CLAIMS

WHAT IS CLAIMED IS:

1. ~~A method of treating cancer in a subject in need thereof~~Use of a pharmaceutical composition in the manufacture of a medicament for the treatment of cancer, wherein the pharmaceutical composition comprises a ~~the method comprising administering to the subject a~~ therapeutic molecule selected from a library of therapeutic molecules~~;~~, wherein the therapeutic molecule has been selected by a method comprising: modifying cancer cells from the subject to knock down or knock out the function of a plurality of genes, each gene in the plurality of genes encoding for a protein target of a therapeutic molecule in the library of therapeutic molecules, whereby the therapeutic molecule has been selected if knocking down or knocking out the function of the gene that encodes for the protein target of the selected therapeutic molecule impairs cancer cell viability.

~~2. The method of claim 1, wherein the library of therapeutic molecules comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 10, or at least 20 therapeutic agents of Tables 2-3.~~

~~3. The method of claim 1 or claim 2, wherein one or more of the plurality of genes encode for a protein of Tables 3-5D.~~

~~4~~2. ~~A method of treating cancer in a subject in need thereof~~Use of a pharmaceutical composition in the manufacture of a medicament for the treatment of cancer, wherein the pharmaceutical composition comprises a ~~the method comprising administering to the subject a~~ therapeutic molecule selected from a library of therapeutic molecules~~;~~, wherein the cancer of the subject has been determined to be susceptible to the selected therapeutic molecule by a method comprising:

(a) contacting a sample of cancer cells from the subject with a library of gene modulatory reagents to generate a plurality of modified cancer cells, wherein each modified cancer cell harbors one or more of the gene modulatory reagents, and each gene modulatory reagent is capable of knocking down or knocking out the function of a gene that encodes a protein target of a therapeutic molecule in the library of therapeutic molecules~~,~~; and

(b) sequencing the plurality of modified cancer cells, wherein a gene modulatory reagent that impairs cell viability will have fewer sequence reads than a gene modulatory reagent that does not impair cell viability, and the gene that is knocked down or knocked out by the gene modulatory reagent that impairs cell viability encodes for the protein targeted by the selected therapeutic molecule.

~~5~~3. The ~~method of claim 4~~ use of claim 2, wherein prior to sequencing, one or more of the plurality of modified cancer cells have been propagated, and wherein the cancer cells are primary cancer cells.

~~6. The method of claim 4 or claim 5, wherein the cancer cells are primary cancer cells.~~

~~7~~4. The ~~method of any one of claims 4-6~~ use of claim 2 or claim 3, wherein contacting comprises introducing the one or more gene modulatory reagents into each modified cancer cell by a viral or non-viral delivery method.

~~8~~5. The ~~method of claim 7~~ use of claim 4, wherein one or more of the gene modulatory reagents in the library are encoded on a viral vector.

96. The ~~method of any one of claims 4-8~~ use of any one of claims 2-5, wherein the library comprises from about 10 to about 2,000, from about 10 to about 500, from about 10 to about 200, from about 10 to about 150, from about 50 to about 500, from about 50 to about 200, from about 50 to about 2,000, from about 100 to about 2,000, or from about 500 to about 2,000 different gene modulatory reagents.

7. The use of any one of claims 2-6, wherein one or more gene modulatory reagents from the library of gene modulatory reagents comprises a gRNA sequence or a shRNA sequence having homology to at least a portion of the gene whose function is knocked out by the gene modulatory reagent wherein the at least a portion is at least about 15 contiguous nucleotides, and wherein the library of therapeutic molecules comprises a drug library comprising at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, at least 95, at least 100, at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, at least 170, at least 180, at least 190, at least 200, at least 210, at least 220, at least 230, at least 240, at least 250, at least 260, at least 270, at least 280, at least 290, or at least 300 therapeutic agents.

8. The use of any one of claims 2-7, wherein one or more of the gene modulatory reagents each comprise a guide RNA (gRNA) sequence comprising homology to at least a portion of the gene that encodes a protein target of a therapeutic molecule in the library of therapeutic molecules, wherein the homology is at least about 90% sequence identity.

~~10. The method of any one of claims 4-9, wherein one or more gene modulatory reagents from the library of gene modulatory reagents comprises a nucleic acid sequence homologous to at least about 15 contiguous nucleotides of a gene encoding a protein of Tables 3-5D.~~

~~11. The method of any one of claims 4-10, wherein the library of therapeutic molecules comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 10, or at least 20 therapeutic agents of Tables 2-3.~~

~~12. The method of any one of claims 4-11, wherein one or more of the gene modulatory reagents each comprise a guide RNA (gRNA) sequence comprising at least about 90% homology to a sequence selected from SEQ ID NOS: 1-2789, 2980-3071.~~

~~13~~9. The ~~method of any one of claims 4-12~~ use of any one of claims 2-8, wherein one or more of the gene modulatory reagents comprise a guide RNA (gRNA) sequence comprising homology to at least a portion of the gene that encodes a protein target of a therapeutic molecule in the library of therapeutic molecules, and wherein the gRNA is positioned within a viral vector.

~~14. The method of any one of claims 4-13, wherein the sample of cancer cells is contacted with an endonuclease.~~

~~15. The method of claim 14, wherein the endonuclease comprises a Cas9 or Cas12a endonuclease.~~

~~16. The method of any one of claims 13-15, wherein the gRNA is positioned within a viral vector.~~

~~17~~10. A method of generating a plurality of modified cancer cells from a subject having cancer, the method comprising delivering a library of gene modulatory reagents to a sample of cancer cells from

the subject to generate the plurality of modified cancer cells; wherein each modified cancer cell harbors one or more of the gene modulatory reagents, and each gene modulatory reagent is capable of knocking down or knocking out the function of a gene that encodes a protein target from a library of protein targets.

~~18~~11. The method of claim ~~17~~10, wherein one or more of the gene modulatory reagents comprises a guide RNA (gRNA) sequence comprising homology to at least a portion of the gene whose function is knocked down or knocked out in the modified cancer cell, wherein the sample of cancer cells comprises primary cancer cells, and wherein the modified cancer cells are propagated.

~~19. The method of claim 17, wherein one or more of the gene modulatory reagents each comprise a guide RNA (gRNA) sequence comprising at least about 90% homology to a sequence selected from SEQ ID NOS: 1-2789, 2980-3071.~~

~~20. The method of any one of claims 17-19, wherein the sample of cancer cells is contacted with an endonuclease.~~

~~21. The method of claim 20, wherein the endonuclease comprises a Cas9 or Cas12a endonuclease.~~

~~22~~12. The method of ~~any one of claims 18-21~~claim 10, wherein one or more of the gene modulatory reagents each comprise a guide RNA (gRNA) sequence comprising homology to at least a portion of the gene that encodes a protein target of a therapeutic molecule in the library of therapeutic molecules, wherein the homology is at least about 90% sequence identity, and wherein the gRNA is positioned within a viral vector.

~~23. The method of any one of claims 17-22, wherein the sample of cancer cells comprises primary cancer cells.~~

~~24~~13. The method of any one of claims ~~17-23~~10-12, wherein the library comprises from about 10 to about 2,000, from about 10 to about 500, from about 10 to about 200, from about 10 to about 150, from about 50 to about 500, from about 50 to about 200, from about 50 to about 2,000, from about 100 to about 2,000, or from about 500 to about 2,000 different gene modulatory reagents; and wherein the library of protein targets comprises all known proteins targeted by known drugs capable of treating a particular disease or condition, wherein the particular disease or condition is cancer.

~~25. The method of any one of claims 17-24, wherein the library of protein targets comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 10, or at least 20 protein targets of Tables 3-5D.~~

~~26. The method of any one of claims 17-25, further comprising propagating the modified cancer cells.~~

~~27~~14. A compilation comprising a plurality of modified cancer cells, wherein each modified cancer cell harbors one or more gene modulatory reagents, and each gene modulatory reagent is capable of knocking down or knocking out the function of a gene that encodes a protein target from a library of protein targets.

~~28. The compilation of claim 27, wherein at least one of the one or more of gene modulatory reagents comprises a sequence at least about 90% homologous to a sequence selected from SEQ ID NOS: 1-2789, 2980-3071.~~

~~29. The compilation of claim 27 or claim 28, wherein the library of protein targets comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 10, or at least 20 protein targets of Tables 3-5D.~~

~~30~~15. The compilation of ~~any of claims 27-29~~claim 14, wherein ~~one of more of the~~ at least one of the one or more of gene modulatory reagents comprises a guide RNA (gRNA) sequence comprising homology to at least a portion of the gene whose function is knocked down or knocked out in the modified cancer cell, wherein the homology is at least about 90% sequence identity; wherein the library of protein targets comprises all known proteins targeted by known drugs capable of treating a particular disease or condition, and wherein the particular disease or condition is cancer.

~~31. The compilation of claim 30, wherein the gRNA comprises homology to about 10 to about 50 contiguous nucleotides of the gene.~~

~~32. The compilation of claim 30 or claim 31, further comprising an endonuclease.~~

~~33. The compilation of claim 32, wherein the endonuclease comprises a Cas9 or Cas12a endonuclease.~~

~~34~~16. The compilation of ~~any one of claims 30-33~~claim 14 or claim 15, wherein the gRNA is positioned within a viral vector, and wherein the modified cancer cells are modified primary cancer cells.

~~35. The compilation of any one of claims 27-34, wherein the modified cancer cells are modified primary cancer cells.~~

~~36~~17. The compilation of any one of claims ~~27-35~~14-16, comprising from about 10 to about 2,000, from about 10 to about 500, from about 10 to about 200, from about 10 to about 150, from about 50 to about 500, from about 50 to about 200, from about 50 to about 2,000, from about 100 to about 2,000, or from about 500 to about 2,000 different gene modulatory reagents; and further comprising from about 10 to about 2,000, from about 10 to about 500, from about 10 to about 200, from about 10 to about 150, from about 50 to about 500, from about 50 to about 200, from about 50 to about 2,000, from about 100 to about 2,000, or from about 500 to about 2,000 different populations of modified cancer cells.

~~37. The compilation of any one of claims 27-36, comprising from about 10 to about 2,000, from about 10 to about 500, from about 10 to about 200, from about 10 to about 150, from about 50 to about 500, from about 50 to about 200, from about 50 to about 2,000, from about 100 to about 2,000, or from about 500 to about 2,000 different populations of modified cancer cells.~~

~~38~~18. A method of evaluating the functional effect of genetically modifying cancer cells from a subject, the method comprising: sequencing a plurality of modified cancer cells, wherein each modified cancer cell harbors one or more gene modulatory reagents, each gene modulatory reagent capable of knocking down or knocking out the function of a gene that encodes a protein target in a library of protein targets; and wherein a gene modulatory reagent that impairs cell viability will have fewer sequence reads than a gene modulatory reagent that does not impair cell viability.

~~39~~19. The method of claim ~~38~~18, further comprising:

a) determining which gene modulatory regents have fewer than a threshold number of sequence reads~~.~~, wherein the threshold number of sequence reads is:

i) an expected number of sequence reads if the gene modulatory reagent did not impair cell viability; or

ii) an average number of sequence reads for each gene modulatory reagent in the plurality of modified cancer cells;

b) correlating each gene modulatory reagent that has fewer than the threshold number of sequence reads to its corresponding protein target in the library of protein targets; and

c) correlating the corresponding protein target to a therapeutic molecule.

~~40. The method of claim 39, wherein the threshold number of sequence reads is an expected number of sequence reads if the gene modulatory reagent did not impair cell viability.~~

~~41. The method of claim 39, wherein the threshold number of sequence reads is an average number of sequence reads for each gene modulatory reagent in the plurality of modified cancer cells.~~

~~42. The method of any one of claims 38-41, further comprising correlating each gene modulatory reagent that has fewer than the threshold number of sequence reads to its corresponding protein target in the library of protein targets.~~

~~43. The method of claim 42, further comprising correlating the corresponding protein target to a therapeutic molecule.~~

~~44. The method of any one of claims 38-43, wherein the library of protein targets comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 10, or at least 20 protein targets of Tables 3-5D.~~

~~45. The method of any one of claims 38-44, wherein one or more of the gene modulatory reagents each comprise a guide RNA (gRNA) sequence comprising at least about 90% homology to a sequence selected from SEQ ID NOS: 1-2789, 2980-3071.~~

20. The method of claim 18 or claim 19, wherein the library of protein targets comprises all known proteins targeted by known drugs capable of treating a particular disease or condition and wherein the disease or condition is cancer; wherein one or more of the gene modulatory reagents each comprise a guide RNA (gRNA) sequence comprising homology to at least a portion of the gene that encodes a protein target of a therapeutic molecule in the library of therapeutic molecules, and wherein the homology is at least about 90% sequence identity.

~~46~~21. A library comprising a plurality of gene modulatory reagents, each gene modulatory reagent capable of knocking down or knocking out the function of a gene that encodes a protein target from a library of protein targets.

~~47~~22. The library of claim ~~46~~21, wherein the plurality of gene modulatory reagents is capable of knocking down or knocking out the function of at least about 50% of the genes that encode for the protein targets in the library; wherein the library of protein targets comprises all known proteins targeted by known drugs capable of treating a particular disease or condition and wherein the disease or condition is cancer; wherein one or more of the gene modulatory reagents each comprise a guide RNA (gRNA) sequence comprising homology to at least a portion of the gene that encodes a protein target of a therapeutic molecule in the library of therapeutic molecules, and wherein the homology is at least about 90% sequence identity.

~~48. The library of claim 46 or claim 47, wherein the library of protein targets comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 10, or at least 20 protein targets of Tables 3-5D.~~

~~49. The library of any one of claims 46-48, wherein one or more of the plurality of gene modulatory reagents each comprise a guide RNA (gRNA) sequence comprising at least about 90% homology to a sequence selected from SEQ ID NOS: 1-2789, 2980-3071.~~

~~50~~23. The library of ~~any one of claims 46-49~~ claim 21 or claim 22, wherein the plurality of gene modulatory reagents is capable of knocking down or knocking out the function of about 10 to about 2,000, from about 10 to about 500, from about 10 to about 200, from about 10 to about 150, from about 50 to about 500, from about 50 to about 200, about 50 to about 2,000, or about 100 to about 2,000 genes; and wherein the library comprises about 10 to about 2,000, from about 10 to about 500, from about 10 to about 200, from about 10 to about 150, from about 50 to about 500, from about 50 to about 200, about 50 to about 2,000, or about 100 to about 2,000 gene modulatory reagents.

~~51. The library of any one of claims 46-50, wherein the library comprises about 10 to about 2,000, from about 10 to about 500, from about 10 to about 200, from about 10 to about 150, from about 50 to about 500, from about 50 to about 200, about 50 to about 2,000, or about 100 to about 2,000 gene modulatory reagents.~~

~~52~~24. The library of any one of claims ~~46-51~~ 21-23, wherein at least one of the gene modulatory reagents is capable of knocking out the function of a gene, wherein at least one of the gene modulatory reagents comprise a gRNA sequence having homology to at least a portion of the gene whose function is knocked out by the gene modulatory reagent, and wherein at least one of the gene modulatory reagents is positioned within a viral vector.

~~53. The library of claim 52, wherein at least one of the gene modulatory reagents comprise a gRNA sequence having homology to at least a portion of the gene whose function is knocked out by the gene modulatory reagent.~~

~~54. The library of any one of claims 46-53, wherein at least one of the gene modulatory reagents is positioned within a viral vector.~~