic compound; and

- an optional patient reporting module 160 for generating reports about the disease (e.g., cancer) status of a test subject.

[0069] In various implementations, one or more of the above identified elements are stored in one or more of the previously mentioned memory devices, and correspond to a set of instructions for performing a function described above. The above identified modules, data, or programs (e.g., sets of instructions) need not be implemented as separate software programs, procedures, datasets, or modules, and thus various subsets of these modules and data may be combined or otherwise re-arranged in various implementations.

[0070] In some implementations, the non-persistent memory 111 optionally stores a subset of the modules and data structures identified above. Furthermore, in some embodiments, the memory stores additional modules and data structures not described above. In some embodiments, one or more of the above identified elements is stored in a computer system, other than that of system 100, that is addressable by system 100 so that system 100 may retrieve all or a portion of such data when needed.

[0071] Although Figure 1 depicts a “system 100,” the figure is intended more as functional description of the various features which may be present in one or more computer systems than as a structural schematic of the implementations described herein. In practice, and as recognized by those of ordinary skill in the art, items shown separately could be combined and some items could be separated. Moreover, although Figure 1 depicts certain data and modules in non-persistent memory 111, some or all of these data and modules may be in persistent memory 112.

[0072] For instance, as depicted in Figure 2, in some embodiments the methods described herein are performed across a distributed diagnostic environment 210, e.g., connected via communication network 212. In some embodiments, one or more biological sample, e.g., one or more tumor biopsy or control sample, is collected from a subject in clinical environment 220, e.g., a doctor’s office, hospital, or medical clinic. In some embodiments, a portion of the sample is processed within the clinical environment using a processing device 224, e.g., a nucleic acid sequencer for obtaining sequencing data, a microscope for obtaining pathology data, a mass spectrometer for obtaining proteomic data, etc. In some embodiments, the biological sample or a portion of the biological sample is sent to one or more external environments, e.g., sequencing lab 230, pathology lab 240, and molecular biology lab 250, each of which includes a processing

device 234, 244, and 254, respectively, to generate biological data about the subject. Each environment includes a communications device 222, 232, 242, and 252, respectively, for communicating biological data about the subject to a processing server 262 and/or database 264, which may be located in yet another environment, e.g., processing/storage center 260. Thus, in some embodiments, different portions of the systems and methods described herein are fulfilled by different processing devices located in different physical environments.

[0073] Classifiers

[0074] In some embodiments, the methods described herein use biological features, e.g., genomic features, of a subject and/or cultured organoid, e.g., a patient-specific tumor organoid, to classify a condition, e.g., cancer, predicted an effect of a particular therapy, etc. Generally, any classifier architecture can be trained for these purposes. Non-limiting examples of classifier types that can be used in conjunction with the methods described herein include a machine learning algorithm, a neural network algorithm, a support vector machine algorithm, a decision tree algorithm, an unsupervised clustering model algorithm, a supervised clustering model algorithm, or a regression model. In some embodiments, the trained classifier is binomial or multinomial.

[0075] In some embodiments, the classifier is implemented as an artificial intelligence engine and may include gradient boosting models, random forest models, neural networks (NN), regression models, Naive Bayes models, and/or machine learning algorithms (MLA). MLAs include supervised algorithms (such as algorithms where the features/classifications in the data set are annotated) using linear regression, logistic regression, decision trees, classification and regression trees, naïve Bayes, nearest neighbor clustering; unsupervised algorithms (such as algorithms where no features/classification in the data set are annotated) using apriori, means clustering, principal component analysis, random forest, adaptive boosting; and semi-supervised algorithms (such as algorithms where an incomplete number of features/classifications in the data set are annotated) using generative approach (such as a mixture of Gaussian distributions, mixture of multinomial distributions, hidden Markov models), low density separation, graph-based approaches (such as mincut, harmonic function, manifold regularization), heuristic approaches, or support vector machines.

[0076] While MLA and neural networks identify distinct approaches to machine learning, the terms may be used interchangeably herein. Thus, a mention of MLA may include a corresponding NN or a mention of NN may include a corresponding MLA unless explicitly stated otherwise. Training may include providing optimized datasets, labeling these traits as they occur in patient records, and training the MLA to predict or classify based on new inputs. Artificial NNs are efficient computing models which have shown their strengths in solving hard problems in artificial intelligence. They have also been shown to be universal approximators, that is, they can represent a wide variety of functions when given appropriate parameters.

[0077] Neural network (NN) algorithms, including convolutional neural network algorithms, that can serve as the classifier for the instant methods are disclosed in See, Vincent *et al.*, 2010, “Stacked denoising autoencoders: Learning useful representations in a deep network with a local denoising criterion,” J Mach Learn Res 11, pp. 3371-3408; Larochelle *et al.*, 2009, “Exploring strategies for training deep neural networks,” J Mach Learn Res 10, pp. 1-40; and Hassoun, 1995, *Fundamentals of Artificial Neural Networks*, Massachusetts Institute of Technology, each of which is hereby incorporated by reference. NNs include conditional random fields, convolutional neural networks, attention based neural networks, deep learning, long short term memory networks, or other neural models.

[0078] Example logistic regression algorithms are disclosed in Agresti, *An Introduction to Categorical Data Analysis*, 1996, Chapter 5, pp. 103-144, John Wiley & Son, New York, which is hereby incorporated by reference.

[0079] Support vector machine (SVM) algorithms that can serve as the classifier for the instant methods are described in Cristianini and Shawe-Taylor, 2000, “An Introduction to Support Vector Machines,” Cambridge University Press, Cambridge; Boser *et al.*, 1992, “A training algorithm for optimal margin classifiers,” in Proceedings of the 5th Annual ACM Workshop on Computational Learning Theory, ACM Press, Pittsburgh, Pa., pp. 142-152; Vapnik, 1998, *Statistical Learning Theory*, Wiley, New York; Mount, 2001, *Bioinformatics: sequence and genome analysis*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Duda, *Pattern Classification*, Second Edition, 2001, John Wiley & Sons, Inc., pp. 259, 262-265; and Hastie, 2001, *The Elements of Statistical Learning*, Springer, New York; and Furey *et al.*, 2000, *Bioinformatics* 16, 906-914, each of which is hereby incorporated by reference in its

entirety. When used for classification, SVMs separate a given set of binary-labeled data training set with a hyper-plane that is maximally distant from the labeled data. For cases in which no linear separation is possible, SVMs can work in combination with the technique of `kernels`, which automatically realizes a non-linear mapping to a feature space. The hyper-plane found by the SVM in feature space corresponds to a non-linear decision boundary in the input space.

[0080] Decision trees (*e.g.*, random forest, boosted trees) that can serve as the classifier for the instant methods are described generally by Duda, 2001, *Pattern Classification*, John Wiley & Sons, Inc., New York, pp. 395-396, which is hereby incorporated by reference. Tree-based methods partition the feature space into a set of rectangles, and then fit a model (like a constant) in each one. In some embodiments, the decision tree is random forest regression. One specific algorithm that can serve as the classifier for the instant methods is a classification and regression tree (CART). Other specific decision tree algorithms that can serve as the classifier for the instant methods include, but are not limited to, ID3, C4.5, MART, and Random Forests. CART, ID3, and C4.5 are described in Duda, 2001, *Pattern Classification*, John Wiley & Sons, Inc., New York, pp. 396-408 and pp. 411-412, which is hereby incorporated by reference. CART, MART, and C4.5 are described in Hastie *et al.*, 2001, *The Elements of Statistical Learning*, Springer-Verlag, New York, Chapter 9, which is hereby incorporated by reference in its entirety. Random Forests are described in Breiman, 1999, "Random Forests--Random Features," Technical Report 567, Statistics Department, U.C. Berkeley, September 1999, which is hereby incorporated by reference in its entirety.

[0081] Biological Samples

[0082] In some embodiments, one or more biological samples collected from a subject is a solid tissue sample, *e.g.*, a solid tumor sample or a solid normal tissue sample. Methods for obtaining solid tissue samples, *e.g.*, of cancerous and/or normal tissue are known in the art, and are dependent upon the type of tissue being sampled. For example, bone marrow biopsies and isolation of circulating tumor cells can be used to obtain samples of blood cancers, endoscopic biopsies can be used to obtain samples of cancers of the digestive tract, bladder, and lungs, needle biopsies (*e.g.*, fine-needle aspiration, core needle aspiration, vacuum-assisted biopsy, and image-guided biopsy, can be used to obtain samples of subdermal tumors, skin biopsies, *e.g.*, shave biopsy, punch biopsy, incisional biopsy, and excisional biopsy, can be used to obtain

samples of dermal cancers, and surgical biopsies can be used to obtain samples of cancers affecting internal organs of a patient. In some embodiments, a solid tissue sample is a formalin-fixed tissue (FFT). In some embodiments, a solid tissue sample is a macro-dissected formalin fixed paraffin embedded (FFPE) tissue. In some embodiments, a solid tissue sample is a fresh frozen tissue sample.

[0083] In some embodiments, one or more of the biological samples collected from a subject is a liquid biological sample, also referred to as a liquid biopsy sample. In some embodiments, one or more of the biological samples obtained from the patient are selected from blood, plasma, serum, urine, vaginal fluid, fluid from a hydrocele (*e.g.*, of the testis), vaginal flushing fluids, pleural fluid, ascitic fluid, cerebrospinal fluid, saliva, sweat, tears, sputum, bronchoalveolar lavage fluid, discharge fluid from the nipple, aspiration fluid from different parts of the body (*e.g.*, thyroid, breast), *etc.* In some embodiments, the liquid biopsy sample includes blood and/or saliva. In some embodiments, the liquid biopsy sample is peripheral blood. In some embodiments, blood samples are collected from patients in commercial blood collection containers, *e.g.*, using a PAXgene® Blood DNA Tubes. In some embodiments, saliva samples are collected from patients in commercial saliva collection containers, *e.g.*, using an Oragene® DNA Saliva Kit.

[0084] Liquid biopsy samples include cell free nucleic acids, including cell-free DNA (cfDNA). As described above, cfDNA isolated from cancer patients includes DNA originating from cancerous cells, also referred to as circulating tumor DNA (ctDNA), cfDNA originating from germline (*e.g.*, healthy or non-cancerous) cells, and cfDNA originating from hematopoietic cells (*e.g.*, white blood cells). The relative proportions of cancerous and non-cancerous cfDNA present in a liquid biopsy sample varies depending on the characteristics (*e.g.*, the type, stage, lineage, genomic profile, *etc.*) of the patient's cancer.

[0085] cfDNA is a particularly useful source of biological data for various implementations of the methods and systems described herein, because it is readily obtained from various body fluids. Advantageously, use of bodily fluids facilitates serial monitoring because of the ease of collection, as these fluids are collectable by non-invasive or minimally-invasive methodologies. This is in contrast to methods that rely upon solid tissue samples, such as biopsies, which often times require invasive surgical procedures. Further, because bodily fluids, such as blood,

circulate throughout the body, the cfDNA population represents a sampling of many different tissue types from many different locations.

[0086] In some embodiments, a liquid biopsy sample is separated into two different samples. For example in some embodiments, a blood sample is separated into a blood plasma sample, containing cfDNA, and a buffy coat preparation, containing white blood cells.

[0087] In some embodiments, a dedicated normal sample is also collected from a subject, for co-processing with a solid or liquid cancer sample. Generally, the normal sample is of a non-cancerous tissue, and can be collected using any tissue collection means described above. In some embodiments, buccal cells collected from the inside of a patient's cheeks are used as a normal sample. Buccal cells can be collected by placing an absorbent material, *e.g.*, a swab, in the subjects mouth and rubbing it against their cheek, *e.g.*, for at least 15 second or for at least 30 seconds. The swab is then removed from the patient's mouth and inserted into a tube, such that the tip of the tube is submerged into a liquid that serves to extract the buccal cells off of the absorbent material. An example of buccal cell recovery and collection devices is provided in U.S. Patent No. 9,138,205, the content of which is hereby incorporated by reference, in its entirety, for all purposes. In some embodiments, the buccal swab DNA is used as a source of normal DNA in circulating heme malignancies.

[0088] The biological samples collected from the patient are, optionally, sent to various analytical environments (*e.g.*, sequencing lab 230, pathology lab 240, and/or molecular biology lab 250) for processing (*e.g.*, data collection) and/or analysis (*e.g.*, feature extraction). Wet lab processing 204 may include cataloguing samples (*e.g.*, accessioning), examining clinical features of one or more samples (*e.g.*, pathology review), and nucleic acid sequence analysis (*e.g.*, extraction, library prep, capture + hybridize, pooling, and sequencing). In some embodiments, the workflow includes clinical analysis of one or more biological samples collected from the subject, *e.g.*, at a pathology lab 240 and/or a molecular and cellular biology lab 250, to generate clinical features such as pathology features 128-3, imaging data 128-3, and/or tissue culture / organoid data 128-3.

[0089] Feature Extraction

[0090] In some embodiments, tissue culture / organoid data includes features identified by evaluation of cultured tissue from the subject. For instance, in some embodiments, tissue

samples obtained from the patients (*e.g.*, tumor tissue, normal tissue, or both) are cultured (*e.g.*, in liquid culture, solid-phase culture, and/or organoid culture) and various features, such as cell morphology, growth characteristics, genomic alterations, and/or drug sensitivity, are evaluated. In some embodiments, tissue culture / organoid data 128-3 includes features determined using machine learning algorithms to evaluate tissue culture / organoid data collected as described herein. Genetic sequencing used to generate the features used in the methods described herein may be done on either a tumor organoid, *e.g.*, cultured from cells of a test subject, or the source tissue (patient biopsy used to generate the TO), *e.g.*, to determine the tumor organoid's (*e.g.*, the subject's cancer) BRCA1/2 mutation status, loss of heterozygosity, bi-allelic loss status, etc.

[0091] In some embodiments of the methods described herein, nucleic acid sequencing of one or more samples, *e.g.*, a biological sample from a subject and/or a tissue culture / organoid sample, is performed. Briefly, nucleic acids, *e.g.*, RNA and/or DNA are extracted (304) from the one or more biological samples. Methods for isolating nucleic acids from biological samples are known in the art, and are dependent upon the type of nucleic acid being isolated (*e.g.*, cfDNA, DNA, and/or RNA) and the type of sample from which the nucleic acids are being isolated (*e.g.*, liquid biopsy samples, white blood cell buffy coat preparations, formalin-fixed paraffin-embedded (FFPE) solid tissue samples, and fresh frozen solid tissue samples). The selection of any particular nucleic acid isolation technique for use in conjunction with the embodiments described herein is well within the skill of the person having ordinary skill in the art, who will consider the sample type, the state of the sample, the type of nucleic acid being sequenced and the sequencing technology being used.

[0092] For instance, many techniques for DNA isolation, *e.g.*, genomic DNA isolation, from a tissue sample are known in the art, such as organic extraction, silica adsorption, and anion exchange chromatography. Likewise, many techniques for RNA isolation, *e.g.*, mRNA isolation, from a tissue sample are known in the art. For example, acid guanidinium thiocyanate-phenol-chloroform extraction (see, for example, Chomczynski and Sacchi, 2006, Nat Protoc, 1(2):581-85, which is hereby incorporated by reference herein), and silica bead/glass fiber adsorption (see, for example, Poeckh, T. *et al.*, 2008, Anal Biochem., 373(2):253-62, which is hereby incorporated by reference herein). The selection of any particular DNA or RNA isolation technique for use in conjunction with the embodiments described herein is well within the skill of the person having ordinary skill in the art, who will consider the tissue type, the state of the

tissue, *e.g.*, fresh, frozen, formalin-fixed, paraffin-embedded (FFPE), and the type of nucleic acid analysis that is to be performed.

[0093] In some embodiments, a nucleic acid library is prepared from the isolated nucleic acids (*e.g.*, cfDNA, DNA, and/or RNA). For example, in some embodiments, DNA libraries (*e.g.*, gDNA and/or cfDNA libraries) are prepared from isolated DNA from the one or more biological samples. In some embodiments, the DNA libraries are prepared using a commercial library preparation kit, *e.g.*, the KAPA Hyper Prep Kit, a New England Biolabs (NEB) kit, or a similar kit.

[0094] In some embodiments, during library preparation, adapters (*e.g.*, UDI adapters, such as Roche SeqCap dual end adapters, or UMI adapters such as full length or stubby Y adapters) are ligated onto the nucleic acid molecules. In some embodiments, the adapters include unique molecular identifiers (UMIs), which are short nucleic acid sequences (*e.g.*, 3-10 base pairs) that are added to ends of DNA fragments during adapter ligation. In some embodiments, UMIs are degenerate base pairs that serve as a unique tag that can be used to identify sequence reads originating from a specific DNA fragment. In some embodiments, *e.g.*, when multiplex sequencing will be used to sequence DNA from a plurality of samples (*e.g.*, from the same or different subjects) in a single sequencing reaction, a patient-specific index is also added to the nucleic acid molecules. In some embodiments, the patient specific index is a short nucleic acid sequence (*e.g.*, 3-20 nucleotides) that are added to ends of DNA fragments during library construction, that serve as a unique tag that can be used to identify sequence reads originating from a specific patient sample. Examples of identifier sequences are described, for example, in Kivioja et al., Nat. Methods 9(1):72-74 (2011) and Islam et al., Nat. Methods 11(2):163-66 (2014), the contents of which are hereby incorporated by reference, in their entireties, for all purposes.

[0095] In some embodiments, an adapter includes a PCR primer landing site, designed for efficient binding of a PCR or second-strand synthesis primer used during the sequencing reaction. In some embodiments, an adapter includes an anchor binding site, to facilitate binding of the DNA molecule to anchor oligonucleotide molecules on a sequencer flow cell, serving as a seed for the sequencing process by providing a starting point for the sequencing reaction. During PCR amplification following adapter ligation, the UMIs, patient indexes, and binding sites are

replicated along with the attached DNA fragment. This provides a way to identify sequence reads that came from the same original fragment in downstream analysis.

[0096] In some embodiments, a sequencing library, or pool of sequencing libraries, is enriched for target nucleic acids, *e.g.*, nucleic acids encompassing loci that are informative for precision oncology and/or used as internal controls for sequencing or bioinformatics processes. In some embodiments, enrichment is achieved by hybridizing target nucleic acids in the sequencing library to probes that hybridize to the target sequences, and then isolating the captured nucleic acids away from off-target nucleic acids that are not bound by the capture probes.

[0097] In some embodiments, *e.g.*, where a whole genome sequencing method will be used, nucleic acid sequencing libraries are not target-enriched prior to sequencing, in order to obtain sequencing data on substantially all of the competent nucleic acids in the sequencing library. Similarly, in some embodiments, *e.g.*, where a whole genome sequencing method will be used, nucleic acid sequencing libraries are not mixed, because of bandwidth limitations related to obtaining significant sequencing depth across an entire genome. However, in other embodiments, *e.g.*, where a low pass whole genome sequencing (LPWGS) methodology will be used, nucleic acid sequencing libraries can still be pooled, because very low average sequencing coverage is achieved across a respective genome, *e.g.*, between about 0.5x and about 5x.

[0098] In some embodiments, the probe set includes probes targeting one or more gene loci, *e.g.*, exon or intron loci. In some embodiments, the probe set includes probes targeting one or more loci not encoding a protein, *e.g.*, regulatory loci, miRNA loci, and other non-coding loci, *e.g.*, that have been found to be associated with cancer. In some embodiments, the plurality of loci include at least 25, 50, 100, 150, 200, 250, 300, 350, 400, 500, 750, 1000, 2500, 5000, or more human genomic loci. In some embodiments, the gene panel is a whole-exome panel that analyzes the exomes of a biological sample. In some embodiments, the gene panel is a whole-genome panel that analyzes the genome of a specimen.

[0099] Sequence reads are then generated from the sequencing library or pool of sequencing libraries. Sequencing data may be acquired by any methodology known in the art. For example, next generation sequencing (NGS) techniques such as sequencing-by-synthesis technology (Illumina), pyrosequencing (454 Life Sciences), ion semiconductor technology (Ion Torrent

sequencing), single-molecule real-time sequencing (Pacific Biosciences), sequencing by ligation (SOLiD sequencing), nanopore sequencing (Oxford Nanopore Technologies), or paired-end sequencing. In some embodiments, massively parallel sequencing is performed using sequencing-by-synthesis with reversible dye terminators. In some embodiments, sequencing is performed using next generation sequencing technologies, such as short-read technologies. In other embodiments, long-read sequencing or another sequencing method known in the art is used.

[00100] In some embodiments, the methods described herein include one or more of obtaining a biological sample, extracting nucleic acids from the biological sample, and sequencing the isolated nucleic acids. In other embodiments, sequencing data or feature data sets obtained therefrom used in the improved systems and methods described herein are obtained by receiving previously generated sequence reads or feature data sets, in electronic form.

[00101] In some embodiments, the methods and systems described herein use additional features to perform the various classifications provided herein. Non-limiting examples of other characteristics that may be used for these purposes include tumor organoid histology, and baseline histology (H&E image; features; see also Patent Application Nos. 62/787,047 and 62/824,039); clinical data (gender, age, family medical history, personal history of cancer, treatment history of cancer, diagnosis, subtype, IHC markers, DNA/RNA sequence); methylation sequence; radiology images/features; comorbidities, geographic location, ancestry, ethnic features, diet, gut microbiome, substance usage, alcohol use, physical exam findings, baseline vitals, vaccination history, religious or cultural beliefs, marital status, sexual orientation, nocturnal/diurnal rhythm, sleep schedule, travel history (including airplane travel), travel to certain regions, presence/absence of parasitic organisms, infectious disease history (including parasitic organisms, viruses, bacteria), germline DNA sequence, biometric features (like distance between eyes, bones), skull shape, physical profile, occupational history, living with pets (including birds), mental health; tumor organoid morphology; viability; and smoking status.

[00102] Evaluation of BRCA1-/BRCA2- cancers for PARPi treatment using tumor organoid cultures

[00103] In one aspect, the disclosure provides methods and systems for performing methods of evaluating a cancer that does not carry either a BRCA1 or a BRCA2 mutation in a test subject

by determining a sensitivity of the cancer to a poly ADP ribose polymerase (PARP) inhibitor. The method includes culturing a plurality of tumor organoids, each respective tumor organoid in the plurality of tumor organoids from one or more cells of a tumor biopsy from the test subject. The method then includes exposing the one or more cultured organoids to one or more amounts of the PARP inhibitor. The method then includes measuring the fitness of cells in the one or more tumor organoids following the exposure to the one or more amounts of the PARP inhibitor. The method then includes correlating the measured fitness of the cells with a sensitivity to the PARP inhibitor.

[00104] In some embodiments, the method also includes providing a recommendation for treatment of the subject. For example, in some embodiments, when the correlated sensitivity to the PARP inhibitor satisfies a sensitivity threshold, the method includes communicating a recommended therapy comprising the PARP inhibitor to the test subject, and when the correlated sensitivity to the PARP inhibitor does not satisfy a sensitivity threshold, the method includes communicating a recommended therapy that does not include the PARP inhibitor to the test subject.

[00105] In some embodiments, the method also includes prescribing therapy for treating the cancer in the subject. For example, in some embodiments, when the correlated sensitivity to the PARP inhibitor satisfies a sensitivity threshold, the method includes prescribing a therapy including the PARP inhibitor to the test subject, and when the correlated sensitivity to the PARP inhibitor does not satisfy a sensitivity threshold, the method includes prescribing a therapy that does not include the PARP inhibitor to the test subject.

[00106] In some embodiments, the method also includes treating the cancer in the subject. For example, in some embodiments, when the correlated sensitivity to the PARP inhibitor satisfies a sensitivity threshold, the method includes administering a therapy including the PARP inhibitor to the test subject, and when the correlated sensitivity to the PARP inhibitor does not satisfy a sensitivity threshold, the method includes administering a therapy that does not include the PARP inhibitor to the test subject.

[00107] In some embodiments, the cancer is a breast cancer. In some embodiments, the cancer is an ovarian cancer. In some embodiments, the cancer is not breast cancer or ovarian cancer. In some embodiments, the test subject has not previously been treated for cancer. That

is, in some embodiments, the PARP inhibitor is administered as a first-line therapy. In some embodiments, the test subject has been previously treated for the cancer. That is, in some embodiments, the PARP inhibitor is administered as a second-line therapy. In some embodiments, the PARP inhibitor is olaparib, veliparib, rucaparib, niraparib, or talazoparib.

[00108] Training a classifier to evaluate PARPi sensitivity

[00109] In one aspect, the disclosure provides methods and systems for performing methods of training a classifier to discriminate between two or more tumor sensitivities to a poly ADP ribose polymerase (PARP) inhibitor. In some embodiments, the method includes obtaining a data set comprising, for each respective tissue sample in a plurality of tissue samples, (i) a corresponding plurality of nucleic acid features of the tissue sample, and (ii) a corresponding indication of the sensitivity of a respective organoid cultured from one or more cells of the tissue sample to the PARP inhibitor. The method then includes training an untrained classifier against at least (i) the corresponding plurality of features and (ii) the corresponding indication of the sensitivity of the organoid to the PARP inhibitor, across the plurality of tissue samples, thereby obtaining a trained classifier that discriminates between the two or more tumor sensitivities to a poly ADP ribose polymerase (PARP) inhibitor.

[00110] In some embodiments, the two or more tumor sensitivities include a first level of sensitivity to a first PARP inhibitor and a second level of sensitivity to the first PARP inhibitor. In some embodiments, the two or more tumor sensitivities include a first sensitivity to a first PARP inhibitor and a second sensitivity to a second PARP inhibitor.

[00111] In some embodiments, the PARP inhibitor is olaparib, veliparib, rucaparib, niraparib, or talazoparib.

[00112] In some embodiments, the classifier is further trained to discriminate between the two or more tumor sensitivities to a PARP inhibitor and one or more tumor sensitivity to a chemotherapeutic drug that is not a PARP inhibitor.

[00113] In some embodiments, each tissue sample in the plurality of tissue samples was obtained from a subject that had not received treatment for cancer prior to the tissue sample being obtained. In some embodiments, each tissue sample in the plurality of tissue samples is a breast cancer biopsy. In some embodiments, each tissue sample in the plurality of tissue samples

is an ovarian cancer biopsy. In some embodiments, each tissue sample in the plurality of tissue samples is a biopsy of a homologous recombination deficient (HRD) cancer. In some embodiments, each tissue sample in the plurality of tissue samples is a biopsy from a cancer other than breast cancer or ovarian cancer.

[00114] In some embodiments, the plurality of nucleic acid features of the tissue sample includes one or more of support for a single nucleotide variant at a genomic location, a methylation status at a genomic location, a relative copy number for a genomic location, an allelic ratio for a genomic location, a relative expression level of a gene, and mathematical combinations thereof. In some embodiments, the plurality of nucleic acid features of the tissue sample comprises a BRCA1 or BRCA2 mutant allele status. In some embodiments, at least a sub-plurality of the plurality of nucleic acid features of the tissue sample are measured from a tumor organoid cultured from one or more cells of the tissue sample.

[00115] In some embodiments, the indication of the sensitivity of the respective organoid to the PARP inhibitor is based at least in part on one or more cellular fitness measurements obtained by: i) culturing one or more organoids, each respective organoid in the one or more organoids from one or more cells of the respective tissue sample, ii) exposing the one or more organoids cultured in i) to one or more amounts of the PARP inhibitor, and iii) measuring the fitness of cells, e.g., using a cellular viability assay or cell death assay, in the one or more organoids following the exposure to the one or more amounts of the PARP inhibitor, thereby obtaining the one or more cellular fitness measurements.

[00116] In some embodiments, the one or more cellular fitness measurements are one or more measurements of cellular apoptosis following exposure to the PARP inhibitor.

[00117] In some embodiments, for at least one respective tissue sample in the plurality of tissue samples, the one or more cultured organoids are exposed to a sensitizing therapy, e.g., radiation or a different chemotherapeutic drug, before being exposed to the PARP inhibitor.

[00118] In some embodiments, the untrained classifier is further trained against, for each respective tissue sample in the plurality of tissue samples, a corresponding cancer classification of the tissue sample in a plurality of cancer classifications. In some embodiments, the plurality of cancer classifications includes cancerous tissue and non-cancerous tissue. In some

embodiments, the plurality of cancer classifications includes a plurality of types of cancer. In some embodiments, the plurality of cancer classifications includes a plurality of stages of cancer.

[00119] In some embodiments, the plurality of tissue samples includes a plurality of tumor biopsies, and the classifier training includes identifying a plurality of genes that are differentially expressed between (i) organoids, cultured from one or more cells of a respective tumor biopsy in the plurality of tumor biopsies, that have a first sensitivity to the PARP inhibitor, e.g., organoids that experience at least a threshold level of cell death upon exposure of the organoid to the PARP inhibitor, and (ii) organoids, cultured from one or more cells of a respective tumor biopsy in the plurality of tumor biopsies, that have a second sensitivity to the PARP inhibitor, e.g., organoids that experience less than a threshold level of cell death upon exposure of the organoid to the PARP inhibitor.

[00120] In some embodiments, the classifier is further trained against, for each respective tissue sample in the plurality of tissue samples, one or more phenotypic characteristics of the tissue sample. In some embodiments, the one or more phenotypic characteristics of the tissue sample include a histologic feature of the tissue sample.

[00121] In some embodiments, the classifier is further trained against, for each respective tissue sample in the plurality of tissue samples, one or more characteristics of the subject from which the tissue sample was obtained, e.g., as described above.

[00122] In some embodiments, the classifier is a multinomial classifier that is trained to provide a plurality of likelihoods, wherein each respective likelihood in the plurality of likelihoods is a likelihood that tumor cells from a test subject with cancer will have a different respective sensitivity in the two or more tumor sensitivities to a PARP inhibitor. In some embodiments, the classifier is a neural network algorithm, a support vector machine algorithm, a Naive Bayes algorithm, a nearest neighbor algorithm, a boosted trees algorithm, a random forest algorithm, a convolutional neural network algorithm, a decision tree algorithm, a regression algorithm, or a clustering algorithm.

[00123] Diagnosing PARPi sensitivity

[00124] In some embodiments, the disclosure provides method and systems for performing methods of recommending therapy for treating a cancer in a subject. In some embodiments, the

method includes obtaining a first test data set comprising a plurality of nucleic acid features of a tumor biopsy from the test subject. The method then includes evaluating the first test data set using a classifier trained to discriminate between two or more tumor sensitivities to a first poly ADP ribose polymerase (PARP) inhibitor. The classifier is trained against, for each respective training tissue sample in a plurality of training tissue samples, at least (i) the plurality of nucleic acid features obtained from the respective training tissue sample, and (ii) a corresponding indication of the sensitivity of a respective organoid cultured from one or more cells of the respective training tissue sample to the first PARP inhibitor. In some embodiments, the sensitivity of the training tissue sample is determined in a two-dimensional cell culture, a three-dimensional cell culture prepared in a matrix, a three-dimensional cell culture prepared in suspension culture, a cell culture suspension, or a xenograft model. A first tumor sensitivity in the two or more tumor sensitivities to the first PARP inhibitor is associated with a first recommendation, in a plurality of recommendations, to treat the cancer in the subject with the first PARP inhibitor. A second tumor sensitivity in the two or more tumor sensitivities to the first PARP inhibitor is associated with a second recommendation, in the plurality of recommendations, not to treat the cancer in the subject with the first PARP inhibitor. In some embodiments, the method includes providing a respective recommendation, in the plurality of recommendations, for treating the cancer in the test subject based on the results of the evaluation.

[00125] In some embodiments, the cancer is a breast cancer. In some embodiments, the cancer is an ovarian cancer. In some embodiments, the cancer is not breast cancer or ovarian cancer. In some embodiments, the test subject has not previously been treated for cancer. That is, in some embodiments, the PARP inhibitor is administered as a first-line therapy. In some embodiments, the test subject has been previously treated for the cancer. That is, in some embodiments, the PARP inhibitor is administered as a second-line therapy. In some embodiments, the PARP inhibitor is olaparib, veliparib, rucaparib, niraparib, or talazoparib.

[00126] In some embodiments, the plurality of nucleic acid features of the tumor biopsy comprise one or more of support for a single nucleotide variant at a genomic location, a methylation status at a genomic location, a relative copy number for a genomic location, an allelic ratio for a genomic location, a relative expression level of a gene, and mathematical combinations thereof. In some embodiments, the plurality of nucleic acid features of the tumor biopsy comprises a BRCA1 or BRCA2 mutant allele status.

[00127] In some embodiments, the first test data set further comprises a cancer status of the test subject in a plurality of cancer statuses, the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, a cancer status of the respective training tissue sample in the plurality of cancer statuses.

[00128] In some embodiments, the plurality of cancer statuses comprises a plurality of types of cancer. In some embodiments, the plurality of cancer statuses comprises a plurality of stages of cancer.

[00129] In some embodiments, the first test data set further comprises one or more phenotypic characteristics of the tumor biopsy, and the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, the one or more phenotypic characteristics of the respective training tissue sample. In some embodiments, the one or more phenotypic characteristics of the tumor biopsy comprise a histologic feature of the tumor biopsy.

[00130] In some embodiments, the first test data set further comprises one or more characteristics of the subject, as described in the feature selection section above, and the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, the one or more characteristics of the subject from which the respective training tissue sample was obtained.

[00131] In some embodiments, the method also includes obtaining a second test data set comprising the plurality of nucleic acid features of a tumor biopsy from the test subject, and evaluating the second test data set using the classifier trained to discriminate between two or more tumor sensitivities to a first poly ADP ribose polymerase (PARP) inhibitor, where the plurality of nucleic acid features in the first test data set are from a first tumor biopsy from the test subject and the second test data set are from a second tumor biopsy from the test subject that is different from the first tumor biopsy. In some embodiments, the first tumor biopsy and the second tumor biopsy are from different sections of a same tumor of the subject. In some embodiments, the first tumor biopsy and the second tumor biopsy are from different tumors of the subject.

[00132] In some embodiments, the classifier is a multinomial classifier that is trained to provide a plurality of likelihoods, wherein each respective likelihood in the plurality of

likelihoods is a likelihood that a different respective treatment regime, in a plurality of treatment regimes, for the cancer in the subject will be more effective than the other treatment regimes in the plurality of treatment regimes.

[00133] In some embodiments, the classifier is a neural network algorithm, a support vector machine algorithm, a Naive Bayes algorithm, a nearest neighbor algorithm, a boosted trees algorithm, a random forest algorithm, a convolutional neural network algorithm, a decision tree algorithm, a regression algorithm, or a clustering algorithm.

[00134] In some embodiments, first PARP inhibitor is selected from the group consisting of olaparib, veliparib, rucaparib, niraparib, and talazoparib. In some embodiments, the classifier is trained to further discriminate between two or more tumor sensitivities to a second PARP inhibitor, wherein the second PARP inhibitor is different than the first PARP inhibitor. In some embodiments, the second PARP inhibitor is selected from the group consisting of olaparib, veliparib, rucaparib, niraparib, and talazoparib. In some embodiments, the classifier is trained to further discriminate between two or more tumor sensitivities to a chemotherapeutic drug that is not a PARP inhibitor.

[00135] In some embodiments, the first recommendation is a recommendation to treat the cancer in the subject with the first PARP inhibitor as a first-line therapy. In some embodiments, the first recommendation is a recommendation to treat the cancer in the subject with the first PARP inhibitor as a second-line therapy.

[00136] In some embodiments, the first recommendation is a recommendation to treat the cancer in the subject with the first PARP inhibitor and without any other chemotherapeutic drug. In some embodiments, after providing, or instead of providing, the first recommendation for treating the cancer in the subject, the method includes administering the first PARP inhibitor to the test subject.

[00137] In some embodiments, the first recommendation is a recommendation to treat the cancer in the subject with the first PARP inhibitor and with a second cancer therapy, e.g., radiation, surgery, a second PARP inhibitor, or a chemotherapeutic drug that is not a PARP inhibitor. In some embodiments, after providing, or instead of providing, the first recommendation for treating the cancer in the subject, the method includes co-administering the

first PARP inhibitor and the second cancer therapy to the test subject. In some embodiments, the second cancer therapy is administration of a chemotherapeutic drug that is not a PARP inhibitor.

[00138] In some embodiments, the second recommendation is a recommendation to treat the cancer in the subject with a second PARP inhibitor. In some embodiments, after providing, or instead of providing, the second recommendation for treating the cancer in the subject, the method includes administering the second PARP inhibitor to the test subject.

[00139] In some embodiments, the plurality of recommendations further comprises a third recommendation to treat the cancer in the subject with a chemotherapeutic drug that is not a PARP inhibitor. In some embodiments, after providing, or instead of providing, the third recommendation for treating the cancer in the subject, the method includes administering the chemotherapeutic drug that is not a PARP inhibitor to the test subject.

[00140] In some embodiments, the second recommendation is a recommendation to treat the cancer in the subject with a therapeutic drug that is not a PARP inhibitor. In some embodiments, after providing, or instead of providing, the second recommendation for treating the cancer in the subject, the method includes administering the chemotherapeutic drug that is not a PARP inhibitor to the test subject.

[00141] In some embodiments, the first recommendation is a recommendation to treat the cancer in the subject with the first PARP inhibitor at a first therapeutic dose, and the plurality of recommendations further comprises a fourth recommendation to treat the cancer in the subject with the first PARP inhibitor at a second therapeutic dose.

[00142] In some embodiments, prior to providing the respective recommendation, the method includes identifying at least two recommendations, in the plurality of recommendations, based on the results of the evaluation, e.g., that are associated with better likelihoods that their associated treatment regimens will be more effective than treatment regimens associated with other recommendations, and determining a sensitivity of the cancer in the subject to each respective therapy regimen, in a plurality of therapy regimens, associated with the at least two recommendations by: a) culturing a plurality of tumor organoids, each respective tumor organoid in the plurality of tumor organoids from one or more cells of a tumor biopsy from the subject, b) exposing the one or more organoids cultured in a) to one or more amounts of a therapeutic agent associated with the respective therapy regimen, and c) measuring the fitness of cells, e.g., using a

cellular viability assay or cell death assay, in the one or more organoids following the exposure to the one or more amounts of the therapeutic agent, thereby obtaining one or more cellular fitness measurements for each respective therapy regimen associated with the two or more recommendations. In this embodiment, the therapeutic recommendation and/or treatment is selected from the two or more recommendations and is based on the one or more cellular fitness measurements obtained for each respective therapy regimen associated with the two or more recommendations.

[00143] In some embodiments, the methods described herein include administering the recommended therapy for treating cancer to the test subject.

[00144] Treatment of BRCA1-/2- cancers with PARPi

[00145] In some embodiments, the disclosure provides methods and systems for performing methods of treating a cancer other than breast cancer or ovarian cancer in a test subject. In some embodiments, the methods include determining a probability or likelihood that the cancer will be sensitive to a poly ADP ribose polymerase (PARP) inhibitor, and treating cancer in the subject. When the probability or likelihood that the cancer will be sensitive to the PARP inhibitor satisfies a threshold likelihood, the method includes administering, or communicating, a recommended therapy comprising the PARP inhibitor to the test subject. When the probability or likelihood that the cancer will be sensitive to the PARP inhibitor does not satisfy a threshold likelihood, the method includes administering, or communicating, a recommended therapy that does not include the PARP inhibitor to the test subject.

[00146] In some embodiments, the cancer is a breast cancer. In some embodiments, the cancer is an ovarian cancer. In some embodiments, the cancer is not breast cancer or ovarian cancer. In some embodiments, the test subject has not previously been treated for cancer. That is, in some embodiments, the PARP inhibitor is administered as a first-line therapy. In some embodiments, the test subject has been previously treated for the cancer. That is, in some embodiments, the PARP inhibitor is administered as a second-line therapy. In some embodiments, the PARP inhibitor is olaparib, veliparib, rucaparib, niraparib, or talazoparib.

[00147] In some embodiments, the probability or likelihood that the cancer will be sensitive to the PARP inhibitor is determined by: 1) culturing a plurality of tumor organoids, each respective tumor organoid in the plurality of tumor organoids from one or more cells of a tumor biopsy

from the test subject, 2) exposing the one or more organoids cultured in a) to one or more amounts of the PARP inhibitor, 3) measuring the fitness of cells, e.g., using a cellular viability assay or cell death assay, in the one or more tumor organoids following the exposure to the one or more amounts of the PARP inhibitor, and 4) correlating the measured fitness of the cells with a probability or likelihood that the cancer will be sensitive to the PARP inhibitor.

[00148] In some embodiments, the probability or likelihood that the cancer will be sensitive to the PARP inhibitor is determined by: 1) obtaining a first test data set comprising a plurality of nucleic acid features of a tumor biopsy from the test subject, 2) evaluating the first test data set using a classifier trained to discriminate between two or more tumor sensitivities to the PARP inhibitor, where the classifier was trained against, for each respective training tissue sample in a plurality of training tissue samples, at least (i) the plurality of nucleic acid features obtained from the respective training tissue sample, and (ii) a corresponding indication of the sensitivity of a respective organoid cultured from one or more cells of the respective training tissue sample to the PARP inhibitor.

[00149] In some embodiments, the plurality of nucleic acid features of the tumor biopsy comprise one or more of support for a single nucleotide variant at a genomic location, a methylation status at a genomic location, a relative copy number for a genomic location, an allelic ratio for a genomic location, a relative expression level of a gene, and mathematical combinations thereof.

[00150] In some embodiments, the first test data set further comprises a cancer status of the test subject in a plurality of cancer statuses, and the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, a cancer status of the respective training tissue sample in the plurality of cancer statuses. In some embodiments, the plurality of cancer statuses comprises a plurality of types of cancer. In some embodiments, the plurality of cancer statuses comprises a plurality of stages of cancer.

[00151] In some embodiments, the first test data set further comprises one or more phenotypic characteristics of the tumor biopsy, and the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, the one or more phenotypic characteristics of the respective training tissue sample. In some embodiments, the

one or more phenotypic characteristics of the tumor biopsy comprise a histologic feature of the tumor biopsy.

[00152] In some embodiments, the first test data set further comprises one or more characteristics of the subject, as described in the feature selection section above, and the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, the one or more characteristics of the subject from which the respective training tissue sample was obtained.

[00153] In some embodiments, the method also includes obtaining a second test data set comprising the plurality of nucleic acid features of a tumor biopsy from the test subject, and evaluating the second test data set using the classifier trained to discriminate between the two or more tumor sensitivities to the PARP inhibitor, where the plurality of nucleic acid features in the first test data set are from a first tumor biopsy from the test subject and the second test data set are from a second tumor biopsy from the test subject that is different from the first tumor biopsy. In some embodiments, the first tumor biopsy and the second tumor biopsy are from different sections of a same tumor of the subject. In some embodiments, the first tumor biopsy and the second tumor biopsy are from different tumors of the subject.

[00154] In some embodiments, the classifier is a multinomial classifier that is trained to provide a plurality of likelihoods, wherein each respective likelihood in the plurality of likelihoods is a likelihood that a different respective treatment regime, in a plurality of treatment regimes, for the cancer in the subject will be more effective than the other treatment regimes in the plurality of treatment regimes.

[00155] In some embodiments, the classifier is a neural network algorithm, a support vector machine algorithm, a Naive Bayes algorithm, a nearest neighbor algorithm, a boosted trees algorithm, a random forest algorithm, a convolutional neural network algorithm, a decision tree algorithm, a regression algorithm, or a clustering algorithm.

[00156] In some embodiments, the methods described herein also include administering the recommended therapy for treating cancer to the test subject.

[00157] Treatment of non-breast/ovarian cancers with PARPi

[00158] In some embodiments, the disclosure provides methods and systems for performing methods of treating a cancer other than breast cancer or ovarian cancer in a test subject. In some embodiments, the methods include determining a probability or likelihood that the cancer will be sensitive to a poly ADP ribose polymerase (PARP) inhibitor, and treating cancer in the subject. When the probability or likelihood that the cancer will be sensitive to the PARP inhibitor satisfies a threshold likelihood, the method includes administering, or communicating, a recommended therapy comprising the PARP inhibitor to the test subject. When the probability or likelihood that the cancer will be sensitive to the PARP inhibitor does not satisfy a threshold likelihood, the method includes administering, or communicating, a recommended therapy that does not include the PARP inhibitor to the test subject.

[00159] In some embodiments, the cancer is a BRCA1-/BRCA2- cancer. In some embodiments, the cancer is a colorectal cancer. In some embodiments, the cancer is a lung cancer. In some embodiments, the cancer is a non-small cell lung cancer. In some embodiments, the cancer is an endometrial cancer. In some embodiments, the test subject has not previously been treated for cancer. In some embodiments, the test subject has previously been treated for the cancer.

[00160] In some embodiments, the PARP inhibitor is selected from the group consisting of olaparib, veliparib, rucaparib, niraparib, and talazoparib.

[00161] In some embodiments, the probability or likelihood that the cancer will be sensitive to the PARP inhibitor is determined by: 1) culturing a plurality of tumor organoids, each respective tumor organoid in the plurality of tumor organoids from one or more cells of a tumor biopsy from the test subject, 2) exposing the one or more organoids cultured in a) to one or more amounts of the PARP inhibitor, 3) measuring the fitness of cells, e.g., using a cellular viability assay or cell death assay, in the one or more tumor organoids following the exposure to the one or more amounts of the PARP inhibitor, and 4) correlating the measured fitness of the cells with a probability or likelihood that the cancer will be sensitive to the PARP inhibitor.

[00162] In some embodiments, the probability or likelihood that the cancer will be sensitive to the PARP inhibitor is determined by: 1) obtaining a first test data set comprising a plurality of nucleic acid features of a tumor biopsy from the test subject, and 2) evaluating the first test data set using a classifier trained to discriminate between two or more tumor sensitivities to the PARP

inhibitor, where the classifier was trained against, for each respective training tissue sample in a plurality of training tissue samples, at least (i) the plurality of nucleic acid features obtained from the respective training tissue sample, and (ii) a corresponding indication of the sensitivity of a respective organoid cultured from one or more cells of the respective training tissue sample to the PARP inhibitor.

[00163] In some embodiments, the plurality of nucleic acid features of the tumor biopsy are selected from the group consisting of support for a single nucleotide variant at a genomic location, a methylation status at a genomic location, a relative copy number for a genomic location, an allelic ratio for a genomic location, a relative expression level of a gene, and a mathematical combination thereof.

[00164] In some embodiments, the first test data set further comprises a cancer status of the test subject in a plurality of cancer statuses, and the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, a cancer status of the respective training tissue sample in the plurality of cancer statuses. In some embodiments, the plurality of cancer statuses comprises a plurality of types of cancer. In some embodiments, the plurality of cancer statuses comprises a plurality of stages of cancer.

[00165] In some embodiments, the first test data set further comprises one or more phenotypic characteristics of the tumor biopsy, and the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, the one or more phenotypic characteristics of the respective training tissue sample. In some embodiments, the one or more phenotypic characteristics of the tumor biopsy comprise a histologic feature of the tumor biopsy.

[00166] In some embodiments, the first test data set further comprises one or more characteristics of the subject, as described in the feature selection section above, and the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, the one or more characteristics of the subject from which the respective training tissue sample was obtained.

[00167] In some embodiments, the method also includes obtaining a second test data set comprising the plurality of nucleic acid features of a tumor biopsy from the test subject, and

[00168] evaluating the second test data set using the classifier trained to discriminate between the two or more tumor sensitivities to the PARP inhibitor, where the plurality of nucleic acid features in the first test data set are from a first tumor biopsy from the test subject and the second test data set are from a second tumor biopsy from the test subject that is different from the first tumor biopsy. In some embodiments, the first tumor biopsy and the second tumor biopsy are from different sections of a same tumor of the subject. In some embodiments, the first tumor biopsy and the second tumor biopsy are from different tumors of the subject.

[00169] In some embodiments, the classifier is a multinomial classifier that is trained to provide a plurality of likelihoods, wherein each respective likelihood in the plurality of likelihoods is a likelihood that a different respective treatment regime, in a plurality of treatment regimes, for the cancer in the subject will be more effective than the other treatment regimes in the plurality of treatment regimes.

[00170] In some embodiments, the classifier is a neural network algorithm, a support vector machine algorithm, a Naive Bayes algorithm, a nearest neighbor algorithm, a boosted trees algorithm, a random forest algorithm, a convolutional neural network algorithm, a decision tree algorithm, a regression algorithm, or a clustering algorithm.

[00171] In some embodiments, the methods described herein also include administering the recommended therapy for treating cancer to the test subject.

[00172] Providing PARPi info on a clinical report

[00173] In some embodiments, the disclosure provides methods and systems for performing methods of providing a clinical report for a cancer patient to a physician. In some embodiments, the methods include obtaining a first test data set comprising features of a transcriptome from a tumor biopsy from the cancer patient. In some embodiments, the methods include evaluating the first test data set using a classifier trained, e.g., as described herein, to discriminate between two or more tumor sensitivities to a first poly ADP ribose polymerase (PARP) inhibitor. The methods then include receiving a recommended therapy for the cancer patient from the classifier, and including the recommended therapy, or sending the recommended therapy to a third party for inclusion, in a clinical report for the cancer patient.

[00174] In some embodiments, the recommended therapy for the cancer patient comprises administration of the PARP inhibitor. In some embodiments, the PARP inhibitor is olaparib, veliparib, rucaparib, niraparib, or talazoparib.

[00175] In some embodiments, the patient has a BRCA1-/BRCA2- cancer. In some embodiments, the patient has a homologous recombination deficient (HRD) cancer. In some embodiments, the patient has a breast cancer. In some embodiments, the patient has an ovarian cancer. In some embodiments, the patient does not have has breast cancer or ovarian cancer. In some embodiments, the patient has a colorectal cancer. In some embodiments, the patient has a lung cancer. In some embodiments, the patient has a non-small cell lung cancer. In some embodiments, the patient has an endometrial cancer. In some embodiments, the patient has not previously been treated for cancer. In some embodiments, the patient has previously been treated for the cancer.

[00176] Research tool for identifying PARPi's

[00177] In some embodiments, the disclosure provides methods and systems for performing methods of identifying an inhibitor of poly ADP ribose polymerase (PARP). In some embodiments, the methods include obtaining a tissue sample that is sensitive to a known PARP inhibitor, and culturing one or more organoids, each respective organoid in the one or more organoids from one or more cells of the tissue sample. The methods then include exposing the one or more of the cultured organoids to one or more concentrations of a candidate molecule, and measuring the fitness of cells in the one or more organoids following the exposure to the one or more concentrations of the candidate molecule. A reduced fitness of cells in the one or more organoids is indicative that the candidate molecule is a PARP inhibitor.

[00178] In some embodiments, the PARP inhibitor is olaparib, veliparib, rucaparib, niraparib, or talazoparib.

[00179] In some embodiments, the tissue sample includes cells from a BRCA1-/BRCA2- cancer. In some embodiments, the tissue sample includes cells from a homologous recombination deficient (HRD) cancer. In some embodiments, the tissue sample includes cells from a breast cancer. In some embodiments, the tissue sample includes cells from an ovarian cancer. In some embodiments, the tissue sample does not includes cells from a breast cancer or ovarian cancer. In some embodiments, the tissue sample includes cells from a colorectal cancer.

In some embodiments, the tissue sample includes cells from a lung cancer. In some embodiments, the tissue sample includes cells from a non-small cell lung cancer. In some embodiments, the tissue sample includes cells from an endometrial cancer. In some embodiments, the tissue sample includes cells from a patient that has not previously been treated for cancer. In some embodiments, the tissue sample includes cells from a patient that has previously been treated for the cancer.

[00180] Research tool for repurposing drugs

[00181] In some embodiments, the disclosure provides methods and systems for performing methods of identifying a new use for a pharmaceutical compound of a first pharmaceutical class. In some embodiments, the methods include obtaining a plurality of tissue samples, wherein each respective tissue sample in the plurality of tissue samples is sensitive to a respective class of pharmaceutical agents in a plurality of classes of pharmaceutical agents that excludes the first pharmaceutical class. The methods then includes culturing, for each respective tissue sample in the plurality of tissue samples, one or more respective organoids from one or more cells of the respective tissue sample, thereby generating a plurality of organoid cultures. The methods then include exposing, for each organoid culture in the plurality of organoid cultures, the respective one or more organoids to one or more concentrations of the pharmaceutical agent, and measuring, for each organoid culture in the plurality of organoid cultures, the fitness of cells in the respective one or more organoids following the exposure to the pharmaceutical agent. Reduced fitness of the cells in the respective one or more organoids is indicative that the pharmaceutical molecule shares pharmacological properties with the class of pharmaceutical compounds in the plurality of classes of pharmaceutical classes to which the tissue sample corresponding to the respective one or more organoids is sensitive.

[00182] In some embodiments, the plurality of tissue samples comprises cells from at least 3 different types of cancers. In some embodiments, the plurality of tissue samples comprises cells from at least 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more different types of cancers.

[00183] Selecting patients for clinical trials with an organoid assay

[00184] In some embodiments, the disclosure provides methods and systems for performing methods of determining the eligibility of a cancer patient for a clinical trial of a candidate cancer pharmaceutical agent. In some embodiments, the methods include obtaining a tumor biopsy

from the cancer patient, and culturing one or more tumor organoids from one or more cells of the tumor biopsy. The methods then include exposing the one or more tumor organoids to one or more concentrations of the candidate cancer pharmaceutical agent, and measuring the fitness of cells in the one or more tumor organoids following the exposure to the one or more concentrations of the candidate cancer pharmaceutical agent. The methods then include determining whether the cancer patient is eligible for the clinical trial based on at least the measured fitness of the cells in the one or more tumor organoids, wherein reduced fitness of the cells in the one or more tumor organoids is indicative that the cancer patient is eligible for the clinical trial. In some embodiments, the candidate cancer pharmaceutical agent is a poly ADP ribose polymerase (PARP) inhibitor.

[00185] In some embodiments, the clinical trial is for the treatment of a BRCA1-/BRCA2-cancer. In some embodiments, the clinical trial is for the treatment of a homologous recombination deficient (HRD) cancer. In some embodiments, the clinical trial is for the treatment of a breast cancer. In some embodiments, the clinical trial is for the treatment of an ovarian cancer. In some embodiments, the clinical trial is for the treatment of a breast cancer or ovarian cancer. In some embodiments, the clinical trial is for the treatment of a colorectal cancer. In some embodiments, the clinical trial is for the treatment of a lung cancer. In some embodiments, the clinical trial is for the treatment of a non-small cell lung cancer. In some embodiments, the clinical trial is for the treatment of an endometrial cancer. In some embodiments, the clinical trial is for the treatment of a patient that has not previously been treated for cancer. In some embodiments, the clinical trial is for the treatment of a patient that has previously been treated for the cancer.

[00186] Selecting patients for clinical trials with a classifier

[00187] In some embodiments, the disclosure provides methods and systems for performing methods of determining the eligibility of a cancer patient for a clinical trial of a candidate cancer pharmaceutical agent. In some embodiments, the methods include obtaining a first test data set comprising a plurality of nucleic acid features of a tumor biopsy from the cancer patient. The methods then include evaluating the first test data set using a classifier trained to discriminate between two or more tumor sensitivities to the candidate cancer pharmaceutical agent. The classifier is trained against, for each respective training tissue sample in a plurality of training

tissue samples, at least (i) the plurality of nucleic acid features obtained from the respective training tissue sample, and (ii) a corresponding indication of the sensitivity of a respective organoid cultured from one or more cells of the respective training tissue sample to the candidate cancer pharmaceutical agent. A first tumor sensitivity in the two or more tumor sensitivities to the candidate cancer pharmaceutical agent is associated with an indication that the cancer patient is eligible for the clinical trial. A second tumor sensitivity in the two or more tumor sensitivities to the candidate cancer pharmaceutical agent is associated with an indication that the cancer patient is eligible for the clinical trial. The methods then include determining whether the cancer patient is eligible for the clinical trial based on at least the evaluation. In some embodiments, the candidate cancer pharmaceutical agent is a poly ADP ribose polymerase (PARP) inhibitor.

[00188] In some embodiments, the clinical trial is for the treatment of a BRCA1-/BRCA2-cancer. In some embodiments, the clinical trial is for the treatment of a homologous recombination deficient (HRD) cancer. In some embodiments, the clinical trial is for the treatment of a breast cancer. In some embodiments, the clinical trial is for the treatment of an ovarian cancer. In some embodiments, the clinical trial is for the treatment of a breast cancer or ovarian cancer. In some embodiments, the clinical trial is for the treatment of a colorectal cancer. In some embodiments, the clinical trial is for the treatment of a lung cancer. In some embodiments, the clinical trial is for the treatment of a non-small cell lung cancer. In some embodiments, the clinical trial is for the treatment of an endometrial cancer. In some embodiments, the clinical trial is for the treatment of a patient that has not previously been treated for cancer. In some embodiments, the clinical trial is for the treatment of a patient that has previously been treated for the cancer.

[00189] In some embodiments, the candidate cancer pharmaceutical agent has previously been found to be effective for the treatment of a cancer having a first clinical marker and the clinical trial is for the treatment of a cancer that does not have the first clinical marker.

[00190] In some embodiments, the plurality of nucleic acid features of the tumor biopsy include one or more of support for a single nucleotide variant at a genomic location, a methylation status at a genomic location, a relative copy number for a genomic location, an allelic ratio for a genomic location, a relative expression level of a gene, and a mathematical combination thereof.

[00191] In some embodiments, the first test data set also includes a cancer status of the test subject in a plurality of cancer statuses, and the classifier was further trained against, for each respective training tissue sample in a plurality of the training tissue samples, a cancer status of the respective training tissue sample in the plurality of cancer statuses. In some embodiments, the plurality of cancer statuses comprises a plurality of types of cancer. In some embodiments, the plurality of cancer statuses comprises a plurality of stages of cancer.

[00192] In some embodiments, the first test data set further comprises one or more phenotypic characteristics of the tumor biopsy, and the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, the one or more phenotypic characteristics of the respective training tissue sample. In some embodiments, the one or more phenotypic characteristics of the tumor biopsy comprise a histologic feature of the tumor biopsy.

[00193] In some embodiments, the first test data set also includes one or more characteristics of the subject, as described in the feature selection section above, and the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, the one or more characteristics of the subject from which the respective training tissue sample was obtained.

[00194] Detecting drug resistance with a classifier

[00195] In some embodiments, the disclosure provides methods and systems for performing methods of identifying a resistance to a chemotherapeutic agent in a patient with cancer. In some embodiments, the methods include obtaining a first test data set comprising a plurality of nucleic acid features of a tumor biopsy from the patient with cancer. The methods then include evaluating the first test data set using a classifier trained to discriminate between two or more tumor sensitivities to the chemotherapeutic agent. The classifier is trained against, for each respective training tissue sample in a plurality of training tissue samples, at least (i) the plurality of nucleic acid features obtained from the respective training tissue sample, and (ii) a corresponding indication of the sensitivity of a respective organoid cultured from one or more cells of the respective training tissue sample to the chemotherapeutic agent. A first tumor sensitivity in the two or more tumor sensitivities to the candidate cancer pharmaceutical agent is associated with an indication that the patient's cancer is resistant to the chemotherapeutic agent.

A second tumor sensitivity in the two or more tumor sensitivities to the candidate cancer pharmaceutical agent is associated with an indication that the patient's cancer is not resistant to the chemotherapeutic agent. The methods then include providing a report indicating whether the patient's cancer is resistant to the chemotherapeutic agent based on at least the results of the evaluation.

[00196] In some embodiments, chemotherapeutic agent is a PARP inhibitor. In some embodiments, the PARP inhibitor is olaparib, veliparib, rucaparib, niraparib, or talazoparib.

[00197] In some embodiments, the patient has a BRCA1-/BRCA2- cancer. In some embodiments, the patient has a cancer that carries a BRCA1 or BRCA2 mutation. In some embodiments, the patient has a homologous recombination deficient (HRD) cancer. In some embodiments, the patient has a breast cancer. In some embodiments, the patient has an ovarian cancer. In some embodiments, the patient does not have has breast cancer or ovarian cancer. In some embodiments, the patient has a colorectal cancer. In some embodiments, the patient has a lung cancer. In some embodiments, the patient has a non-small cell lung cancer. In some embodiments, the patient has an endometrial cancer. In some embodiments, the patient has not previously been treated for cancer. In some embodiments, the patient has previously been treated for the cancer.

[00198] In some embodiments, the chemotherapeutic agent has been previously associated with a clinical marker and the cancer has the clinical marker.

[00199] In some embodiments, the plurality of nucleic acid features includes one or more of support for a single nucleotide variant at a genomic location, a methylation status at a genomic location, a relative copy number for a genomic location, an allelic ratio for a genomic location, a relative expression level of a gene, and a mathematical combination thereof. In some embodiments, the plurality of nucleic acid features comprises a BRCA1 or BRCA2 mutant allele status.

[00200] In some embodiments, the first test data set also includes one or more phenotypic characteristics of the tumor biopsy, and the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, the one or more phenotypic characteristics of the respective training tissue sample. In some embodiments, the

one or more phenotypic characteristics of the tumor biopsy comprise a histologic feature of the tumor biopsy.

[00201] In some embodiments, the first test data set also includes one or more characteristics of the subject, as described in the feature selection section above, and the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, the one or more characteristics of the subject from which the respective training tissue sample was obtained.

[00202] Evaluating effect of cancer drug using tumor organoid assay and Assigning cancer treatment based on tumor organoid assay

[00203] In some aspects, the present disclosure provides systems and methods for large scale analysis of therapeutic responses using tumor organoids (TOs).

[00204] In certain aspects, a drug screening platform may be utilized to assess the effectiveness of drugs or other agents on tumor organoids. In one embodiment, tumor organoids are first dissociated into single cells, which are then seeded into individual partitions in the presence of tumor organoid culture media. Tumor organoids used in the methods provided herein can be derived from cells from any suitable cancer including, but not limited to, an anal cancer, a basal cell skin cancer, a squamous cancer, a breast cancer, a bladder cancer, a cervical cancer, a colon cancer, an endometrial cancer, a head and neck cancer, a hepatobiliary cancer, a kidney cancer, a gastric cancer, a lung cancer, a mesothelial cancer of the pleural cavity, a mesothelial cancer of the peritoneal cavity, an ovarian cancer, prostate cancer, a rectal cancer.

[00205] Tumor organoids that subsequently form from the individual cells in each partition exhibit tumor organoid heterogeneity. In some embodiments, the single cells are seeded in a multi-well plate (e.g., 24-, 48-, 96-, 384-well). Preferably, individual cells are seeded at a density to allow sufficient number of TOs to form while not overcrowding the plate so that TOs do not overlap or touch allowing for easy identification of individual TOs.

[00206] Any suitable tumor organoid medium can be used to culture the tumor organoids. In some embodiments, the tumor organoid medium includes one or more tumor organoid growth factors. Organoid growth factors include an epidermal growth factor (EGF), a fibroblast growth factor (FGF), a hepatocyte growth factor (HGF), a Wnt, Noggin, an R-spondin, Gastrin,

Prostaglandin, and Neuregulin. Organoid growth factors include ligands (natural, semi-synthetic, or synthetic, agonist or antagonist) of EGF family receptors, HGF family of receptors, Wnt family receptors, NOTCH family receptors, LRP receptor, Frizzled receptor, LGR5 receptor, insulin receptor, neuregulin family of receptors, or any growth factor receptor tyrosine kinase family member. Exemplary organoid culture media are summarized below in Table 1. In particular embodiments, the organoid culture medium is free of R-spondins. In some embodiments, the organoid culture medium is free of Wnt.

[00207] Table 1

Organoid Culture Medium	Growth Factors	Molecular Inhibitors	Additional Components
B	Noggin	Rho kinase inhibitor, transforming growth factor-beta inhibitor, and MAP kinase inhibitor	a chemically-defined, minimal culture medium; L-glutamine; a serum replacement supplement; N-acetyl-L-cysteine; and nicotinamide
C	EGF and Noggin	Rho kinase inhibitor, transforming growth factor-beta inhibitor, and MAP kinase inhibitor	a chemically-defined, minimal culture medium; L-glutamine; a serum replacement supplement; N-acetyl-L-cysteine; and nicotinamide
D	EGF, Noggin, FGF7 and FGF10	Rho kinase inhibitor, transforming growth factor-beta inhibitor, and MAP kinase inhibitor	a chemically-defined, minimal culture medium; L-glutamine; a serum replacement supplement; N-acetyl-L-cysteine; and nicotinamide

[00208] In one example, the TOs are also cultured in the presence of one or more extracellular matrix (ECM) components that function as a substrate for culturing the TO. In particular embodiments, the substrate is a Matrigel.

[00209] In one example, one or more agents (e.g., a therapeutic agent) are applied to the TOs in the partitions. Exemplary therapeutic agents include, but are not limited to, a molecular inhibitors, antibodies, recombinant nucleic acids (e.g., antisense oligonucleotides) and engineered immune cells (e.g., CAR T-cells and NK cells). Exemplary therapeutic agents include, but are not limited to, Paclitaxel, Gemcitabine, Cisplatin, Carboplatin, Oxaliplatin, Capecitabine, SN-38 (CPT-11), 5-FU, MTX (methotrexate), Docetaxel, Bortezomib, Everolimus, Ulixertinib, Dasatinib, Vinblastine, Nelarabine, Epirubicin, Afatinib, Lapatinib, Cytarabine, Cladribine, Doxorubicin, Azacitidine, and Staurosporine. Other examples include classes of drugs including but not limited to: taxanes, platinating agents, vinca alkaloids, alkylating agents, and anthracyclines. One or more molecular inhibitors may be applied to the TOs in the well plates. Molecular inhibitors may be selected by name, target, pathway, formula, or other known characterizations. Additional exemplary therapeutic agents that can be used with the methods described herein are provided in Table 2.

[00210] Table 2

Agent	Target	Pathway	Formula
Veliparib (ABT-888)	PARP	DNA Damage	C ₁₃ H ₁₆ N ₄ O
Selumetinib (AZD6244)	MEK	MAPK	C ₁₇ H ₁₅ BrClFN ₄ O ₃
PD184352 (CI-1040)	MEK	MAPK	C ₁₇ H ₁₄ ClF ₂ IN ₂ O ₂
PD0325901	MEK	MAPK	C ₁₆ H ₁₄ F ₃ IN ₂ O ₄
Tozasertib (VX-680, MK-0457)	Aurora Kinase	Cell Cycle	C ₂₃ H ₂₈ N ₈ OS
Y-27632 2HCl	Autophagy,ROCK	Cell Cycle	C ₁₄ H ₂₃ Cl ₂ N ₃ O
Olaparib (AZD2281, Ku-0059436)	PARP	DNA Damage	C ₂₄ H ₂₃ FN ₄ O ₃
SL-327	MEK	MAPK	C ₁₆ H ₁₂ F ₃ N ₃ S
SB431542	TGF-beta/Smad	TGF-beta/Smad	C ₂₂ H ₁₆ N ₄ O ₃
MK-2206 2HCl	Akt	PI3K/Akt/mTOR	C ₂₅ H ₂₃ Cl ₂ N ₅ O

Agent	Target	Pathway	Formula
Refametinib (RDEA119, Bay 86-9766)	MEK	MAPK	C ₁₉ H ₂₀ F ₃ IN ₂ O ₅ S
KU-55933 (ATM Kinase Inhibitor)	ATM/ATR	DNA Damage	C ₂₁ H ₁₇ NO ₃ S ₂
GSK1904529A	IGF-1R	Protein Tyrosine Kinase	C ₄₄ H ₄₇ F ₂ N ₉ O ₅ S
PF-04217903	c-Met	Protein Tyrosine Kinase	C ₁₉ H ₁₆ N ₈ O
U0126-EtOH	MEK	MAPK	C ₂₀ H ₂₂ N ₆ OS ₂
BI 2536	PLK	Cell Cycle	C ₂₈ H ₃₉ N ₇ O ₃
JNJ-38877605	c-Met	Protein Tyrosine Kinase	C ₁₉ H ₁₃ F ₂ N ₇
Odanacatib (MK-0822)	Cysteine Protease	Proteases	C ₂₅ H ₂₇ F ₄ N ₃ O ₃ S
Alisertib (MLN8237)	Aurora Kinase	Cell Cycle	C ₂₇ H ₂₀ ClFN ₄ O ₄
Barasertib (AZD1152-HQPA)	Aurora Kinase	Cell Cycle	C ₂₆ H ₃₀ FN ₇ O ₃
CP-724714	EGFR,HER2	Protein Tyrosine Kinase	C ₂₇ H ₂₇ N ₅ O ₃
TGX-221	PI3K	PI3K/Akt/mTOR	C ₂₁ H ₂₄ N ₄ O ₂
WZ4002	EGFR	Protein Tyrosine Kinase	C ₂₅ H ₂₇ ClN ₆ O ₃
BIBR 1532	Telomerase	DNA Damage	C ₂₁ H ₁₇ NO ₃
Anastrozole	Aromatase	Endocrinology & Hormones	C ₁₇ H ₁₉ N ₅
Aprepitant	Substance P	Others	C ₂₃ H ₂₁ F ₇ N ₄ O ₃
TAK-700 (Orteronel)	P450 (e.g. CYP17)	Metabolism	C ₁₈ H ₁₇ N ₃ O ₂

Agent	Target	Pathway	Formula
PFI-1 (PF-6405761)	Epigenetic Reader Domain	Epigenetics	C16H17N3O4S
KU-0063794	mTOR	PI3K/Akt/mTOR	C25H31N5O4
CHIR-99021 (CT99021)	GSK-3	PI3K/Akt/mTOR	C22H18Cl2N8
WYE-354	mTOR	PI3K/Akt/mTOR	C24H29N7O5
TG100-115	PI3K	PI3K/Akt/mTOR	C18H14N6O2
Aurora A Inhibitor I	Aurora Kinase	Cell Cycle	C31H31ClFN7O2
Ispinesib (SB-715992)	Kinesin	Cytoskeletal Signaling	C30H33ClN4O2
Zibotentan (ZD4054)	Endothelin Receptor	GPCR & G Protein	C19H16N6O4S
Safinamide Mesylate	MAO	Metabolism	C18H23FN2O5S
GSK429286A	ROCK	Cell Cycle	C21H16F4N4O2
Pimasertib (AS-703026)	MEK	MAPK	C15H15FIN3O3
Tadalafil	PDE	Metabolism	C22H19N3O4
Adavosertib (MK-1775)	Wee1	Cell Cycle	C27H32N8O2
CP-673451	PDGFR	Protein Tyrosine Kinase	C24H27N5O2
Selisistat (EX 527)	Sirtuin	Epigenetics	C13H13ClN2O
Dapagliflozin	SGLT	GPCR & G Protein	C21H25ClO6
Nebivolol HCl	Adrenergic Receptor	Neuronal Signaling	C22H26ClF2NO4
Pimobendan	PDE	Metabolism	C19H18N4O2
AZD8055	mTOR	PI3K/Akt/mTOR	C25H31N5O4

Agent	Target	Pathway	Formula
KU-60019	ATM/ATR	DNA Damage	C30H33N3O5S
Tie2 kinase inhibitor	Tie-2	ProteinTyrosine Kinase	C26H21N3O2S
Apixaban	Factor Xa	Metabolism	C25H25N5O4
Raltegravir (MK-0518)	Integrase	Microbiology	C20H21FN6O5
PCI-34051	HDAC	Epigenetics	C17H16N2O3
Ambrisentan	Endothelin Receptor	GPCR & G Protein	C22H22N2O4
SB743921 HCl	Kinesin	Cytoskeletal Signaling	C31H34Cl2N2O3
AST-1306	EGFR	Protein Tyrosine Kinase	C ₃₁ H ₂₆ ClFN ₄ O ₅ S
Sapitinib (AZD8931)	EGFR,HER2	Protein Tyrosine Kinase	C23H25ClFN5O3
GSK461364	PLK	Cell Cycle	C27H28F3N5O2S
Mubritinib (TAK 165)	HER2	Protein Tyrosine Kinase	C25H23F3N4O2
UK 383367	Procollagen C Proteinase	Metabolism	C15H24N4O4
Cryptotanshinone	STAT	JAK/STAT	C19H20O3
Icariin	PDE	Metabolism	C33H40O15
OSI-027	mTOR	PI3K/Akt/mTOR	C21H22N6O3
Rabusertib (LY2603618)	Chk	Cell Cycle	C18H22BrN5O3
URB597	FAAH	Metabolism	C20H22N2O3
A66	PI3K	PI3K/Akt/mTOR	C17H23N5O2S2

Agent	Target	Pathway	Formula
ICG-001	Wnt/beta-catenin	Stem Cells & Wnt	C33H32N4O4
PF-3845	FAAH	Metabolism	C24H23F3N4O2
Trametinib (GSK1120212)	MEK	MAPK	C26H23FIN5O4
Ibrutinib (PCI-32765)	BTK	Angiogenesis	C25H24N6O2
CHIR-124	Chk	Cell Cycle	C23H22ClN5O
Mardepodect (PF-2545920)	PDE	Metabolism	C25H20N4O
WAY-600	mTOR	PI3K/Akt/mTOR	C28H30N8O
Nepicastat (SYN-117) HCl	Hydroxylase	Metabolism	C14H16ClF2N3S
RS-127445	5-HT Receptor	Neuronal Signaling	C17H16FN3
CP-91149	Phosphorylase	Metabolism	C21H22ClN3O3
SB415286	GSK-3	PI3K/Akt/mTOR	C16H10ClN3O5
GSK1070916	Aurora Kinase	Cell Cycle	C30H33N7O
Niraparib (MK-4827)	PARP	DNA Damage	C19H20N4O
CHIR-98014	GSK-3	PI3K/Akt/mTOR	C20H17Cl2N9O2
AMG-458	c-Met	Protein Tyrosine Kinase	C30H29N5O5
Tivantinib (ARQ 197)	c-Met	Protein Tyrosine Kinase	C23H19N3O2
Canagliflozin	SGLT	GPCR & G Protein	C24H25FO5S
NVP-BVU972	c-Met	Protein Tyrosine Kinase	C20H16N6
MK-5108 (VX-689)	Aurora Kinase	Cell Cycle	C22H21ClFN3O3S

Agent	Target	Pathway	Formula
SB705498	TRPV	Others	C ₁₇ H ₁₆ BrF ₃ N ₄ O
Vistusertib (AZD2014)	mTOR	PI3K/Akt/mTOR	C ₂₅ H ₃₀ N ₆ O ₃
A-803467	Sodium Channel	Transmembrane Transporters	C ₁₉ H ₁₆ ClNO ₄
Sirtinol	Sirtuin	Epigenetics	C ₂₆ H ₂₂ N ₂ O ₂
Ipatasertib (GDC-0068)	Akt	PI3K/Akt/mTOR	C ₂₄ H ₃₂ ClN ₅ O ₂
Sapanisertib (INK 128, MLN0128)	mTOR	PI3K/Akt/mTOR	C ₁₅ H ₁₅ N ₇ O
Tyrphostin AG 879	HER2	Protein Tyrosine Kinase	C ₁₈ H ₂₄ N ₂ OS
JNJ-1661010	FAAH	Metabolism	C ₁₉ H ₁₉ N ₅ OS
CTEP (RO4956371)	GluR	Neuronal Signaling	C ₁₉ H ₁₃ ClF ₃ N ₃ O
Alogliptin (SYK-322) benzoate	DPP-4	Proteases	C ₁₈ H ₂₁ N ₅ O ₂
T0070907	PPAR	DNA Damage	C ₁₂ H ₈ ClN ₃ O ₃
GW441756	Trk receptor	Protein Tyrosine Kinase	C ₁₇ H ₁₃ N ₃ O
SB742457	5-HT Receptor	Neuronal Signaling	C ₁₉ H ₁₉ N ₃ O ₂ S
ZM 323881 HCl	VEGFR	Protein Tyrosine Kinase	C ₂₂ H ₁₉ ClFN ₃ O ₂
GNF-2	Bcr-Abl	Angiogenesis	C ₁₈ H ₁₃ F ₃ N ₄ O ₂
Lumiracoxib	COX	Neuronal Signaling	C ₁₅ H ₁₃ ClFNO ₂
JNJ-7777120	Histamine Receptor	Neuronal	C ₁₄ H ₁₆ ClN ₃ O

Agent	Target	Pathway	Formula
		Signaling	
IOX2	HIF	Angiogenesis	C ₁₉ H ₁₆ N ₂ O ₅
PF-4981517	P450 (e.g. CYP17)	Metabolism	C ₂₆ H ₃₂ N ₈
CHIR-99021 (CT99021) HCl	GSK-3	PI3K/Akt/mTOR	C ₂₂ H ₁₉ Cl ₃ N ₈
Rivaroxaban	Factor Xa	Metabolism	C ₁₉ H ₁₈ ClN ₃ O ₅ S
Linagliptin	DPP-4	Proteases	C ₂₅ H ₂₈ N ₈ O ₂
Azilsartan Medoxomil	RAAS	Endocrinology & Hormones	C ₃₀ H ₂₄ N ₄ O ₈
Sulfaphenazole	P450 (e.g. CYP17)	Metabolism	C ₁₅ H ₁₄ N ₄ O ₂ S
Sitagliptin phosphate monohydrate	DPP-4	Proteases	C ₁₆ H ₂₀ F ₆ N ₅ O ₆ P
Avanafil	PDE	Metabolism	C ₂₃ H ₂₆ ClN ₇ O ₃
Eprosartan Mesylate	RAAS	Endocrinology & Hormones	C ₂₄ H ₂₈ N ₂ O ₇ S ₂
Carprofen	COX	Neuronal Signaling	C ₁₅ H ₁₂ ClNO ₂
Saxagliptin hydrate	DPP-4	Proteases	C ₁₈ H ₂₇ N ₃ O ₃
Daminozide	Histone Demethylase	Epigenetics	C ₆ H ₁₂ N ₂ O ₃
Bedaquiline fumarate	Anti-infection	Microbiology	C ₃₆ H ₃₅ BrN ₂ O ₆
JZL184	Lipase	Metabolism	C ₂₇ H ₂₄ N ₂ O ₉
SC-514	I κ B/IKK	NF- κ B	C ₉ H ₈ N ₂ O ₅ S ₂
(R)-Nepicastat HCl	Hydroxylase	Metabolism	C ₁₄ H ₁₆ ClF ₂ N ₃ S
Asunaprevir	HCV Protease	Proteases	C ₃₅ H ₄₆ ClN ₅ O ₉ S
Trelagliptin succinate	DPP-4	Proteases	C ₂₂ H ₂₆ FN ₅ O ₆

Agent	Target	Pathway	Formula
Dabrafenib Mesylate	Raf	MAPK	C ₂₄ H ₂₄ F ₃ N ₅ O ₅ S ₃
Argatroban Monohydrate	Thrombin	Others	C ₂₃ H ₃₈ N ₆ O ₆ S
Sitagliptin	DPP-4	Proteases	C ₁₆ H ₁₅ F ₆ N ₅ O
Raltegravir potassium	Integrase,HIV Protease	Microbiology	C ₂₀ H ₂₀ FKN ₆ O ₅
Alogliptin	DPP-4	Proteases	C ₁₈ H ₂₁ N ₅ O ₂
Dasabuvir(ABT-333)	HCV Protease	Proteases	C ₂₆ H ₂₇ N ₃ O ₅ S
Ertugliflozin	SGLT2	Ion-Channel	C ₂₂ H ₂₅ ClO ₇
Rolapitant	NK1-receptor	GPCR	C ₂₅ H ₂₆ F ₆ N ₂ O ₂
Dapagliflozin propanediol monohydrate	SGLT	GPCR & G Protein	C ₂₄ H ₃₅ ClO ₉
Bedaquiline	tuberculosis	Immunology	C ₃₂ H ₃₁ BrN ₂ O ₂
Fruquintinib	VEGFRs	VEGFR	C ₂₁ H ₁₉ N ₃ O ₅
JNJ0966	Others	Others	C ₁₆ H ₁₆ N ₄ O ₂ S ₂
acalisib (GS-9820)	PI3K	PI3K/Akt/mTOR	C ₂₁ H ₁₆ FN ₇ O
BRL-50481	PDE	Metabolism	C ₉ H ₁₂ N ₂ O ₄ S
Canagliflozin hemihydrate	SGLT	GPCR & G Protein	C ₄₈ H ₅₂ F ₂ O ₁₁ S ₂
JANEX-1	JAK	JAK/STAT	C ₁₆ H ₁₅ N ₃ O ₃
Anagliptin	DPP-4	Proteases	C ₁₉ H ₂₅ N ₇ O ₂
GSK 5959	Epigenetic Reader Do	Epigenetics	C ₂₂ H ₂₆ N ₄ O ₃
Pitolisant hydrochloride	Histamine Receptor	Neuronal Signaling	C ₁₇ H ₂₇ Cl ₂ NO
K 858	Kinesin	Cytoskeletal Signaling	C ₁₃ H ₁₅ N ₃ O ₂ S

Agent	Target	Pathway	Formula
BAY-61-3606	Syk	Angiogenesis	C ₂₀ H ₂₀ Cl ₂ N ₆ O ₃
Stattic	STAT	JAK/STAT	C ₈ H ₅ NO ₄ S
GSK2656157	PERK	Apoptosis	C ₂₃ H ₂₁ FN ₆ O
XL388	mTOR	PI3K/Akt/mTOR	C ₂₃ H ₂₂ FN ₃ O ₄ S
LY2090314	GSK-3	PI3K/Akt/mTOR	C ₂₈ H ₂₅ FN ₆ O ₃
MK-8745	Aurora Kinase	Cell Cycle	C ₂₀ H ₁₉ ClFN ₅ OS
Tepotinib (EMD 1214063)	c-Met	Protein Tyrosine Kinase	C ₂₉ H ₂₈ N ₆ O ₂
SGC 0946	Histone Methyltransferase	Epigenetics	C ₂₈ H ₄₀ BrN ₇ O ₄
GSK2334470	PDK	PI3K/Akt/mTOR	C ₂₅ H ₃₄ N ₈ O
IPA-3	PAK	Cytoskeletal Signaling	C ₂₀ H ₁₄ O ₂ S ₂
VE-822	ATM/ATR	PI3K/Akt/mTOR	C ₂₄ H ₂₅ N ₅ O ₃ S
(+)-JQ1	Epigenetic Reader Domain	Epigenetics	C ₂₃ H ₂₅ ClN ₄ O ₂ S
PYR-41	E1 Activating	Ubiquitin	C ₁₇ H ₁₃ N ₃ O ₇
TCID	DUB	Ubiquitin	C ₉ H ₂ Cl ₄ O ₂
DMH1	TGF-beta/Smad	TGF-beta/Smad	C ₂₄ H ₂₀ N ₄ O
ML347	TGF-beta/Smad, ALK	TGF-beta/Smad	C ₂₂ H ₁₆ N ₄ O
UNC1999	Histone Methyltransferase	Epigenetics	C ₃₃ H ₄₃ N ₇ O ₂
SSR128129E	FGFR	Angiogenesis	C ₁₈ H ₁₅ N ₂ NaO ₄
Spebrutinib (CC-292, AVL-292)	BTK	Angiogenesis	C ₂₂ H ₂₂ FN ₅ O ₃

Agent	Target	Pathway	Formula
SKI II	S1P Receptor	GPCR & G Protein	C ₁₅ H ₁₁ ClN ₂ OS
PF-543	S1P Receptor	GPCR & G Protein	C ₂₇ H ₃₁ NO ₄ S
CID755673	Serine/threonin kinase, CaMK	Apoptosis	C ₁₂ H ₁₁ NO ₃
1-Azakenpaullone	GSK-3	PI3K/Akt/mTOR	C ₁₅ H ₁₀ BrN ₃ O
CNX-2006	EGFR	Protein Tyrosine Kinase	C ₂₆ H ₂₇ F ₄ N ₇ O ₂
Bisindolylmaleimide I (GF109203X)	PKC	TGF-beta/Smad	C ₂₅ H ₂₄ N ₄ O ₂
Thiamet G	Others	Others	C ₉ H ₁₆ N ₂ O ₄ S
Alvelestat (AZD9668)	Serine Protease	Proteases	C ₂₄ H ₂₀ F ₃ N ₅ O ₄ S
RGFP966	HDAC	Epigenetics	C ₂₁ H ₁₉ FN ₄ O
UNC0642	Histone Methyltransferase	Epigenetics	C ₂₉ H ₄₄ F ₂ N ₆ O ₂
NVP-TNKS656	PARP	DNA Damage	C ₂₇ H ₃₄ N ₄ O ₅
AGI-6780	Dehydrogenase	Metabolism	C ₂₁ H ₁₈ F ₃ N ₃ O ₃ S ₂
Ro3280	PLK	Cell Cycle	C ₂₇ H ₃₅ F ₂ N ₇ O ₃
NMS-P937 (NMS1286937)	PLK	Cell Cycle	C ₂₄ H ₂₇ F ₃ N ₈ O ₃
CNX-774	BTK	Angiogenesis	C ₂₆ H ₂₂ FN ₇ O ₃
AZD1981	GPR	Endocrinology & Hormones	C ₁₉ H ₁₇ ClN ₂ O ₃ S
SRPIN340	Others	Others	C ₁₈ H ₁₈ F ₃ N ₃ O
4μ8C	Others	Others	C ₁₁ H ₈ O ₄

Agent	Target	Pathway	Formula
NMS-E973	HSP (e.g. HSP90)	Cytoskeletal Signaling	C ₂₂ H ₂₂ N ₄ O ₇
PFI-2 HCl	Histone Methyltransferase	Epigenetics	C ₂₃ H ₂₅ F ₄ N ₃ O ₃ S
GSK2606414	PERK	Apoptosis	C ₂₄ H ₂₀ F ₃ N ₅ O
IPI-3063	PI3K	PI3K/Akt/mTOR	C ₂₅ H ₂₅ N ₇ O ₂
Atglistatin	Lipase	Metabolism	C ₁₇ H ₂₁ N ₃ O
CGP 57380	MNK	MAPK	C ₁₁ H ₉ FN ₆
SB-3CT	MMP	Proteases	C ₁₅ H ₁₄ O ₃ S ₂
AR-A014418	GSK-3	PI3K/Akt/mTOR	C ₁₂ H ₁₂ N ₄ O ₄ S
NH125	CaMK	Neuronal Signaling	C ₂₇ H ₄₅ IN ₂
XEN445	Lipase	Metabolism	C ₁₈ H ₁₇ F ₃ N ₂ O ₃ R
LDC000067	CDK	Cell Cycle	C ₁₈ H ₁₈ N ₄ O ₃ S
PI-1840	Proteasome	Proteases	C ₂₂ H ₂₆ N ₄ O ₃
FTI 277 HCl	Transferase	Metabolism	C ₂₂ H ₃₀ ClN ₃ O ₃ S ₂
Nexturastat A	HDAC	DNA Damage	C ₁₉ H ₂₃ N ₃ O ₃
ESI-09	cAMP	GPCR & G Protein	C ₁₆ H ₁₅ ClN ₄ O ₂
HJC0350	cAMP	GPCR & G Protein	C ₁₅ H ₁₉ NO ₂ S
HO-3867	STAT	JAK/STAT	C ₂₈ H ₃₀ F ₂ N ₂ O ₂
JNK Inhibitor IX	JNK	MAPK	C ₂₀ H ₁₆ N ₂ OS
Trelagliptin	DPP-4	Proteases	C ₁₈ H ₂₀ FN ₅ O ₂
XMD8-92	ERK	MAPK	C ₂₆ H ₃₀ N ₆ O ₃

Agent	Target	Pathway	Formula
A-366	Histone Methyltransferase	Epigenetics	C ₁₉ H ₂₇ N ₃ O ₂
GSK-LSD1 2HCl	Histone Demethylase	Epigenetics	C ₁₄ H ₂₂ Cl ₂ N ₂
LLY-507	Histone Methyltransferase	Epigenetics	C ₃₆ H ₄₂ N ₆ O
Santacruzamate A (CAY10683)	HDAC	DNA Damage	C ₁₅ H ₂₂ N ₂ O ₃
CAY10603	HDAC	DNA Damage	C ₂₂ H ₃₀ N ₄ O ₆
GSK1324726A (I-BET726)	Epigenetic Reader Domain	Epigenetics	C ₂₅ H ₂₃ ClN ₂ O ₃
SD-208	TGF-beta/Smad	TGF-beta/Smad	C ₁₇ H ₁₀ ClFN ₆
TH588	MTH1	DNA Damage	C ₁₃ H ₁₂ Cl ₂ N ₄
SB225002	CXCR	GPCR & G Protein	C ₁₃ H ₁₀ BrN ₃ O ₄
CPI-360	Histone Methyltransferase	Epigenetics	C ₂₅ H ₃₁ N ₃ O ₄
Picropodophyllin (PPP)	IGF-1R	Protein Tyrosine Kinase	C ₂₂ H ₂₂ O ₈
Savolitinib(AZD6094, HMPL-504)	c-Met	Protein Tyrosine Kinase	C ₁₇ H ₁₅ N ₉
SP2509	Histone Demethylase	Epigenetics	C ₁₉ H ₂₀ ClN ₃ O ₅ S
VX-11e	ERK	MAPK	C ₂₄ H ₂₀ Cl ₂ FN ₅ O ₂
SBE 13 HCl	PLK	Cell Cycle	C ₂₄ H ₂₈ Cl ₂ N ₂ O ₄
BLZ945	CSF-1R	Protein Tyrosine Kinase	C ₂₀ H ₂₂ N ₄ O ₃ S
LFM-A13	BTK	Angiogenesis	C ₁₁ H ₈ Br ₂ N ₂ O ₂

Agent	Target	Pathway	Formula
EPZ015666(GSK3235025)	Histone Methyltransferase	Epigenetics	C ₂₀ H ₂₅ N ₅ O ₃
VER155008	HSP (e.g. HSP90)	Cytoskeletal Signaling	C ₂₅ H ₂₃ Cl ₂ N ₇ O ₄
BPTES	Glutaminase	Proteases	C ₂₄ H ₂₄ N ₆ O ₂ S ₃
AZ6102	PPAR	DNA Damage	C ₂₅ H ₂₈ N ₆ O
Erlotinib	EGFR	Protein Tyrosine Kinase	C ₂₂ H ₂₃ N ₃ O ₄
ORY-1001 (RG-6016) 2HCl	Histone Demethylase	Epigenetics	C ₁₅ H ₂₄ Cl ₂ N ₂
EPZ020411 2HCl	Histone Methyltransferase	Epigenetics	C ₂₅ H ₄₀ Cl ₂ N ₄ O ₃
I-BRD9	Epigenetic Reader Domain	Epigenetics	C ₂₂ H ₂₂ F ₃ N ₃ O ₃ S ₂
SirReal2	Sirtuin	Epigenetics	C ₂₂ H ₂₀ N ₄ OS ₂
BDA-366	Bcl-2	Apoptosis	C ₂₄ H ₂₉ N ₃ O ₄
NVP-CGM097	Mdm2	Apoptosis	C ₃₈ H ₄₇ ClN ₄ O ₄
CC-223	mTOR	PI3K/Akt/mTOR	C ₂₁ H ₂₇ N ₅ O ₃
PFI-4	Epigenetic Reader Domain	Epigenetics	C ₂₁ H ₂₄ N ₄ O ₃
BIO-acetoxime	GSK-3	PI3K/Akt/mTOR	C ₁₈ H ₁₂ BrN ₃ O ₃
GSK2292767	PI3K	PI3K/Akt/mTOR	C ₂₄ H ₂₈ N ₆ O ₅ S
SIS3 HCl	TGF-beta/Smad	TGF-beta/Smad	C ₂₈ H ₂₈ ClN ₃ O ₃
Larotrectinib (LOXO-101) sulfate	Trk receptor	Protein Tyrosine Kinase	C ₂₁ H ₂₄ F ₂ N ₆ O ₆ S
PLX7904	Raf	MAPK	C ₂₄ H ₂₂ F ₂ N ₆ O ₃ S

Agent	Target	Pathway	Formula
VPS34-IN1	PI3K	PI3K/Akt/mTOR	C ₂₁ H ₂₄ ClN ₇ O
A-196	Histone Methyltransferase	Epigenetics	C ₁₈ H ₁₆ Cl ₂ N ₄
LDC4297 (LDC044297)	CDK	Cell Cycle	C ₂₃ H ₂₈ N ₈ O
SMI-4a	Pim	JAK/STAT	C ₁₁ H ₆ F ₃ NO ₂ S
Empagliflozin (BI 10773)	SGLT	GPCR & G Protein	C ₂₃ H ₂₇ ClO ₇
TCS 359	FLT3	Angiogenesis	C ₁₈ H ₂₀ N ₂ O ₄ S
NSC 23766	Rho	Cell Cycle	C ₂₄ H ₃₈ Cl ₃ N ₇
GDC-0349	mTOR	PI3K/Akt/mTOR	C ₂₄ H ₃₂ N ₆ O ₃
Cobimetinib (GDC-0973, RG7420)	MEK	MAPK	C ₂₁ H ₂₁ F ₃ IN ₃ O ₂
GW2580	CSF-1R	Protein Tyrosine Kinase	C ₂₀ H ₂₂ N ₄ O ₃
BMS-345541	I κ B/IKK	NF- κ B	C ₁₄ H ₁₇ N ₅
Dynasore	Dynamin	Cytoskeletal Signaling	C ₁₈ H ₁₄ N ₂ O ₄
Venetoclax (ABT-199, GDC-0199)	Bcl-2	Apoptosis	C ₄₅ H ₅₀ ClN ₇ O ₇ S
ICI-118551 Hydrochloride	Adrenergic Receptor	GPCR & G Protein	C ₁₇ H ₂₈ ClNO ₂
AMG 337	c-Met	Protein Tyrosine Kinase	C ₂₃ H ₂₂ FN ₇ O ₃
PF-CBP1 HCl	Epigenetic Reader Domain	Epigenetics	C ₂₉ H ₃₇ ClN ₄ O ₃
CPI-637	Epigenetic Reader Domain	Epigenetics	C ₂₂ H ₂₂ N ₆ O

Agent	Target	Pathway	Formula
BI-78D3	JNK	MAPK	C ₁₃ H ₉ N ₅ O ₅ S ₂
SB366791	TRPV	Transmembrane Transporters	C ₁₆ H ₁₄ ClNO ₂
Thiomyristoyl	Sirtuin	DNA Damage	C ₃₄ H ₅₁ N ₃ O ₃ S
CCT245737	Chk	Cell Cycle	C ₁₆ H ₁₆ F ₃ N ₇ O
GSK6853	Epigenetic Reader Domain	Epigenetics	C ₂₂ H ₂₇ N ₅ O ₃
SHP099 dihydrochloride	phosphatase	Others	C ₁₆ H ₂₁ Cl ₄ N ₅
Selonsertib (GS-4997)	ASK	Apoptosis	C ₂₄ H ₂₄ FN ₇ O
KYA1797K	Wnt/beta-catenin	Stem Cells & Wnt	C ₁₇ H ₁₁ KN ₂ O ₆ S ₂
IPI-549	PI3K	PI3K/Akt/mTOR	C ₃₀ H ₂₄ N ₈ O ₂
SGC2085	Histone Methyltransferase	Epigenetics	C ₁₉ H ₂₄ N ₂ O ₂
Irbinitinib (ARRY-380, ONT-380)	HER2	Protein Tyrosine Kinase	C ₂₆ H ₂₄ N ₈ O ₂
NMS-P118	PARP	DNA Damage	C ₂₀ H ₂₄ F ₃ N ₃ O ₂
BAY-876	GLUT	Metabolism	C ₂₄ H ₁₆ F ₄ N ₆ O ₂
VPS34 inhibitor 1 (Compound 19, PIK-III analogue)	PI3K	PI3K/Akt/mTOR	C ₂₁ H ₂₅ N ₇ O
UK-371804 HCl	Serine Protease	Proteases	C ₁₄ H ₁₇ Cl ₂ N ₅ O ₄ S
GSK'872 (GSK2399872A)	Serine/threonin kinase	Apoptosis	C ₁₉ H ₁₇ N ₃ O ₂ S ₂
LLY-283	Histone Methyltransferase	Epigenetics	C ₁₇ H ₁₈ N ₄ O ₄
GSK180736A (GSK180736)	ROCK	Cell Cycle	C ₁₉ H ₁₆ FN ₅ O ₂

Agent	Target	Pathway	Formula
PD-166866 (PD166866)	FGFR	Angiogenesis	C ₂₀ H ₂₄ N ₆ O ₃
BLU-554 (BLU554)	FGFR	Angiogenesis	C ₂₄ H ₂₄ Cl ₂ N ₄ O ₄
LY3214996	ERK	MAPK	C ₂₂ H ₂₇ N ₇ O ₂ S
PF-06651600	JAK	JAK/STAT	C ₁₅ H ₁₉ N ₅ O
FM-381	JAK	JAK/STAT	C ₂₄ H ₂₄ N ₆ O ₂
AZ31	ATM/ATR	DNA Damage	C ₂₄ H ₂₈ N ₄ O ₃
Tofogliflozin(CSG 452)	SGLT	GPCR & G Protein	C ₂₂ H ₂₈ O ₇
Omarigliptin (MK-3102)	DPP-4	Proteases	C ₁₇ H ₂₀ F ₂ N ₄ O ₃ S
Serabelisib (INK-1117,MLN-1117,TAK-117)	PI3K	PI3K/Akt/mTOR	C ₁₉ H ₁₇ N ₅ O ₃
FX1	Bcl-6	Apoptosis	C ₁₄ H ₉ ClN ₂ O ₄ S ₂
Pamiparib (BGB-290)	PARP	DNA Damage	C ₁₆ H ₁₅ FN ₄ O
NCT-503	Dehydrogenase	Metabolism	C ₂₀ H ₂₃ F ₃ N ₄ S
Chk2 Inhibitor II (BML-277)	Chk	Cell Cycle	C ₂₀ H ₁₄ ClN ₃ O ₂
Ipragliflozin (ASP1941)	SGLT	GPCR & G Protein	C ₂₁ H ₂₁ FO ₅ S
Nec-1s (7-Cl-O-Nec1)	TNF-alpha	Apoptosis	C ₁₃ H ₁₂ ClN ₃ O ₂
GSK'963	NF-κB, TNF-alpha	NF-κB	C ₁₄ H ₁₈ N ₂ O
GNF-6231	Wnt/beta-catenin	Stem Cells & Wnt	C ₂₄ H ₂₅ FN ₆ O ₂
Skp2 inhibitor C1 (SKPin C1)	CDK	Cell Cycle	C ₁₈ H ₁₃ BrN ₂ O ₄ S ₂
PF-06840003	IDO	Metabolism	C ₁₂ H ₉ FN ₂ O ₂
GI254023X	Immunology &	Immunology &	C ₂₁ H ₃₃ N ₃ O ₄

Agent	Target	Pathway	Formula
	Inflammation related	Inflammation	
BAY 1895344 (BAY-1895344)	ATM/ATR	DNA Damage	C ₂₀ H ₂₂ ClN ₇ O
Tenalisib (RP6530)	PI3K	PI3K/Akt/mTOR	C ₂₃ H ₁₈ FN ₅ O ₂
H3B-6527	FGFR	Protein Tyrosine Kinase	C ₂₉ H ₃₄ Cl ₂ N ₈ O ₄
Cu-CPT22	TLR	Immunology & Inflammation	C ₁₉ H ₂₂ O ₇
AZD1390	ATM/ATR	PI3K/Akt/mTOR	C ₂₇ H ₃₂ FN ₅ O ₂
Atuveciclib (BAY-1143572)	CDK	Cell Cycle	C ₁₈ H ₁₈ FN ₅ O ₂ S
LXH254	Raf	MAPK	C ₂₅ H ₂₅ F ₃ N ₄ O ₄
WM-1119	Histone Acetyltransf	Epigenetics	C ₁₈ H ₁₃ F ₂ N ₃ O ₃ S
Evobrutinib	BTK	Protein Tyrosine Kinase	C ₂₅ H ₂₇ N ₅ O ₂
LIT-927	CXCR	Immunology & Inflammation	C ₁₇ H ₁₃ ClN ₂ O ₃
4-Hydroxyquinazoline	Others	antiplatelet	C ₈ H ₆ N ₂ O
Valbenazine tosylate	VMAT2	Others	C ₃₈ H ₅₄ N ₂ O ₁₀ S ₂
SAR125844	c-Met	Protein Tyrosine Kinase	C ₂₅ H ₂₃ FN ₈ O ₂ S ₂
dBET1	Epigenetic Reader Do	Epigenetics	C ₃₈ H ₃₇ ClN ₈ O ₇ S
GSK'547	TNF-alpha	Apoptosis	C ₂₀ H ₁₈ F ₂ N ₆ O
Palbociclib (PD-0332991) HCl	CDK	Cell Cycle	C ₂₄ H ₃₀ ClN ₇ O ₂
Palbociclib (PD0332991) Isethionate	CDK	Cell Cycle	C ₂₆ H ₃₅ N ₇ O ₆ S

Agent	Target	Pathway	Formula
LDN-193189 2HCl	TGF-beta/Smad	TGF-beta/Smad	C ₂₅ H ₂₃ ClN ₆
MCC950(CP-456773)	Immunology & Inflammation related	Immunology & Inflammation	C ₂₀ H ₂₃ N ₂ NaO ₅ S
bpV (HOpic)	PTEN	Others	C ₆ H ₄ K ₂ NO ₈ V
Erlotinib HCl (OSI-744)	Autophagy,EGFR	Protein Tyrosine Kinase	C ₂₂ H ₂₄ ClN ₃ O ₄
SGX-523	c-Met	Protein Tyrosine Kinase	C ₁₈ H ₁₃ N ₇ S
(-)-Huperzine A (HupA)	GluR,AChR	Neuronal Signaling	C ₁₅ H ₁₈ N ₂ O
GSK256066	PDE	Metabolism	C ₂₇ H ₂₆ N ₄ O ₅ S
CCT137690	Aurora Kinase	Cell Cycle	C ₂₆ H ₃₁ BrN ₈ O
Capmatinib (INCB28060)	c-Met	Protein Tyrosine Kinase	C ₂₃ H ₁₇ FN ₆ O
EPZ005687	Histone Methyltransferase	Epigenetics	C ₃₂ H ₃₇ N ₅ O ₃
GSK126	Histone Methyltransferase	Epigenetics	C ₃₁ H ₃₈ N ₆ O ₂
Tazemetostat (EPZ-6438)	Histone Methyltransferase	Epigenetics	C ₃₄ H ₄₄ N ₄ O ₄
ISRIB (trans-isomer)	PERK	Apoptosis	C ₂₂ H ₂₄ Cl ₂ N ₂ O ₄
A-1210477	Bcl-2	Apoptosis	C ₄₆ H ₅₅ N ₇ O ₇ S
Otenabant (CP-945598) HCl	Cannabinoid Receptor	GPCR & G Protein	C ₂₅ H ₂₆ Cl ₃ N ₇ O
FGF401	FGFR	Protein Tyrosine Kinase	C ₂₅ H ₃₀ N ₈ O ₄
Lazertinib (YH25448,GNS-	EGFR	Protein Tyrosine	C ₃₀ H ₃₄ N ₈ O ₃

Agent	Target	Pathway	Formula
1480)		Kinase	

[00211] In some embodiments, different concentrations of the therapeutic agent are applied to the TOs in each partition or a group of replicate partitions. In certain embodiments, different therapeutic agents or combination of therapeutic agents are applied to the TOs in each partition or a group of replicate partitions. In certain embodiments, tumor organoids derived from different subjects are assessed.

[00212] Concurrently with or following the application of the therapeutic agent(s) to the TOs in the partitions, the TOs are contacted with one or more detection agents to assess for changes in morphological features or cell viability of individual cells in the TOs. In exemplary embodiments, the detection agents include fluorescent markers that can be visualized by fluorescent confocal imaging analysis. In particular embodiments, the markers include two or more markers for dead/apoptotic cells. Markers and assays useful for assessing dead apoptotic cells include, but are not limited to, IncuCyte® Caspase-3/7 Green Apoptosis Assay Reagent (Essen Biosciences cat # 4440), and TO-PRO™-3 Iodide (642/661) (Fisher Scientific cat # T3605) and Annexin V assay (Abcam, ab14085).

[00213] In some embodiments, an additional detection agent is used to identify total cells in the tumor organoid sample. In some examples, all cells per organoid are measured by Hoechst 33342 staining, apoptotic cells per organoid are measured by Caspase 3/7 staining and dead/dying cells may be by TO-PRO-3 staining. Utilizing fluorescent markers for all cells and two markers for dead/apoptotic cells permits analysis of TOs at the single cell level and also permits generation of an absolute number of live and dead cells per organoid. This TO by TO analysis provides more information than simply calculating a relative value of viable cells from an entire well. Maintaining TO heterogeneity allows for determination of whether all cells are dying at a constant rate or if there is a mix of susceptible and resistant cells to a given treatment based on the distribution of viable cells per organoid. The aspects, such as the number of partitions (e.g., wells), types of plates, and types of cultures disclosed here, are exemplary in nature. Other aspects known in the art may be used instead or in combination with those aspects disclosed herein.

[00214] Following contact with the detection agents, the TOs are imaged and a tumor organoid profile is obtained from each image. In particular embodiments, the tumor organoid profile includes the number of TOs per image, the total number of cells (live and dead) present in each TO, the number of dead and dying cells present, and/or a cell viability value indicating the percentage of viable cells/tumor organoid. In some embodiments, the TOs are imaged on an inverted confocal microscope using the light microscopy and multiple fluorescent channels with varying wave-length excitation sources (e.g. laser or LED) and emission filters. In exemplary embodiments, an analysis module in a computer system is used to derive the tumor organoid profile from the images.

[00215] In some embodiments, tumor organoid profiles are acquired from at least 5, 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, 1×10^3 , 1×10^4 , or 1×10^5 tumor organoids per condition (e.g., a particular therapeutic dosage). In certain embodiments, the tumor organoid profiles are acquired at different time points. In some embodiments, the tumor organoid profiles acquired are used to generate a dose-response curve (see, e.g., Example 2 and Figure 2, below). In particular embodiments, a particular therapeutic dosage is assigned to a patient based on the dose-response curve. Also provided herein are methods for treating a patient having a cancer with a therapeutic agent based on a dosage derived using the subject method provided herein.

[00216] The high number of measurements recorded per condition (e.g., dose) allows the use of more complex statistical methods that would otherwise be unable to be used with a low-throughput dose response assay. In some embodiments, the tumor organoid profiles obtained are adjusted for one or more confounding technical effect. Use of a linear model allows for inclusion of covariates to adjust for potential confounding technical effects including initial TO viability, differences in growth rates between TOs derived from different patients, and different cancer types, and leverages all of the TO data to gain better statistical power. In some embodiments, a linear model is applied to determine differences between patients, or between drugs, at equivalent therapeutic concentrations (or doses).

[00217] In some embodiments, a brightfield image of the TO sample is obtained for each sample in addition to the cell death/viability data. In particular embodiments, tumor organoid profiles that include cell viability data (e.g., the percentage of viable cells in TO) and corresponding brightfield images for each TO sample at particular conditions (e.g., drug dosage

conditions) are inputted into a classified trainer. In particular embodiments, the trainer is used to predict morphological changes (e.g., cell death or apoptosis) in a tumor organoid sample in response to a particular condition (e.g., dose of a therapeutic agent) based on a brightfield image of the sample (see Example 2 and Figure 3).

[00218] In another aspect, provided herein are computer systems for carrying out the subject methods for assessing the effects of an agent (e.g., a therapeutic agent) on tumor organoids (TOs). In some embodiments, the computer system includes at least one processor and a memory storing at least one program for execution by the at least one processor, the at least one program includes instructions for carrying out one or more steps of the subject methods.

[00219] In some embodiments, the disclosure provides methods and systems for evaluating an effect of a cancer therapeutic agent. In some embodiments, the method includes providing a plurality of tumor organoids cultured in a tumor organoid culture medium, where the plurality of tumor organoids is divided into a plurality of tumor organoid subsets. The method then includes contacting each subset in the plurality of tumor organoid subsets with a cancer therapeutic agent. The method also includes contacting each subset in the plurality of tumor organoid subsets with one or more cell death detection agents and a total cell detection agent. The method then includes obtaining a tumor organoid profile for each subset in the plurality of subset. In some embodiments, the tumor organoid profile includes a cell viability value for every tumor organoid in the subset. The method then includes assessing the effect of the cancer therapeutic agent based on the tumor organoid profiles.

[00220] In some embodiments, the disclosure provides methods and systems for assigning a treatment dosage of a cancer therapeutic agent for a subject in need thereof. In some embodiments, the method includes providing a plurality of tumor organoids cultured in a tumor organoid culture medium, where the tumor organoids are derived from a subject, and where the plurality of tumor organoids is divided into a plurality of tumor organoid subsets. The method then includes contacting each subset in the plurality of tumor organoid subsets with a different dosage of a cancer therapeutic agent. The method also includes contacting each subset in the plurality of tumor organoid subsets with one or more cell death detection agents and a total cell detection agent. The method then includes obtaining a tumor organoid profile for each subset in

the plurality of subset, where the tumor organoid profile includes a cell viability value for every tumor organoid in the subset. The method then includes determining a therapeutic agent dosage curve from the tumor organoid profiles. The method then includes assigning a treatment dosage of the cancer therapeutic agent to the subject based on the therapeutic agent dosage curve.

[00221] *Examples*

[00222] *Example 1 – PARPi Sensitivity of BRCA1⁻/BRCA2⁻ Tumors*

[00223] Tumor biopsies were collected from four individuals: UK1393, 10423-12001, 10524-12001, and 10941-12002. The tumors were genotyped and determined not carry BRCA1 or BRCA2 mutations. As such, according to conventional guidance, the tumors are not indicated for first-line therapy with a PARP inhibitor. In order to test whether these tumors may still be sensitive to PARP inhibitors, despite not carrying a BRCA1 or BRCA2 mutation, tumor organoids were cultured using cells from the biopsied material.

[00224] Briefly, cells within each of the biopsied tumor samples were dissociated and cultured to establish a tumor organoid culture line from the tumor of each individual. Single tumor organoids from the tumor organoid culture lines were collected and plated, individually, into wells of a multiwell plate with culture medium. A series of six concentrations of olaparib, in a 5-fold serial dilution, were established for the tumor organoids derived from each individual, as well as negative controls. After incubation with olaparib, a caspase 3/7 apoptosis assay was performed in each well, to determine the sensitivity of each tumor organoid to the respective concentration of olaparib. Results of the caspase dilution series experiments are plotted in Figure 3.

[00225] As expected, since the tumors did not carry a BRCA1 or BRCA2 mutation, three of the tumor organoid culture lines were fairly insensitive to olaparib: 10423-12001 (Figure 3B), 10524-12001 (Figure 3C), and 10941-12002 (Figure 3D). However, unexpectedly, tumor organoid culture line UK1393 was more than 100-fold more sensitive to olaparib, despite being derived from a tumor that did not carry a BRCA1 or BRCA2 mutation. These results suggest that the cancer in this individual may be effectively treated with a PARP inhibitor as a first-line therapy, despite not carrying a biomarker conventionally associated with PARP inhibitor efficacy.

[00226] *Example 2 – Drug Screening Platform*

[00227] Patient derived Tumor Organoids (TOs) are emerging as patient-representative models that recapitulate clinical responses to candidate therapeutics. Yet standard methods of interpreting in vitro treatments of TOs have been developed from monoclonal, rapidly proliferating 2D cell lines that are not amenable for TOs which have limited biomass and intra-tumoral clonal heterogeneity. To address these challenges, a drug screening platform more applicable for the unique characteristics of TOs was developed and optimized. The platform couples high content fluorescent confocal imaging analysis with a robust statistical analytical approach to measure hundreds of discrete data points of TO viability from as few as 10^3 cells. This approach was validated through evaluating responses to hundreds of small molecule inhibitors as well as a panel of chemotherapeutic agents in TO models derived from different patients.

[00228] The platform was highly reproducible with minimal intra- and inter-assay variance (well:well variance = ns, plate:plate variance = ns, by ANOVA). QC of TOs was performed to remove outlier TOs by size and remaining TOs were normalized by mean vehicle proportion survival. To compare differential therapeutic toxicity between TOs from different patients, a linear model was developed to evaluate differences in proportion of surviving cells across equivalent therapeutic concentrations, identifying highly significant differences ($P < 10^{-12}$). Intriguingly, the linear model not only uncovered heterogeneity of responses between TOs derived from different patients, but also identified organoid clonal populations derived from the same patient with differential drug response offering a window into uncovering functional intratumoral heterogeneity.

[00229] Lastly, throughput was substantially increased by applying a machine learning algorithm to predict therapeutic response via TO morphological changes from light microscopy. Employing this algorithm eliminated the need for fluorescent labeling leading to increased assay throughput by 3-4 fold amounting to 96 and 384 well plate acquisitions in as little as 5 and 15 minutes respectively. As such provided herein is a high-throughput capable of measuring TO therapeutic response with high statistical confidence and exquisite inter-assay reproducibility. This approach can be utilized in research settings to elucidate heterogeneity of therapeutic

responses within and among patients, and may be utilized in the clinical laboratory to potentially guide precision oncology treatments.

[00230] Cell Plating

[00231] A drug screening platform may be utilized to assess the effectiveness of drugs or other agents on tumor organoids (TOs). Single cell suspensions of tumor organoid cells are generated as described below.

1. Gently remove the medium from the tumor organoids (TOs) by pipetting out the medium. Do not use vacuum to aspirate the medium.
2. Add 500 μ L of TrypLE Express to each well, attempting to disrupt the organoid containing Matrigel domes.
3. Scrape the remaining Matrigel off the bottom of the wells with 1 mL pipette.
4. Place the dissociated organoids into a 15 ml low-binding centrifuge tube.
5. Place the tube in the 37°C water bath for 15 min.
6. Spin the cells at 200 g for 5 min. in a 4°C centrifuge.
7. Carefully pipet out and discard the supernatant.
8. Add 1 ml of DPBS to the cell pellet.
9. Centrifuge the cell containing tube at 4°C at 200 g for 5 min.
10. Pipet out the supernatant and add any medium/reagent required for your following procedure.

[00232] In one example, TOs from 24-well plate culture are dissociated to single cells and seeded in 384-well plates in a mix of 30% Matrigel and 70% media. This allows TOs to form from individual cells for the assay, maintaining TO heterogeneity in each well. The cells are seeded at 2,000 cells per well allowing a sufficient number of TOs to form while not overcrowding the plate so that TOs do not overlap or touch allowing for easy identification of individual TOs.

[00233] Drug and Detection Reagent Application

[00234] Caspase 3/7 Green Apoptosis Assay Reagent (Essen Biosciences cat # 4440) is diluted in media to 2.5 μ M. Drugs are diluted to 20 μ M in media with Caspase 3/7 reagent and 10-fold serial dilutions are prepared in a separate 384-well plate. Diluted drugs are added to the

384-well assay plate by pipetting 20 μ l of the diluted drug, media, Caspase 3/7 mix to the appropriate wells using an Integra Viaflo automated pipetting system.

[00235] In one example, one or more chemotherapeutic agents may be applied to the TOs in the well plates. Example agents include Paclitaxel, Gemcitabine, Cisplatin, Carboplatin, Oxaliplatin, Capecitabine, SN-38 (CPT-11), 5-FU, MTX (methotrexate), Docetaxel, Bortezomib, Everolimus, Ulixertinib, Dasatinib, Vinblastine, Nelarabine, Epirubicin, Afatinib, Lapatinib, Cytarabine, Cladribine, Doxorubicin, Azacitidine, and Staurosporine. Other examples include classes of drugs including but not limited to: taxanes, platinating agents, vinca alkaloids, alkylating agents, and anthracyclines.

[00236] *Example 3 - High Content Fluorescent Confocal Imaging Analysis*

[00237] TOs may be stained using common vital dyes to measure cellular behaviors amenable for high content fluorescent confocal imaging analysis. In one example, TOs are stained with Hoechst 33342 (Fisher Scientific cat # H3570), IncuCyte® Caspase-3/7 Green Apoptosis Assay Reagent (Essen Biosciences cat # 4440), and TO-PRO™-3 Iodide (642/661) (Fisher Scientific cat # T3605) in multi-well tissue culture plates (e.g. 24, 48, 96, 384, etc.). After staining, TOs are imaged on an inverted confocal microscope using the light microscopy and multiple fluorescent channels with varying wave-length excitation sources (e.g. laser or LED) and emission filters. Each channel (i.e. light microscopy, Hoechst nuclear stain, FITC, Cy5) is acquired through an objective lens, in one example a 10x objective lens is used to take images at two sites per well with a stack of images in the Z plane ranging from 1-100 heights in the Z-plane with increments ranging from submicron to as high as 15 micron per Z-plane height. Z-stack images are projected to 2D and analyzed using image analysis software with parameters to identify TOs based on the pixel intensities in the nuclear stain channel (i.e. Hoechst 33342 channel) and the size of the object by measurements in 2D space of the object as well as number of nuclei. All cell nuclei are identified by Hoechst 33342 staining. TOs are identified by clusters of nuclei identified by Hoechst 33342 staining. Apoptotic cells are identified by Caspase 3/7 staining and dead cells are identified by TO-PRO-3 staining overlapping with Hoechst 33342 staining. The analysis module is used to enumerate TOs per image, how many cells (live and dead) are present in each TO, and how many dead and dying cells are present in

each TO by Caspase 3/7 and TO-PRO-3 staining separately. The Caspase 3/7 and TO-PRO-3 stains provide two independent counts of dead and dying cells.

[00238] Utilizing fluorescent markers for all cells and two markers for dead/apoptotic cells permits analysis of TOs at the single cell level and also permits generation of an absolute number of live and dead cells per organoid. This TO by TO analysis provides more information than simply calculating a relative value of viable cells from an entire well. Maintaining TO heterogeneity allows for determination of whether all cells are dying at a constant rate or if there is a mix of susceptible and resistant cells to a given treatment based on the distribution of viable cells per organoid. The aspects, such as the number of wells, types of plates, and types of cultures disclosed here, are exemplary in nature. Other aspects known in the art may be used instead or in combination with those aspects disclosed herein.

[00239] Figure 4 shows an example image of high-content fluorescent confocal imaging analysis. (A-D) Brightfield (gray scale), Hoechst 33342 (blue), Caspase 3/7 (green), and TO-PRO-3 (red) staining is shown for vehicle control (A and B) and staurosporine treated (C and D) gastric cancer tumor organoids. (B and D) Overlays of the fluorescent channels and the result of image analysis are shown side by side. Light-blue coloring on the image analysis indicates the area of a given tumor organoid. Live (purple) and dead (red) cells are shown in the image analysis panels. Scale bars represent 100 microns.

[00240] *Example 4 – Statistical Approach*

[00241] Since data are collected from hundreds of TOs per drug condition, hundreds of data points may be analyzed within an experimental replicate usually a single culture well in a multiwell plate, rather than just collecting one data point from each experimental replicate well as is typically done in drug screens. The percent viable cells (which may be determined by a lack of Caspase 3/7 and/or TO-PRO-3 staining) is calculated for each individual TO. The percent viable cells per organoid from each technical replicate is then averaged together for the actual percent viable cells per drug concentration. These values are normalized to the percent viable cells per TO of the DMSO treated vehicle control and plotted to generate a dose-response curve.

[00242] Table 3 contains a small example of the data output. Each row of the table represents an individual organoid. The columns contain information for which well of the

culture plate was imaged (Well Name), the size of the TO (TO area), the total number of cells in the organoid (All cells (TO-PRO-3) and All cells (Caspase 3/7)), the number of dead cells by positive TO-PRO-3 staining (Dead cells (TO-PRO-3)), the number of live cells by negative TO-PRO-3 staining (Live cells (TO-PRO-3)), the number of dead cells by positive Caspase 3/7 staining (Dead cells (Caspase 3/7)), and the number of live cells by negative Caspase 3/7 staining (Live cells (Caspase 3/7)).

[00243] Table 3

Well Name	TO Area	All cells (TO-PRO-3)	Live cells (TO-PRO-3)	Dead cells (TO-PRO-3)	All cells (Caspase 3/7)	Live cells (Caspase 3/7)	Dead cells (Caspase 3/7)
O02	410.178223	2	1	1	2	2	0
O02	152.455612	1	0	1	1	0	1
O02	319.430817	1	0	1	1	0	1
O02	3570.00244	0	0	0	0	0	0
O02	212.348892	1	0	1	1	0	1
O02	246.832901	1	0	1	1	0	1
O02	348.469971	1	0	1	1	0	1
O02	2268.68481	7	0	7	7	0	7
O02	132.491196	1	0	1	1	0	1
O02	1969.21838	7	1	6	7	0	7
O02	1069.00427	4	1	3	4	1	3
O02	373.879242	1	0	1	1	0	1
O02	176.049942	1	0	1	1	0	1
O02	1386.62012	4	0	4	4	0	4
O02	214.163849	1	0	1	1	0	1
O02	397.473572	1	0	1	1	0	1
O02	343.025146	1	0	1	1	0	1
O02	346.655029	1	0	1	1	0	1

Well Name	TO Area	All cells (TO-PRO-3)	Live cells (TO-PRO-3)	Dead cells (TO-PRO-3)	All cells (Caspase 3/7)	Live cells (Caspase 3/7)	Dead cells (Caspase 3/7)
O02	840.320862	2	1	1	2	1	1
O02	208.718994	1	0	1	1	0	1
O02	264.982391	1	0	1	1	0	1
O02	464.626648	1	0	1	1	0	1
O02	947.402771	2	0	2	4	0	4
O02	446.477173	1	0	1	1	0	1
O02	568.078674	3	0	3	3	0	3
O02	431.957581	2	0	2	2	0	2
O02	713.274475	2	0	2	2	0	2
O02	110.711815	1	0	1	1	0	1
O02	980.071838	3	1	2	3	0	3

[00244] Figure 5 shows an example dose-response curve for cell viability by TO-PRO-3 (left) and Caspase 3/7 (right) after treatment with staurosporine on tumor organoids derived from a gastric cancer. Each point represents the mean $\pm$ SD from 450-1200 individual organoids.

[00245] Hundreds of discrete measurements of TO viability per drug concentration can be made, beginning with as few as 6×10^5 cells. These measurements may be performed for every cell of every organoid. There are hundreds of organoids per experimental condition such as incubation with a drug at a specific concentration with approximately between 3-15 cells per organoid.

[00246] Additionally, high reproducibility with minimal inter- and intra-assay variance can be achieved using the subject platform.

[00247] Due to the large number of TOs being measured per well, there is a high number of sampling occurring to identify the true mean. Just as one arrives at a high precision of 0.1666666 probability of rolling a six on a standard die if one rolls the die 600 times instead of

just 6 times. The high accuracy of finding the true mean means that interwell reproducibility is high and inter assay reproducibility is also high.

[00248] To compare differential therapeutic toxicity between TOs from different patients, a linear model was developed to evaluate survival across equivalent therapeutic concentrations.

[00249] The high number of measurements recorded per dose allows the use of more complex statistical or non-statistical methods that would otherwise be unable to be used with a low-throughput dose response assay. Therefore, a linear model was used (Galton, 1886, "Regression Towards Mediocrity in Hereditary Stature," The Journal of the Anthropological Institute of Great Britain and Ireland 15: 246-263) to determine differences between patients, or between drugs, at equivalent therapeutic concentrations (or doses). Use of a linear model allows for inclusion of covariates to adjust for potential confounding technical effects including initial TO viability, differences in growth rates between TOs derived from different patients, and different cancer types, and leverages all of the TO data to gain better statistical power.

[00250] For example, to determine differences in viability to a 10uM dose of staurosporine compared to a 10uM dose of Olaparib that were run on different experiments (plates), we would run the following model and estimate the betas and corresponding p-values for each main effect (x_{drug} , and x_{plate}). Since we estimate both the drug and plate effect, we can control for potential experimental noise generated by the experiment being run on different plates.

[00251] $Y_{\text{normalized viability proportion}} \sim \beta_{X_{\text{drug}}} + \beta_{X_{\text{plate}}} + \epsilon$

[00252] In a different example, differences in viability to a 1 nM dose of Dapagliflozin between TO cell lines that were run by different technicians is determined. Here, differences between TO lines (estimating the beta for $x_{\text{cell line}}$) and determined, with control for experimental noise that might be due to different technicians running the assay :

[00253] $Y_{\text{normalized viability proportion}} \sim \beta_{X_{\text{cell line}}} + \beta_{X_{\text{technician}}} + \epsilon$

[00254] Lastly, throughput was substantially increased by applying a deep learning algorithm to the high-dimensional dataset. The algorithm was adapted from the Generative Adversarial Network Pix2pix by Phillip Isola *et al*, 2018, "Image-to-Image Translation with

Conditional Adversarial Networks,” arXiv:1611.07004v3 [cs.CV] 26Nove2018, which is hereby incorporated by reference. The GAN architecture makes use of a generator model that is trained to generate meaningful data (typically from noise), and a discriminator model that is trained to distinguish between what’s real and what’s been generated.

[00255] Pix2pix is a specific type of GAN designed for general purpose Image-to-Image translation involving the controlled conversion of a source image to target image. Here, pix2pix was employed to transfer the style of brightfield images to fluorescent images.

[00256] Figure 6 shows two examples of predictions on a test set where the brightfield image is shown in the far left column, the model generated fluorescent image is shown in the middle column, and is the actual fluorescent image is shown in the far right column. As shown in Figure 6, the trained model accurately predicted morphological responses to pharmacological agents based on brightfield images, thus potentially reducing a 384 well plate reading to as little as several minutes.

[00257] In summary, the systems and methods described here may measure therapeutic response in patient derived tumor organoids with high statistical confidence and technical reproducibility. The approach may be utilized in both research and clinical settings to better understand heterogeneity of therapeutic responses within and among patients, and further guide precision oncologic decision-making.

[00258] *Example 5 – Example PARP inhibitor Drug Screen*

[00259] A recent development in precision oncology is the use of poly (ADP-ribose) polymerase (PARP) inhibitors in patients whose tumors exhibit evidence of homologous recombination deficiency (HRD), especially in cases of somatic loss of BRCA1 or BRCA2, or LOH of functional BRCA1/2 alleles in patients with inherited non-functional BRCA1/2 alleles.

[00260] To advance TO utility for drug development and precision medicine, a universal label-free TO drug screening assay was developed. Without sacrificing throughput, this neural network prediction of drug response from light microscopy achieved high reproducibility compared to previously described metabolic-based assays (Tiriatic et al., Cancer Discov 8, 1112-1129 (2018); Tiriatic et al., Isolation and Characterization of Patient-derived Pancreatic Ductal Adenocarcinoma Organoid Models. J Vis Exp. (2020); Vlachogiannis et al., Science 359, 920-

926 (2018)). Importantly, the network identified clinically relevant drug responses across a broad range of cancer-types including PARPi response from HRD-positive and -negative TOs. Notably, the PARPi responses in this small cohort of organoids were generally higher in HRD-positive TOs, with certain drugs exhibiting more potency in vitro than others. These observations confirm previous studies in patients (Coleman et al., *Lancet* 390, 1949-1961 (2017)) and short-term ovarian cancer organoid cultures (Hill et al., *Cancer Discov* 8, 1404-1421 (2018)). In addition, the RCA network identified response to afatinib associated with a high ERBB2 (HER2) amplification in the gastric TO. The distribution of response among the TO populations was demonstrative of functional intratumoral heterogeneity, as evidenced by a subset of the organoid population exhibiting resistance to afatinib. When correlated with the patient's clinical course, we found that the biopsy used to establish this TO line was obtained during disease progression on anti-ERBB2 therapy. Identifying heterogeneity in drug response has the potential to uncover mechanisms of primary resistance to novel therapeutics and may also provide predictive and prognostic information for treatment response in personalized clinical assays

[00261] To provide proof-of-concept for this precision medicine approach, we evaluated ten TO lines (breast, endometrial, colon, ovarian, and NSCLC) based on HRD status. We assessed genome-wide LOH and categorized TOs as either HRD-positive or HRD-negative (Timms et al., *Breast Cancer Research* 16, 475 (2014); Yi et al., *International Journal of Cancer* 145, 1209-1220 (2019)). We then exposed TOs to a panel of FDA-approved PARP inhibitors and compared predicted drug responses, based on a trained neural network, to fluorescent-based readouts. We found the network predictions were strongly correlated with fluorescent-based drug responses across all cancer types (Figure 7). Additionally, the classifier's measurement of PARPi response was able to discriminate between HRD-positive and HRD-negative organoids, comparable to fluorescent-based measurements (Figure 8). Thus, the network-based assay accurately predicts clinically relevant drug responses.

[00262] Methodology

[00263] Tumor specimens were received from multiple institutions and processed for organoid culture at a CLIA-certified, CAP-accredited laboratory. All specimens were collected from consented patients under protocols monitored by the Institutional Review Board at each institution. The specimens were placed into iced RPMI (Lifetech) and processed the same day or

after overnight storage at 4°C. The specimens were then digested to single-cell suspension with a GentleMACs instrument using the Miltenyi Tumor Dissociation kit (Miltenyi Biotec) according to the manufacturer's protocol.

[00264] Development and culture of tumor organoids

[00265] Tumor organoids (TOs) were developed using an adapted epithelial cell-only submerged Matrigel culture technique (Sato et al., Nature 459, 262-265 (2009)). Briefly, cells were seeded at a density of 400 cells/ μ l in a 50 μ l droplet of GFR Matrigel matrix (Corning) and grown in 24-well, flat-bottom tissue culture plates (Eppendorf). Growth medium was changed twice weekly and TOs were passaged every 1-2 weeks as needed. For passaging, growth media was removed, and Matrigel domes were disrupted mechanically by adding 500 μ l TrypLE Express Enzyme (GIBCO) per well. TOs were transferred to 15-ml conical tubes and incubated in a 37°C water bath for 10 minutes. Cells were then centrifuged at 200 x g for 3 minutes and resuspended in 2 ml DPBS. Cells were resuspended in GFR Matrigel Matrix at 400 cells per microliter, and 50 μ l domes were plated in pre-warmed, 24-well, flat-bottom plates and left to polymerize in a 37°C incubator before the addition of 500 μ l growth medium and 10 μ M Y-27632 (Bio-technique) per well.

[00266] TO cultures were maintained in serum-free defined media conditions based on the consensus of previous reports for anticipated tumor types; namely, Advanced DMEM/F12 nutrient mix supplemented with B27, nicotinamide, n-acetylcysteine, recombinant growth factors EGF, Noggin, R-spondin1 (RSPO-1), Wnt-3A, FGF-2, 7, and 10, and small molecule inhibitors of p38 MAPK and TGF-beta.

[00267] Tumor organoid growth analysis

[00268] Two independent observers determined organoid formation and serial passaging. Cells that demonstrated exponential increase in biomass on serial passage were deemed high proliferation and were cryopreserved in Recovery Cell Culture Freezing media (Gibco) when biomass reached $>10^6$ cells, following the manufacturer's protocol.

[00269] For quantitative organoid growth measurements, bright-field images of 24-well plates containing TOs were captured at a minimum of two different time points for the same passage using the ImageXpress Micro Confocal high-content imaging system (Molecular Devices, CA).

The average total area sum of TOs was then plotted by time and the maximum growth rate was calculated by linear regression.

[00270] Pathologic Evaluation

[00271] Cultures were intermittently selected for formalin fixation and were prepared for H&E staining either via paraffin embedding or cyto-spin. H&E stains of organoid cultures from 320 patients were interpreted by a board-certified pathologist (GK) using histologic and cytologic features. Due to the use of growth factors, cultures displayed features of cellular proliferation including nuclear hyperchromasia with slight pleomorphism, increase in nuclear to cytoplasmic ratio and conspicuous nucleoli. Mitotic figures were frequent with few atypical forms. As such, the identification of malignant cells on the basis of cytologic features was obscured and was therefore based on the degree of these changes and the presence of marked loss of polarity and macronucleoli.

[00272] Sample processing and nucleic acid isolation

[00273] Organoids were dissociated using TrypLE Express Enzyme (GIBCO) and dissociated cells were immediately lysed in 350 μ L of buffer RLT from the Allprep DNA/RNA Micro Kit (Qiagen) and stored at -80°C. Upon thawing, cell lysates were homogenized using QIAshredder spin columns (Qiagen). DNA and RNA were isolated using the Allprep DNA/RNA Micro Kit per manufacturer's instructions.

[00274] HLA-typed PBMC controls used for flow cytometry analysis were commercially procured as cryovials. PBMCs were thawed, washed, and resuspended in RPMI growth medium containing 10% FBS and 2 mM L-glutamine. Cells were left to recover for 1 hr at 37°C.

[00275] Next-generation sequencing

[00276] Next-generation sequencing was conducted as previously described (Beaubier et al., 2019a; Beaubier et al., 2019b). Briefly, nucleic acids were library prepped using a targeted gene panel as previously described, loaded on an Illumina HiSeq 4000 (Illumina), and DNA tumor libraries were sequenced to an average unique on-target depth of 500x. Similarly, RNA was prepared using an exome-capture RNA-Seq protocol and sequenced on the Illumina HiSeq 4000 platform. Variant calls and other molecular signatures, such as copy-number alterations, fusion

events, and mRNA gene expression levels, were called and reported based on established guidelines.

[00277] Associations of clinical and molecular features with sustainable growth

[00278] Clinical features including tissue site, T stage, N stage, M stage, race (caucasian, Asian, African-american, other), sex, neoadjuvant therapy treatment, and biospecimen features such as cell count and cold ischemia time (>12 hours) were structured as binary values and evaluated as a generalized linear model. Logistic regression was then applied to model high-proliferation organoid growth as a linear combination of clinical features using the stats R package (v3.5.2).

[00279] Analysis of single-nucleotide variants (SNV), copy-number alterations (CNA), and transcriptome profiling was performed using a NGS assay (Beaubier et al., Nat Biotechnol 37, 1351-1360 (2019); Beaubier et al., Oncotarget 10, 2384-2396 (2019)). The combined effect of pathogenic variants within a gene classified by the assay platform were then tested using a gene-based test as implemented in SKAT-o (Lee et al., American journal of human genetics 91, 224-237 (2012)). Gene-based tests were performed within each cancer and combined across cancers. Transcriptome analysis was performed as described below.

[00280] Copy-number calls were determined for paired probes by an internal algorithm that considered tumor purity and sequencing depth in a two-pass approach. First, segmentation of chromosomal regions was performed to define chromosomal regions with specific copy numbers using panel coverage data and transformed to provide probe-level copy-number values. Next, the segment data was used in a second algorithm to approximate integer copy-number values for major (total observed copies at a given loci) and minor allele count as well as estimates for tumor purity, ploidy, and B-allele frequencies (BAF). Following segmentation, copy number and tumor purity were assessed using a grid search methodology. Starting from the initial estimate of the tumor purity lower bound, a copy-number state matrix was generated containing total/minor copy state combinations and their expected log ratios and logBAF given the initialized tumor purity. Each segment was projected into the matrix and the log probability that it belonged to each analyzed copy state was computed based on drawing from a normal probability density function defined by the expected log-ratio and the pre-computed log ratio standard deviation. The same process was applied for logBAF with a sliding-weight scale based on the number of

heterozygous germline variants observed within the segment. This was done to account for noise in logBAF in the context of sparse observations.

[00281] Drug screen and image analysis

[00282] A 320-drug panel was purchased from Selleckchem, which is detailed on their website (found online at [selleckchem.com/screening/selective-library.html](https://www.selleckchem.com/screening/selective-library.html)). Additional compounds are included in STAR methods.

[00283] TOs were dissociated as described above and resuspended in a 30:70% mix of GFR Matrigel:growth media at a concentration of 100 cells/ μ l. The solution was added to 384-well assay plates (Corning) at 20 μ l per well for a final concentration of 2,000 cells per well. Assay plates were covered with a Breathe-Easy sealing membrane (Sigma Aldrich) to prevent evaporation. TOs were grown for 72 hours before drug addition. Drugs were prepared in growth media with 2.5 μ M Caspase-3/7 Green Apoptosis Assay Reagent (Essen Bioscience). Serial dilutions of each molecule were prepared in 384-well polystyrene plates (Nunc). Diluted drug was added to the assay plate using an Integra Viaflo pipette (Integra) mounted on an Integra Assist Plus Pipetting Robot (Integra). Assay plates were again covered with a Breathe-Easy sealing membrane and TOs were exposed to drugs for another 72 hours before imaging.

[00284] Prior to imaging, TOs were incubated with 4 μ M Hoechst 33342 (Fisher Scientific) and 300 nM TO-PRO-3 Iodide (642/661) (Invitrogen) for 1.5-2 hours. Assay plates were imaged using an ImageXpress Micro Confocal (Molecular Devices) at 10x magnification so that ~100-200 TOs were imaged per well. Images were acquired as 4 x 15 μ m Z-stacks and the 2D projections were analyzed to assess cell viability. Confocal images were analyzed using the MetaXpress software (Molecular Devices) custom module editor feature to design an analysis module that identified TOs by clusters of Hoechst 33342 staining, individual cells by Hoechst 33342 staining, and dead/dying cells by either TO-PRO-3 or Caspase-3/7 staining. The result of this analysis module is a spreadsheet detailing the number of live and dead cells for every individual organoid.

[00285] The percentage of viable cells per organoid was calculated based on the image analysis described above. Organoids with fewer than three cells and larger than the top one percent by size were excluded from analysis. The mean viability for all organoids at a given drug concentration was used in dose-response curves to calculate AUC. AUC was calculated using the

computeAUC function using settings for “actual” AUC of the R Package PharmacGx (v1.17.1). Heatmaps of AUC values were generated using the Pheatmap package (v1.0.12) in R. Scatterplots of AUC values were generated using the ggplot2 package (v3.3.0) in R.

[00286] Response and Label-Free prediction from brightfield images

[00287] The multiplexed fluorescence images were 1024x1024x3 RGB images, where red corresponds to dead cells (TO-PRO-3), green to apoptotic cells (Caspase-3/7), and blue to nuclei (Hoechst 33342). All wavelength channels underwent a simple intensity rescaling contrast enhancement technique to brighten and sharpen the TOs/cells as well as remove background noise. The mean viability for all organoids per site was obtained from the MetaXpress software readout.

[00288] *Example 6 – PARPi sensitivity in colorectal cancer tumor organoids*

[00289] In this example, PARPi sensitivity was measured for two colorectal tumor organoids (TOs). Each TO or source tissue associated with the TO (for example, a patient biopsy used to generate the TO) were genetically sequenced to determine whether the TO had variants (mutations) in homologous recombination proteins (including BRCA1 and BRCA2) and whether the TO was likely to have homologous recombination deficiency (HRD), according to an HRD engine.

[00290] None of the TOs had pathogenic single nucleotide variants (SNVs) in BRCA1 or BRCA2. None of the TOs had loss of heterozygosity (LOH) of BRCA1 or BRCA2. None of the TOs had bi-allelic loss of BRCA1 or BRCA2 (both a pathogenic SNV and LOH) and traditionally would not have been expected to have HRD or respond to PARPi therapy. BRCA mutation and LOH status was based on sequencing analysis of either the TO or the source tissue.

[00291] Regardless of the lack of bi-allelic BRCA loss, some of the TOs were analyzed by an HRD engine and predicted to have HRD based on other criteria.

[00292] The HRD engine is described in U.S. Patent App. No. 16/789,363, titled “An Integrated Machine-Learning Framework To Predict Homologous Recombination Deficiency”, filed February 12, 2020, the contents of which are incorporated by reference herein in their entirety for any and all purposes. In another embodiment, the HRD score or likelihood of PARPi sensitivity may be determined by the trained classifier disclosed herein.

[00293] One of the TOs had a low HRD score (unlikely to have HRD or be sensitive to PARPi) and one of the TOs had a high HRD score (likely to have HRD and be sensitive to PARPi).

[00294] All of the TOs were grown in culture wells and each well was exposed for 96 hours to either a negative control (1% DMSO) or one of multiple PARPi therapies (Rucaparib, Niraparib, Pamiparib, Talazoparib, Olaparib, or Veliparib) at one of three concentrations (1 nM, 100 nM, or 10,000 nM). Some of the organoids were exposed to additional concentrations as noted in the x-axis of the figures.

[00295] At 96 hours the cells were stained by either caspase 3/7 or TO-PRO-3 (caspase 3/7 stained dying, apoptic cells and TO-PRO-3 stained dead cells). For each PARPi therapy, efficacy was measured at each concentration by detecting the proportion of viable cells that were not stained and normalizing that proportion to the proportion of viable cells in the negative control well, where the proportion of negative control well cells that were viable was adjusted to 100% or 75% (indicated in the figure). This normalization resulted in some experimental wells having a proportion of viable cells that was greater than 100% (or 75%). A best fit curve was generated for the viability proportion at each concentration and an inverse AUC was calculated as the area between actual cell viability (the best fit curve) and 100% (or 75%) cell viability. The inverse AUC served as an additional measure of PARPi efficacy, where a higher inverse AUC indicated a higher drug efficacy.

[00296] The PARPi therapies were hypothesized to be more effective against TOs with a high HRD score than TOs with a low HRD score.

[00297] The viability data shown below indicate that the PARPi therapies were more effective against TOs with a high HRD score than TOs with a low HRD score.

[00298] For organoids that were PARPi sensitive, PARPi therapies were effective against TOs that did not have BRCA1 or BRCA2 mutations or biallelic inactivation of BRCA1 or BRCA2.

[00299] FIGS. 9A and 9B illustrate viability data for a tumor organoid having a low HRD score, a pathogenic BRCA1 mutation, no BRCA2 mutations and no BRCA1 or BRCA2 LOH. The x-axis label indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control).

[00300] FIGS. 10A and 10B illustrate viability data for a colorectal tumor organoid having a high HRD score, no BRCA1 or BRCA2 mutations and no BRCA1 or BRCA2 LOH. The x-axis label indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control).

[00301] *Example 7 – PARPi sensitivity in breast cancer tumor organoids*

[00302] In this example, PARPi sensitivity was measured for three tumor organoids (TOs). Each TO or source tissue associated with the TO (for example, a patient biopsy used to generate the TO) were genetically sequenced to determine whether the TO had variants (mutations) in homologous recombination proteins (including BRCA1 and BRCA2) and whether the TO was likely to have homologous recombination deficiency (HRD), according to an HRD engine.

[00303] None of the TOs had pathogenic single nucleotide variants (SNVs) in BRCA1 or BRCA2. None of the TOs had loss of heterozygosity (LOH) of BRCA1 or BRCA2. None of the TOs had bi-allelic loss of BRCA1 or BRCA2 (both a pathogenic SNV and LOH) and traditionally would not have been expected to have HRD or respond to PARPi therapy. BRCA mutation and LOH status was based on sequencing analysis of either the TO or the source tissue.

[00304] Regardless of the lack of bi-allelic BRCA loss, some of the TOs were analyzed by an HRD engine and predicted to have HRD based on other criteria.

[00305] The HRD engine is described in U.S. Patent App. No. 16/789,363, titled “An Integrated Machine-Learning Framework To Predict Homologous Recombination Deficiency”, filed February 12, 2020, the contents of which are incorporated by reference herein in their entirety for any and all purposes. In another embodiment, the HRD score or likelihood of PARPi sensitivity may be determined by the trained classifier disclosed herein.

[00306] Two of the TOs had a low HRD score (unlikely to have HRD or be sensitive to PARPi) and one of the TOs had a high HRD score (likely to have HRD and be sensitive to PARPi).

[00307] All of the TOs were grown in culture wells and each well was exposed for 96 hours to either a negative control (1% DMSO) or one of multiple PARPi therapies (Rucaparib, Niraparib, Pamiparib, Talazoparib, Olaparib, or Veliparib) at one of three concentrations (1 nM, 100 nM, or

10,000 nM). Some of the organoids were exposed to additional concentrations as noted in the x-axis of the figures.

[00308] At 96 hours the cells were stained by either caspase 3/7 or TO-PRO-3. Caspase 3/7 stained dying, apoptotic cells and TO-PRO-3 stained dead cells. For each PARPi therapy, efficacy was measured at each concentration by detecting the proportion of viable cells that were not stained and normalizing that proportion to the proportion of viable cells in the negative control well, where the proportion of negative control well cells that were viable was adjusted to 100% or 75% (indicated in the figure). This normalization resulted in some experimental wells having a proportion of viable cells that was greater than 100% (or 75%). A best fit curve was generated for the viability proportion at each concentration and an inverse AUC was calculated as the area between actual cell viability (the best fit curve) and 100% (or 75%) cell viability. The inverse AUC served as an additional measure of PARPi efficacy, where a higher inverse AUC indicated a higher drug efficacy.

[00309] The PARPi therapies were hypothesized to be more effective against TOs with a high HRD score than TOs with a low HRD score.

[00310] The viability data shown below indicate that the PARPi therapies were more effective against TOs with a high HRD score than TOs with a low HRD score.

[00311] For organoids that were PARPi sensitive, PARPi therapies were effective against TOs that did not have BRCA1 or BRCA2 mutations or biallelic inactivation of BRCA1 or BRCA2.

[00312] FIGS. 11A and 11B illustrate viability data for a tumor organoid having a low HRD score, no BRCA1 or BRCA2 mutations and no BRCA1 or BRCA2 LOH. The x-axis label indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control).

[00313] FIGS. 12A and 12B illustrate viability data for a tumor organoid having a low HRD score, no BRCA1 or BRCA2 mutations and no BRCA1 or BRCA2 LOH. The x-axis label indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control).

[00314] FIGS. 13A and 13B illustrate viability data for a tumor organoid having a high HRD score, no BRCA1 or BRCA2 mutations and no BRCA1 or BRCA2 LOH. The x-axis label

indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control).

[00315] *Example 8 – PARPi sensitivity in ovarian cancer tumor organoids*

[00316] In this example, PARPi sensitivity was measured for two ovarian cancer tumor organoids (TOs). Each TO or source tissue associated with the TO (for example, a patient biopsy used to generate the TO) were genetically sequenced to determine whether the TO had variants (mutations) in homologous recombination proteins (including BRCA1 and BRCA2) and whether the TO was likely to have homologous recombination deficiency (HRD), according to an HRD engine.

[00317] None of the TOs had pathogenic single nucleotide variants (SNVs) in BRCA1 or BRCA2. One of the TOs had loss of heterozygosity (LOH) of BRCA1 or BRCA2 and had only one functioning copy of the gene. None of the TOs had bi-allelic loss of BRCA1 or BRCA2 (both a pathogenic SNV and LOH) and traditionally would not have been expected to have HRD or respond to PARPi therapy. BRCA mutation and LOH status was based on sequencing analysis of either the TO or the source tissue.

[00318] Regardless of the lack of bi-allelic BRCA loss, some of the TOs were analyzed by an HRD engine and predicted to have HRD based on other criteria.

[00319] The HRD engine is described in U.S. Patent App. No. 16/789,363, titled “An Integrated Machine-Learning Framework To Predict Homologous Recombination Deficiency”, filed February 12, 2020, the contents of which are incorporated by reference herein in their entirety for any and all purposes. In another embodiment, the HRD score or likelihood of PARPi sensitivity may be determined by the trained classifier disclosed herein.

[00320] One of the TOs had a low HRD score (unlikely to have HRD or be sensitive to PARPi) and one of the TOs had a high HRD score (likely to have HRD and be sensitive to PARPi).

[00321] All of the TOs were grown in culture wells and each well was exposed for 96 hours to either a negative control (1% DMSO) or one of multiple PARPi therapies (Rucaparib, Niraparib, Pamiparib, Talazoparib, Olaparib, or Veliparib) at one of three concentrations (1 nM, 100 nM, or

10,000 nM). Some of the organoids were exposed to additional concentrations as noted in the x-axis of the figures.

[00322] At 96 hours the cells were stained by either caspase 3/7 or TO-PRO-3. Caspase 3/7 stained dying, apoptotic cells and TO-PRO-3 stained dead cells. For each PARPi therapy, efficacy was measured at each concentration by detecting the proportion of viable cells that were not stained and normalizing that proportion to the proportion of viable cells in the negative control well, where the proportion of negative control well cells that were viable was adjusted to 100% or 75% (indicated in the figure). This normalization resulted in some experimental wells having a proportion of viable cells that was greater than 100% (or 75%). A best fit curve was generated for the viability proportion at each concentration and an inverse AUC was calculated as the area between actual cell viability (the best fit curve) and 100% (or 75%) cell viability. The inverse AUC served as an additional measure of PARPi efficacy, where a higher inverse AUC indicated a higher drug efficacy.

[00323] The PARPi therapies were hypothesized to be more effective against TOs with a high HRD score than TOs with a low HRD score.

[00324] The viability data shown below indicate that the PARPi therapies were more effective against TOs with a high HRD score than TOs with a low HRD score.

[00325] For organoids that were PARPi sensitive, PARPi therapies were effective against TOs that did not have BRCA1 or BRCA2 mutations or biallelic inactivation of BRCA1 or BRCA2.

[00326] FIGS. 14A and 14B illustrate viability data for a tumor organoid having a low HRD score, no BRCA1 or BRCA2 mutations, BRCA1 LOH, and no BRCA2 LOH. The x-axis label indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control).

[00327] FIGS. 15A and 15B illustrate viability data for a tumor organoid having a high HRD score, no BRCA1 or BRCA2 mutations and no BRCA1 or BRCA2 LOH. The x-axis label indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control). Lower doses of PARPi were more effective for this organoid than the low HRD shown in the previous figures organoid.

[00328] *Example 9 – PARPi sensitivity in non-small cell lung cancer cancer tumor organoids*

[00329] In this example, PARPi sensitivity was measured for two non-small cell lung cancer tumor organoids (TOs). Each TO or source tissue associated with the TO (for example, a patient biopsy used to generate the TO) were genetically sequenced to determine whether the TO had variants (mutations) in homologous recombination proteins (including BRCA1 and BRCA2) and whether the TO was likely to have homologous recombination deficiency (HRD), according to an HRD engine.

[00330] None of the TOs had pathogenic single nucleotide variants (SNVs) in BRCA1 or BRCA2. None of the TOs had loss of heterozygosity (LOH) of BRCA1 or BRCA2. None of the TOs had bi-allelic loss of BRCA1 or BRCA2 (both a pathogenic SNV and LOH) and traditionally would not have been expected to have HRD or respond to PARPi therapy. BRCA mutation and LOH status was based on sequencing analysis of either the TO or the source tissue.

[00331] Regardless of the lack of bi-allelic BRCA loss, some of the TOs were analyzed by an HRD engine and predicted to have HRD based on other criteria.

[00332] The HRD engine is described in U.S. Patent App. No. 16/789,363, titled “An Integrated Machine-Learning Framework To Predict Homologous Recombination Deficiency”, filed February 12, 2020, the contents of which are incorporated by reference herein in their entirety for any and all purposes. In another embodiment, the HRD score or likelihood of PARPi sensitivity may be determined by the trained classifier disclosed herein.

[00333] One of the TOs had a low HRD score (unlikely to have HRD or be sensitive to PARPi) and one of the TOs had a high HRD score (likely to have HRD and be sensitive to PARPi).

[00334] All of the TOs were grown in culture wells and each well was exposed for 96 hours to either a negative control (1% DMSO) or one of multiple PARPi therapies (Rucaparib, Niraparib, Pamiparib, Talazoparib, Olaparib, or Veliparib) at one of three concentrations (1 nM, 100 nM, or 10,000 nM). Some of the organoids were exposed to additional concentrations as noted in the x-axis of the figures.

[00335] At 96 hours the cells were stained by either caspase 3/7 or TO-PRO-3. Caspase 3/7 stained dying, apoptic cells and TO-PRO-3 stained dead cells. For each PARPi therapy, efficacy was measured at each concentration by detecting the proportion of viable cells that were not stained and normalizing that proportion to the proportion of viable cells in the negative control well, where the proportion of negative control well cells that were viable was adjusted to 100% or 75% (indicated in the figure). This normalization resulted in some experimental wells having a proportion of viable cells that was greater than 100% (or 75%). A best fit curve was generated for the viability proportion at each concentration and an inverse AUC was calculated as the area between actual cell viability (the best fit curve) and 100% (or 75%) cell viability. The inverse AUC served as an additional measure of PARPi efficacy, where a higher inverse AUC indicated a higher drug efficacy.

[00336] The PARPi therapies were hypothesized to be more effective against TOs with a high HRD score than TOs with a low HRD score.

[00337] The viability data shown below indicate that the PARPi therapies were more effective against TOs with a high HRD score than TOs with a low HRD score.

[00338] For organoids that were PARPi sensitive, PARPi therapies were effective against TOs that did not have BRCA1 or BRCA2 mutations or biallelic inactivation of BRCA1 or BRCA2.

[00339] FIGS. 16A and 16B illustrate viability data for a tumor organoid having a low HRD score, no BRCA1 or BRCA2 mutations and no BRCA1 or BRCA2 LOH. The x-axis label indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control).

[00340] FIGS. 17A and 17B illustrate viability data for a tumor organoid having a high HRD score, no BRCA1 or BRCA2 mutations, no BRCA1 LOH and having BRCA2 LOH. The x-axis label indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control).

[00341] *Example 10 – PARPi sensitivity in endometrial tumor organoids*

[00342] In this example, PARPi sensitivity was measured for one endometrial tumor organoid (TOs). Each TO or source tissue associated with the TO (for example, a patient biopsy used to generate the TO) were genetically sequenced to determine whether the TO had variants

(mutations) in homologous recombination proteins (including BRCA1 and BRCA2) and whether the TO was likely to have homologous recombination deficiency (HRD), according to an HRD engine.

[00343] None of the TOs had pathogenic single nucleotide variants (SNVs) in BRCA1 or BRCA2. None of the TOs had loss of heterozygosity (LOH) of BRCA1 or BRCA2. None of the TOs had bi-allelic loss of BRCA1 or BRCA2 (both a pathogenic SNV and LOH) and traditionally would not have been expected to have HRD or respond to PARPi therapy. BRCA mutation and LOH status was based on sequencing analysis of either the TO or the source tissue.

[00344] The TO was analyzed by an HRD engine and predicted not to have HRD.

[00345] The HRD engine is described in U.S. Patent App. No. 16/789,363, titled “An Integrated Machine-Learning Framework To Predict Homologous Recombination Deficiency”, filed February 12, 2020, the contents of which are incorporated by reference herein in their entirety for any and all purposes. In another embodiment, the HRD score or likelihood of PARPi sensitivity may be determined by the trained classifier disclosed herein.

[00346] One of the TOs had a low HRD score (unlikely to have HRD or be sensitive to PARPi).

[00347] All of the TOs were grown in culture wells and each well was exposed for 96 hours to either a negative control (1% DMSO) or one of multiple PARPi therapies (Rucaparib, Niraparib, Pamiparib, Talazoparib, Olaparib, or Veliparib) at one of three concentrations (1 nM, 100 nM, or 10,000 nM). Some of the organoids were exposed to additional concentrations as noted in the x-axis of the figures.

[00348] At 96 hours the cells were stained by either caspase 3/7 or TO-PRO-3. Caspase 3/7 stained dying, apoptic cells and TO-PRO-3 stained dead cells. For each PARPi therapy, efficacy was measured at each concentration by detecting the proportion of viable cells that were not stained and normalizing that proportion to the proportion of viable cells in the negative control well, where the proportion of negative control well cells that were viable was adjusted to 100% or 75% (indicated in the figure). This normalization resulted in some experimental wells having a proportion of viable cells that was greater than 100% (or 75%). A best fit curve was generated for the viability proportion at each concentration and an inverse AUC was calculated as the area

between actual cell viability (the best fit curve) and 100% (or 75%) cell viability. The inverse AUC served as an additional measure of PARPi efficacy, where a higher inverse AUC indicated a higher drug efficacy.

[00349] The viability data shown below indicate that the PARPi therapies were not effective against a TO with a low HRD score.

[00350] FIGS. 18A and 18B illustrate viability data for a tumor organoid having a low HRD score, no BRCA1 or BRCA2 mutations and no BRCA1 or BRCA2 LOH. The x-axis label indicates the PARP-i therapy and dose. The y-axis indicates the percent of cells in the well that were viable, normalized to the DMSO mock condition (negative control).

REFERENCES CITED AND ALTERNATIVE EMBODIMENTS

[00351] All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

[00352] The present invention can be implemented as a computer program product that comprises a computer program mechanism embedded in a non-transitory computer readable storage medium. For instance, the computer program product could contain the program modules shown in any combination in Figure 1 and/or as described elsewhere within the application. These program modules can be stored on a CD-ROM, DVD, magnetic disk storage product, USB key, or any other non-transitory computer readable data or program storage product.

[00353] The methods and systems described above may be utilized in combination with or as part of a digital and laboratory health care platform that is generally targeted to medical care and research. It should be understood that many uses of the methods and systems described above, in combination with such a platform, are possible. One example of such a platform is described in U.S. Patent Application No. 16/657,804, titled "Data Based Cancer Research and Treatment Systems and Methods", and filed 10/18/2019, which is incorporated herein by reference and in its entirety for all purposes.

[00354] Many modifications and variations of this disclosure can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only. The embodiments were chosen and described in order to best explain the principles of the invention and its practical applications, to thereby enable others skilled in the art to best utilize the invention and various embodiments with various modifications as are suited to the particular use contemplated. The disclosure is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

What is claimed is:

1. A method for evaluating a cancer that does not carry either a BRCA1 or a BRCA2 mutation in a test subject, the method comprising determining a sensitivity of the cancer to a poly ADP ribose polymerase (PARP) inhibitor by:

A) culturing a plurality of tumor organoids, each respective tumor organoid in the plurality of tumor organoids from one or more cells of a tumor biopsy from the test subject;

B) exposing the one or more organoids cultured in A) to one or more amounts of the PARP inhibitor;

C) measuring the fitness of cells in the one or more tumor organoids following the exposure to the one or more amounts of the PARP inhibitor; and

D) correlating the measured fitness of the cells with a sensitivity to the PARP inhibitor.

2. The method of claim 1, further comprising:

treating cancer in the subject by:

when the correlated sensitivity to the PARP inhibitor satisfies a sensitivity threshold, communicating a recommended therapy comprising the PARP inhibitor to the test subject, and

when the correlated sensitivity to the PARP inhibitor does not satisfy a sensitivity threshold, communicating a recommended therapy that does not include the PARP inhibitor to the test subject.

3. The method of claim 1 or 2, further comprising:

treating cancer in the subject by:

when the correlated sensitivity to the PARP inhibitor satisfies a sensitivity threshold, administering a therapy comprising the PARP inhibitor to the test subject, and

when the correlated sensitivity to the PARP inhibitor does not satisfy a sensitivity threshold, administering a therapy that does not include the PARP inhibitor to the test subject.

4. The method of any one of claims 1-3, wherein the cancer is a breast cancer.

5. The method of any one of claims 1-3, wherein the cancer is an ovarian cancer.

6. The method of any one of claims 1-3, wherein the cancer is not breast cancer or ovarian cancer.
7. The method of any one of claims 1-6, wherein the test subject has not previously been treated for cancer.
8. The method of any one of claims 1-7, wherein the PARP inhibitor is selected from the group consisting of olaparib, veliparib, rucaparib, niraparib, and talazoparib.
9. A method for training a classifier to discriminate between two or more tumor sensitivities to a poly ADP ribose polymerase (PARP) inhibitor, the method comprising:
 - at a computer system comprising least one processor and a memory storing at least one program for execution by the at least one processor:
 - (A) obtaining a data set comprising, for each respective tissue sample in a plurality of tissue samples, (i) a corresponding plurality of nucleic acid features of the tissue sample, and (ii) a corresponding indication of the sensitivity of a respective organoid cultured from one or more cells of the tissue sample to the PARP inhibitor; and
 - (B) training an untrained classifier against at least (i) the corresponding plurality of features and (ii) the corresponding indication of the sensitivity of the organoid to the PARP inhibitor, across the plurality of tissue samples, thereby obtaining a trained classifier that discriminates between the two or more tumor sensitivities to a poly ADP ribose polymerase (PARP) inhibitor.
10. The method of claim 9, wherein the two or more tumor sensitivities include a first level of sensitivity to a first PARP inhibitor and a second level of sensitivity to the first PARP inhibitor.
11. The method of claim 9 or 10, wherein the two or more tumor sensitivities include a first sensitivity to a first PARP inhibitor and a second sensitivity to a second PARP inhibitor.
12. The method of any one of claims 9-11, wherein the PARP inhibitor is selected from the group consisting of olaparib, veliparib, rucaparib, niraparib, and talazoparib.

13. The method of any one of claims 9-12, wherein the classifier is further trained to discriminate between the two or more tumor sensitivities to a PARP inhibitor and one or more tumor sensitivity to a chemotherapeutic drug that is not a PARP inhibitor.

14. The method of any one of claims 9-13, wherein each tissue sample in the plurality of tissue samples was obtained from a subject that had not received treatment for cancer prior to the tissue sample being obtained.

15. The method of any one of claims 9-14, wherein each tissue sample in the plurality of tissue samples comprises a breast cancer biopsy.

16. The method of any one of claims 9-14, wherein each tissue sample in the plurality of tissue samples comprises an ovarian cancer biopsy.

17. The method of any one of claims 9-14, wherein each tissue sample in the plurality of tissue samples comprises a biopsy of a homologous recombination deficient (HRD) cancer.

18. The method of any one of claims 9-14, wherein each tissue sample in the plurality of tissue samples comprises a biopsy from a cancer other than breast cancer or ovarian cancer.

19. The method of any one of claims 9-18, wherein the plurality of nucleic acid features of the tissue sample comprise one or more of support for a single nucleotide variant at a genomic location, a methylation status at a genomic location, a relative copy number for a genomic location, an allelic ratio for a genomic location, a relative expression level of a gene, and mathematical combinations thereof.

20. The method of claim 19, wherein the plurality of nucleic acid features of the tissue sample comprises a BRCA1 or BRCA2 mutant allele status.

21. The method of any one of claims 9-20, wherein at least a sub-plurality of the plurality of nucleic acid features of the tissue sample are measured from a tumor organoid cultured from one or more cells of the tissue sample.

22. The method of any one of claims 9-21, wherein the indication of the sensitivity of the respective organoid to the PARP inhibitor is based at least in part on one or more cellular fitness measurements obtained by:

i) culturing one or more organoids, each respective organoid in the one or more organoids from one or more cells of the respective tissue sample;

ii) exposing the one or more organoids cultured in i) to one or more amounts of the PARP inhibitor; and

iii) measuring the fitness of cells in the one or more organoids following the exposure to the one or more amounts of the PARP inhibitor, thereby obtaining the one or more cellular fitness measurements.

23. The method of claim 22, wherein the one or more cellular fitness measurements are one or more measurements of cellular apoptosis following exposure to the PARP inhibitor.

24. The method of claim 22 or 23, wherein, for at least one respective tissue sample in the plurality of tissue samples, the one or more organoids cultured in i) are exposed to a sensitizing therapy before being exposed to the PARP inhibitor.

25. The method of any one of claims 9-24, wherein the untrained classifier is further trained against, for each respective tissue sample in the plurality of tissue samples, (iii) a corresponding cancer classification of the tissue sample in a plurality of cancer classifications.

26. The method of claim 25, wherein the plurality of cancer classifications comprises cancerous tissue and non-cancerous tissue.

27. The method of claim 25 or 26, wherein the plurality of cancer classifications comprises a plurality of types of cancer.

28. The method of any one of claims 25-27, wherein the plurality of cancer classifications comprises a plurality of stages of cancer.

29. The method of any one of claims 9-28, wherein:

the plurality of tissue samples comprise a plurality of tumor biopsies; and
the training (B) comprises identifying a plurality of genes that are differentially expressed between (i) organoids, cultured from one or more cells of a respective tumor biopsy in the plurality of tumor biopsies, that have a first sensitivity to the PARP inhibitor, and (ii) organoids, cultured from one or more cells of a respective tumor biopsy in the plurality of tumor biopsies, that have a second sensitivity to the PARP inhibitor.

30. The method of any one of claims 9-29, wherein the untrained classifier is further trained against, for each respective tissue sample in the plurality of tissue samples, (iv) one or more phenotypic characteristics of the tissue sample.

31. The method of claim 30, wherein the one or more phenotypic characteristics of the tissue sample comprise a histologic feature of the tissue sample.

32. The method of any one of claims 9-31, wherein the untrained classifier is further trained against, for each respective tissue sample in the plurality of tissue samples, (v) one or more characteristics of the subject from which the tissue sample was obtained.

33. The method of any one of claims 9-32, wherein the classifier is a multinomial classifier that is trained to provide a plurality of likelihoods, wherein each respective likelihood in the plurality of likelihoods is a likelihood that tumor cells from a test subject with cancer will have a different respective sensitivity in the two or more tumor sensitivities to a PARP inhibitor.

34. The method of any one of claims 9-33, wherein the classifier is a neural network algorithm, a support vector machine algorithm, a Naive Bayes algorithm, a nearest neighbor algorithm, a boosted trees algorithm, a random forest algorithm, a convolutional neural network algorithm, a decision tree algorithm, a regression algorithm, or a clustering algorithm.

35. A method for recommending therapy for treating a cancer in a subject, the method comprising:

A) obtaining a first test data set comprising a plurality of nucleic acid features of a tumor biopsy from the test subject;

B) evaluating the first test data set using a classifier trained to discriminate between two or more tumor sensitivities to a first poly ADP ribose polymerase (PARP) inhibitor, wherein:

the classifier was trained against, for each respective training tissue sample in a plurality of training tissue samples, at least (i) the plurality of nucleic acid features obtained from the respective training tissue sample, and (ii) a corresponding indication of the sensitivity of a respective organoid cultured from one or more cells of the respective training tissue sample to the first PARP inhibitor,

a first tumor sensitivity in the two or more tumor sensitivities to the first PARP inhibitor is associated with a first recommendation, in a plurality of recommendations, to treat the cancer in the subject with the first PARP inhibitor, and

a second tumor sensitivity in the two or more tumor sensitivities to the first PARP inhibitor is associated with a second recommendation, in the plurality of recommendations, not to treat the cancer in the subject with the first PARP inhibitor; and

C) providing a respective recommendation, in the plurality of recommendations, for treating the cancer in the test subject based on the results of the evaluating in step B).

36. The method of claim 35, wherein the cancer is breast cancer.

37. The method of claim 35, wherein the cancer is ovarian cancer.

38. The method of claim 35, wherein the cancer is a homologous recombination deficient (HRD) cancer.

39. The method of claim 35, wherein the cancer is not breast cancer or ovarian cancer.

40. The method of any one of claims 35-39, wherein the cancer is a BRCA1⁺/BRCA2⁺ cancer.

41. The method of any one of claims 35-40, wherein the plurality of nucleic acid features of the tumor biopsy comprise one or more of support for a single nucleotide variant at a genomic location, a methylation status at a genomic location, a relative copy number for a genomic location, an allelic ratio for a genomic location, a relative expression level of a gene, and mathematical combinations thereof.

42. The method of claim 41, wherein the plurality of nucleic acid features of the tumor biopsy comprises a BRCA1 or BRCA2 mutant allele status.

43. The method of any one of claims 35-42, wherein:

the first test data set further comprises a cancer status of the test subject in a plurality of cancer statuses; and

the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, a cancer status of the respective training tissue sample in the plurality of cancer statuses.

44. The method of claim 43, wherein the plurality of cancer statuses comprises a plurality of types of cancer.

45. The method of claim 43 or 44, wherein the plurality of cancer statuses comprises a plurality of stages of cancer.

46. The method of any one of claims 35-45, wherein:

the first test data set further comprises one or more phenotypic characteristics of the tumor biopsy; and

the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, the one or more phenotypic characteristics of the respective training tissue sample.

47. The method of claim 46, wherein the one or more phenotypic characteristics of the tumor biopsy comprise a histologic feature of the tumor biopsy.

48. The method of any one of claims 35-47, wherein:

the first test data set further comprises one or more characteristics of the subject selected from the group consisting of tumor organoid histology, baseline histology, gender, age, family medical history, personal history of cancer, treatment history of cancer, diagnosis, subtype, IHC markers, DNA/RNA sequence, a genomic methylation pattern, a radiologic image or feature, a comorbidity, a geographic location, ancestry, an ethnicity, diet, gut microbiome, substance

usage, alcohol use, physical exam findings, baseline vitals, vaccination history, religious or cultural beliefs, marital status, sexual orientation, nocturnal/diurnal rhythm, sleep schedule, a travel history, a presence or absence of parasitic organisms, an infectious disease history, a germline DNA sequence, a biometric feature, skull shape, physical profile, occupational history, a pet history, a mental health history, a tumor organoid morphology, a tumor organoid, and a smoking history; and

the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, the one or more characteristics of the subject from which the respective training tissue sample was obtained.

49. The method of any one of claims 35-48, further comprising:

obtaining a second test data set comprising the plurality of nucleic acid features of a tumor biopsy from the test subject; and

evaluating the second test data set using the classifier trained to discriminate between two or more tumor sensitivities to a first poly ADP ribose polymerase (PARP) inhibitor,

wherein the plurality of nucleic acid features in the first test data set are from a first tumor biopsy from the test subject and the second test data set are from a second tumor biopsy from the test subject that is different from the first tumor biopsy.

50. The method of claim 49, wherein the first tumor biopsy and the second tumor biopsy are from different sections of a same tumor of the subject.

51. The method of claim 49, wherein the first tumor biopsy and the second tumor biopsy are from different tumors of the subject.

52. The method of any one of claims 35-51, wherein the classifier is a multinomial classifier that is trained to provide a plurality of likelihoods, wherein each respective likelihood in the plurality of likelihoods is a likelihood that a different respective treatment regime, in a plurality of treatment regimes, for the cancer in the subject will be more effective than the other treatment regimes in the plurality of treatment regimes.

53. The method of any one of claims 35-49, wherein the classifier is a neural network algorithm, a support vector machine algorithm, a Naive Bayes algorithm, a nearest neighbor algorithm, a boosted trees algorithm, a random forest algorithm, a convolutional neural network algorithm, a decision tree algorithm, a regression algorithm, or a clustering algorithm.

54. The method of any one of claims 35-53, wherein the first PARP inhibitor is selected from the group consisting of olaparib, veliparib, rucaparib, niraparib, and talazoparib.

55. The method of any one of claims 35-54, wherein the classifier is trained to further discriminate between two or more tumor sensitivities to a second PARP inhibitor, wherein the second PARP inhibitor is different than the first PARP inhibitor.

56. The method of claim 55, wherein the second PARP inhibitor is selected from the group consisting of olaparib, veliparib, rucaparib, niraparib, and talazoparib.

57. The method of any one of claims 35-56, wherein the classifier is trained to further discriminate between two or more tumor sensitivities to a chemotherapeutic drug that is not a PARP inhibitor.

58. The method of any one of claims 35-57, wherein the first recommendation is a recommendation to treat the cancer in the subject with the first PARP inhibitor as a first-line therapy.

59. The method of any one of claims 35-57, wherein the first recommendation is a recommendation to treat the cancer in the subject with the first PARP inhibitor as a second-line therapy.

60. The method of any one of claims 35-59, wherein the first recommendation is a recommendation to treat the cancer in the subject with the first PARP inhibitor and without any other chemotherapeutic drug.

61. The method of claim 60, further comprising, after providing the first recommendation for treating the cancer in the subject, administering the first PARP inhibitor to the test subject.

62. The method of any of one claims 35-59, wherein the first recommendation is a recommendation to treat the cancer in the subject with the first PARP inhibitor and with a second cancer therapy.

63. The method of claim 60, further comprising, after providing the first recommendation for treating the cancer in the subject, co-administering the first PARP inhibitor and the second cancer therapy to the test subject.

64. The method of claim 62 or 63, wherein the second cancer therapy is administration of a chemotherapeutic drug that is not a PARP inhibitor.

65. The method of any one of claims 35-64, wherein the second recommendation is a recommendation to treat the cancer in the subject with a second PARP inhibitor.

66. The method of claim 65, further comprising, after providing the second recommendation for treating the cancer in the subject, administering the second PARP inhibitor to the test subject.

67. The method of claim 65 or 66, wherein the plurality of recommendations further comprises a third recommendation to treat the cancer in the subject with a chemotherapeutic drug that is not a PARP inhibitor.

68. The method of claim 67, further comprising, after providing the third recommendation for treating the cancer in the subject, administering the chemotherapeutic drug that is not a PARP inhibitor to the test subject.

69. The method of any one of claims 35-64, wherein the second recommendation is a recommendation to treat the cancer in the subject with a therapeutic drug that is not a PARP inhibitor.

70. The method of claim 69, further comprising, after providing the second recommendation for treating the cancer in the subject, administering the chemotherapeutic drug that is not a PARP inhibitor to the test subject.

71. The method of any one of claims 35-70, wherein:

the first recommendation is a recommendation to treat the cancer in the subject with the first PARP inhibitor at a first therapeutic dose; and

the plurality of recommendations further comprises a fourth recommendation to treat the cancer in the subject with the first PARP inhibitor at a second therapeutic dose.

72. The method of any one of claims 35-71, wherein:

prior to providing C) the respective recommendation, the method further comprises:

identifying at least two recommendations, in the plurality of recommendations, based on the results of the evaluating in step B), and

determining a sensitivity of the cancer in the subject to each respective therapy regimen, in a plurality of therapy regimens, associated with the at least two recommendations by:

a) culturing a plurality of tumor organoids, each respective tumor organoid in the plurality of tumor organoids from one or more cells of a tumor biopsy from the subject;

b) exposing the one or more organoids cultured in a) to one or more amounts of a therapeutic agent associated with the respective therapy regimen; and

c) measuring the fitness of cells in the one or more organoids following the exposure to the one or more amounts of the therapeutic agent, thereby obtaining one or more cellular fitness measurements for each respective therapy regimen associated with the two or more recommendations; and

the recommendation provided in C) is selected from the two or more recommendations and is based on the one or more cellular fitness measurements obtained for each respective therapy regimen associated with the two or more recommendations.

73. The method of any one of claims 35-72, further comprising administering the recommended therapy for treating cancer to the test subject.

74. A method for treating a BRCA1⁻/BRCA2⁻ cancer in a test subject, the method comprising:

A) determining a probability or likelihood that the cancer will be sensitive to a poly ADP ribose polymerase (PARP) inhibitor; and

B) treating cancer in the subject by:

when the probability or likelihood that the cancer will be sensitive to the PARP inhibitor satisfies a sensitivity threshold, communicating a recommended therapy comprising the PARP inhibitor to the test subject, and

when the probability or likelihood that the cancer will be sensitive to the PARP inhibitor does not satisfy a sensitivity threshold, communicating a recommended therapy that does not include the PARP inhibitor to the test subject.

75. The method of claim 74, wherein the cancer is breast cancer.

76. The method of claim 74, wherein the cancer is ovarian cancer.

77. The method of claim 74, wherein the cancer is not breast cancer or ovarian cancer.

78. The method of any one of claims 74-77, wherein the test subject has not previously been treated for cancer.

79. The method of any one of claims 74-78, wherein the PARP inhibitor is selected from the group consisting of olaparib, veliparib, rucaparib, niraparib, and talazoparib.

80. The method of any one of claims 74-79, wherein the probability or likelihood that the cancer will be sensitive to the PARP inhibitor is determined by:

1) culturing a plurality of tumor organoids, each respective tumor organoid in the plurality of tumor organoids from one or more cells of a tumor biopsy from the test subject;

2) exposing the one or more organoids cultured in a) to one or more amounts of the PARP inhibitor;

3) measuring the fitness of cells in the one or more tumor organoids following the exposure to the one or more amounts of the PARP inhibitor, and

4) correlating the measured fitness of the cells with a probability or likelihood that the cancer will be sensitive to the PARP inhibitor.

81. The method of any one of claims 74-79, wherein the probability or likelihood that the cancer will be sensitive to the PARP inhibitor is determined by:

1) obtaining a first test data set comprising a plurality of nucleic acid features of a tumor biopsy from the test subject;

2) evaluating the first test data set using a classifier trained to discriminate between two or more tumor sensitivities to the PARP inhibitor, wherein:

the classifier was trained against, for each respective training tissue sample in a plurality of training tissue samples, at least (i) the plurality of nucleic acid features obtained from the respective training tissue sample, and (ii) a corresponding indication of the sensitivity of a respective organoid cultured from one or more cells of the respective training tissue sample to the PARP inhibitor.

82. The method of claim 81, wherein the plurality of nucleic acid features of the tumor biopsy comprise one or more of support for a single nucleotide variant at a genomic location, a methylation status at a genomic location, a relative copy number for a genomic location, an allelic ratio for a genomic location, a relative expression level of a gene, and mathematical combinations thereof.

83. The method of claim 81 or 82, wherein:

the first test data set further comprises a cancer status of the test subject in a plurality of cancer statuses; and

the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, a cancer status of the respective training tissue sample in the plurality of cancer statuses.

84. The method of claim 83, wherein the plurality of cancer statuses comprises a plurality of types of cancer.

85. The method of claim 83 or 84, wherein the plurality of cancer statuses comprises a plurality of stages of cancer.

86. The method of any one of claims 81-85, wherein:

the first test data set further comprises one or more phenotypic characteristics of the tumor biopsy; and

the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, the one or more phenotypic characteristics of the respective training tissue sample.

87. The method of claim 86, wherein the one or more phenotypic characteristics of the tumor biopsy comprise a histologic feature of the tumor biopsy.

88. The method of any one of claims 81-87, wherein:

the first test data set further comprises one or more characteristics of the subject selected from the group consisting of tumor organoid histology, baseline histology, gender, age, family medical history, personal history of cancer, treatment history of cancer, diagnosis, subtype, IHC markers, DNA/RNA sequence, a genomic methylation pattern, a radiologic image or feature, a comorbidity, a geographic location, ancestry, an ethnicity, diet, gut microbiome, substance usage, alcohol use, physical exam findings, baseline vitals, vaccination history, religious or cultural beliefs, marital status, sexual orientation, nocturnal/diurnal rhythm, sleep schedule, a travel history, a presence or absence of parasitic organisms, an infectious disease history, a germline DNA sequence, a biometric feature, skull shape, physical profile, occupational history, a pet history, a mental health history, a tumor organoid morphology, a tumor organoid, and a smoking history; and

the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, the one or more characteristics of the subject from which the respective training tissue sample was obtained.

89. The method of any one of claims 81-88, further comprising:

obtaining a second test data set comprising the plurality of nucleic acid features of a tumor biopsy from the test subject; and

evaluating the second test data set using the classifier trained to discriminate between the two or more tumor sensitivities to the PARP inhibitor,

wherein the plurality of nucleic acid features in the first test data set are from a first tumor biopsy from the test subject and the second test data set are from a second tumor biopsy from the test subject that is different from the first tumor biopsy.

90. The method of claim 89, wherein the first tumor biopsy and the second tumor biopsy are from different sections of a same tumor of the subject.

91. The method of claim 89, wherein the first tumor biopsy and the second tumor biopsy are from different tumors of the subject.

92. The method of any one of claims 81-91, wherein the classifier is a multinomial classifier that is trained to provide a plurality of likelihoods, wherein each respective likelihood in the plurality of likelihoods is a likelihood that a different respective treatment regime, in a plurality of treatment regimes, for the cancer in the subject will be more effective than the other treatment regimes in the plurality of treatment regimes.

93. The method of any one of claims 81-92, wherein the classifier is a neural network algorithm, a support vector machine algorithm, a Naive Bayes algorithm, a nearest neighbor algorithm, a boosted trees algorithm, a random forest algorithm, a convolutional neural network algorithm, a decision tree algorithm, a regression algorithm, or a clustering algorithm.

94. The method of any one of claims 74-93, further comprising administering the recommended therapy for treating cancer to the test subject.

95. A method for treating a cancer other than breast cancer or ovarian cancer in a test subject, the method comprising:

A) determining a probability or likelihood that the cancer will be sensitive to a poly ADP ribose polymerase (PARP) inhibitor; and

B) treating cancer in the subject by:

when the probability or likelihood that the cancer will be sensitive to the PARP inhibitor satisfies a threshold likelihood, communicating a recommended therapy comprising the PARP inhibitor to the test subject, and

when the probability or likelihood that the cancer will be sensitive to the PARP inhibitor does not satisfy a threshold likelihood, communicating a recommended therapy that does not include the PARP inhibitor to the test subject.

96. The method of claim 95, wherein the cancer is a BRCA1⁺/BRCA2⁺ cancer.

97. The method of claim 95 or 96, wherein the test subject has not previously been treated for cancer.

98. The method of any one of claims 95-97, wherein the PARP inhibitor is selected from the group consisting of olaparib, veliparib, rucaparib, niraparib, and talazoparib.

99. The method of any one of claims 95-98, wherein the probability or likelihood that the cancer will be sensitive to the PARP inhibitor is determined by:

1) culturing a plurality of tumor organoids, each respective tumor organoid in the plurality of tumor organoids from one or more cells of a tumor biopsy from the test subject;

2) exposing the one or more organoids cultured in a) to one or more amounts of the PARP inhibitor; and

3) measuring the fitness of cells in the one or more tumor organoids following the exposure to the one or more amounts of the PARP inhibitor, and

4) correlating the measured fitness of the cells with a probability or likelihood that the cancer will be sensitive to the PARP inhibitor.

100. The method of any one of claims 95-98, wherein the probability or likelihood that the cancer will be sensitive to the PARP inhibitor is determined by:

1) obtaining a first test data set comprising a plurality of nucleic acid features of a tumor biopsy from the test subject; and

2) evaluating the first test data set using a classifier trained to discriminate between two or more tumor sensitivities to the PARP inhibitor, wherein:

the classifier was trained against, for each respective training tissue sample in a plurality of training tissue samples, at least (i) the plurality of nucleic acid features obtained from the respective training tissue sample, and (ii) a corresponding indication of the sensitivity of a

respective organoid cultured from one or more cells of the respective training tissue sample to the PARP inhibitor.

101. The method of claim 100, wherein the plurality of nucleic acid features of the tumor biopsy are selected from the group consisting of support for a single nucleotide variant at a genomic location, a methylation status at a genomic location, a relative copy number for a genomic location, an allelic ratio for a genomic location, a relative expression level of a gene, and a mathematical combination thereof.

102. The method of claim 100 or 101, wherein:

the first test data set further comprises a cancer status of the test subject in a plurality of cancer statuses; and

the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, a cancer status of the respective training tissue sample in the plurality of cancer statuses.

103. The method of claim 102, wherein the plurality of cancer statuses comprises a plurality of types of cancer.

104. The method of claim 102 or 103, wherein the plurality of cancer statuses comprises a plurality of stages of cancer.

105. The method of any one of claims 100-104, wherein:

the first test data set further comprises one or more phenotypic characteristics of the tumor biopsy; and

the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, the one or more phenotypic characteristics of the respective training tissue sample.

106. The method of claim 105, wherein the one or more phenotypic characteristics of the tumor biopsy comprise a histologic feature of the tumor biopsy.

107. The method of any one of claims 100-106, wherein:

the first test data set further comprises one or more characteristics of the subject selected from the group consisting of tumor organoid histology, baseline histology, gender, age, family medical history, personal history of cancer, treatment history of cancer, diagnosis, subtype, IHC markers, DNA/RNA sequence, a genomic methylation pattern, a radiologic image or feature, a comorbidity, a geographic location, ancestry, an ethnicity, diet, gut microbiome, substance usage, alcohol use, physical exam findings, baseline vitals, vaccination history, religious or cultural beliefs, marital status, sexual orientation, nocturnal/diurnal rhythm, sleep schedule, a travel history, a presence or absence of parasitic organisms, an infectious disease history, a germline DNA sequence, a biometric feature, skull shape, physical profile, occupational history, a pet history, a mental health history, a tumor organoid morphology, a tumor organoid, and a smoking history; and

the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, the one or more characteristics of the subject from which the respective training tissue sample was obtained.

108. The method of any one of claims 100-107, further comprising:

obtaining a second test data set comprising the plurality of nucleic acid features of a tumor biopsy from the test subject; and

evaluating the second test data set using the classifier trained to discriminate between the two or more tumor sensitivities to the PARP inhibitor,

wherein the plurality of nucleic acid features in the first test data set are from a first tumor biopsy from the test subject and the second test data set are from a second tumor biopsy from the test subject that is different from the first tumor biopsy.

109. The method of claim 108, wherein the first tumor biopsy and the second tumor biopsy are from different sections of a same tumor of the subject.

110. The method of claim 108, wherein the first tumor biopsy and the second tumor biopsy are from different tumors of the subject.

111. The method of any one of claims 100-110, wherein the classifier is a multinomial classifier that is trained to provide a plurality of likelihoods, wherein each respective likelihood in the plurality of likelihoods is a likelihood that a different respective treatment regime, in a plurality of treatment regimes, for the cancer in the subject will be more effective than the other treatment regimes in the plurality of treatment regimes.

112. The method of any one of claims 100-111, wherein the classifier is a neural network algorithm, a support vector machine algorithm, a Naive Bayes algorithm, a nearest neighbor algorithm, a boosted trees algorithm, a random forest algorithm, a convolutional neural network algorithm, a decision tree algorithm, a regression algorithm, or a clustering algorithm.

113. The method of any one of claims 95-112, further comprising administering the recommended therapy for treating cancer to the test subject.

114. A method for providing a clinical report for a cancer patient to a physician, comprising:

(A) obtaining a first test data set comprising features of a transcriptome from a tumor biopsy from the cancer patient;

(B) evaluating the first test data set using a classifier trained, according to the method of any one of claims 9-34, to discriminate between two or more tumor sensitivities to a first poly ADP ribose polymerase (PARP) inhibitor;

(C) receiving a recommended therapy for the cancer patient from the classifier; and

(D) including the recommended therapy, or sending the recommended therapy to a third party for inclusion, in a clinical report for the cancer patient.

115. The method of claim 114, wherein the recommended therapy for the cancer patient comprises administration of the PARP inhibitor.

116. The method of claim 114, wherein the cancer patient has breast cancer.

117. The method of claim 114, wherein the cancer patient has ovarian cancer.

118. The method of claim 114, wherein the cancer patient has a homologous recombination deficient (HRD) cancer.

119. The method of claim 114, wherein the cancer patient does not have breast cancer or ovarian cancer.

120. The method of any one of claims 114-119, wherein the cancer patient has a BRCA1⁻/BRCA2⁻ cancer.

121. A method for identifying an inhibitor of poly ADP ribose polymerase (PARP), the method comprising:

- A) obtaining a tissue sample that is sensitive to a known PARP inhibitor;
- B) culturing one or more organoids, each respective organoid in the one or more organoids from one or more cells of the tissue sample;
- C) exposing the one or more organoids cultured in B) to one or more concentrations of a candidate molecule; and
- D) measuring the fitness of cells in the one or more organoids following the exposure to the one or more concentrations of the candidate molecule, wherein reduced fitness of cells in the one or more organoids is indicative that the candidate molecule is a PARP inhibitor.

122. The method of claim 121, wherein the tissue sample comprises breast cancer cells.

123. The method of claim 121 or 122, wherein the tissue sample comprises ovarian cancer cells.

124. The method of any one of claims 121-123, wherein the tissue sample comprises cells from a cancer other than breast cancer or ovarian cancer.

125. The method of any one of claims 121-124, wherein the tissue sample comprises cells from a cancer that does not carry a BRCA1 or BRCA2 variant allele.

126. The method of any one of claims 121-124, wherein the tissue sample comprises cells from a cancer that carries a BRCA1 or BRCA2 variant allele.

127. The method of any one of claims 121-126, wherein the tissue sample comprises cells from a homologous recombination deficient (HRD) cancer.

128. A method for identifying a new use for a pharmaceutical compound of a first pharmaceutical class, the method comprising:

A) obtaining a plurality of tissue samples, wherein each respective tissue sample in the plurality of tissue samples is sensitive to a respective class of pharmaceutical agents in a plurality of classes of pharmaceutical agents that excludes the first pharmaceutical class;

B) culturing, for each respective tissue sample in the plurality of tissue samples, one or more respective organoids from one or more cells of the respective tissue sample, thereby generating a plurality of organoid cultures;

C) exposing, for each organoid culture in the plurality of organoid cultures, the respective one or more organoids to one or more concentrations of the pharmaceutical agent; and

D) measuring, for each organoid culture in the plurality of organoid cultures, the fitness of cells in the respective one or more organoids following the exposure to the pharmaceutical agent, wherein reduced fitness of the cells in the respective one or more organoids is indicative that the pharmaceutical molecule shares pharmacological properties with the class of pharmaceutical compounds in the plurality of classes of pharmaceutical classes to which the tissue sample corresponding to the respective one or more organoids is sensitive.

129. The method of claim 128, wherein the plurality of tissue samples comprises cells from at least 3 different types of cancers.

130. A method for determining the eligibility of a cancer patient for a clinical trial of a candidate cancer pharmaceutical agent, the method comprising:

A) obtaining a tumor biopsy from the cancer patient;

B) culturing one or more tumor organoids from one or more cells of the tumor biopsy,

C) exposing the one or more tumor organoids to one or more concentrations of the candidate cancer pharmaceutical agent; and

D) measuring the fitness of cells in the one or more tumor organoids following the exposure to the one or more concentrations of the candidate cancer pharmaceutical agent;

E) determining whether the cancer patient is eligible for the clinical trial based on at least the measured fitness of the cells in the one or more tumor organoids, wherein reduced fitness of the cells in the one or more tumor organoids is indicative that the cancer patient is eligible for the clinical trial.

131. A method for determining the eligibility of a cancer patient for a clinical trial of a candidate cancer pharmaceutical agent, the method comprising:

A) obtaining a first test data set comprising a plurality of nucleic acid features of a tumor biopsy from the cancer patient;

B) evaluating the first test data set using a classifier trained to discriminate between two or more tumor sensitivities to the candidate cancer pharmaceutical agent, wherein:

the classifier was trained against, for each respective training tissue sample in a plurality of training tissue samples, at least (i) the plurality of nucleic acid features obtained from the respective training tissue sample, and (ii) a corresponding indication of the sensitivity of a respective organoid cultured from one or more cells of the respective training tissue sample to the candidate cancer pharmaceutical agent,

a first tumor sensitivity in the two or more tumor sensitivities to the candidate cancer pharmaceutical agent is associated with an indication that the cancer patient is eligible for the clinical trial, and

a second tumor sensitivity in the two or more tumor sensitivities to the candidate cancer pharmaceutical agent is associated with an indication that the cancer patient is eligible for the clinical trial; and

C) determining whether the cancer patient is eligible for the clinical trial based on at least the evaluating B).

132. The method of claim 130 or 131, wherein the candidate cancer pharmaceutical agent is a poly ADP ribose polymerase (PARP) inhibitor.

133. The method of any one of claims 130-132, wherein the clinical trial is for the treatment of a breast cancer.

134. The method of any one of claims 130-132, wherein the clinical trial is for the treatment of an ovarian cancer.

135. The method of any one of claims 130-132, wherein the clinical trial is for the treatment of a cancer other than a breast cancer or an ovarian cancer.

136. The method of any one of claims 130-135, wherein the clinical trial is for the treatment of a cancer that does not carry a BRCA1 or BRCA2 variant allele.

137. The method of any one of claims 130-135, wherein the clinical trial is for the treatment of a cancer that carries a BRCA1 or BRCA2 variant allele.

138. The method of any one of claims 130-137, wherein the clinical trial is for the treatment of a homologous recombination deficient (HRD) cancer.

139. The method of any one of claims 130-138, wherein the candidate cancer pharmaceutical agent has previously been found to be effective for the treatment of a cancer having a first clinical marker and the clinical trial is for the treatment of a cancer that does not have the first clinical marker.

140. The method of any one of claims 131-139, wherein the plurality of nucleic acid features of the tumor biopsy are selected from the group consisting of support for a single nucleotide variant at a genomic location, a methylation status at a genomic location, a relative copy number for a genomic location, an allelic ratio for a genomic location, a relative expression level of a gene, and a mathematical combination thereof.

141. The method of any one of claims 131-140, wherein:

the first test data set further comprises a cancer status of the test subject in a plurality of cancer statuses; and

the classifier was further trained against, for each respective training tissue sample in a plurality of the training tissue samples, a cancer status of the respective training tissue sample in the plurality of cancer statuses.

142. The method of claim 141, wherein the plurality of cancer statuses comprises a plurality of types of cancer.

143. The method of claim 141 or 142, wherein the plurality of cancer statuses comprises a plurality of stages of cancer.

144. The method of any one of claims 131-143, wherein:

the first test data set further comprises one or more phenotypic characteristics of the tumor biopsy; and

the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, the one or more phenotypic characteristics of the respective training tissue sample.

145. The method of claim 144, wherein the one or more phenotypic characteristics of the tumor biopsy comprise a histologic feature of the tumor biopsy.

146. The method of any one of claims 131-145, wherein:

the first test data set further comprises one or more characteristics of the subject selected from the group consisting of tumor organoid histology, baseline histology, gender, age, family medical history, personal history of cancer, treatment history of cancer, diagnosis, subtype, IHC markers, DNA/RNA sequence, a genomic methylation pattern, a radiologic image or feature, a comorbidity, a geographic location, ancestry, an ethnicity, diet, gut microbiome, substance usage, alcohol use, physical exam findings, baseline vitals, vaccination history, religious or cultural beliefs, marital status, sexual orientation, nocturnal/diurnal rhythm, sleep schedule, a travel history, a presence or absence of parasitic organisms, an infectious disease history, a germline DNA sequence, a biometric feature, skull shape, physical profile, occupational history, a pet history, a mental health history, a tumor organoid morphology, a tumor organoid, and a smoking history; and

the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, the one or more characteristics of the subject from which the respective training tissue sample was obtained.

147. A method for identifying a resistance to a chemotherapeutic agent in a patient with cancer, the method comprising:

A) obtaining a first test data set comprising a plurality of nucleic acid features of a tumor biopsy from the patient with cancer;

B) evaluating the first test data set using a classifier trained to discriminate between two or more tumor sensitivities to the chemotherapeutic agent, wherein:

the classifier was trained against, for each respective training tissue sample in a plurality of training tissue samples, at least (i) the plurality of nucleic acid features obtained from the respective training tissue sample, and (ii) a corresponding indication of the sensitivity of a respective organoid cultured from one or more cells of the respective training tissue sample to the chemotherapeutic agent,

a first tumor sensitivity in the two or more tumor sensitivities to the candidate cancer pharmaceutical agent is associated with an indication that the patient's cancer is resistant to the chemotherapeutic agent, and

a second tumor sensitivity in the two or more tumor sensitivities to the candidate cancer pharmaceutical agent is associated with an indication that the patient's cancer is not resistant to the chemotherapeutic agent; and

C) providing a report indicating whether the patient's cancer is resistant to the chemotherapeutic agent based on at least the results of the evaluating B).

148. The method of claim 147, wherein the chemotherapeutic agent is a poly ADP ribose polymerase (PARP) inhibitor.

149. The method of claim 148, wherein the PARP inhibitor is selected from the group consisting of olaparib, veliparib, rucaparib, niraparib, and talazoparib.

150. The method of any one of claims 147-149, wherein the cancer is breast cancer.

151. The method of any one of claims 147-149, wherein the cancer is ovarian cancer.

152. The method of any one of claims 147-149, wherein the cancer is a homologous recombination deficient (HRD) cancer.

153. The method of any one of claims 147-149, wherein the cancer is not breast cancer or ovarian cancer.

154. The method of any one of claims 147-153, wherein the cancer does not carry either a BRCA1 or a BRCA2 mutation.

155. The method of any one of claims 147-153, wherein the cancer carries a BRCA1 or a BRCA2 mutation.

156. The method of any one of claims 147-155, wherein the chemotherapeutic agent has been previously associated with a clinical marker and the cancer has the clinical marker.

157. The method of any one of claims 147-156, wherein the plurality of nucleic acid features comprise one or more features selected from the group consisting of support for a single nucleotide variant at a genomic location, a methylation status at a genomic location, a relative copy number for a genomic location, an allelic ratio for a genomic location, a relative expression level of a gene, and mathematical combinations thereof.

158. The method of any one of claims 147-157, wherein the plurality of nucleic acid features comprises a BRCA1 or BRCA2 mutant allele status.

159. The method of any one of claims 147-158, wherein the first test data set further comprises one or more characteristics of the subject selected from the group consisting of tumor organoid histology, baseline histology, gender, age, family medical history, personal history of cancer, treatment history of cancer, diagnosis, subtype, IHC markers, DNA/RNA sequence, a genomic methylation pattern, a radiologic image or feature, a comorbidity, a geographic location, ancestry, an ethnicity, diet, gut microbiome, substance usage, alcohol use, physical exam findings, baseline vitals, vaccination history, religious or cultural beliefs, marital status, sexual orientation, nocturnal/diurnal rhythm, sleep schedule, a travel history, a presence or absence of parasitic organisms, an infectious disease history, a germline DNA sequence, a biometric feature, skull shape, physical profile, occupational history, a pet history, a mental health history, a tumor organoid morphology, a tumor organoid, and a smoking history; and

the classifier was further trained against, for each respective training tissue sample in a plurality of training tissue samples, the one or more characteristics of the subject from which the respective training tissue sample was obtained.

160. A method of evaluating an effect of a cancer therapeutic agent, the method comprising:

a) providing a plurality of tumor organoids cultured in a tumor organoid culture medium, wherein the plurality of tumor organoids is divided into a plurality of tumor organoid subsets;

b) contacting each subset in the plurality of tumor organoid subsets with a cancer therapeutic agent;

c) contacting each subset in the plurality of tumor organoid subsets with one or more cell death detection agents and a total cell detection agent;

d) obtaining a tumor organoid profile for each subset in the plurality of subset, wherein the tumor organoid profile comprises a cell viability value for every tumor organoid in the subset; and

e) assessing the effect of the cancer therapeutic agent based on the tumor organoid profiles.

161. A method for assigning a treatment dosage of a cancer therapeutic agent for a subject in need thereof, the method comprising:

a) providing a plurality of tumor organoids cultured in a tumor organoid culture medium, wherein the tumor organoids are derived from a subject, and wherein the plurality of tumor organoids is divided into a plurality of tumor organoid subsets;

b) contacting each subset in the plurality of tumor organoid subsets with a different dosage of a cancer therapeutic agent;

c) contacting each subset in the plurality of tumor organoid subsets with one or more cell death detection agents and a total cell detection agent;

d) obtaining a tumor organoid profile for each subset in the plurality of subset, wherein the tumor organoid profile comprises a cell viability value for every tumor organoid in the subset;

e) determining a therapeutic agent dosage curve from the tumor organoid profiles; and

f) assigning a treatment dosage of the cancer therapeutic agent to the subject based on the therapeutic agent dosage curve.

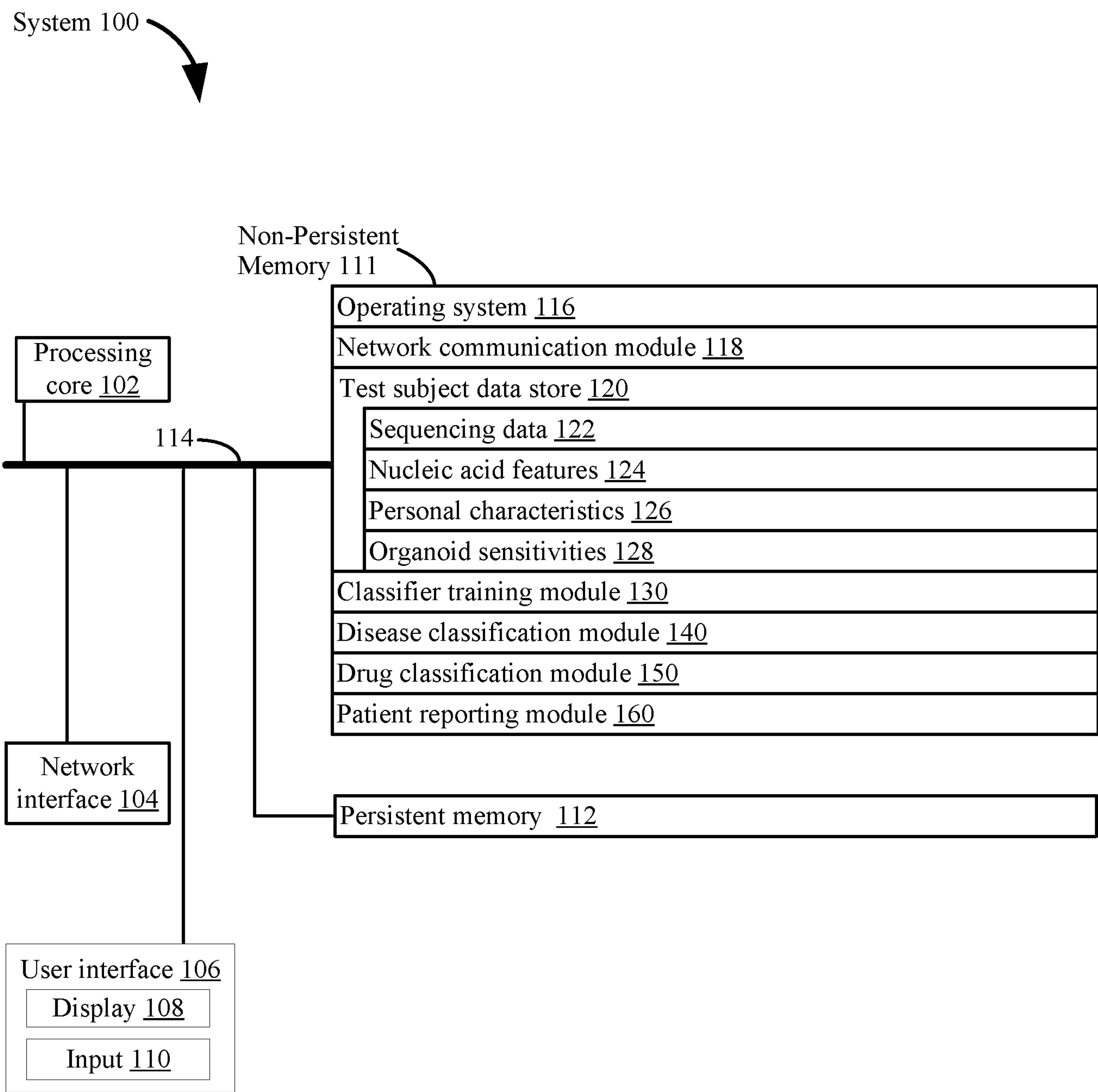


FIG. 1

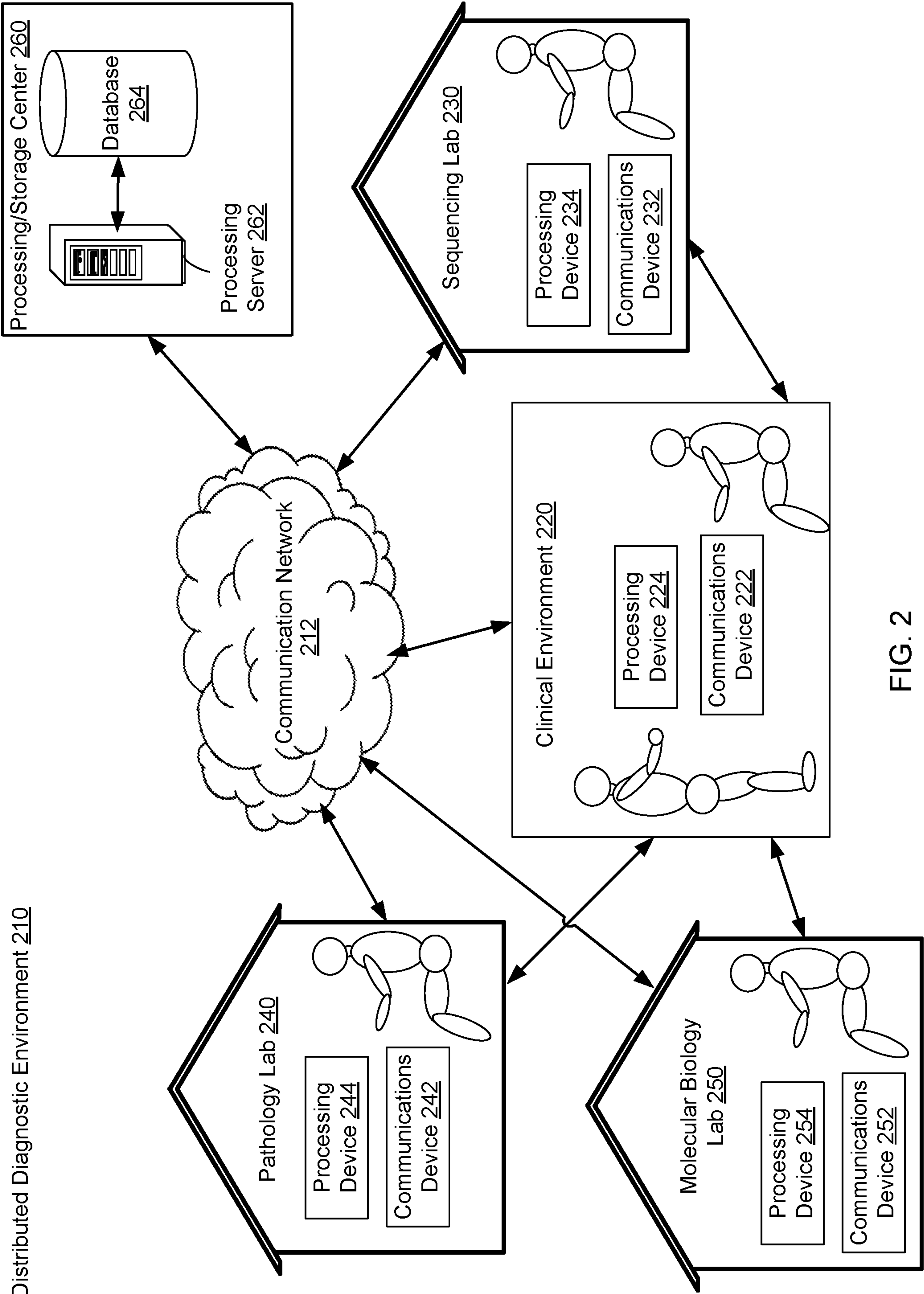


FIG. 2

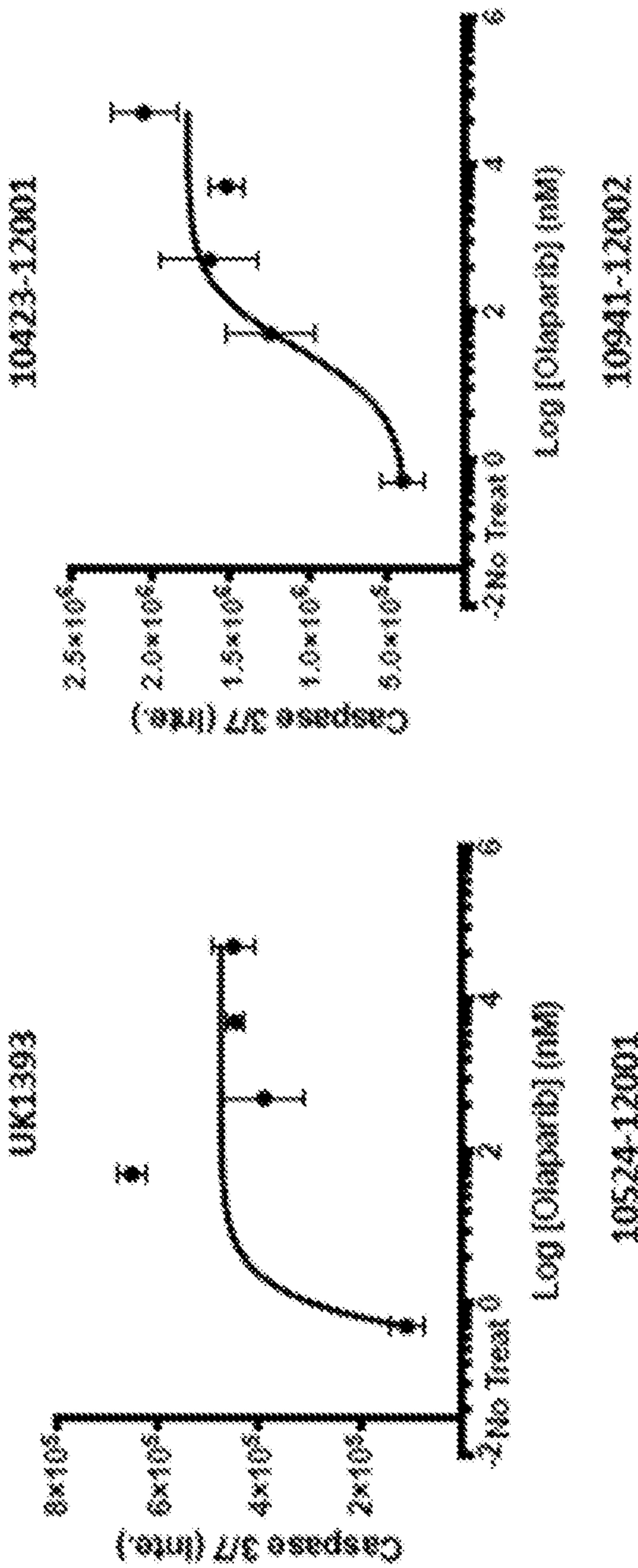


FIG. 3B

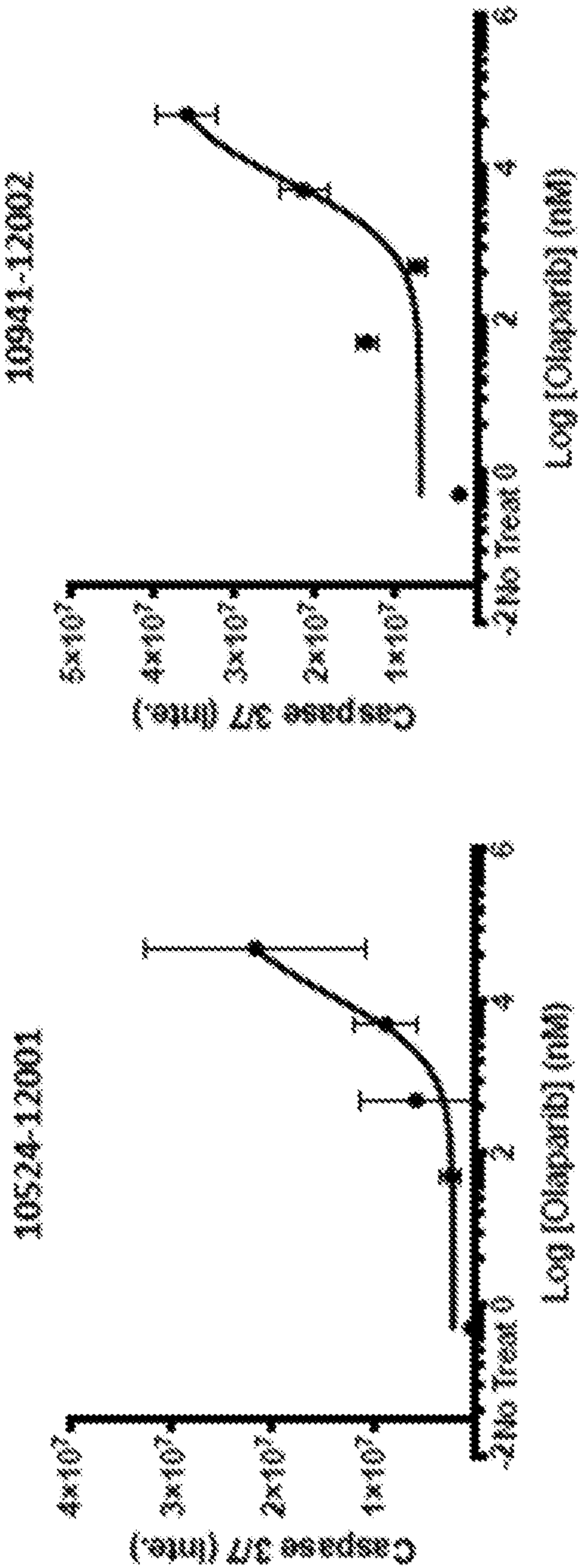


FIG. 3D

FIG. 3C

Vehicle

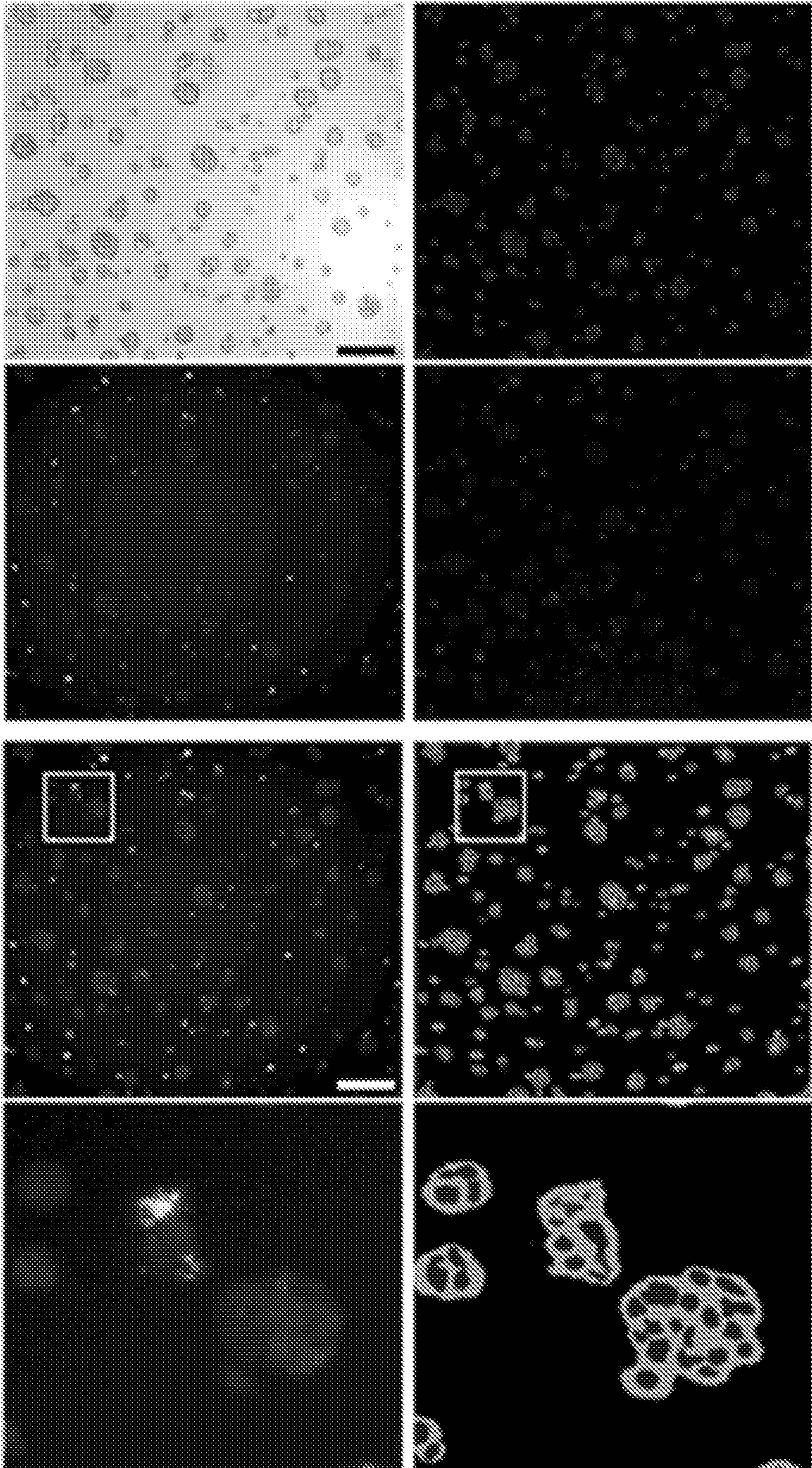


FIG. 4A

10 μ M Staurosporine

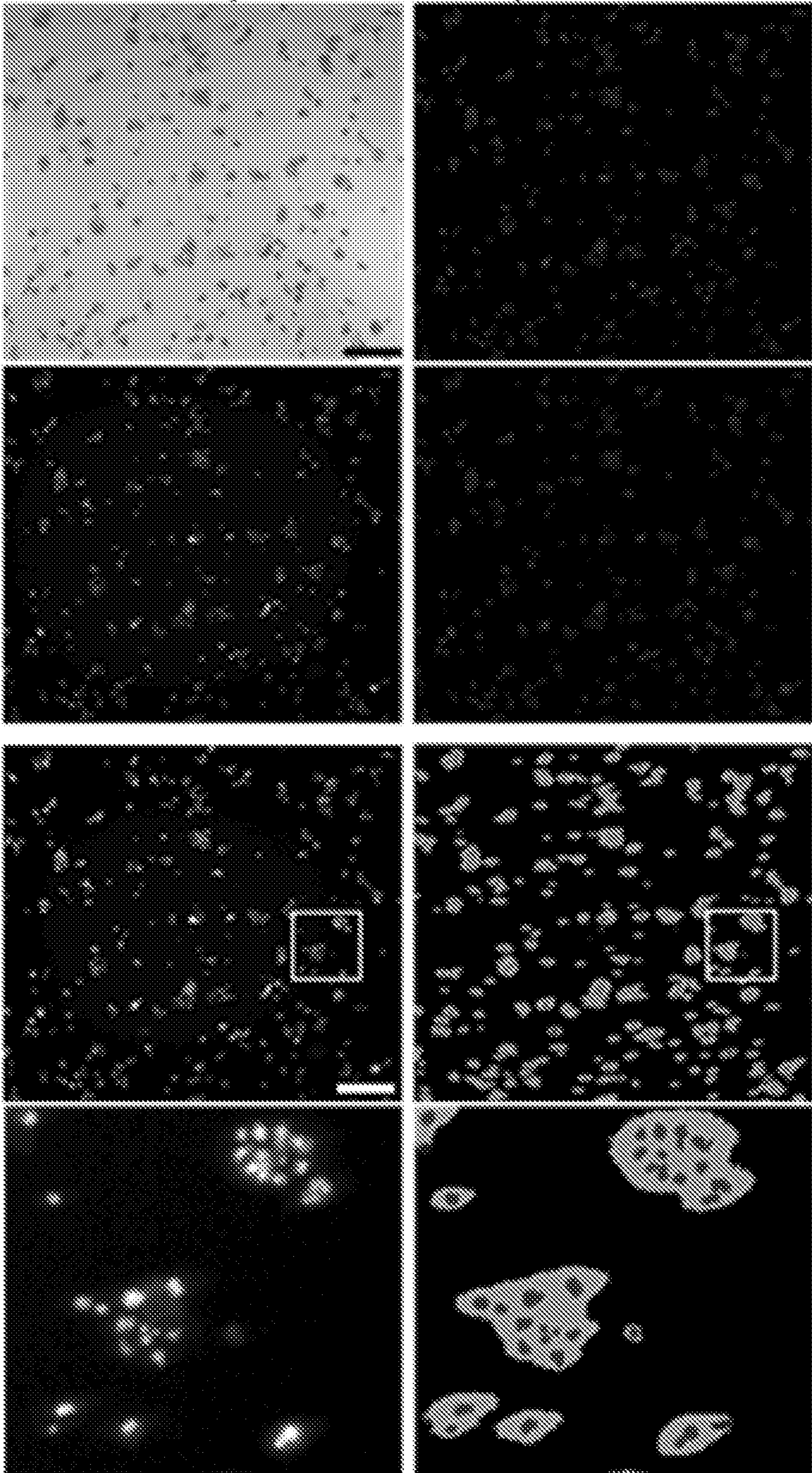


FIG. 4B

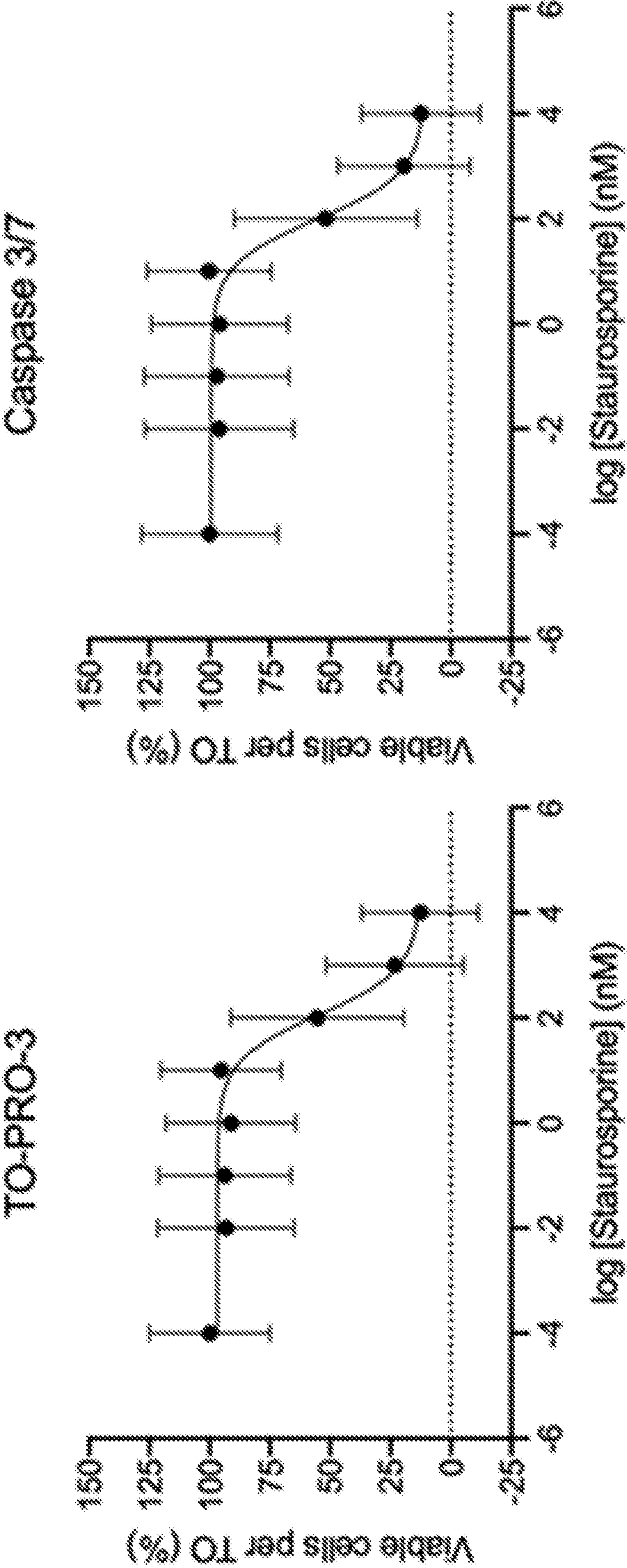


FIG. 5B

FIG. 5A

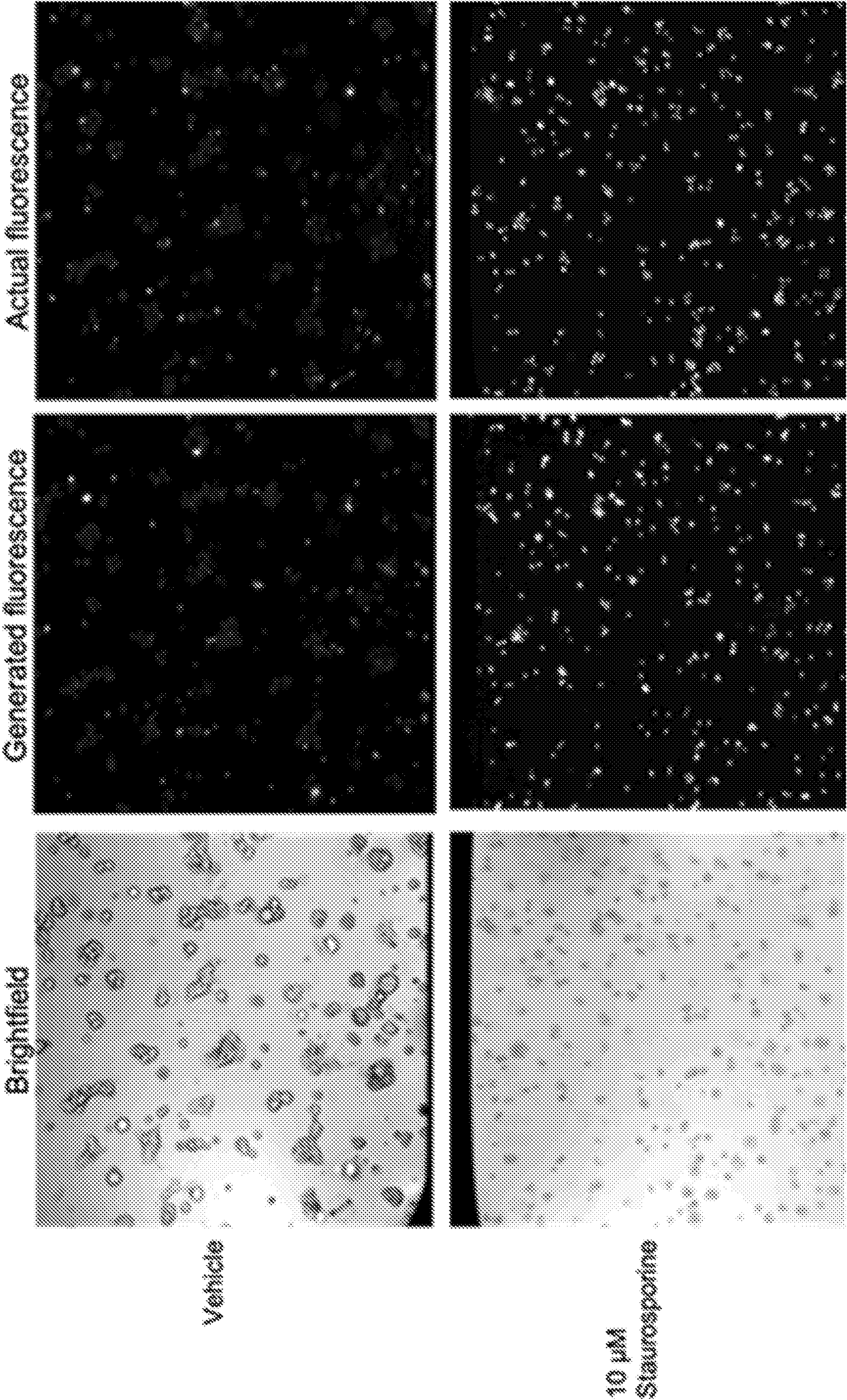


FIG. 6

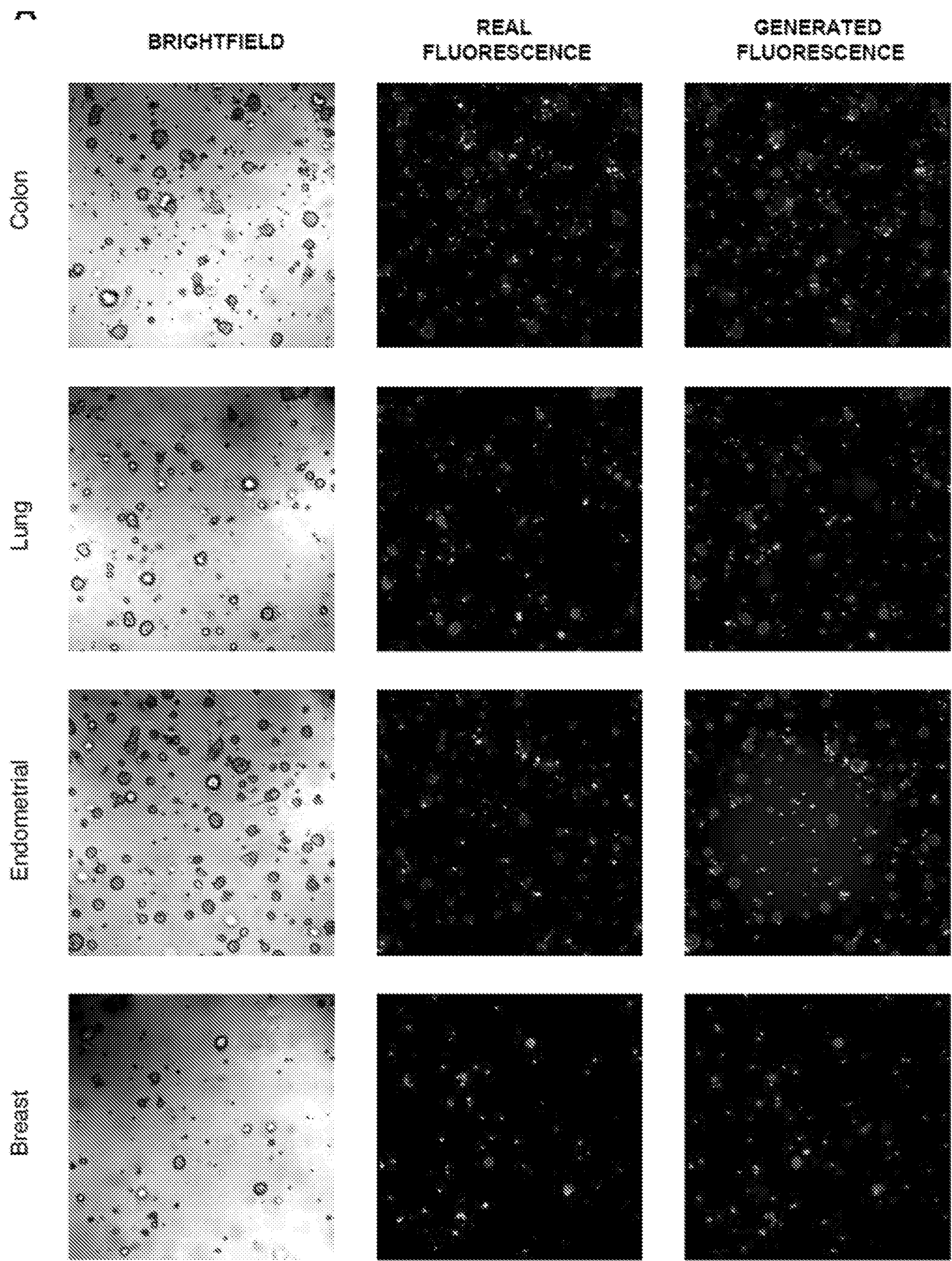


FIG. 7A

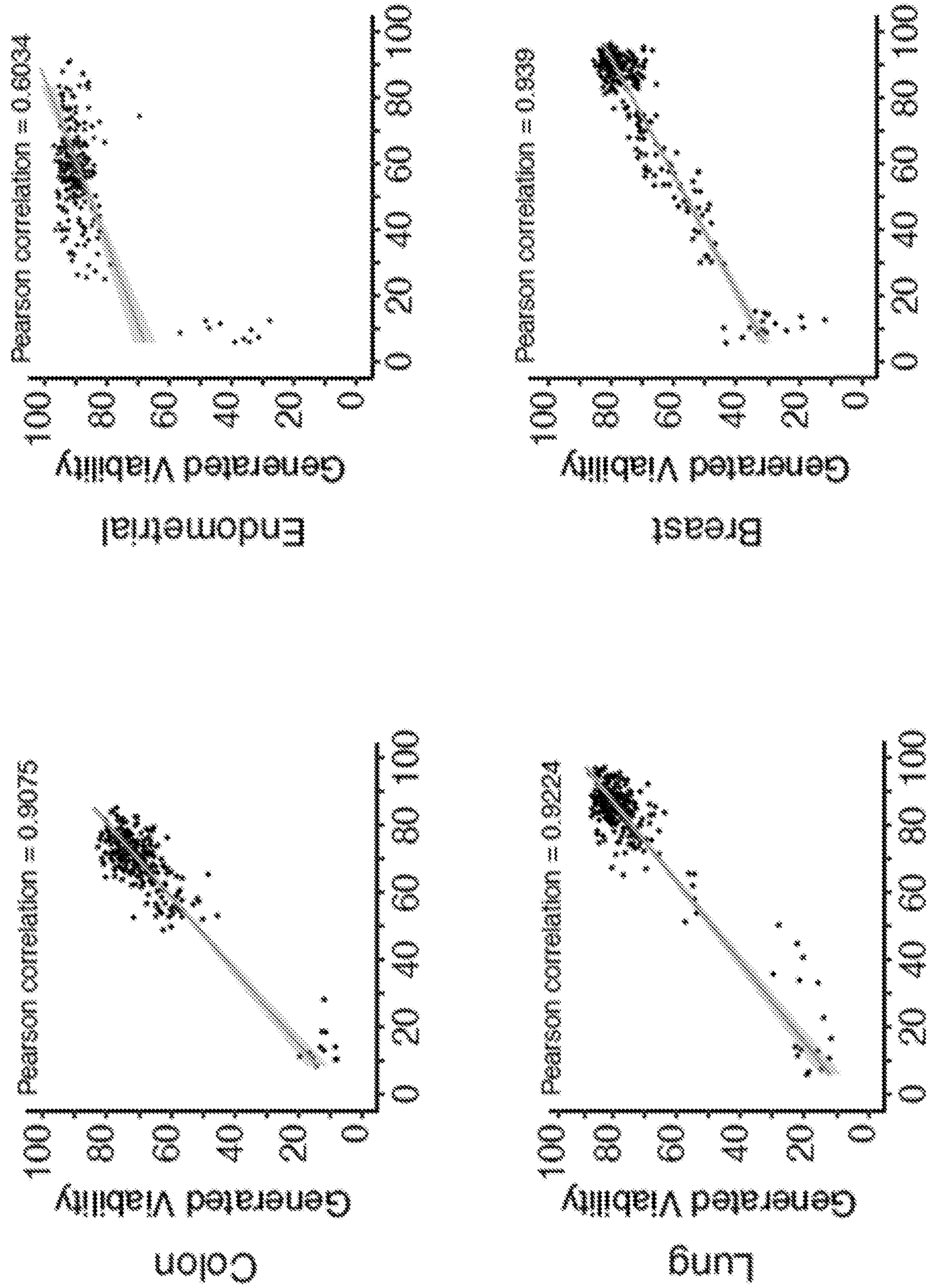


FIG. 7B

ROC OF GENERATED AND REAL AUC

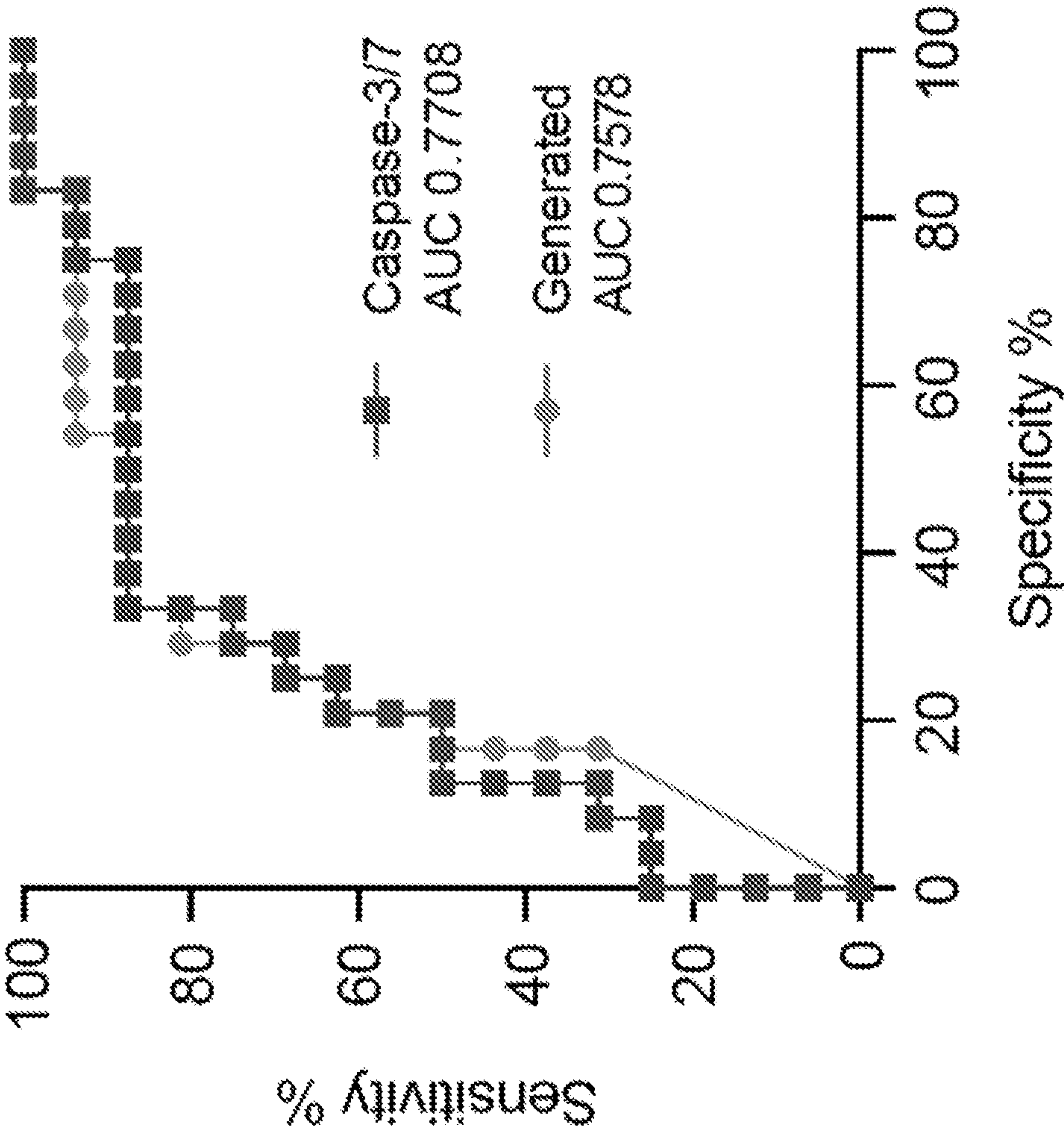


FIG. 8

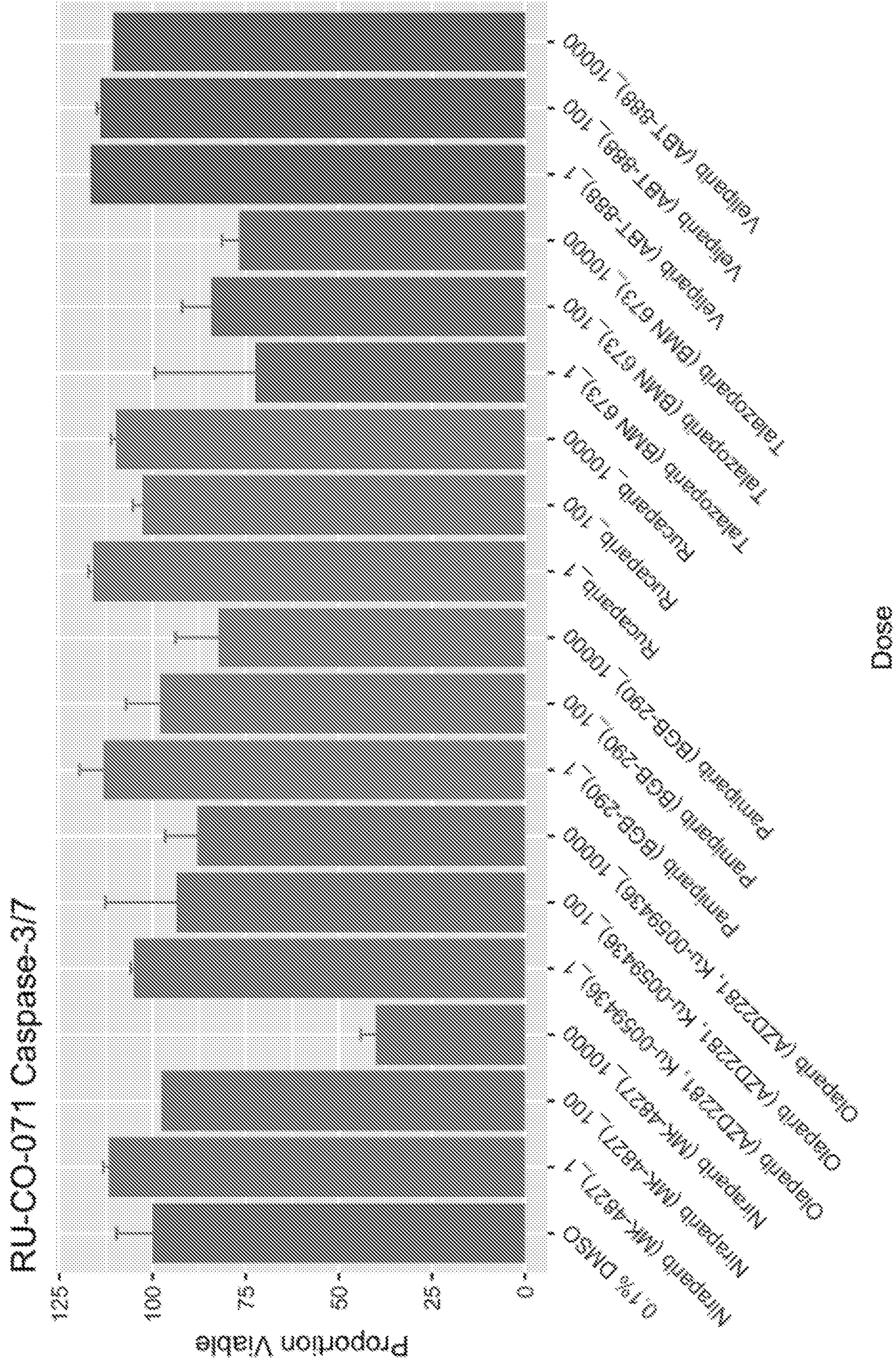


FIG. 9A

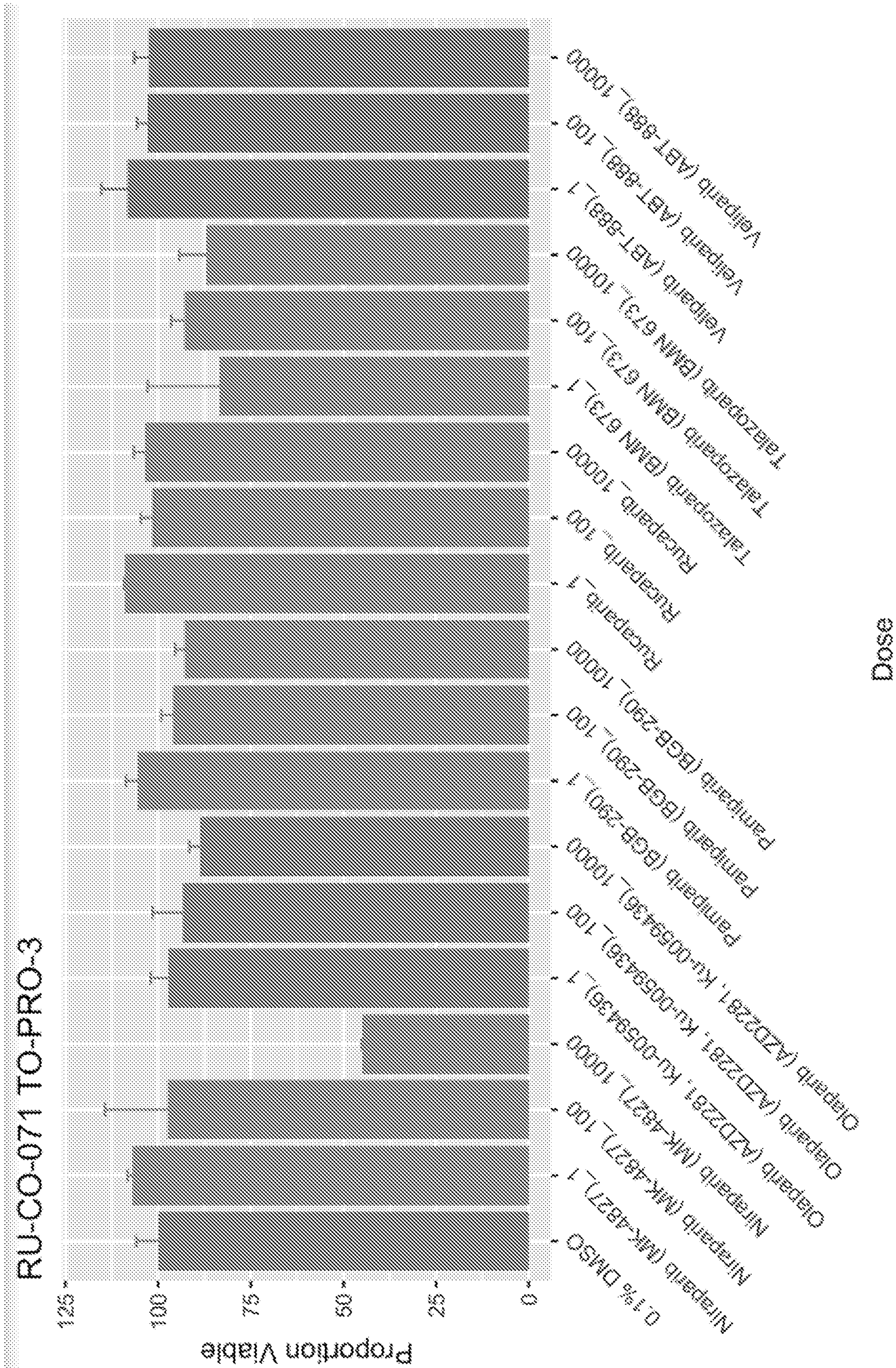


FIG. 9B

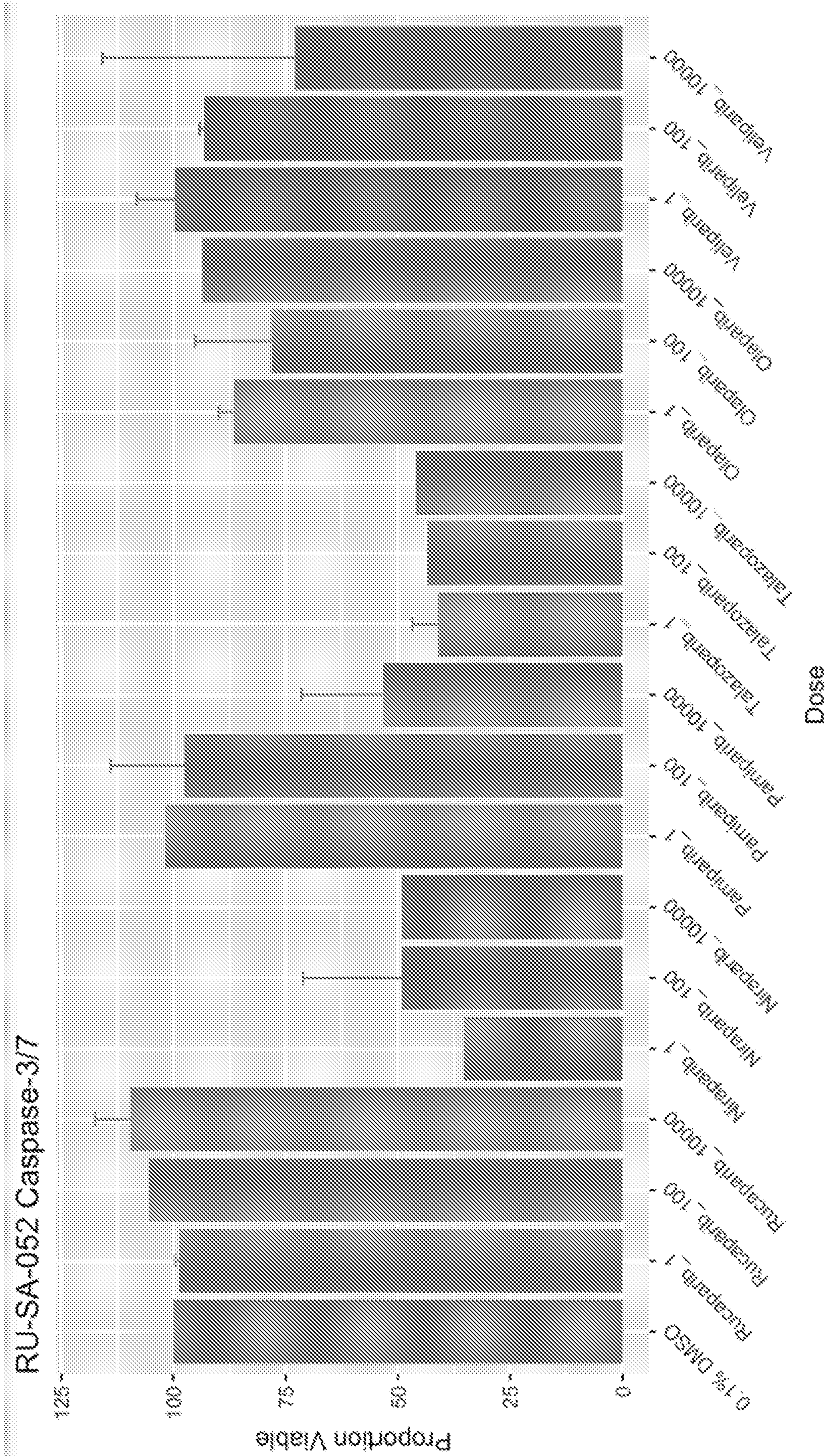


FIG. 10A

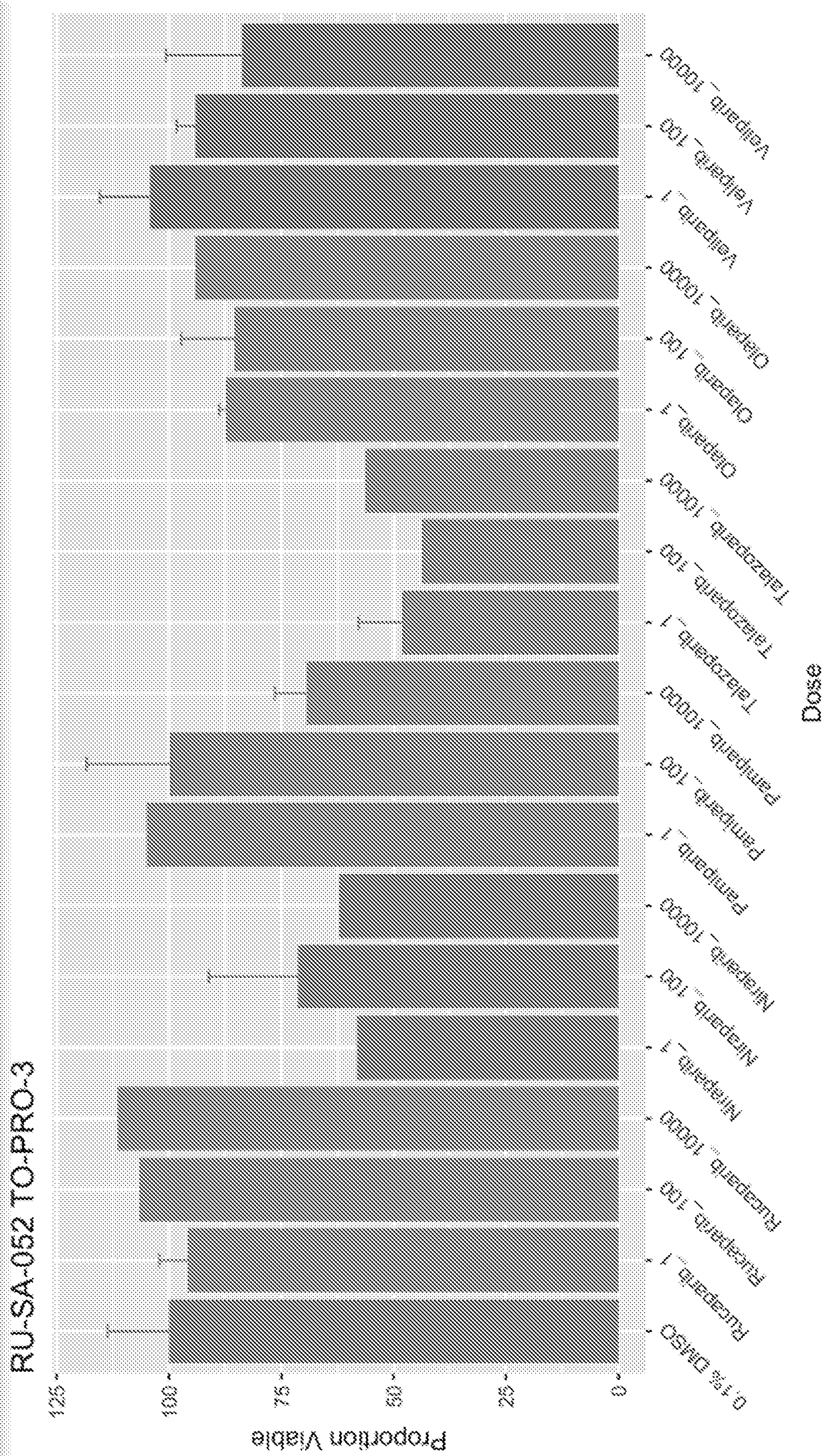


FIG. 10B

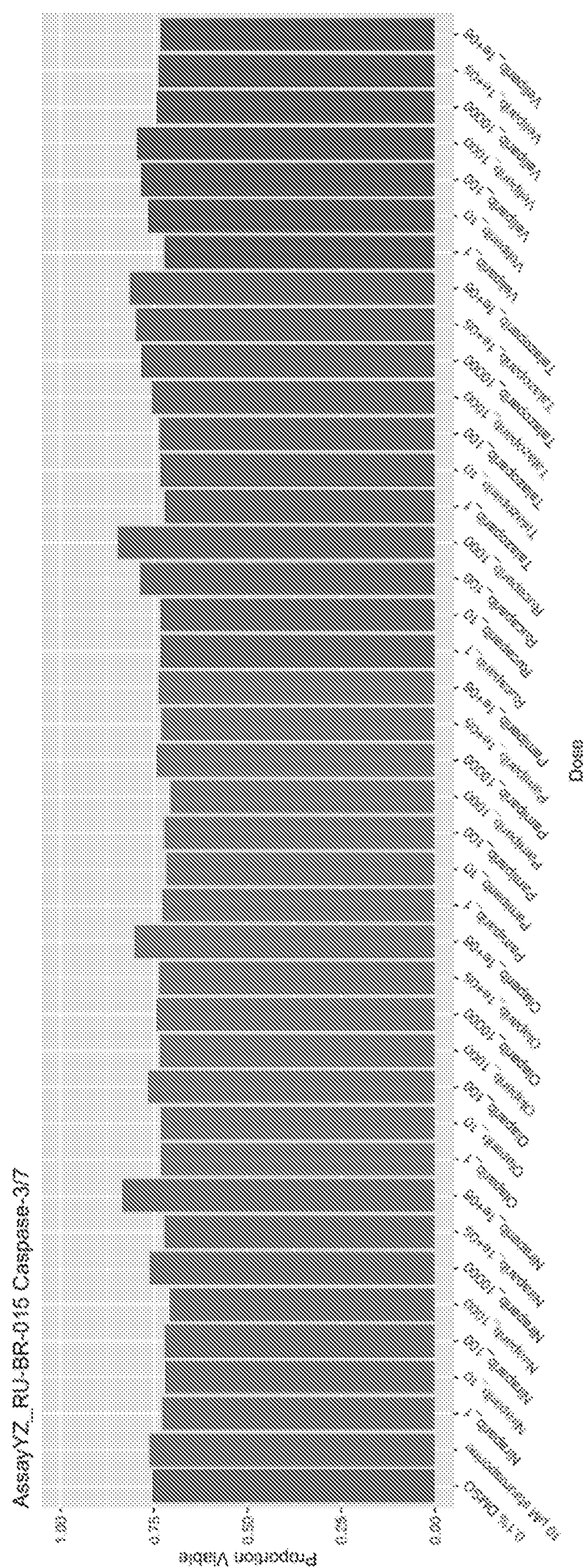


FIG. 11A

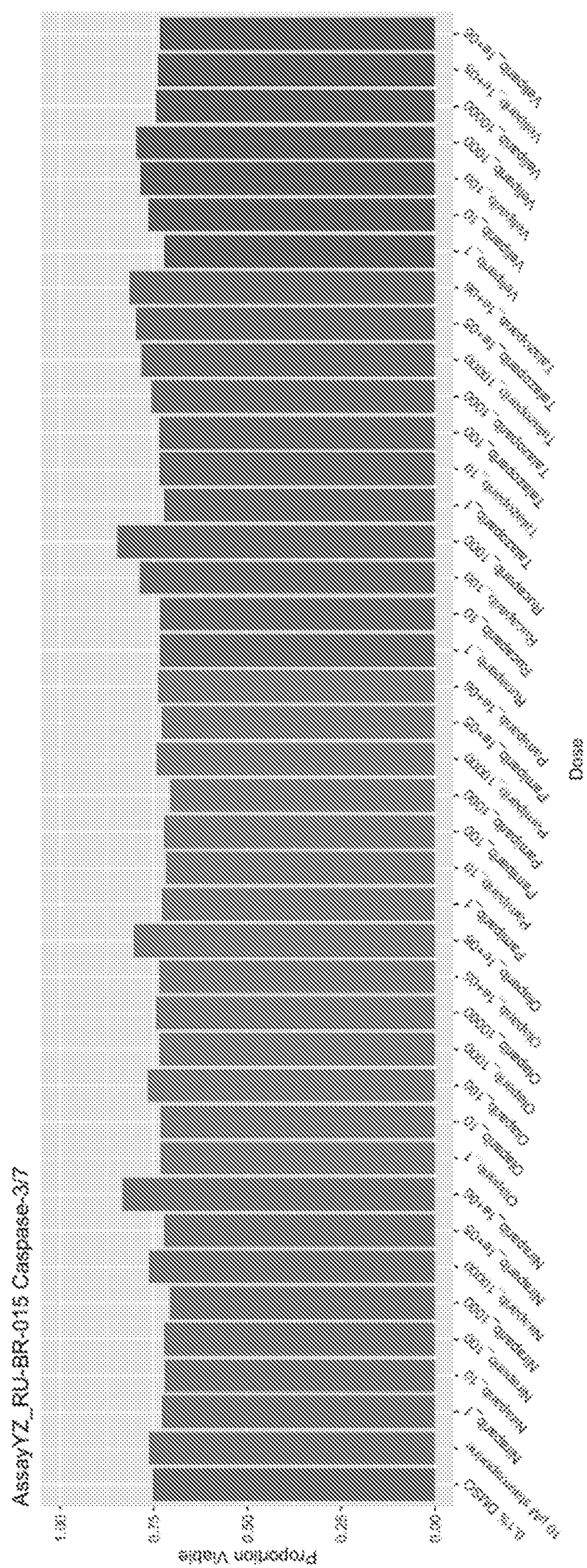


FIG. 11B

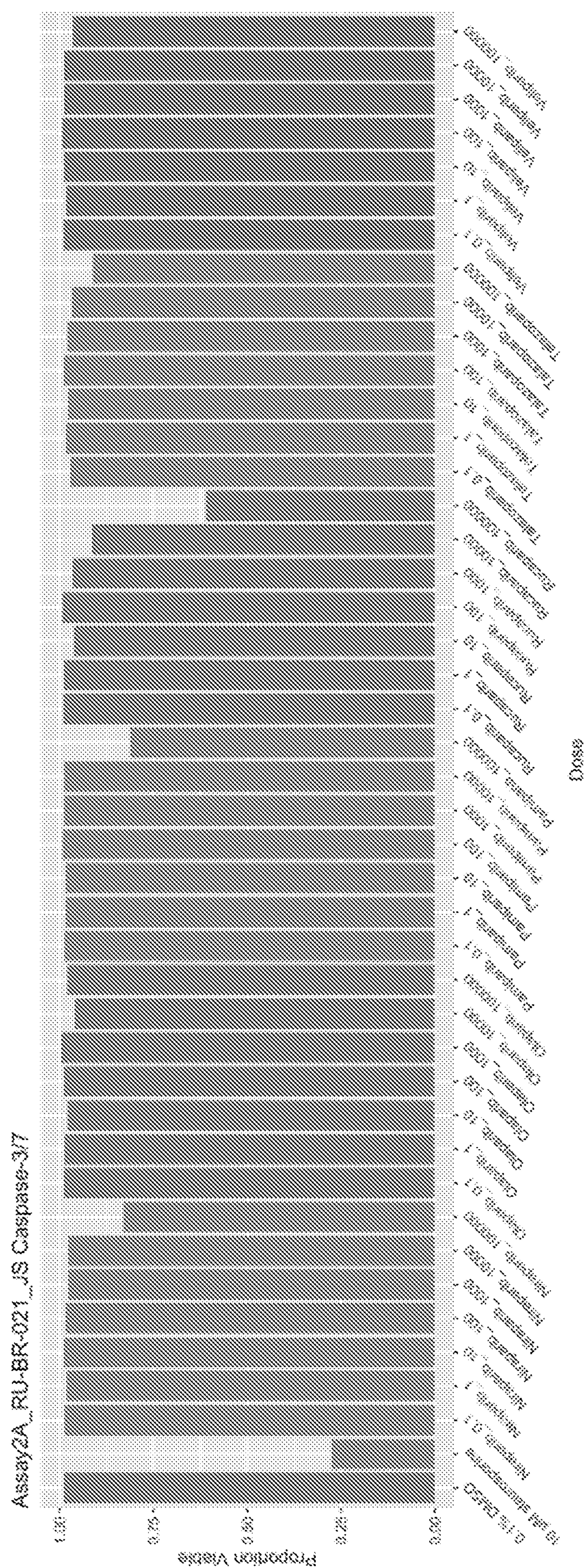


FIG. 12A

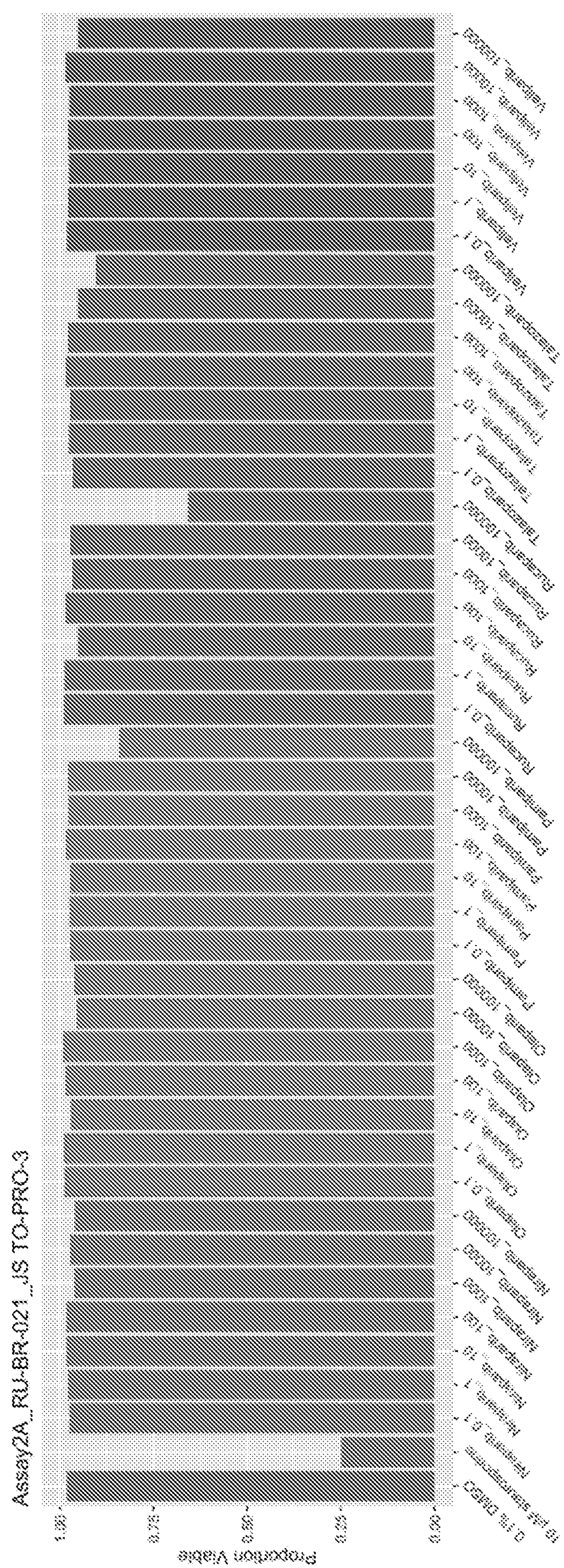


FIG. 12B

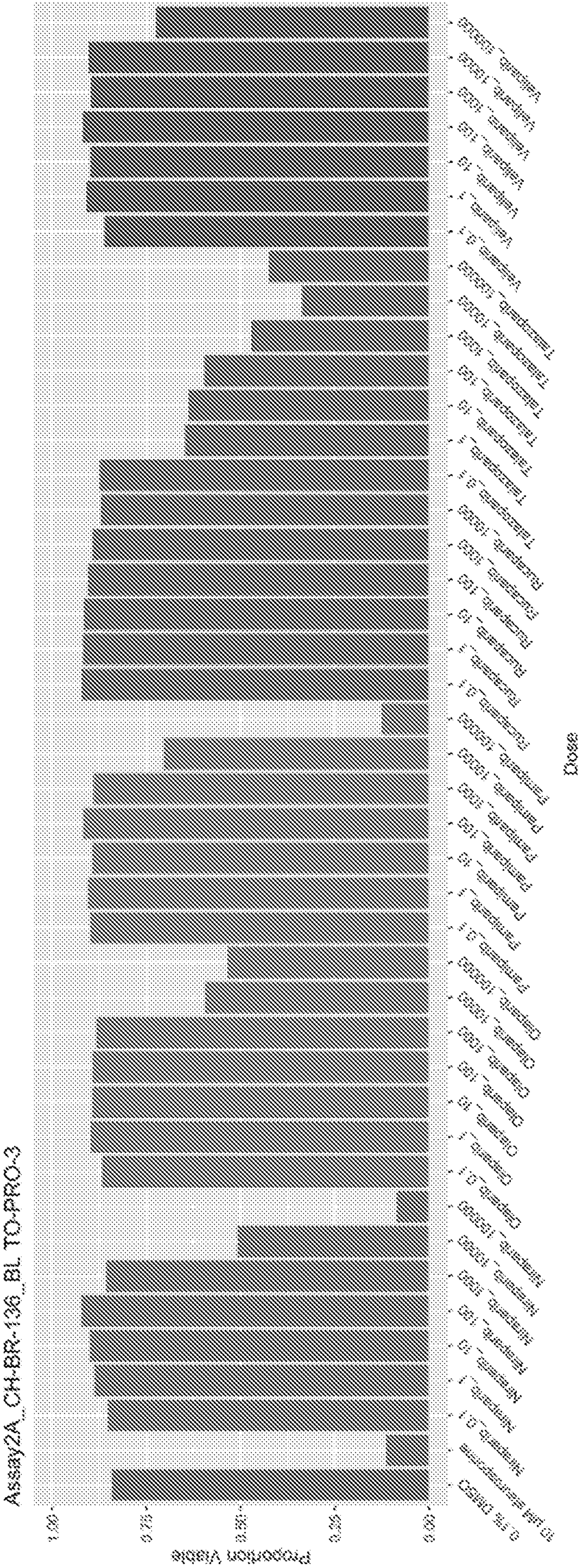


FIG. 13B

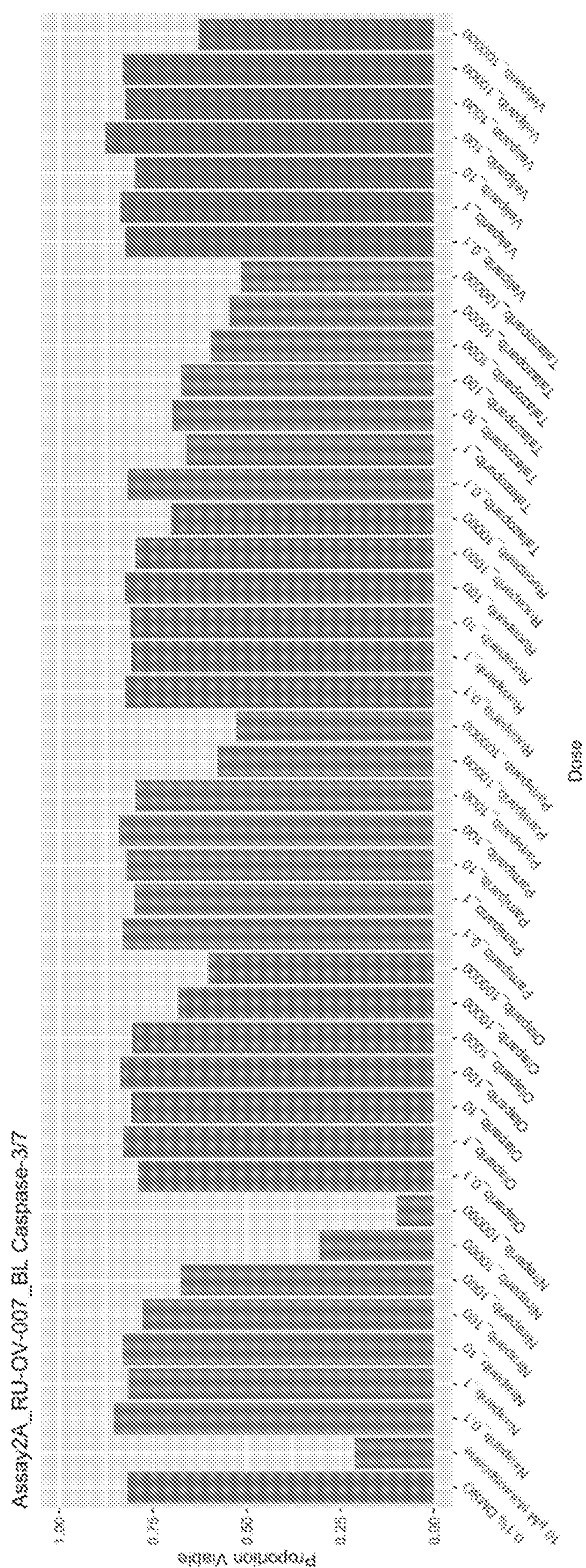


FIG. 14A

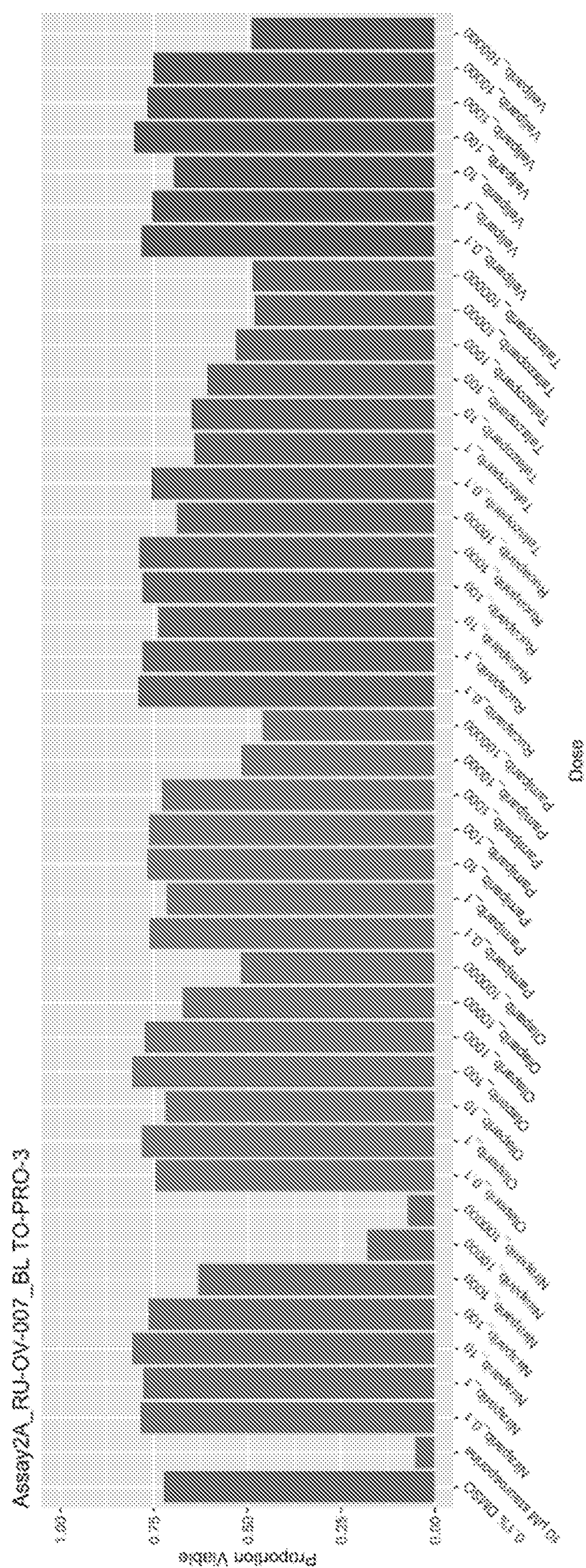


FIG. 14B

CH-OV-202 Caspase-3/7

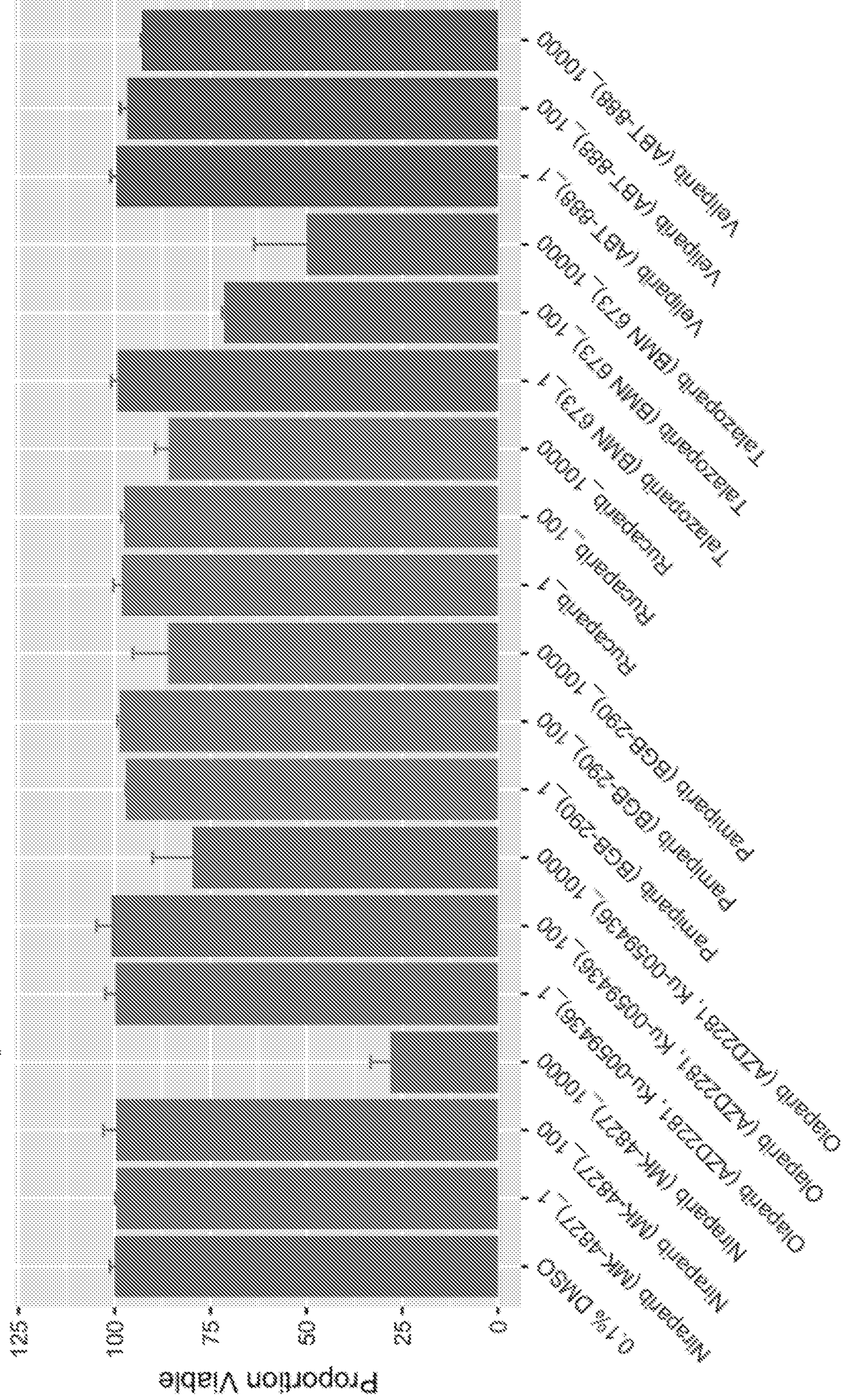


FIG. 15A

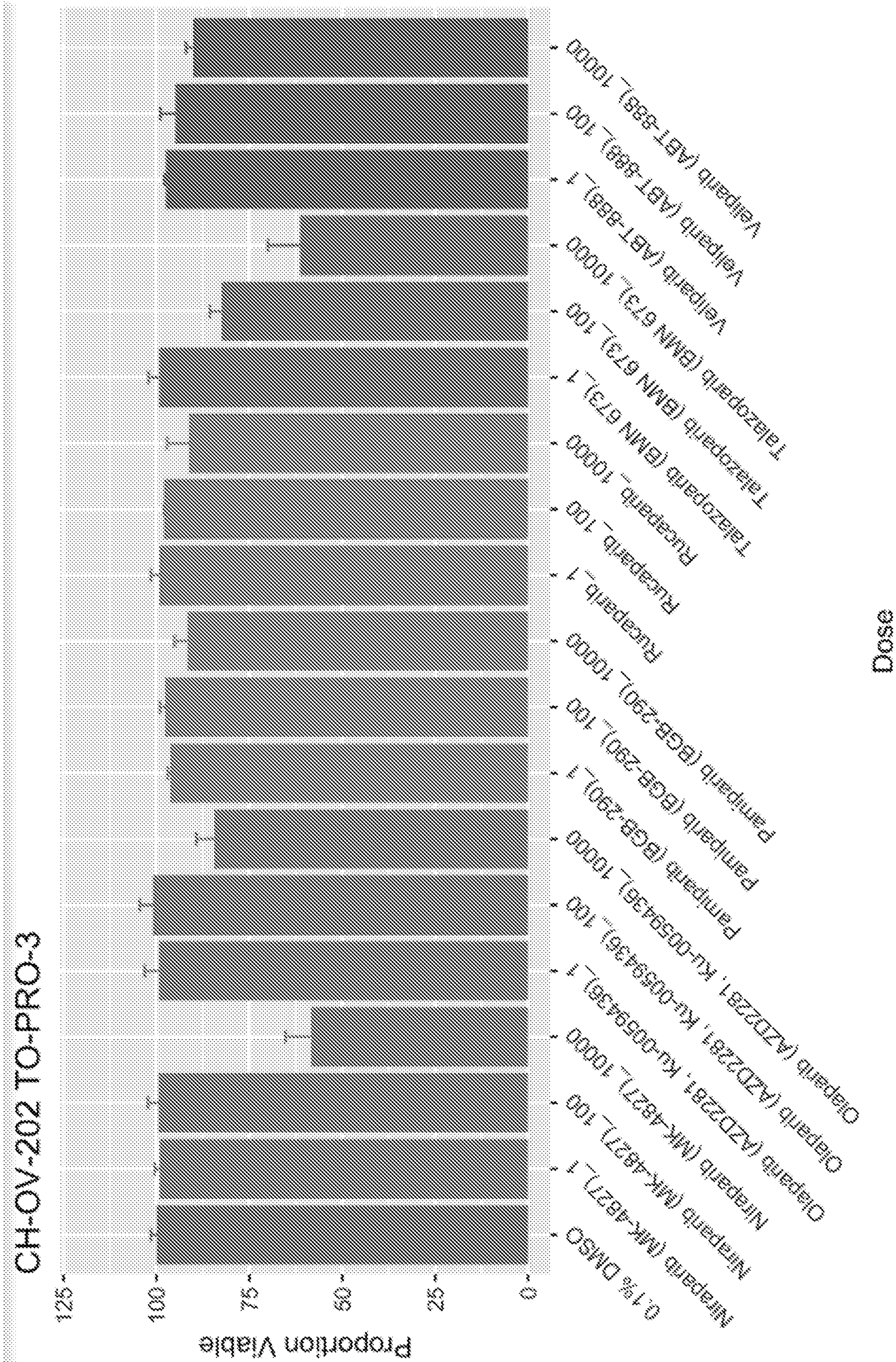


FIG. 15B

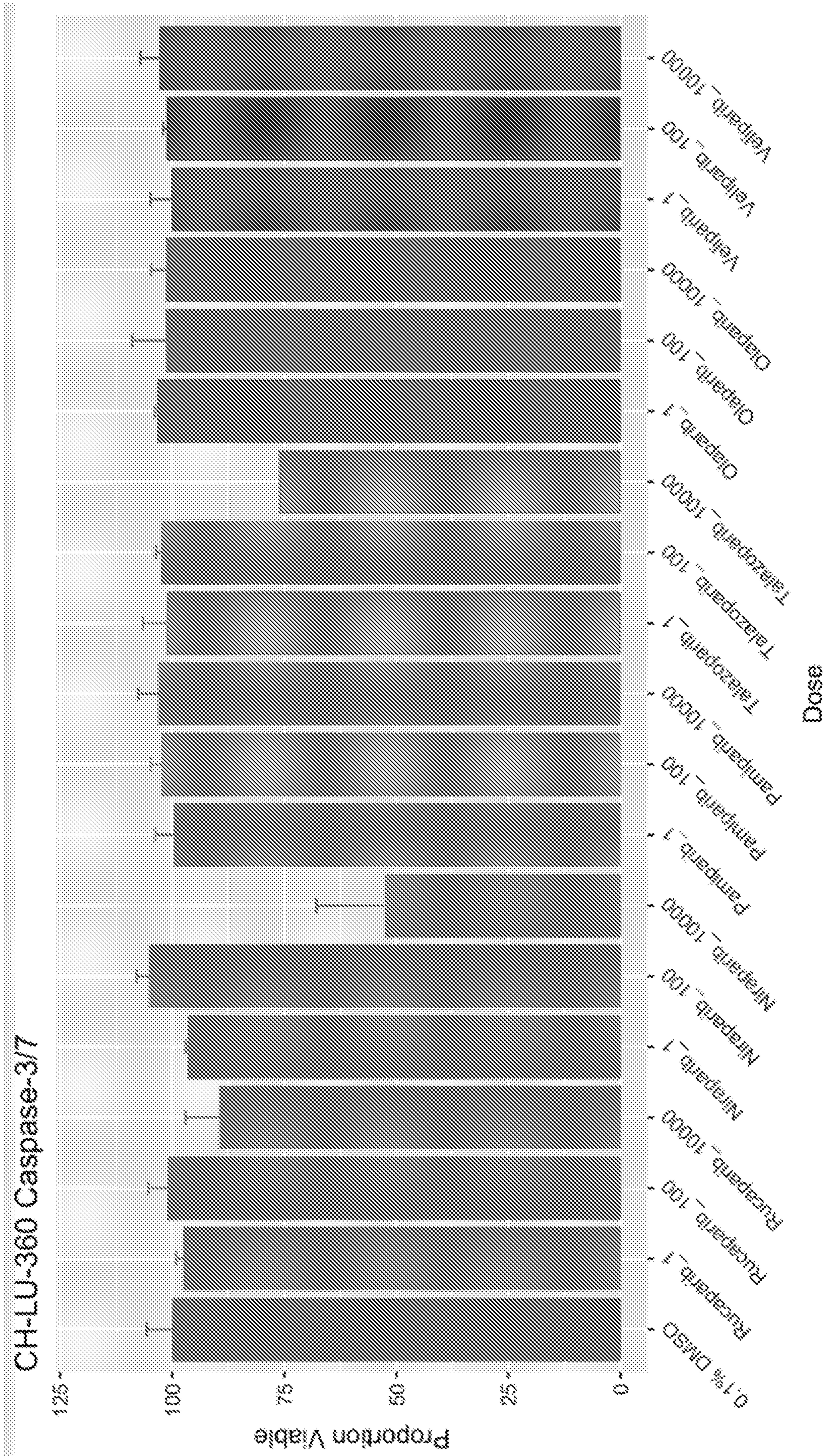


FIG. 16A

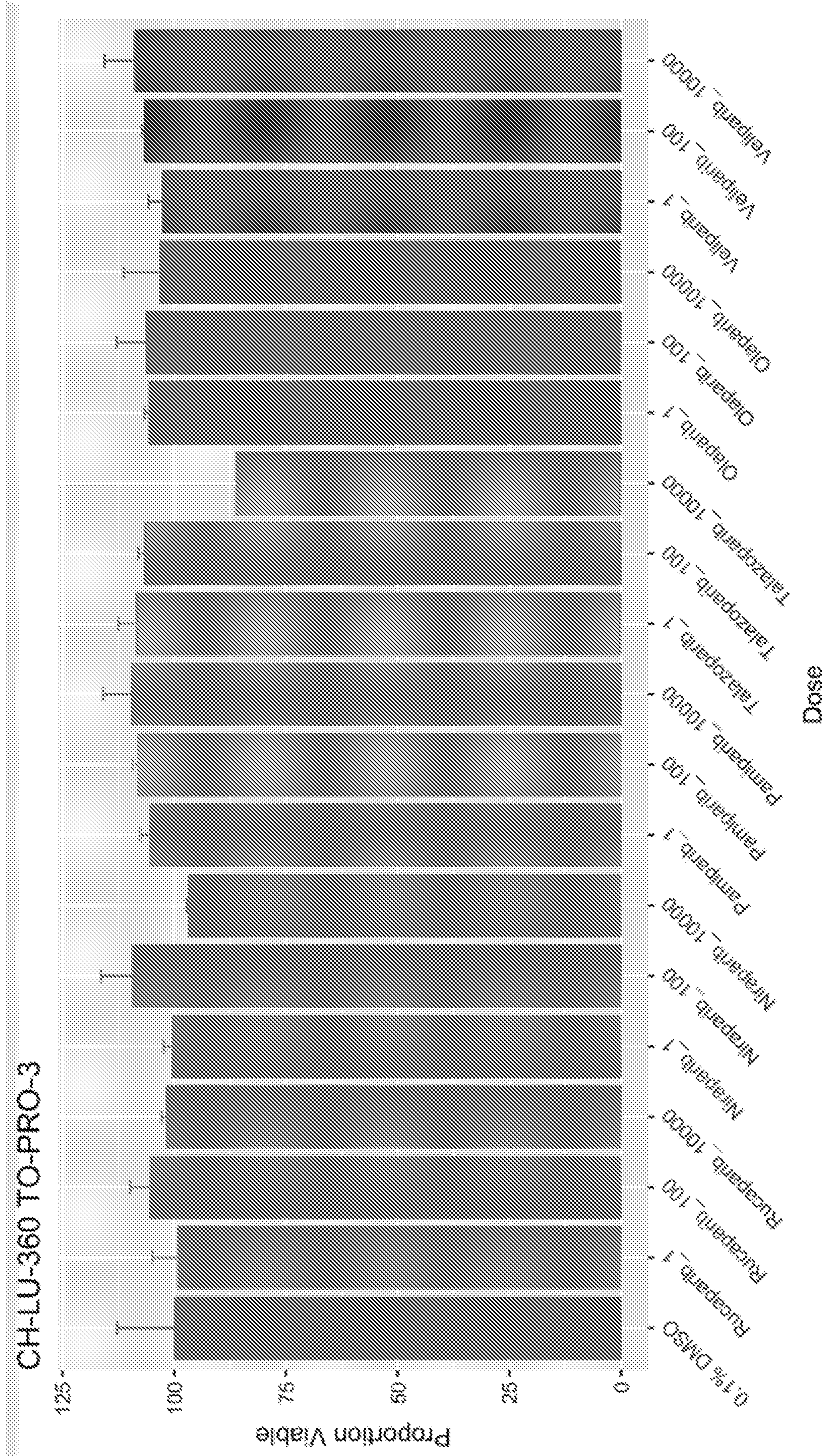


FIG. 16B

RU-LU-165-TO Caspase-3/7

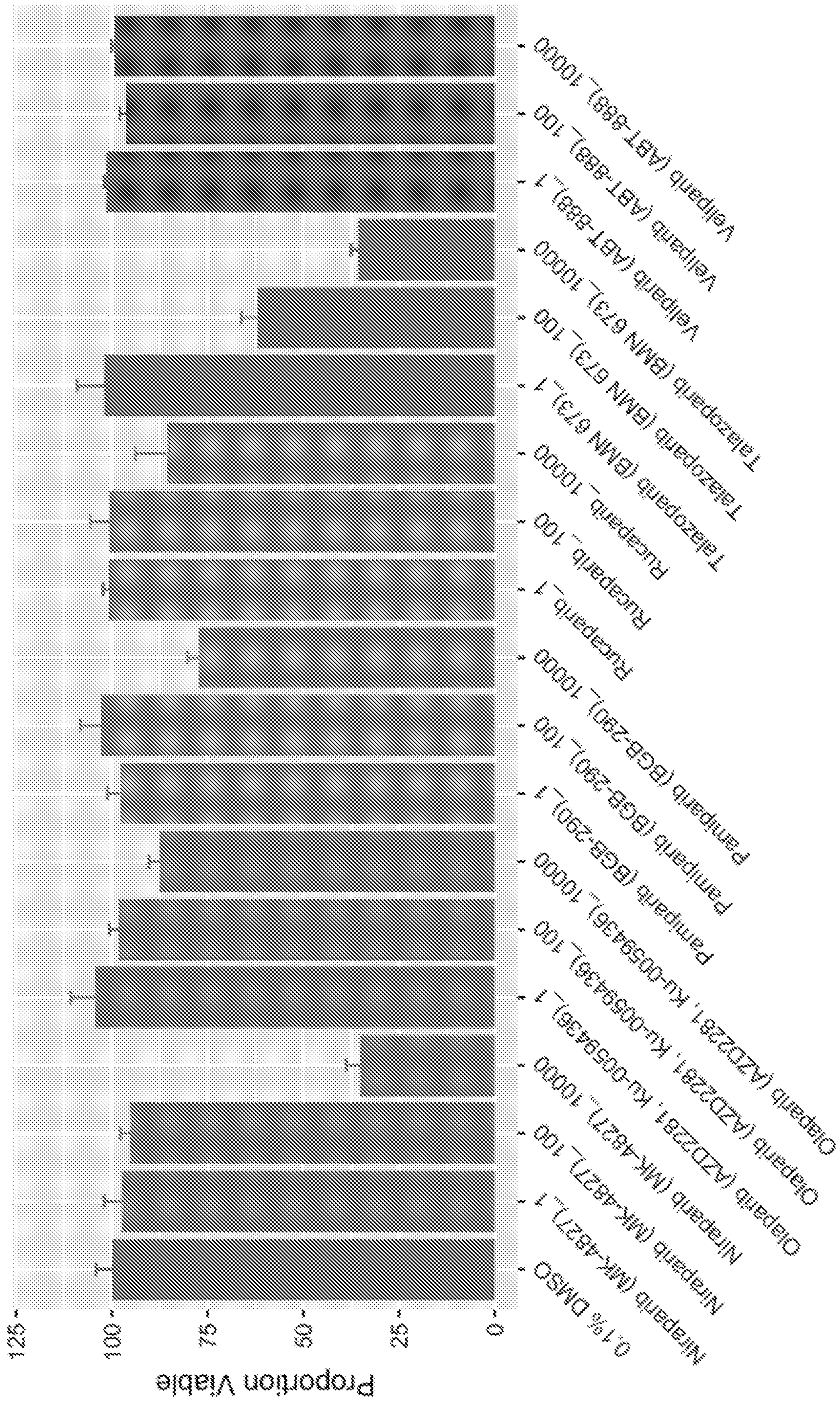


FIG. 17A

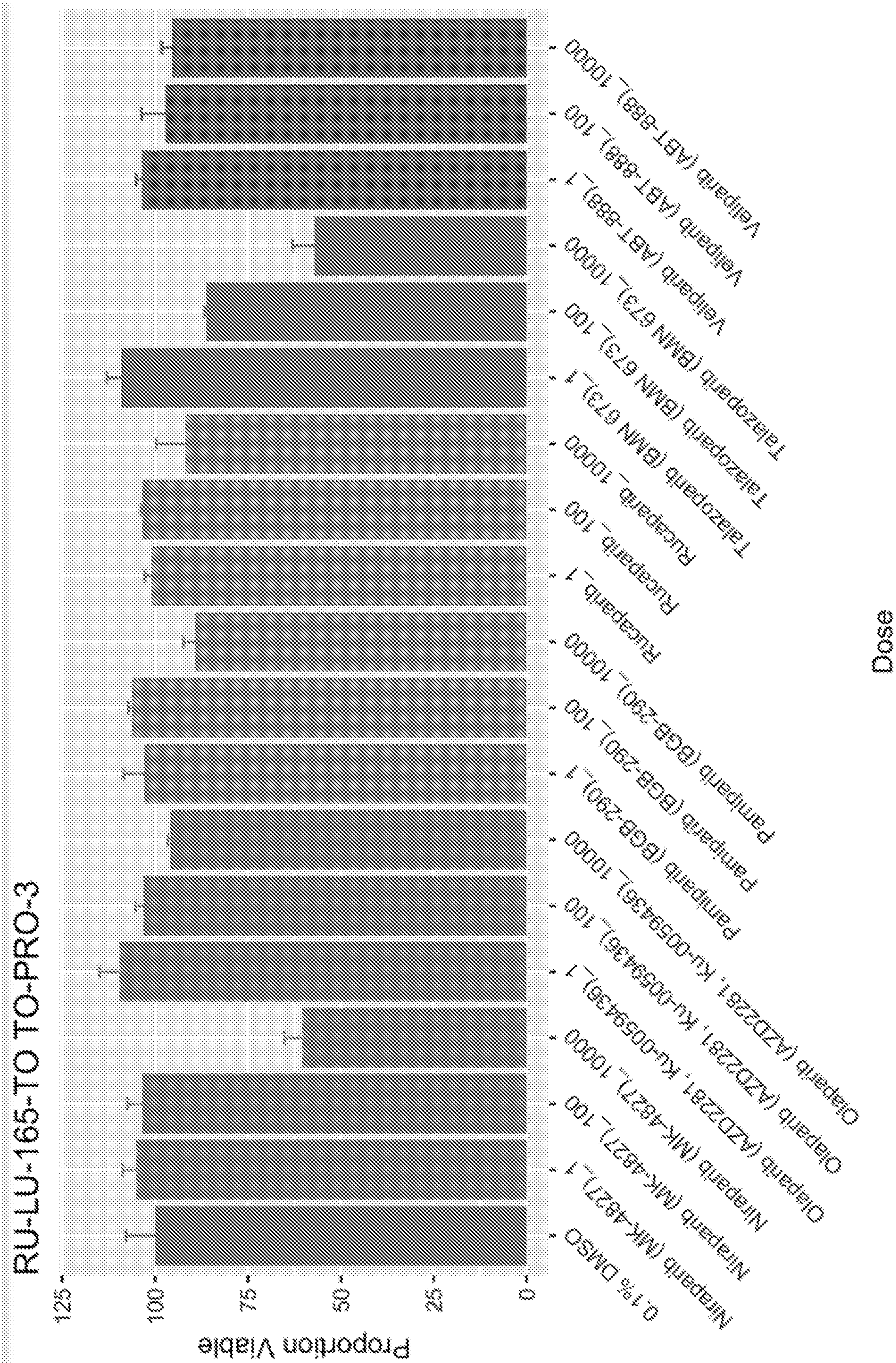


FIG. 17B

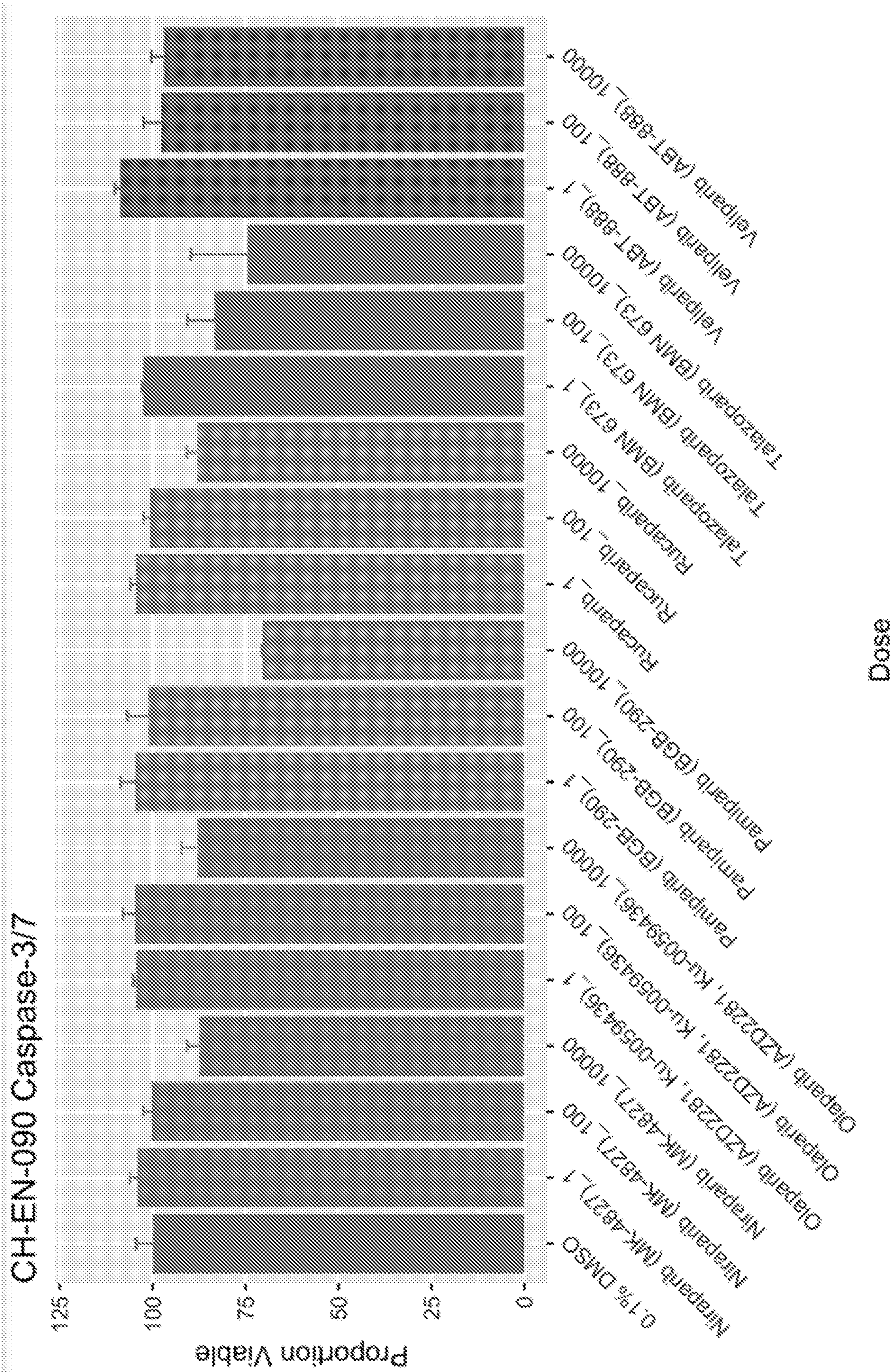


FIG. 18A

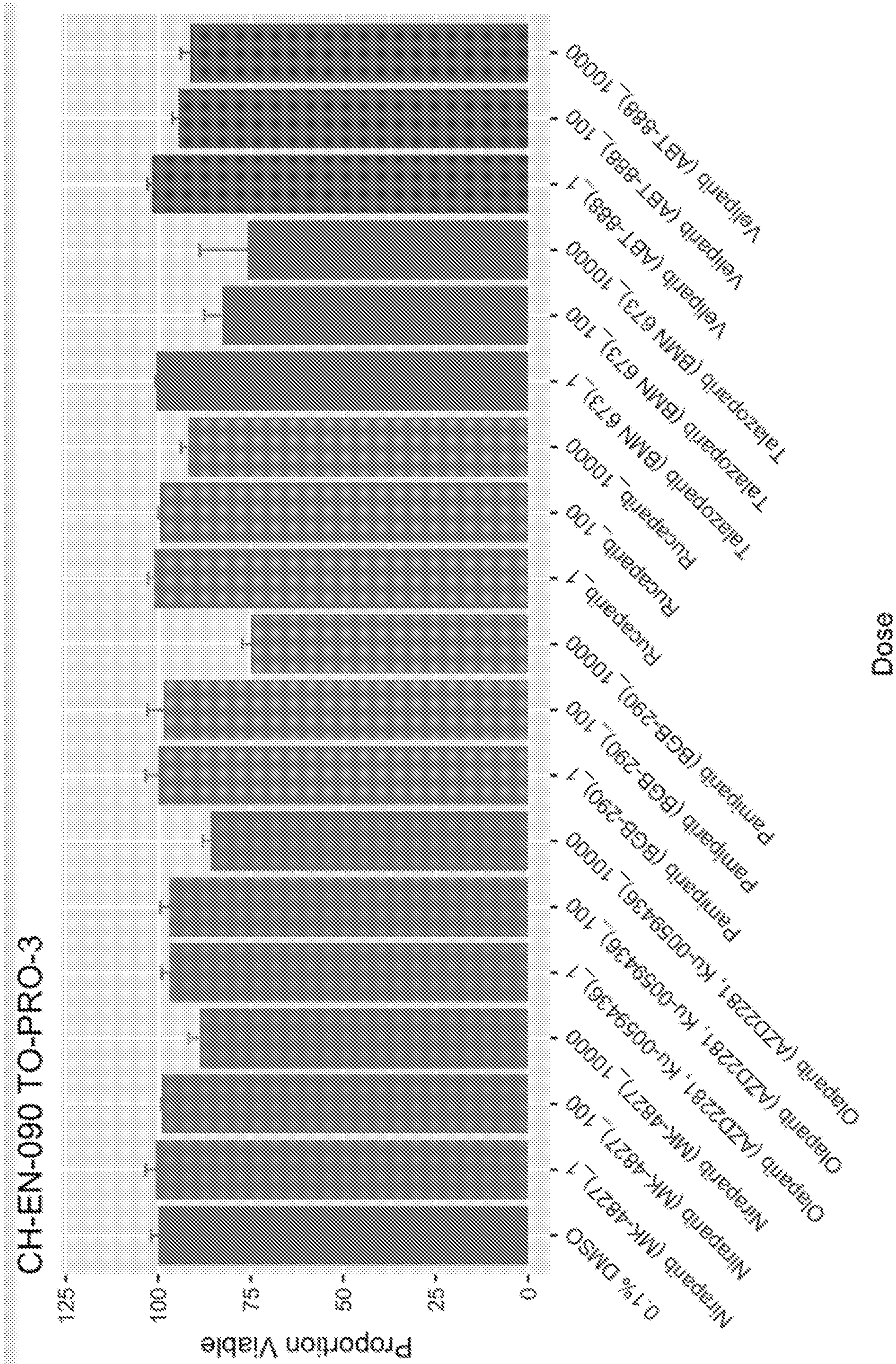


FIG. 18B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/56930

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12Q 1/68, C12Q 1/6886, A61B 5/00, G01N 33/50 (2021.01)

CPC - G01N 33/5008, C12Q 1/6886, C12Q 2600/112

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)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DUARTE et al. BRCA-deficient mouse mammary tumor organoids to study cancer-drug resistance. Nature methods. February 2018, Vol 15, No 2, pg 134. Entire document, especially abstract; p. 134, col 2, para 2; pg 135, col 2, para 2; p. 136, col 2, para 1-2; p. 137, col 1, para 1-2; pg 138, col 2, para 1; p. 142, col 2, para 2; Figure 2 Legend; Figure 2	1-3, 74-78, 95-97, 130, 132/130
A	BARAZAS et al. Radiosensitivity Is an Acquired Vulnerability of PARPi-Resistant BRCA1-Deficient Tumors. Cancer Res. 01 February 2019, Vol 79, No 3, pg 452. Entire document, especially abstract; p. 453, col 1	1-3, 74-78, 95-97, 130, 132/130

☐ 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

"D" document cited by the applicant in the international application

"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

12 February 2021

Date of mailing of the international search report

02 MAR 2021

Name and mailing address of the ISA/US

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Facsimile No. 571-273-8300

Authorized officer

Lee Young

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/56930

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-8, 12-34, 41-73, 79-94, 98-120, 124-127, 133-146, 154-159
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-3, 74-78, 95-97, 130, 132(in part), directed to a method for evaluating a cancer in a test subject.

Group II, claims 9-11, 35-40, 131, 132(in part), 147-153, directed to a method for training a classifier, and use of a classifier in a method for recommending therapy, determining eligibility for a clinical trial, or identifying a resistance to a chemotherapeutic agent.

Group III, claims 121-123, 128, 129, 160, 161, directed to a method of identifying or evaluating the effect of a candidate therapeutic agent/compound, or effective dose thereof.

--continued on extra sheet--

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 74-78, 95-97, 130, 132(in part)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/56930

--continued from: Box No. III Observations where unity of invention is lacking--

The inventions listed as Groups I-III do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features:

Group I has the special technical feature of measuring the fitness of cells in the one or more tumor organoids following the exposure to the one or more amounts of the PARP inhibitor, and correlating the measured fitness of the cells with a sensitivity to the PARP inhibitor, that is not required by Group II or III.

Group II has the special technical feature of (use of) a classifier trained on a plurality of nucleic acid features of the tissue sample, that is not required by Group I or III.

Group III has the special technical feature of identifying a candidate agent as effective based on measured fitness of cells exposed to the candidate agent, that is not required by Group I or II.

Common technical features:

Groups I-III share the common technical feature of a method for recommending therapy for a cancer subject, determining eligibility of a cancer subject for a clinical trial, or identifying resistance or effect of an agent on a cancer subject biopsy sample.

Groups I and III further share the common technical feature of a method comprising

A) culturing a plurality of tumor organoids, each respective tumor organoid in the plurality of tumor organoids from one or more cells of a tumor biopsy from the test subject;

B) exposing the one or more organoids cultured in A) to one or more amounts of the PARP inhibitor, measuring the fitness of cells in the one or more tumor organoids following the exposure to the one or more amounts of the PARP inhibitor; and

C) correlating the measured fitness of the cells with a sensitivity to the PARP inhibitor.

However, these shared technical features do not represent a contribution over prior art, because these shared technical features are previously made obvious over the article entitled "BRCA-deficient mouse mammary tumor organoids to study cancer-drug resistance" by Duarte et al. (hereinafter "Duarte").

Duarte teaches a method for evaluating a cancer in a test subject, the method comprising determining a sensitivity of the cancer to a poly ADP ribose polymerase (PARP) inhibitor (abstract; pg 134, col 2, para 2 "We adapted a previously described culture system to both fresh and cryopreserved mammary tumor tissues, yielding mammary tumor organoid cultures that could be rapidly expanded and cultured in vitro"; pg 136, col 2, para 1 "organoids derived from KB1P/KB2P tumors were more sensitive to treatment with the clinical PARP inhibitor olaparib than homologous recombination (HR)-proficient KP(M) organoids") by:

A) culturing a plurality of tumor organoids, each respective tumor organoid in the plurality of tumor organoids from one or more cells of a tumor biopsy from the test subject (abstract "We developed three-dimensional cancer organoids derived from genetically engineered mouse models (GEMM s) for BRC A1- and BRC A2-deficient cancers...organoid cultures can be efficiently derived and rapidly expanded in vitro");

B) exposing the one or more organoids cultured in A) to one or more amounts of the PARP inhibitor (Figure 2a; Figure 2 Legend - "Comparison of PARPi response of...tumor-derived organoids...In vitro responses of BRCA-deficient and BRCA-proficient organoids to the PARP inhibitor olaparib.... In vivo and in vitro PARPi response of models derived from KB1P4 (d) and KB1PM7 (e) donor tumors");

C) measuring the fitness of cells in the one or more tumor organoids following the exposure to the one or more amounts of the PARP inhibitor (Figure 2 legend "in vitro olaparib response of...organoids, as determined by a viability assay... value was calculated...Kaplan-Meier curves showing survival of mice transplanted with the same organoids"; pg 135, col 2, para 2 - "To examine the cellular fitness of organoids in vivo and the clonal evolution of organoid-derived tumors, we barcoded individual cells in organoids by...transduction with a GFP-encoding lentivirus, we found that a multiplicity of infection (MOI, the number of viral particles per cell) of 1 resulted in about 30% of cells expressing GFP (Supplementary Fig. 5). This value increased to 68% when we applied an MOI of 5. We introduced a lentiviral library of 20,000 barcodes in the PARPi-naive KB1PM7 organoid line (ORG-KB1PM7N.1) at an MOI of 1."; pg 136, col 2, para 1 - "We determined the PARPi sensitivity of organoid cultures using a standard cytotoxicity assay. Consistent with the concept of synthetic lethal interaction between PARP1 inhibition and BRCA1 and BRCA2 deficiency"; p. 142, col 2, para 2); and

D) correlating the measured fitness of the cells with a sensitivity to the PARP inhibitor (pg 136, col 2, para 1 "organoids derived from KB1P/KB2P tumors were more sensitive to treatment with the clinical PARP inhibitor olaparib than homologous recombination (HR)-proficient KP(M) organoids"). Duarte does not specifically teach said cancer is one that does not carry either a BRCA1 or a BRCA2 mutation. It was common practice in the art of medical research at the time to apply a cancer drug efficacy assay to different types of cancer (BRCA1/2 vs non BRCA1/2), in order to determine if the cancer drug would be effective against the other type of cancer. Since Duarte does teach comparing the response of BRCA-deficient and BRCA-proficient organoids to a PARP inhibitor (Figure 2 Legend "In vitro responses of BRCA-deficient and BRCA-proficient organoids to the PARP inhibitor olaparib"), it would have been obvious to one of ordinary skill in the art to have applied the PARP inhibitor efficacy assay taught by Duarte to samples from human cancer patients with a cancer carrying a BRCA1 or BRCA2 mutation, in order to determine if a PARP inhibitor would be effective against BRCA1/2 cancers.

--continued on next sheet--

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Duarte teaches a method for treating a BRCA1-/BRCA2- cancer in a test subject (abstract; pg 134, col 2, para 2 "We adapted a previously described culture system to both fresh and cryopreserved mammary tumor tissues, yielding mammary tumor organoid cultures that could be rapidly expanded and cultured in vitro"; pg 136, col 2, para 1 - "Consistent with the concept of synthetic lethal interaction between PARP1 inhibition and BRCA1 and BRCA2 deficiency, organoids derived from KB1P/KB2P tumors were more sensitive to treatment with the clinical PARP inhibitor olaparib than homologous recombination (HR)-proficient KP(M) organoids"), the method comprising:

A) determining a probability or likelihood that the cancer will be sensitive to a poly ADP ribose polymerase (PARP) inhibitor (pg 136, col 2, para 1 - "We determined the PARPi sensitivity of organoid cultures using a standard cytotoxicity assay...organoids derived from KB1P/KB2P tumors were more sensitive to treatment with the clinical PARP inhibitor olaparib than homologous recombination (HR)-proficient KP(M) organoids"; Figure 2 Legend - "Comparison of PARPi response of original GEMM tumors, tumor-derived organoids and organoid-derived tumors...In vitro responses of BRCA-deficient and BRCA-proficient organoids to the PARP inhibitor olaparib.... In vivo and in vitro PARPi response of models derived from KB1P4 (d) and KB1PM7 (e) donor tumors. Left, Kaplan-Meier curves showing the survival of mice bearing the original tumors treated with either vehicle or olaparib ...P values were calculated by log-rank test (Mantel-Cox)...in vitro olaparib response of...organoids, as determined by a viability assay... value was calculated...Kaplan-Meier curves showing survival of mice transplanted with the same organoids"). Duarte does not teach B) treating cancer in the subject by: when the probability or likelihood that the cancer will be sensitive to the PARP inhibitor satisfies a sensitivity threshold, communicating a recommended therapy comprising the PARP inhibitor to the test subject, and when the probability or likelihood that the cancer will be sensitive to the PARP inhibitor does not satisfy a sensitivity threshold, communicating a recommended therapy that does not include the PARP inhibitor to the test subject. However, application of sensitivity tests in animal models of a disease were commonly used at the time to establish drug resistance based based therapy selection methods for human patients. Therefore, given that Duarte specifically teaches the method of culturing tumor organoid cells with and without BRCA1/2 mutations and determining sensitivity to PARP inhibitor by measuring fitness responses in treated, untreated and/or positive control samples is uniquely useful to determine drug resistance in samples from patients with different tumor types (Abstract - "Poly(ADP-ribose) polymerase inhibition (PARPi) is a promising new therapeutic approach for the treatment of cancers...Despite the success of PARPi in targeting HRD in tumors that lack the tumor suppressor function of BRC A1 or BRC A2, drug resistance poses a major obstacle. We developed three-dimensional cancer organoids derived from genetically engineered mouse models (GEMM s) for BRC A1- and BRC A2-deficient cancers. Unlike conventional cell lines or mammospheres, organoid cultures can be efficiently derived and rapidly expanded in vitro."; pg 135, col 2, para 1-2; pg 136, col 2, para 1 - "We determined the PARPi sensitivity of organoid cultures using a standard cytotoxicity assay"; pg 137, col 1, para 1-2 - "Similar to the original KB1P4 olaparib-naive and olaparib-resistant tumors, ORG-KB1P4N.1 and its resistant derivative retained their differential sensitivities to PARPi treatment both in vitro and in vivo"; pg 138, col 2, para 1 - "we inactivated Trp53bp1 in PARPi-naive BRCA1-deficient mouse mammary tumor organoids (ORG-KB1PM7N.1) and examined the response to olaparib treatment in vivo. ...tumors derived from transplantation of Trp53bp1-targeted ORG-KB1PM7N.1 organoids exhibited a limited response to olaparib compared with the control tumors (derived from ORG-KB1PM7N.1 organoids transduced with a non-targeting (NT) guide RNA (gRNA)), which were highly sensitive"), one with ordinary skill in the art would have found it obvious to have applied the PARP inhibitor efficacy assay taught by Duarte to samples from human cancer patients with a cancer carrying a BRCA1 or BRCA2 mutation, in order to determine if a PARP inhibitor would be effective against BRCA1/2 cancers by comparing with threshold and recommending other anti-cancer agents to effectively treat a BRCA1/BRCA2 deficient, PARP inhibitor-resistant cancer in a patient in need thereof.

Duarte does not specifically teach a method of determining the eligibility of a cancer patient for a clinical trial of a candidate cancer pharmaceutical agent; comprising E) determining whether the cancer patient is eligible for the clinical trial based on at least the measured fitness of the cells in the one or more tumor organoids, wherein reduced fitness of the cells in the one or more tumor organoids is indicative that the cancer patient is eligible for the clinical trial. Given that Duarte specifically teaches the method of determining cellular fitness of tumor organoid cells with and without BRCA1/2 mutations and determining sensitivity to PARP inhibitor, one with ordinary skill in the art would have found it obvious that PARP inhibitor efficacy can be assessed in cells from cultured tumor organoids modeled to validate clinical safety or predict clinical trial outcomes with Duarte's method of determining tumor organoid's cellular fitness correlating with sensitivity to PARP inhibitor to predict cancer patient's eligibility for the clinical trial if there is reduced fitness of the cells in the tumor organoids.

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I-III inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

NOTE, continuation of number 4 above: claims 4-8, 12-34, 41-73, 79-94, 98-120, 124-127, 133-146, 154-159 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).