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(71) Applicant: **JANSSEN BIOTECH, INC.** [US/US]; 800/850 Ridgeview Drive, Horsham, Pennsylvania 19044 (US).

(72) Inventors: **SMIRNOV, Denis A.**; 1400 McKean Road, Spring House, Pennsylvania 19477 (US). **BHARGAVA, Vipul**; 1400 McKean Road, Spring House, Pennsylvania 19477 (US). **RAJPUROHIT, Yashoda Rani**; 1400 McKean Road, Spring House, Pennsylvania 19477 (US). **WILKINSON, Patrick**; 1400 McKean Road, Spring House, Pennsylvania 19477 (US). **FU, Kai**; 1400 McKean Road, Spring House, Pennsylvania 19477 (US). **SEPULVEDA, Manuel Alejandro**; 1400 McKean Road, Spring House, Pennsylvania 19477 (US).

(74) Agent: **BAKER & HOSTETLER LLP**; 2929 Arch Street, Cira Centre, 12th Floor, Philadelphia, Pennsylvania 19104-2891 (US).

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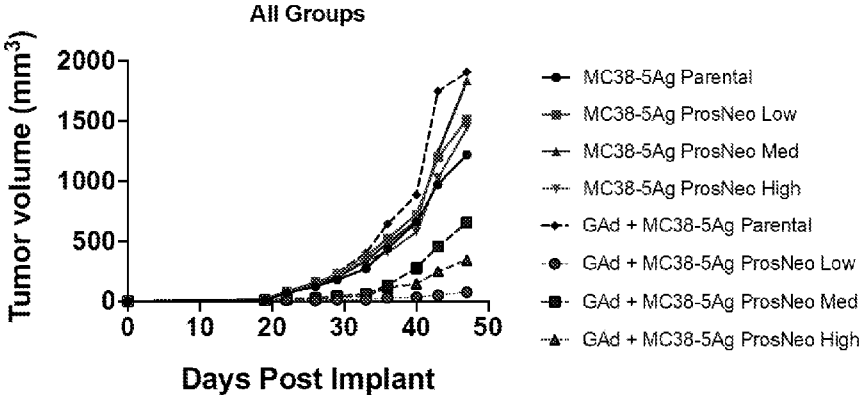
Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

(54) **Title:** A METHOD FOR DETERMINING RESPONSIVENESS TO PROSTATE CANCER TREATMENT

FIG. 14



(57) **Abstract:** Disclosed herein are methods of diagnosing and treating a subject with prostate cancer, as well as methods of monitoring the responsiveness of a subject having prostate cancer to a therapeutic agent.

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[Continued on next page]

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A METHOD FOR DETERMINING RESPONSIVENESS TO PROSTATE CANCER TREATMENT

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 63/048,463, filed July 6, 2020, the disclosure of which is hereby incorporated by reference in its entirety.

SEQUENCE LISTING

[0002] This application contains a Sequence Listing submitted via EFS-Web, the entire content of which is incorporated herein by reference in its entirety. The ASCII text file, created on 30 June 2020, is named 103693.002548_SL.txt and is 554 kilobytes in size.

BACKGROUND

[0003] Prostate cancer is the most common non-cutaneous malignancy in men and the second leading cause of death in men from cancer in the western world. Prostate cancer results from the uncontrolled growth of abnormal cells in the prostate gland. Once a prostate cancer tumor develops, androgens such as testosterone promote prostate cancer growth. At its early stages, localized prostate cancer is often curable with local therapy including, for example, surgical removal of the prostate gland and radiotherapy. However, when local therapy fails to cure prostate cancer, as it does in up to a third of men, the disease progresses into incurable metastatic disease.

[0004] For many years, the established standard of care for men with malignant castration-resistant prostate cancer (mCRPC) was docetaxel chemotherapy. More recently, abiraterone acetate (ZYTIGA®) in combination with prednisone has been approved for treating metastatic castrate resistant prostate cancer. Androgen receptor (AR)-targeted agents, such as enzalutamide (XTANDI®) have also entered the market for treating metastatic castrate resistant prostate cancer. Platinum-based chemotherapy has been tested in a number of clinical studies in molecularly unselected prostate cancer patients with limited results and significant toxicities. However, there remains a subset of patients who either do not respond initially or become refractory (or resistant) to these treatments. No approved therapeutic options are available for such patients.

BRIEF SUMMARY

[0005] Provided herein are methods of diagnosing a subject with prostate cancer, the methods comprising: evaluating the presence of one or more prostate cancer neoantigens in a sample from the subject, the one or more prostate cancer neoantigens comprising the amino acid sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55,

57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, fragments of the preceding sequences, or any combination thereof, wherein the presence of the one or more prostate cancer neoantigens is indicative of prostate cancer in the subject.

[0006] Also disclosed are methods of treating prostate cancer in a subject, the methods comprising:

a) evaluating the presence of one or more prostate cancer neoantigens in a sample from the subject, the one or more prostate cancer neoantigens comprising the amino acid sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, fragments of the preceding sequences, or any combination thereof; and

b) administering a therapeutically effective amount of a prostate cancer vaccine to the subject to thereby treat the prostate cancer.

[0007] Also disclosed are methods of treating prostate cancer in a subject, the methods comprising:

a) evaluating the presence of one or more prostate cancer neoantigens in a sample from the subject, the one or more prostate cancer neoantigens comprising the amino acid sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149,

151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, fragments of the preceding sequences, or any combination thereof; and;

b) evaluating expression of one or more prostate cancer biomarkers in a sample from the subject, wherein the one or more prostate cancer biomarkers comprise: RCN1, STEAP1, PITX2, TIMP1, KLK4, KRT18, KLK3, ACPP, KLK2, TSPAN1, ATF3, SPDEF, NPY, SPINK1, HOXB13, FOXA1, KRT17, FOLH1, TNFRSF19, GREB1, KRT8, FLNC, GRHL2, RAB3B, JCHAIN, TMEFF2, AGR2, ACADL, AZGP1, MUC1, STEAP2, UGT2B17, METTL7A, HPN, NKX3-1, GNMT, ADAMTS15, HSD3B2, EPHA3, KCNN2, LGR5, IDO1, GPR39, C9orf152, MYBPC1, THBS2, ETV7, COL1A1, FGFR4, NR0B1, AR, ARv3, TMPRSS2:ERG, ROR1, FGF8, NKX2-2, EDIL3, RELN, FGF9, AKR1C4, CLUL1, KISS1R, CYP3A5, CYP17A1, SFRP4, HNF1A, CALCR, SYP, MSLN, or any combination thereof; and

c) administering a therapeutically effective amount of a prostate cancer vaccine to the subject.

[0008] Methods for monitoring responsiveness of a subject having prostate cancer to a therapeutic agent are also provided. The methods comprise:

a) evaluating expression of one or more prostate cancer biomarkers, wherein the one or more prostate cancer biomarkers comprise: RCN1, STEAP1, PITX2, TIMP1, KLK4, KRT18, KLK3, ACPP, KLK2, TSPAN1, ATF3, SPDEF, NPY, SPINK1, HOXB13, FOXA1, KRT17, FOLH1, TNFRSF19, GREB1, KRT8, FLNC, GRHL2, RAB3B, JCHAIN, TMEFF2, AGR2, ACADL, AZGP1, MUC1, STEAP2, UGT2B17, METTL7A, HPN, NKX3-1, GNMT, ADAMTS15, HSD3B2, EPHA3, KCNN2, LGR5, IDO1, GPR39, C9orf152, MYBPC1, THBS2, ETV7, COL1A1, FGFR4, NR0B1, AR, ARv3, TMPRSS2:ERG, ROR1, FGF8, NKX2-2, EDIL3, RELN, FGF9, AKR1C4, CLUL1, KISS1R, CYP3A5, CYP17A1, SFRP4, HNF1A, CALCR, SYP, MSLN, or combinations thereof;

b) administering a therapeutic agent to the subject; and

c) evaluating the expression of the one or more prostate cancer biomarkers evaluated in step a), wherein a decrease in the expression of the one or more prostate cancer biomarkers compared to the expression in step a) is indicative of responsiveness to the therapeutic agent.

[0009] Further provided are methods for preparing a cDNA from a subject with prostate cancer useful for analyzing an expression of prostate cancer neoantigens, the method comprising:

a) extracting RNA from a sample from the subject;

b) producing amplified cDNA from the RNA extracted in step a) by: (i) reverse transcribing the extracted RNA to produce the cDNA, and (ii) amplifying the cDNA; and

c) analyzing the amplified cDNA produced in step b) for one or more prostate cancer neoantigens, wherein the cDNA encodes an amino acid sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, fragments of the preceding sequences, or any combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] The summary, as well as the following detailed description, is further understood when read in conjunction with the appended drawings. For the purpose of illustrating the disclosed methods, there are shown in the drawings exemplary embodiments of the methods; however, the methods are not limited to the specific embodiments disclosed. In the drawings:

[0011] **FIG. 1** depicts an exemplary chimeric read-through fusion between Gene A and Gene B. Neoantigenic peptide sequences arise at the breakpoint junction.

[0012] **FIG. 2** depicts an exemplary gene fusion resulting from chromosomal alteration, such as DNA translocations.

[0013] **FIG. 3** depicts exemplary splice variants with alternative 5' or 3' splice sites, retained introns, excluded exons, or alternative terminations or insertions.

[0014] **FIG. 4** depicts an exemplary approach of identifying splice variants.

[0015] **FIG. 5A** illustrates a flow cytometry dot plot depicting $TNF\alpha^{+}IFN\gamma^{+}CD8^{+}$ T cell frequencies in PBMC samples after no stimulation (DMSO)

[0016] **FIG. 5B** illustrates a flow cytometry dot plot depicting $TNF\alpha^{+}IFN\gamma^{+}CD8^{+}$ T cell frequencies in PBMC samples after stimulating with CEF peptide.

[0017] **FIG. 5C** illustrates a flow cytometry dot plot depicting $TNF\alpha^{+}IFN\gamma^{+}CD8^{+}$ T cell frequencies in PBMC samples after stimulation with P16.

[0018] **FIG. 5D** illustrates a flow cytometry dot plot depicting $TNF\alpha^+IFN\gamma^+CD8^+$ T cell frequencies in PBMC samples after stimulation with P98.

[0019] **FIG. 5E** illustrates a flow cytometry dot plot depicting $TNF\alpha^+IFN\gamma^+CD8^+$ T cell frequencies in PBMC samples after stimulation with P3 self-antigen.

[0020] **FIG. 6** illustrates the number of prostate cancer patients whose PBMC samples demonstrated a positive immune response to the specified neoantigens. P3, P6, P7, P9 and P92 represent self-antigens.

[0021] **FIG. 7** illustrates the number of prostate cancer patients whose PBMC samples demonstrated a positive $CD8^+$ immune response to the specified neoantigens.

[0022] **FIG. 8** illustrates the number of prostate cancer patients whose PBMC samples demonstrated a positive $CD4^+$ immune response to the specified neoantigens.

[0023] **FIG. 9** illustrates an exemplary embodiment of the disclosed methods for monitoring responsiveness of a subject having prostate cancer to a therapeutic agent.

[0024] **FIG. 10** illustrates the genes from exosome samples with AUC values larger than 0.55.

[0025] **FIG. 11** illustrates the mean and standard deviation (error bar) of the accuracy, sensitivity, and specificity for the exosome samples.

[0026] **FIG. 12** illustrates the genes from PAXgene samples with AUC values larger than 0.55.

[0027] **FIG. 13** illustrates the mean and standard deviation (error bar) of the accuracy, sensitivity, and specificity for the PAXgene samples.

[0028] **FIG. 14** illustrates the MC38 tumor volume (mm^3) in mice immunized with GAd20-PCaNeoAg compared to mice that did not receive GAd20-PCaNeoAg immunization and mice implanted with the parental MC38 cell line that did not express the 10 prostate neoantigens.

DETAILED DESCRIPTION

[0029] The disclosed methods may be understood more readily by reference to the following detailed description taken in connection with the accompanying figures, which form a part of this disclosure. It is to be understood that the disclosed methods are not limited to the specific methods described and/or shown herein, and that the terminology used herein is for the purpose of describing particular embodiments by way of example only and is not intended to be limiting of the claimed methods.

[0030] All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as though fully set forth.

[0031] Although any methods and materials similar or equivalent to those described herein may be used in the practice for testing of the present disclosure, exemplary materials and methods are described herein. In describing and claiming the present disclosure, the following terminology will be used.

[0032] Unless specifically stated otherwise, any description as to a possible mechanism or mode of action or reason for improvement is meant to be illustrative only, and the disclosed methods are not to be constrained by the correctness or incorrectness of any such suggested mechanism or mode of action or reason for improvement.

[0033] Throughout this text, the descriptions refer to methods of diagnosis and methods of treatment. Where the disclosure describes or claims a feature or embodiment associated with a method of diagnosis, such a feature or embodiment is equally applicable to the methods of treatment. Likewise, where the disclosure describes or claims a feature or embodiment associated with a method of treatment, such a feature or embodiment is equally applicable to the method of diagnosis.

[0034] It is to be appreciated that certain features of the disclosed methods which are, for clarity, described herein in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the disclosed methods that are, for brevity, described in the context of a single embodiment, may also be provided separately or in any subcombination.

[0035] Various terms relating to aspects of the description are used throughout the specification and claims. Such terms are to be given their ordinary meaning in the art unless otherwise indicated. Other specifically defined terms are to be construed in a manner consistent with the definitions provided herein.

[0036] As used herein, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a cell" includes a combination of two or more cells, and the like.

[0037] The term "comprising" is intended to include examples encompassed by the terms "consisting essentially of" and "consisting of"; similarly, the term "consisting essentially of" is intended to include examples encompassed by the term "consisting of."

[0038] As used herein, the phrase "and fragments thereof" when appended to a list includes fragments of one or more members of the associated list. The list may comprise a Markush group so that, as an example, the phrase "the group consisting of peptides A, B, and C, and fragments thereof" specifies or recites a Markush group including A, B, C, fragments of A, fragments of B, and/or fragments of C.

[0039] "Isolated" refers to a homogenous population of molecules (such as synthetic polynucleotides or polypeptides) which have been substantially separated and/or purified away from other components of the system the molecules are produced in, such as a recombinant cell, as well as a protein that has been subjected to at least one purification or isolation step. "Isolated" refers to a molecule that is substantially free of other cellular material and/or chemicals and encompasses molecules that are isolated

to a higher purity, such as to 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% purity.

[0040] "Immunogenic fragment" refers to a polypeptide that is recognized by cytotoxic T lymphocytes, helper T lymphocytes, or B cells when the fragment is in complex with MHC class I or MHC class II molecules.

[0041] "In-frame" refers to the reading frame of codons in a first polynucleotide being the same as the reading frame of codons in a second polynucleotide which are joined together to form a polynucleotide. In-frame polynucleotide encodes a polypeptide encoded by both the first polynucleotide and the second polynucleotide.

[0042] "Immunogenic" refers to a polypeptide that comprises one or more immunogenic fragments.

[0043] "Heterologous" refers to two or more polynucleotides or two or more polypeptides that are not found in the same relationship to each other in nature.

[0044] "Heterologous polynucleotide" refers to a non-naturally occurring polynucleotide that encodes two or more neoantigens as described herein.

[0045] "Heterologous polypeptide" refers to a non-naturally occurring polypeptide comprising two or more neoantigen polypeptides as described herein.

[0046] "Non-naturally occurring" refers to a molecule that does not exist in nature.

[0047] "Neoantigen" refers to a polypeptide that is present in prostate tumor tissue that has at least one alteration that makes it distinct from the corresponding wild-type polypeptide present in non-malignant tissue, e.g., via mutation in a tumor cell or post-translational modification specific to a tumor cell. A mutation can include a frameshift or nonframeshift insertion or deletion, missense or nonsense substitution, splice site alteration, aberrant splice variants, genomic rearrangement or gene fusion, or any genomic or expression alteration giving rise to the neoantigen.

[0048] "Recombinant" refers to polynucleotides, polypeptides, vectors, viruses and other macromolecules that are prepared, expressed, created or isolated by recombinant means.

[0049] "Vaccine" refers to a composition that comprises one or more immunogenic polypeptides, immunogenic polynucleotides or fragments, or any combination thereof intentionally administered to induce acquired immunity in the recipient (e.g. subject).

[0050] "Treat," "treating," or "treatment" of a disease or disorder such as cancer refers to accomplishing one or more of the following: reducing the severity and/or duration of the disorder, inhibiting worsening of symptoms characteristic of the disorder being treated, limiting or preventing recurrence of the disorder in subjects that have previously had the disorder, or limiting or preventing recurrence of symptoms in subjects that were previously symptomatic for the disorder.

[0051] "Prevent," "preventing," "prevention," or "prophylaxis" of a disease or disorder means preventing that a disorder occurs in subject.

[0052] "Therapeutically effective amount" refers to an amount effective, at doses and for periods of time necessary, to achieve a desired therapeutic result. A therapeutically effective amount may vary depending on factors such as the disease state, age, sex, and weight of the individual, and the ability of a therapeutic or a combination of therapeutics to elicit a desired response in the individual. Exemplary indicators of an effective therapeutic or combination of therapeutics that include, for example, improved well-being of the patient.

[0053] "Relapsed" refers to the return of a disease or the signs and symptoms of a disease after a period of improvement after prior treatment with a therapeutic.

[0054] "Refractory" refers to a disease that does not respond to a treatment. A refractory disease can be resistant to a treatment before or at the beginning of the treatment, or a refractory disease can become resistant during a treatment.

[0055] "Subject" includes any human or nonhuman animal. "Nonhuman animal" includes all vertebrates, *e.g.,* mammals and non-mammals, such as nonhuman primates, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, etc. The terms "subject" and "patient" can be used interchangeably herein.

[0056] "In combination with" means that two or more therapeutic agents are administered to a subject together in a mixture, concurrently as single agents or sequentially as single agents in any order.

[0057] "Enhance" or "induce" when in reference to an immune response refers to increasing the scale and/or efficiency of an immune response or extending the duration of the immune response. The terms are used interchangeably with "augment."

[0058] "Immune response" refers to any response to an immunogenic polypeptide or polynucleotide or fragment by the immune system of a vertebrate subject. Exemplary immune responses include local and systemic cellular as well as humoral immunity, such as cytotoxic T lymphocyte (CTL) responses, including antigen-specific induction of $CD8^+$ CTLs, helper T-cell responses including T-cell proliferative responses and cytokine release, and B-cell responses including antibody response.

[0059] "Variant," "mutant," or "altered" refers to a polypeptide or a polynucleotide that differs from a reference polypeptide or a reference polynucleotide by one or more modifications, for example one or more substitutions, insertions, or deletions.

[0060] "About" means within an acceptable error range for the particular value as determined by one of skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. Unless explicitly stated otherwise within the Examples or elsewhere in the Specification in the context of a particular assay, result or embodiment, "about" means within one standard deviation per the practice in the art, or a range of up to 5%, whichever is larger.

[0061] "Prime-boost" or "prime-boost regimen" refers to a method of treating a subject involving priming a T-cell response with a first vaccine followed by boosting the immune response with a second vaccine. The first vaccine and the second vaccine are typically distinct. These prime-boost immunizations elicit immune responses of greater height and breadth than can be achieved by priming

and boosting with the same vaccine. The priming step initiates memory cells and the boost step expands the memory response. Boosting can occur once or multiple times.

[0062] Cancer cells produce neoantigens that result from genomic alterations and aberrant transcriptional programs. Neoantigen burden in patients has been associated with response to immunotherapy (Snyder *et al.*, N Engl J Med. 2014 Dec 4;371(23):2189-2199. doi: 10.1056/NEJMoa1406498. Epub 2014 Nov 19; Le *et al.*, N Engl J Med. 2015 Jun 25;372(26):2509-20. doi: 10.1056/NEJMoa1500596. Epub 2015 May 30; Rizvi *et al.*, Science. 2015 Apr 3;348(6230):124-8. doi: 10.1126/science.aaa1348. Epub 2015 Mar 12; Van Allen *et al.*, Science. 2015 Oct 9;350(6257):207-211. doi: 10.1126/science.aad0095. Epub 2015 Sep 10). The disclosure is based, at least in part, on the identification of prostate neoantigens that are common in prostate cancer patients and hence can be utilized in diagnosing a subject with prostate cancer, treating prostate cancer, and monitoring responsiveness of a subject having prostate cancer to a therapeutic agent. One or more neoantigens or polynucleotides encoding the neoantigens of the disclosure may also be used for diagnostic or prognostic purposes.

Methods of diagnosis

[0063] Disclosed herein are methods of diagnosing a subject with prostate cancer. The methods comprise evaluating the presence of one or more prostate cancer neoantigens in a sample from the subject, the one or more prostate cancer neoantigens comprising the amino acid sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, fragments of the preceding sequences, or any combination thereof, wherein the presence of the one or more prostate cancer neoantigens is indicative of prostate cancer in the subject.

[0064] The methods can comprise evaluating the presence of one or more prostate cancer neoantigens comprising the amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, fragments of the preceding sequences, or any combination thereof.

[0065] In some embodiments, the methods comprise evaluating the presence of one or more prostate cancer neoantigens comprising the amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, fragments of the preceding sequences, or any combination thereof.

[0066] In some embodiments, the methods comprise evaluating the presence of each of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, and 223.

[0067] The presence of the one or more prostate cancer neoantigens can be evaluated by, for example, PCR, quantitative PCR (qPCR), various forms of nucleic acid sequencing (including but not limited to Illumina, Ion Torrent, Pacific Bioscience, Oxford Nanopore platforms), and various hybridization-based approaches (including not limited to Affymetrix Gene Chip or Nanostring platforms).

[0068] In some embodiments, the presence of the one or more prostate cancer neoantigens is evaluated by qPCR. In some aspects, the methods further comprise, prior to performing the qPCR, extracting RNA from the sample from the subject and synthesizing cDNA from the extracted RNA. The RNA extracted from the subject can correspond to a polynucleotide sequence comprising SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 380, 382, 384, 386, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 519, 520, 521, 522, 523, 524, 525, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, fragments of the preceding sequences, or any combination thereof.

[0069] As used herein, "RNA . . . can correspond to a polynucleotide sequence comprising" refers to an RNA transcript generated from the DNA encoding the RNA or the RNA complement of a cDNA, wherein the DNA or cDNA comprise the listed sequence (i.e. SEQ ID NO).

[0070] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 1 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 1, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 2 or a fragment thereof.

[0071] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 3 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 3, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 4 or a fragment thereof.

[0072] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 5 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 5, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 6 or a fragment thereof.

[0073] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 7 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 7, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 8 or a fragment thereof.

[0074] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 9 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 9, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 10 or a fragment thereof.

[0075] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 11 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 11, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 12 or a fragment thereof.

[0076] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 13 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 13, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 14 or a fragment thereof.

[0077] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 15 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 15, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 16 or a fragment thereof.

[0078] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 17 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 17, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 18 or a fragment thereof.

[0079] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 19 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 19, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 20 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 19, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 497 or a fragment thereof. In some

embodiments, the polypeptide of SEQ ID NO: 19, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 538 or a fragment thereof.

[0080] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 21 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 21, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 22 or a fragment thereof.

[0081] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 23 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 23, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 24 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 23, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 498 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 23, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 539 or a fragment thereof.

[0082] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 25 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 25, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 26 or a fragment thereof.

[0083] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 27 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 27, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 28 or a fragment thereof.

[0084] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 29 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 29, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 30 or a fragment thereof.

[0085] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 31 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 31, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 32 or a fragment thereof.

[0086] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 33 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 33, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 34 or a fragment thereof.

[0087] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 35 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 35, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 36 or a fragment thereof.

[0088] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 37 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 37, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 38 or a fragment thereof.

[0089] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 39 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 39, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 40 or a fragment thereof.

[0090] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 41 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 41, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 42 or a fragment thereof.

[0091] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 43 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 43, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 44 or a fragment thereof.

[0092] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 45 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 45, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 46 or a fragment thereof.

[0093] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 47 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 47, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 48 or a fragment thereof.

[0094] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 49 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 49, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 50 or a fragment thereof.

[0095] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 51 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 51, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 52 or a fragment thereof.

[0096] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 53 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 53, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 54 or a fragment thereof.

[0097] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 55 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 55, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 56 or a fragment thereof.

[0098] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 57 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 57, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 58 or a fragment thereof.

[0099] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 59 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 59, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 60 or a fragment thereof.

[0100] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 61 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 61, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 62 or a fragment thereof.

[0101] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 63 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 63, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 64 or a fragment thereof.

[0102] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 65 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 65, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 66 or a fragment thereof.

[0103] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 67 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 67, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 68 or a fragment thereof.

[0104] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 69 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 69, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 70 or a fragment thereof.

[0105] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 71 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 71, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 72 or a fragment thereof.

[0106] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 73 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 73, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 74 or a fragment thereof.

[0107] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 75 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 75, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 76 or a fragment thereof.

[0108] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 77 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 77, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 78 or a fragment thereof.

[0109] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 79 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 79, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 80 or a fragment thereof.

[0110] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 81 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 81, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 82 or a fragment thereof.

[0111] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 83 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 83, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 84 or a fragment thereof.

[0112] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 85 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 85, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 86 or a fragment thereof.

[0113] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 87 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 87, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 88 or a fragment thereof.

[0114] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 89 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 89, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 90 or a fragment thereof.

[0115] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 91 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 91, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 92 or a fragment thereof.

[0116] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 93 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 93, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 94 or a fragment thereof.

[0117] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 95 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 95, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 96 or a fragment thereof.

[0118] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 97 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 97, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 98 or a fragment thereof.

[0119] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 99 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 99, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 100 or a fragment thereof.

[0120] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 101 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 101, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 102 or a fragment thereof.

[0121] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 103 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 103, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 104 or a fragment thereof.

[0122] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 105 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 105, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 106 or a fragment thereof.

[0123] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 107 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 107, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 108 or a fragment thereof.

[0124] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 109 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 109, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 110 or a fragment thereof.

[0125] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 111 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 111, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 112 or a fragment thereof.

[0126] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 113 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 113, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 114 or a fragment thereof.

[0127] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 115 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 115, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 116 or a fragment thereof.

[0128] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 117 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 117, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 118 or a fragment thereof.

[0129] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 119 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 119, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 120 or a fragment thereof.

[0130] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 121 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 121, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 122 or a fragment thereof.

[0131] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 123 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 123, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 124 or a fragment thereof.

[0132] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 125 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 125, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 126 or a fragment thereof.

[0133] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 127 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 127, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 128 or a fragment thereof.

[0134] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 129 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 129, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 130 or a fragment thereof.

[0135] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 131 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 131, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 132 or a fragment thereof.

[0136] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 133 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 133, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 134 or a fragment thereof.

[0137] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 135 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 135, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 136 or a fragment thereof.

[0138] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 137 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 137, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 138 or a fragment thereof.

[0139] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 139 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 139, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 140 or a fragment thereof.

[0140] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 141 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 141, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 142 or a fragment thereof.

[0141] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 143 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 143, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 144 or a fragment thereof.

[0142] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 145 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 145, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 146 or a fragment thereof.

[0143] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 147 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 147, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 148 or a fragment thereof.

[0144] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 149 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 149, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 150 or a fragment thereof.

[0145] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 151 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 151, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 152 or a fragment thereof.

[0146] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 153 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 153, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 154 or a fragment thereof.

[0147] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 155 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 155, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 156 or a fragment thereof.

[0148] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 157 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 157, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 158 or a fragment thereof.

[0149] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 159 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 159, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 160 or a fragment thereof.

[0150] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 161 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 161, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 162 or a fragment thereof.

[0151] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 163 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 163, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 164 or a fragment thereof.

[0152] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 165 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 165, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 166 or a fragment thereof.

[0153] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 167 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 167, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 168 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 167, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 495 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 167, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 536 or a fragment thereof.

[0154] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 169 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 169, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 170 or a fragment thereof.

[0155] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 171 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 171, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 172 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 171, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 496 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 171, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 537 or a fragment thereof.

[0156] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 173 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 173, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 174 or a fragment thereof.

[0157] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 175 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 175, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 176 or a fragment thereof.

[0158] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 177 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 177, or a fragment thereof, is encoded by the polynucleotide of SEQ ID

NO: 178 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 177, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 499 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 177, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 540 or a fragment thereof.

[0159] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 179 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 179, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 180 or a fragment thereof.

[0160] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 181 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 181, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 182 or a fragment thereof.

[0161] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 183 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 183, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 184 or a fragment thereof.

[0162] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 185 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 185, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 186 or a fragment thereof.

[0163] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 187 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 187, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 188 or a fragment thereof.

[0164] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 189 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 189, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 190 or a fragment thereof.

[0165] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 191 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 191, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 192 or a fragment thereof.

[0166] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 193 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 193, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 194 or a fragment thereof.

[0167] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 195 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 195, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 196 or a fragment thereof.

[0168] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 197 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 197, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 198 or a fragment thereof.

[0169] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 199 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 199, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 200 or a fragment thereof.

[0170] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 201 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 201, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 202 or a fragment thereof.

[0171] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 203 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 203, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 204 or a fragment thereof.

[0172] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 205 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 205, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 206 or a fragment thereof.

[0173] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 207 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 207, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 208 or a fragment thereof.

[0174] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 209 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 209, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 210 or a fragment thereof.

[0175] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 211 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 211, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 212 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 211, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 484 or a fragment thereof. In some

embodiments, the polypeptide of SEQ ID NO: 211, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 525 or a fragment thereof.

[0176] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 213 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 213, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 214 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 213, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 486 or a fragment thereof.

[0177] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 215 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 215, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 216 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 215, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 487 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 215, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 528 or a fragment thereof.

[0178] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 217 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 217, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 218 or a fragment thereof.

[0179] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 219 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 219, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 220 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 219, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 489 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 219, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 530 or a fragment thereof.

[0180] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 221 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 221, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 222 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 221, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 488 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 221, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 529 or a fragment thereof.

[0181] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 223 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 223, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 224 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 223, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 494 or a fragment thereof. In some

embodiments, the polypeptide of SEQ ID NO: 223, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 535 or a fragment thereof.

[0182] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 225 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 225, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 226 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 225, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 490 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 225, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 531 or a fragment thereof.

[0183] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 227 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 227, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 228 or a fragment thereof.

[0184] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 229 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 229, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 230 or a fragment thereof.

[0185] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 231 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 231, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 232 or a fragment thereof.

[0186] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 233 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 233, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 234 or a fragment thereof.

[0187] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 235 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 235, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 236 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 235, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 493 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 235, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 534 or a fragment thereof.

[0188] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 237 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 237, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 238 or a fragment thereof.

[0189] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 239 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 239, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 240 or a fragment thereof.

[0190] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 241 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 241, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 242 or a fragment thereof.

[0191] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 243 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 243, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 244 or a fragment thereof.

[0192] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 245 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 245, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 246 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 245, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 470 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 245, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 511 or a fragment thereof.

[0193] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 247 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 247, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 248 or a fragment thereof.

[0194] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 249 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 249, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 250 or a fragment thereof.

[0195] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 251 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 251, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 252 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 251, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 469 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 251, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 510 or a fragment thereof.

[0196] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 253 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 253, or a fragment thereof, is encoded by the polynucleotide of SEQ ID

NO: 254 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 253, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 464 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 253, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 505 or a fragment thereof.

[0197] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 255 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 255, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 256 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 255, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 474 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 255, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 515 or a fragment thereof.

[0198] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 257 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 257, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 258 or a fragment thereof.

[0199] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 259 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 259, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 260 or a fragment thereof.

[0200] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 261 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 261, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 262 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 261, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 471 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 261, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 512 or a fragment thereof.

[0201] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 263 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 263, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 264 or a fragment thereof.

[0202] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 265 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 265, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 266 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 265, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 472 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 265, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 513 or a fragment thereof.

[0203] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 267 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 267, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 268 or a fragment thereof.

[0204] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 269 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 269, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 270 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 269, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 463 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 269, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 504 or a fragment thereof.

[0205] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 271 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 271, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 272 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 271, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 465 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 271, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 508 or a fragment thereof.

[0206] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 273 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 273, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 274 or a fragment thereof.

[0207] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 275 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 275, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 276 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 275, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 459 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 275, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 500 or a fragment thereof.

[0208] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 277 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 277, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 278 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 277, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 475 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 277, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 516 or a fragment thereof.

[0209] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 279 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 279, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 280 or a fragment thereof.

[0210] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 281 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 281, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 282 or a fragment thereof.

[0211] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 283 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 283, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 284 or a fragment thereof.

[0212] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 285 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 285, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 286 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 285, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 477 or a fragment thereof.

[0213] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 287 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 287, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 288 or a fragment thereof.

[0214] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 289 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 289, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 290 or a fragment thereof.

[0215] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 291 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 291, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 292 or a fragment thereof.

[0216] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 293 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 293, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 294 or a fragment thereof.

[0217] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 295 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 295, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 296 or a fragment thereof.

[0218] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 297 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 297, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 298 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 297, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 476 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 297, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 517 or a fragment thereof.

[0219] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 299 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 299, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 300 or a fragment thereof.

[0220] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 301 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 301, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 302 or a fragment thereof.

[0221] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 303 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 303, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 304 or a fragment thereof.

[0222] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 305 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 305, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 306 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 305, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 468 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 305, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 509 or a fragment thereof.

[0223] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 307 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 307, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 308 or a fragment thereof.

[0224] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 309 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 309, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 310 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 309, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 465 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 309, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 506 or a fragment thereof.

[0225] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 311 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 311, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 312 or a fragment thereof.

[0226] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 313 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 313, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 314 or a fragment thereof.

[0227] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 315 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 315, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 316 or a fragment thereof.

[0228] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 317 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 317, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 318 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 317, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 473 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 317, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 514 or a fragment thereof.

[0229] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 319 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 319, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 320 or a fragment thereof.

[0230] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 321 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 321, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 322 or a fragment thereof.

[0231] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 323 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 323, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 324 or a fragment thereof.

[0232] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 325 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 325, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 326 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 325, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 466 or a fragment thereof. In some

embodiments, the polypeptide of SEQ ID NO: 325, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 507 or a fragment thereof.

[0233] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 327 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 327, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 328 or a fragment thereof.

[0234] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 329 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 329, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 330 or a fragment thereof.

[0235] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 331 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 331, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 332 or a fragment thereof.

[0236] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 333 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 333, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 334 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 333, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 461 or a fragment thereof.

[0237] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 335 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 335, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 336 or a fragment thereof.

[0238] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 337 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 337, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 338 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 337, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 462 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 337, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 503 or a fragment thereof.

[0239] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 339 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 339, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 340 or a fragment thereof.

[0240] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 341 or a fragment thereof. In some embodiments,

the polypeptide of SEQ ID NO: 341, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 342 or a fragment thereof.

[0241] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 343 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 343, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 344 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 343, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 483 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 343, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 524 or a fragment thereof.

[0242] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 345 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 345, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 346 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 345, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 491 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 345, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 532 or a fragment thereof.

[0243] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 347 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 347, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 348 or a fragment thereof.

[0244] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 349 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 349, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 350 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 349, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 485 or a fragment thereof.

[0245] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 351 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 351, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 352 or a fragment thereof.

[0246] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 353 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 353, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 354 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 353, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 492 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 353, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 533 or a fragment thereof.

[0247] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 355 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 355, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 356 or a fragment thereof.

[0248] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 357 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 357, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 358 or a fragment thereof.

[0249] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 359 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 359, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 360 or a fragment thereof.

[0250] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 361 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 361, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 362 or a fragment thereof.

[0251] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 363 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 363, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 364 or a fragment thereof.

[0252] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 365 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 365, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 366 or a fragment thereof.

[0253] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 367 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 367, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 368 or a fragment thereof.

[0254] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 369 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 369, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 370 or a fragment thereof.

[0255] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 371 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 371, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 372 or a fragment thereof.

[0256] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 373 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 373, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 374 or a fragment thereof.

[0257] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 375 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 375, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 376 or a fragment thereof.

[0258] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 379 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 379, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 380 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 379, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 482 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 379, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 523 or a fragment thereof.

[0259] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 381 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 381, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 382 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 381, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 460 or a fragment thereof.. In some embodiments, the polypeptide of SEQ ID NO: 381, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 501 or a fragment thereof.

[0260] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 383 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 383, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 384 or a fragment thereof.

[0261] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 385 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 385, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 386 or a fragment thereof.

[0262] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 387 or a fragment thereof.

[0263] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 388 or a fragment thereof.

[0264] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 389 or a fragment thereof.

[0265] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 390 or a fragment thereof.

[0266] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 391 or a fragment thereof.

[0267] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 392 or a fragment thereof.

[0268] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 393 or a fragment thereof.

[0269] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 394 or a fragment thereof.

[0270] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 395 or a fragment thereof.

[0271] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 396 or a fragment thereof.

[0272] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 397 or a fragment thereof.

[0273] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 398 or a fragment thereof.

[0274] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 399 or a fragment thereof.

[0275] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 400 or a fragment thereof.

[0276] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 401 or a fragment thereof.

[0277] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 402 or a fragment thereof.

[0278] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 403 or a fragment thereof.

[0279] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 404 or a fragment thereof.

[0280] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 405 or a fragment thereof.

[0281] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 406 or a fragment thereof.

[0282] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 407 or a fragment thereof.

[0283] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 408 or a fragment thereof.

[0284] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 426 or a fragment thereof.

[0285] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 427 or a fragment thereof.

[0286] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 428 or a fragment thereof.

[0287] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 429 or a fragment thereof.

[0288] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 430 or a fragment thereof.

[0289] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 431 or a fragment thereof.

[0290] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 432 or a fragment thereof.

[0291] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 433 or a fragment thereof.

[0292] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 434 or a fragment thereof.

[0293] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 435 or a fragment thereof.

[0294] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 436 or a fragment thereof.

[0295] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 437 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 437, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 448 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 437, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 478 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 437, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 519 or a fragment thereof.

[0296] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 438 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 438, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 449 or a fragment thereof.

[0297] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 439 or a fragment thereof. In some embodiments,

the polypeptide of SEQ ID NO: 439, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 450 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 439, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 479 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 439, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 520 or a fragment thereof.

[0298] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 440 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 440, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 451 or a fragment thereof.

[0299] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 441 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 441, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 452 or a fragment thereof.

[0300] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 442 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 442, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 453 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 442, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 480 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 442, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 521 or a fragment thereof.

[0301] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 443 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 443, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 454 or a fragment thereof.

[0302] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 444 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 444, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 455 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 444, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 481 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 444, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 522 or a fragment thereof.

[0303] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 445 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 445, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 456 or a fragment thereof.

[0304] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 446 or a fragment thereof. In some embodiments,

the polypeptide of SEQ ID NO: 446, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 457 or a fragment thereof.

[0305] The methods can comprise evaluating the presence of the prostate cancer neoantigen comprising the amino acid sequence of SEQ ID NO: 447 or a fragment thereof. In some embodiments, the polypeptide of SEQ ID NO: 447, or a fragment thereof, is encoded by the polynucleotide of SEQ ID NO: 458 or a fragment thereof.

[0306] The fragments of the prostate cancer neoantigens can comprise at least 6 amino acids. In some embodiments, the fragments comprise at least 7 amino acids In some embodiments, the fragments comprise at least 8 amino acids. In some embodiments, the fragments comprise at least 9 amino acids. In some embodiments, the fragments comprise at least 10 amino acids. In some embodiments, the fragments comprise at least 11 amino acids. In some embodiments, the fragments comprise at least 12 amino acids. In some embodiments, the fragments comprise at least 13 amino acids. In some embodiments, the fragments comprise at least 14 amino acids. In some embodiments, the fragments comprise at least 15 amino acids. In some embodiments, the fragments comprise at least 16 amino acids. In some embodiments, the fragments comprise at least 17 amino acids. In some embodiments, the fragments comprise at least 18 amino acids. In some embodiments, the fragments comprise at least 19 amino acids. In some embodiments, the fragments comprise at least 20 amino acids. In some embodiments, the fragments comprise at least 21 amino acids. In some embodiments, the fragments comprise at least 22 amino acids. In some embodiments, the fragments comprise at least 23 amino acids. In some embodiments, the fragments comprise at least 24 amino acids. In some embodiments, the fragments comprise at least 25 amino acids. In some embodiments, the fragments comprise about 6-25 amino acids. In some embodiments, the fragments comprise about 7-25 amino acids. In some embodiments, the fragments comprise about 8-25 amino acids. In some embodiments, the fragments comprise about 8-24 amino acids. In some embodiments, the fragments comprise about 8-23 amino acids. In some embodiments, the fragments comprise about 8-22 amino acids. In some embodiments, the fragments comprise about 8-21 amino acids. In some embodiments, the fragments comprise about 8-20 amino acids. In some embodiments, the fragments comprise about 8-19 amino acids. In some embodiments, the fragments comprise about 8-18 amino acids. In some embodiments, the fragments comprise about 8-17 amino acids. In some embodiments, the fragments comprise about 8-16 amino acids. In some embodiments, the fragments comprise about 8-15 amino acids. In some embodiments, the fragments comprise about 8-14 amino acids. In some embodiments, the fragments comprise about 9-14 amino acids. In some embodiments, the fragments comprise about 9-13 amino acids. In some embodiments, the fragments comprise about 9-12 amino acids. In some embodiments, the fragments comprise about 9-11 amino acids. In some embodiments, the fragments comprise about 9-10 amino acids.

[0307] In some embodiments, the fragments comprise at least 18 nucleotides. In some embodiments, the fragments comprise at least 21 nucleotides. In some embodiments, the fragments

comprise at least 24 nucleotides. In some embodiments, the fragments comprise at least 27 nucleotides. In some embodiments, the fragments comprise at least 30 nucleotides. In some embodiments, the fragments comprise at least 33 nucleotides. In some embodiments, the fragments comprise at least 36 nucleotides. In some embodiments, the fragments comprise at least 39 nucleotides. In some embodiments, the fragments comprise at least 42 nucleotides. In some embodiments, the fragments comprise at least 45 nucleotides. In some embodiments, the fragments comprise at least 48 nucleotides. In some embodiments, the fragments comprise at least 51 nucleotides. In some embodiments, the fragments comprise at least 54 nucleotides. In some embodiments, the fragments comprise at least 57 nucleotides. In some embodiments, the fragments comprise at least 60 nucleotides. In some embodiments, the fragments comprise at least 63 nucleotides. In some embodiments, the fragments comprise at least 66 nucleotides. In some embodiments, the fragments comprise at least 69 nucleotides. In some embodiments, the fragments comprise at least 72 nucleotides. In some embodiments, the fragments comprise at least 75 nucleotides. In some embodiments, the fragments comprise about 18-75 nucleotides. In some embodiments, the fragments comprise about 21-75 nucleotides. In some embodiments, the fragments comprise about 24-75 nucleotides. In some embodiments, the fragments comprise about 24-72 nucleotides. In some embodiments, the fragments comprise about 24-69 nucleotides. In some embodiments, the fragments comprise about 24-66 nucleotides. In some embodiments, the fragments comprise about 24-63 nucleotides. In some embodiments, the fragments comprise about 24-60 nucleotides. In some embodiments, the fragments comprise about 24-57 nucleotides. In some embodiments, the fragments comprise about 24-54 nucleotides. In some embodiments, the fragments comprise about 24-51 nucleotides. In some embodiments, the fragments comprise about 24-48 nucleotides. In some embodiments, the fragments comprise about 24-45 nucleotides. In some embodiments, the fragments comprise about 24-42 nucleotides. In some embodiments, the fragments comprise about 27-42 nucleotides. In some embodiments, the fragments comprise about 27-39 nucleotides. In some embodiments, the fragments comprise about 27-36 nucleotides. In some embodiments, the fragments comprise about 27-33 nucleotides. In some embodiments, the fragments comprise about 27-30 nucleotides.

[0308] In some embodiments, the fragments comprise one or more of SEQ ID NOs: 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, or 621.

[0309] In some embodiments, the fragments comprise one or more of SEQ ID NOs: 377, 378, 415,417, 418, 420, 502, 518, 526, 527, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 74, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789,

790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 487, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, or 548.

[0310] The methods can comprise evaluating the presence of any combination of the above prostate cancer neoantigens, fragments of the prostate cancer neoantigens, polynucleotides encoding the prostate cancer neoantigens, and/or fragments of the polynucleotides encoding the prostate cancer neoantigens.

[0311] The sample from the subject can comprise any biological sample known to contain or suspected of containing tumor material. In some embodiments, the sample can comprise a prostate cancer tissue sample. In some embodiments, the sample can contain other types of materials containing cancer cells or biological derivatives from cancer cells (exosomes, apoptotic modies, circulating nucleic acids, etc.).

[0312] The disclosed methods can be used to diagnose a subject with any form of prostate cancer. "Prostate cancer" as used herein is meant to include all types of cancerous growths within prostate or oncogenic processes, metastatic tissues or malignantly transformed cells, tissues, or organs, irrespective of histopathology type or stage of invasiveness. The disclosed methods can be used to diagnose, for example, a localized prostate adenocarcinoma, a relapsed prostate cancer, a refractory prostate cancer, a metastatic prostate cancer, a castration resistant prostate cancer, or any combination thereof. In some embodiments, the prostate cancer is an adenocarcinoma. In some embodiments, the prostate cancer is a metastatic prostate cancer. In some embodiments, the prostate cancer has metastasized to rectum, lymph node or bone, or any combination thereof. In some embodiments, the prostate cancer is a relapsed or a refractory prostate cancer. In some embodiments, the prostate cancer is a castration resistant prostate cancer. In some embodiments, the prostate cancer is sensitive to an androgen deprivation therapy. In some embodiments, the prostate cancer is insensitive to the androgen deprivation therapy.

[0313] In some embodiments, the subject is treatment naïve. In some embodiments, the subject has received androgen deprivation therapy. In some embodiments, the subject has an elevated level of prostate specific antigen (PSA). PSA is elevated in a subject when the level is typically about $\geq$4.0 ng/mL. In some instances, elevated PSA may refer to level of $\geq$ 3.0 ng/mL. PSA levels may also be compared to post-androgen deprivation therapy levels.

Methods of treatment, uses and administration

[0314] Methods of treating prostate cancer in a subject are also provided. The methods can comprise administering a therapeutically effective amount of a prostate cancer vaccine to the subject to thereby treat the prostate cancer, wherein the prostate cancer vaccine comprises a polynucleotide encoding one or more polypeptides selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, fragments of the preceding sequences, or any combination thereof.

[0315] The methods of treating prostate cancer in a subject can comprise evaluating the presence of any one or more of the prostate cancer neoantigens listed in the methods of diagnosis section above and administering a therapeutically effective amount of a prostate cancer vaccine to the subject to thereby treat the prostate cancer. In some embodiments, the methods can comprise:

a) evaluating the presence of one or more prostate cancer neoantigens in a sample from the subject, the one or more prostate cancer neoantigens comprising the amino acid sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441,

442, 443, 444, 445, 446, 447, fragments of the preceding sequences, or any combination thereof; and

b) administering a therapeutically effective amount of a prostate cancer vaccine to the subject to thereby treat the prostate cancer.

[0316] The methods can comprise evaluating the presence of one or more prostate cancer neoantigens comprising the amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, fragments of the preceding sequences, or any combination thereof.

[0317] The methods can comprise evaluating the presence of one or more prostate cancer neoantigens comprising the amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, fragments of the preceding sequences, or any combination thereof.

[0318] The methods can comprise evaluating the presence of each of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, and 223.

[0319] The sample from the subject from which the neoantigens are evaluated can comprise any biological sample known to contain or suspected of containing tumor material including, for example, a prostate cancer tissue sample or other types of materials containing cancer cells or biological derivatives from cancer cells (exosomes, apoptotic modies, circulating nucleic acids, etc.). In some embodiments, the sample from the subject from which the neoantigens are evaluated can comprise a prostate cancer tissue sample.

[0320] The presence of the one or more prostate cancer neoantigens can be evaluated by, for example, PCR, qPCR, various forms of nucleic acid sequencing (including but not limited to Illumina, Ion Torrent, Pacific Bioscience, Oxford Nanopore platforms), and various hybridization based approaches (including not limited to Affymetrix Gene Chip or Nanostring platforms). In some embodiments, the presence of the one or more prostate cancer neoantigens is evaluated by qPCR. In some aspects, the methods further comprise, prior to performing the qPCR, extracting RNA from the sample from the subject and synthesizing cDNA from the extracted RNA. The RNA extracted from the subject can correspond to a polynucleotide sequence comprising SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334,

336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 380, 382, 384, 386, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 519, 520, 521, 522, 523, 524, 525, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, fragments of the preceding sequences, or any combination thereof.

[0321] In some embodiments, the methods of treating prostate cancer in a subject can further comprise, prior to administering the therapeutically effective amount of a prostate cancer vaccine, evaluating the expression of one or more prostate cancer biomarkers in a sample from the subject. The one or more prostate cancer biomarkers in a sample from the subject can comprise: RCN1, STEAP1, PITX2, TIMP1, KLK4, KRT18, KLK3, ACPP, KLK2, TSPAN1, ATF3, SPDEF, NPY, SPINK1, HOXB13, FOXA1, KRT17, FOLH1, TNFRSF19, GREB1, KRT8, FLNC, GRHL2, RAB3B, JCHAIN, TMEFF2, AGR2, ACADL, AZGP1, MUC1, STEAP2, UGT2B17, METTL7A, HPN, NKX3-1, GNMT, ADAMTS15, HSD3B2, EPHA3, KCNN2, LGR5, IDO1, GPR39, C9orf152, MYBPC1, THBS2, ETV7, COL1A1, FGFR4, NR0B1, AR, ARv3, TMPRSS2:ERG, ROR1, FGF8, NKX2-2, EDIL3, RELN, FGF9, AKR1C4, CLUL1, KISS1R, CYP3A5, CYP17A1, SFRP4, HNF1A, CALCR, SYP, MSLN, or any combination thereof. Accordingly, the methods of treating prostate cancer in a subject can comprise:

a) evaluating the presence of one or more prostate cancer neoantigens in a sample from the subject, the one or more prostate cancer neoantigens comprising the amino acid sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, fragments of the preceding sequences, or any combination thereof; and;

b) evaluating expression of one or more prostate cancer biomarkers in a sample from the subject, wherein the one or more prostate cancer biomarkers comprise: RCN1, STEAP1, PITX2, TIMP1, KLK4, KRT18, KLK3, ACPP, KLK2, TSPAN1, ATF3, SPDEF, NPY,

SPINK1, HOXB13, FOXA1, KRT17, FOLH1, TNFRSF19, GREB1, KRT8, FLNC, GRHL2, RAB3B, JCHAIN, TMEFF2, AGR2, ACADL, AZGP1, MUC1, STEAP2, UGT2B17, METTL7A, HPN, NKX3-1, GNMT, ADAMTS15, HSD3B2, EPHA3, KCNN2, LGR5, IDO1, GPR39, C9orf152, MYBPC1, THBS2, ETV7, COL1A1, FGFR4, NR0B1, AR, ARv3, TMPRSS2:ERG, ROR1, FGF8, NKX2-2, EDIL3, RELN, FGF9, AKR1C4, CLUL1, KISS1R, CYP3A5, CYP17A1, SFRP4, HNF1A, CALCR, SYP, MSLN, or any combination thereof; and

c) administering a therapeutically effective amount of a prostate cancer vaccine to the subject.

[0322] The sample from the subject from which the prostate cancer biomarkers is evaluated can comprise any biological sample known to contain or suspected of containing tumor material including, for example, a prostate cancer tissue sample or other types of materials containing cancer cells or biological derivatives from cancer cells (exosomes, apoptotic modies, circulating nucleic acids, etc.). In some embodiments, the sample is a plasma sample. In some aspects, the sample is from plasma exosomes. In some embodiments, the sample is a blood sample.

[0323] The one or more prostate cancer biomarkers can comprise: RCN1, STEAP1, PITX2, TIMP1, KLK4, KRT18, KLK3, ACPP, KLK2, TSPAN1, ATF3, SPDEF, NPY, SPINK1, HOXB13, FOXA1, KRT17, FOLH1, TNFRSF19, GREB1, KRT8, FLNC, GRHL2, RAB3B, JCHAIN, TMEFF2, AGR2, ACADL, AZGP1, MUC1, STEAP2, UGT2B17, METTL7A, HPN, NKX3-1, GNMT, ADAMTS15, HSD3B2, EPHA3, KCNN2, LGR5, IDO1, GPR39, C9orf152, MYBPC1, THBS2, ETV7, COL1A1, FGFR4, NR0B1, AR, ARv3, TMPRSS2:ERG, or combinations thereof. In some embodiments, the one or more prostate cancer biomarkers are from a plasma sample. In some aspects, the one or more prostate cancer biomarkers are from plasma exosomes.

[0324] The one or more prostate cancer biomarkers can comprise: HPN, ROR1, FLNC, GPR39, FGF8, NKX2-2, MUC1, NKX3-1, EDIL3, LGR5, FGFR4, STEAP1, ATF3, RELN, UGT2B17, KLK3, C9orf152, GNMT, METTL7A, FGF9, SPDEF, FOXA1, AKR1C4, GREB1, CLUL1, TMEFF2, HOXB13, KLK2, NPY, GRHL2, STEAP2, THBS2, KISS1R, KRT8, TNFRSF19, CYP3A5, KLK4, IDO1, FOLH1, NR0B1, EPHA3, CYP17A1, SFRP4, KRT18, TSPAN1, HNF1A, ADAMTS15, ACPP, CALCR, SYP, AZGP1, AR, ARv3, MSLN, TMPRSS2:ERG, and combinations thereof. In some embodiments, the one or more prostate cancer biomarkers are from a blood sample.

[0325] The presence of the one or more prostate cancer biomarkers can be evaluated by, for example, PCR, qPCR, various forms of nucleic acid sequencing (including but not limited to Illumina, Ion Torrent, Pacific Bioscience, Oxford Nanopore platforms), and various hybridization based approaches (including not limited to Affymetrix Gene Chip or Nanostring platforms). In some embodiments, the presence of the one or more prostate cancer biomarkers is evaluated by qPCR. In some aspects, the methods further comprise, prior to performing the qPCR, extracting RNA from the sample from the subject and synthesizing cDNA from the extracted RNA.

[0326] In some embodiments, the methods can further comprise, after administering the therapeutically effective amount of the prostate cancer vaccine, evaluating the expression of the one or more prostate cancer biomarkers evaluated in step b), wherein a decrease in expression compared to the expression in step b) is indicative of responsiveness to the prostate cancer vaccine. In such embodiments, the expression of the one or more prostate cancer biomarkers detected in step b) is the baseline expression of the cancer biomarker. Evaluating the expression of the one or more prostate cancer biomarkers after administering the therapeutically effective amount of the prostate cancer vaccine can provide an indication of responsiveness/therapeutic efficacy. In some embodiments, the collective expression of the biomarkers can determine whether the patient has responded to treatment. In some embodiments, a decrease in expression of the one or more prostate cancer biomarkers after administering the prostate cancer vaccine compared to the expression prior to administering the prostate cancer vaccine is indicative of responsiveness to the prostate cancer vaccine.

[0327] The prostate cancer vaccine can comprise one or more polynucleotides, one or more polypeptides, and/or one or more recombinant viruses. The prostate cancer vaccine can comprise one or more polynucleotides selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 380, 382, 384, 386, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 519, 520, 521, 522, 523, 524, 525, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, and fragments of the preceding sequences.

[0328] In some embodiments, the prostate cancer vaccine comprises:

a) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 276, 382, 334, 338, 270, 254, 310, 326, 272, 306, 252, 246, 262, 266, 318, 256, 278, 298, 286, 448, 450, 453, 455, 380, 344, 212, 350, 214, 216, 222, 220, 226, 346, 354, 236, 224, 168, 172, 20, 24, 178, and fragments thereof;

b) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, and fragments thereof; or

c) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 500, 501, 461, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 477, 519, 520, 521, 522, 523, 524, 525, 485, 486, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, and fragments thereof.

[0329] In some embodiments, the prostate cancer vaccine comprises a polynucleotide sequence of SEQ ID NOs: 542, 551, 544, or 553.

[0330] The prostate cancer vaccine can comprise a polynucleotide encoding one or more polypeptides selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, and fragments of the preceding sequences.

[0331] Through the validation process, 41 neoantigens (SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, and 177) were identified as particularly useful to be included into a prostate cancer vaccine based on their expression profile, prevalence, and *in vitro* immunogenicity. Thus, in some embodiments, the prostate cancer vaccine can comprise a polynucleotide encoding one or more polypeptides selected from the group consisting of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, and fragments thereof. In some embodiments, the prostate cancer vaccine can comprise a polynucleotide encoding a polypeptide of any one of SEQ ID NOs: 541, 550, 554, 555, 556, 623, 624, 543, 552, 557, 558, 559, 625, or 626. It is expected that any combination of the 41 neoantigens can be utilized to generate a prostate cancer vaccine that can be delivered to a subject via any available delivery vehicles and any form available, such as peptides, DNA, RNA, replicons, or using viral delivery. The 41 neoantigens may be assembled into polynucleotides encoding polypeptides in any neoantigen order, and the neoantigen order may differ between the various delivery options. In general, assembly of the neoantigens into a particular order may be based on generating a minimum number of junctional epitopes utilizing known algorithms. Exemplary orders of the neoantigens are provided as SEQ ID NOs: 541, 550,

554, 555, 556, 623, 624, 543, 552, 557, 558, 559, 625 or 626 as described herein and throughout the examples.

[0332] The polynucleotide can be DNA or RNA. Suitable RNA molecules include mRNA or self-replicating RNA. In some embodiments, the polynucleotide comprises a promoter, an enhancer, a polyadenylation site, a Kozak sequence, a stop codon, a T cell enhancer (TCE), or any combination thereof. In some embodiments, the promoter comprises a CMV promoter or a vaccinia P7.5 promoter. In some embodiments, the TCE is encoded by a polynucleotide of SEQ ID NO: 546, the CMV promoter comprises a polynucleotide of SEQ ID NO: 628, the vaccinia P7.5 promoter comprises a polynucleotide of SEQ ID NO: 630, and the polyadenylation site comprises a bovine growth hormone polyadenylation site of SEQ ID NO: 629.

[0333] The prostate cancer vaccine can comprise one or more polypeptides selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, and fragments of the preceding sequences. In some embodiments, the prostate cancer vaccine can comprise one or more polypeptides selected from the group consisting of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, and fragments thereof. In some embodiments, the prostate cancer vaccine can comprise a polypeptide comprising the amino acid sequence of any one of SEQ ID NOs: 541, 550, 554, 555, 556, 623, 624, 543, 552, 557, 558, 559, 625, or 626.

[0334] The polypeptides and polynucleotides may be attached to nanoparticles for delivery to a subject. Delivery of the polypeptides and polynucleotides using nanoparticles may eliminate the need to include a virus or an adjuvant in the vaccine composition. The polynucleotide may be DNA or RNA. The nanoparticles may contain immune danger signals that help to effectively induce an immune response to the peptides. The nanoparticles may induce dendritic cell (DC) activation and maturation, required for a robust immune response. The nanoparticles may contain non-self components that improve uptake of the nanoparticles and thus the peptides by cells, such as antigen presenting cells.

[0335] The nanoparticles are typically from about 1 nm to about 100 nm in diameter, such as about 20 nm to about 40 nm. Nanoparticles with a mean diameter of 20 to 40 nm may facilitate uptake of the nanoparticle to the cytosol (see. e.g. WO2019/135086). Exemplary nanoparticles are polymeric nanoparticles, inorganic nanoparticles, liposomes, lipid nanoparticles (LNP), an immune stimulating complex (ISCOM), a virus-like particle (VLP), or a self-assembling protein. The nanoparticles may be calcium phosphate nanoparticles, silicon nanoparticles or gold nanoparticles. The polymeric nanoparticles may comprise one or more synthetic polymers, such as poly(d,l-lactide-co-glycolide) (PLG), poly(d,l-lactic-coglycolic acid) (PLGA), poly(g-glutamic acid) (g-PGA)m poly(ethylene glycol) (PEG), or polystyrene or one or more natural polymers such as a polysaccharide, for example pullulan, alginate, inulin, and chitosan. The use of a polymeric nanoparticles may be advantageous due to the properties of the polymers that may be include in the nanoparticle. For instance, the natural and synthetic polymers recited above may have good biocompatibility and biodegradability, a non-toxic nature, and/or the ability to be manipulated into desired shapes and sizes. The polymeric nanoparticle may also form hydrogel nanoparticles, hydrophilic three-dimensional polymer networks with favorable properties including flexible mesh size, large surface area for multivalent conjugation, high water content, and high loading capacity for antigens. Polymers such as Poly(L-lactic acid) (PLA), PLGA, PEG, and polysaccharides are suitable for forming hydrogel nanoparticles. Inorganic nanoparticles typically have a rigid structure and comprise a shell in which an antigen is encapsulated or a core to which the antigen may be covalently attached. The core may comprise one or more atoms such as gold (Au), silver (Ag), copper (Cu) atoms, Au/Ag, Au/Cu, Au/Ag/Cu, Au/Pt, Au/Pd or Au/Ag/Cu/Pd or calcium phosphate (CaP).

[0336] The nanoparticles may be liposomes. Liposomes are typically formed from biodegradable, non-toxic phospholipids and comprise a self-assembling phospholipid bilayer shell with an aqueous core. Liposomes may be an unilamellar vesicle comprising a single phospholipid bilayer, or a multilamellar vesicle that comprises several concentric phospholipid shells separated by layers of water. As a consequence, liposomes may be tailored to incorporate either hydrophilic molecules into the aqueous core or hydrophobic molecules within the phospholipid bilayers. Liposomes may encapsulate antigens such as the disclosed polypeptides or fragments thereof within the core for delivery. Liposomes and liposomal formulations can be prepared according to standard methods and are well known in the art, see, e.g., Remington's; Akimaru, 1995, Cytokines Mol. Ther. 1: 197-210; Alving, 1995, Immunol. Rev. 145: 5-31; Szoka, 1980, Ann. Rev. Biophys. Bioeng. 9: 467; U.S. Pat. No. 4,235,871; U.S. Pat. No. 4,501,728; and U.S. Pat. No. 4,837,028. The liposomes may comprise a targeting molecule for targeting liposome complexes to a particular cell type. Targeting molecule may comprise a binding partner (e.g., a ligand or receptor) for a biomolecule (e.g., a receptor or ligand) on the surface of a blood vessel or a cell found in a target tissue. Liposome charge is an important determinant in liposome clearance from the blood, with negatively charged liposomes being taken up more rapidly by the reticuloendothelial system (Juliano, 1975, Biochem. Biophys. Res. Commun. 63: 651) and thus having shorter half-lives in the bloodstream.

Incorporating phosphatidylethanolamine derivatives enhances the circulation time by preventing liposomal aggregation. For example, incorporation of N-(omega-carboxy)acylamidophosphatidylethanolamines into large unilamellar vesicles of L-alpha-distearoylphosphatidylcholine dramatically increases the *in vivo* liposomal circulation lifetime (see, e.g., Ahl, 1997, Biochim. Biophys. Acta 1329: 370-382). Typically, liposomes are prepared with about 5 to 15 mole percent negatively charged phospholipids, such as phosphatidylglycerol, phosphatidylserine, or phosphatidyl-inositol. Added negatively charged phospholipids, such as phosphatidylglycerol, also serve to prevent spontaneous liposome aggregation, and thus minimize the risk of undersized liposomal aggregate formation. Membrane-rigidifying agents, such as sphingomyelin or a saturated neutral phospholipid, at a concentration of at least about 50 mole percent, and 5 to 15 mole percent of monosialylganglioside can also impart desirably liposome properties, such as rigidity (see, e.g., U.S. Pat. No. 4,837,028). Additionally, the liposome suspension can include lipid-protective agents which protect lipids against free-radical and lipid-peroxidative damages on storage. Lipophilic free-radical quenchers, such as alpha- tocopherol and water-soluble iron-specific chelators, such as ferrioxianine, are preferred.

[0337] The nanoparticles may be lipid nanoparticles (LNP). LNPs are similar to liposomes but have slightly different function and composition. LNPs are designed toward encapsulating polynucleotides, such as DNA, mRNA, siRNA, and sRNA. Traditional liposomes contain an aqueous core surrounded by one or more lipid bilayers. LNPs may assume a micelle-like structure, encapsulating drug molecules in a non-aqueous core. LNPs typically contain a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle (e.g., a PEG-lipid conjugate). LNPs are useful for systemic applications, as they exhibit extended circulation lifetimes following intravenous (i.e.) injection and accumulate at distal sites (e.g., sites physically separated from the administration site). The LNPs may have a mean diameter of about 50 nm to about 150 nm, such as about 60 nm to about 130 nm, or about 70 nm to about 110 nm, or about 70 nm to about 90 nm, and are substantially nontoxic. Preparation of polynucleotide loaded LNPs are disclosed in, e.g., U.S. Patent Nos. 5,976,567; 5,981,501; 6,534,484; 6,586,410; 6,815,432; and PCT Publication No. WO 96/40964. Polynucleotide containing LNPs are described for example in WO2019/191780.

[0338] The polynucleotides, and polypeptides of the disclosure can include multilamellar vesicles of heterogeneous sizes. For example, vesicle-forming lipids can be dissolved in a suitable organic solvent or solvent system and dried under vacuum or an inert gas to form a thin lipid film. If desired, the film can be redissolved in a suitable solvent, such as tertiary butanol, and then lyophilized to form a more homogeneous lipid mixture which is in a more easily hydrated powder like form. This film is covered with an aqueous solution of the polypeptide complex and allowed to hydrate, typically over a 15 to 60 minute period with agitation. The size distribution of the resulting multilamellar vesicles can be shifted toward smaller sizes by hydrating the lipids under more vigorous agitation conditions or by adding solubilizing detergents such as deoxycholate. The hydration medium may comprise the nucleic acid at a concentration which is desired in the interior volume of the liposomes in the final liposome suspension.

Suitable lipids that may be used to form multilamellar vesicles include DOTMA (Feigner, *et al.*, 1987, Proc. Natl. Acad. Sci. USA 84: 7413-7417), DOGS or Transfectain™ (Behr, *et al.*, 1989, Proc. Natl. Acad. Sci. USA 86: 6982- 6986), DNERIE or DORIE (Feigner, *et al.*, Methods 5: 67-75), DC-CHOL (Gao and Huang, 1991, BBRC 179: 280-285), DOTAP™ (McLachlan, *et al.*, 1995, Gene Therapy 2: 674-622), Lipofectamine™, and glycerolipid compounds (see, e.g., EP901463 and W098/37916).

[0339] The nanoparticle may be an immune-stimulating complex (ISCOM). ISCOMs are cage-like particles which are typically formed from colloidal saponin-containing micelles. ISCOMs may comprise cholesterol, phospholipid (such as phosphatidylethanolamine or phosphatidylcholine), and saponin (such as Quil A from the tree Quillaia saponaria).

[0340] The nanoparticle may be a virus-like particle (VLP). VLPs are self-assembling nanoparticles that lack infectious nucleic acid, which are formed by self-assembly of biocompatible capsid protein. VLPs are typically about 20 to about 150 nm, such as about 20 to about 40 nm, about 30 to about 140 nm, about 40 to about 130 nm, about 50 to about 120 nm, about 60 to about 110 nm, about 70 to about 100 nm, or about 80 to about 90 nm in diameter. VLPs advantageously harness the power of evolved viral structure, which is naturally optimized for interaction with the immune system. The naturally-optimized nanoparticle size and repetitive structural order means that VLPs induce potent immune responses, even in the absence of adjuvant.

[0341] The nanoparticles may contain replicons that encode the polypeptides of the disclosure. The replicons may be DNA or RNA. "Replicon" refers to a viral nucleic acid that is capable of directing the generation of copies of itself and includes RNA as well as DNA. For example, double-stranded DNA versions of arterivirus genomes can be used to generate a single-stranded RNA transcript that constitutes an arterivirus replicon. Generally, a viral replicon contains the complete genome of the virus. "Sub-genomic replicon" refers to a viral nucleic acid that contains something less than the full complement of genes and other features of the viral genome, yet is still capable of directing the generation of copies of itself. For example, the sub-genomic replicons of arterivirus may contain most of the genes for the non-structural proteins of the virus, but are missing most of the genes coding for the structural proteins. Sub-genomic replicons are capable of directing the expression of all of the viral genes necessary for the replication of the viral sub-genome (replication of the sub-genomic replicon), without the production of viral particles.

[0342] "RNA replicon," "self-replication RNA," or "self-replicating RNA" refers to RNA which contains all of the genetic information required for directing its own amplification or self-replication within a permissive cell. To direct its own replication, the RNA molecule: 1) encodes polymerase, replicase, or other proteins which may interact with viral or host cell-derived proteins, nucleic acids or ribonucleoproteins to catalyze the RNA amplification process; and 2) contain cis-acting RNA sequences required for replication and transcription of the replicon-encoded RNA. Self-replicating RNA is typically derived from the genomes of positive strand RNA viruses and can be used as a basis of introducing foreign sequences to host cells by replacing viral sequences encoding structural or non-

structural genes or inserting the foreign sequences 5' or 3' of the sequences encoding the structural or non-structural genes. Foreign sequences may also be introduced into the subgenomic regions of alphaviruses. Self-replicating RNA may be packaged into recombinant virus particles, such as recombinant alphavirus particles or alternatively delivered to the host using lipid nanoparticles (LNP). Self-replicating RNA may be at least 1 kb or at least 2 kb or at least 3 kb or at least 4 kb or at least 5 kb or at least 6 kb or at least 7 kb or at least 8 kb or at least 10 kb or at least 12 kb or at least 15 kb or at least 17 kb or at least 19 kb or at least 20 kb in size, or can be 100 bp-8 kb or 500 bp-8 kb or 500 bp-7 kb or 1-7 kb or 1-8 kb or 2-15 kb or 2-20 kb or 5-15 kb or 5-20 kb or 7-15 kb or 7-18 kb or 7-20 kb in size. Self-replicating RNAs are described, for example, in WO2017/180770, WO2018/075235, and WO2019143949A2.

[0343] Other molecules suitable for complexing with the polynucleotides or the polypeptides of the disclosure include cationic molecules, such as, polyamidoamine (Haensler and Szoka, 1993, Bioconjugate Chem. 4: 372-379), dendritic polylysine (Int. Pat. Publ. No. WO1995/24221), polyethylene irinine or polypropylene h-nine (Int. Pat. Publ. No. WO1996/02655), polylysine (U.S. Pat. No. 5,595,897), chitosan (U.S. Pat. No. 5,744,166), DNA-gelatin coarcervates (see, e.g., U.S. Pat. No. 6,207,195; U.S. Pat. No. 6,025,337; U.S. Pat. No. 5,972,707) or DEAE dextran (Lopata, et al., 1984, Nucleic Acid Res. 12: 5707-5717), dendrimers (see, e.g., WO1996/19240), or polyethylenimine (PEI) (see, e.g., Sun *et al.*, 2014, Mol Med Rep. 10(5):2657–2662).

[0344] In some embodiments, the prostate cancer vaccine comprises one or more recombinant viruses. Suitable recombinant viruses can be derived from an adenovirus (Ad), a poxvirus, an adeno-associated virus (AAV), or a retrovirus.

[0345] Adenoviruses may be derived from human adenovirus (Ad) but also from adenoviruses that infect other species, such as bovine adenovirus (e.g. bovine adenovirus 3, BAdV3), a canine adenovirus (e.g. CAdV2), a porcine adenovirus (e.g. PAdV3 or 5), or great apes, such as Chimpanzee (*Pan*), Gorilla (*Gorilla*), Orangutan (*Pongo*), Bonobo (*Pan paniscus*) and common chimpanzee (*Pan troglodytes*). Typically, naturally occurring great ape adenoviruses are isolated from stool samples of the respective great ape.

[0346] Human adenoviruses may be derived from various adenovirus serotypes, for example, from human adenovirus serotypes hAd5, hAd7, hAd11, hAd26, hAd34, hAd35, hAd48, hAd49, or hAd50 (the serotypes are also referred to as Ad5, Ad7, Ad11, Ad26, Ad34, Ad35, Ad48, Ad49, or Ad50).

[0347] Great ape adenoviruses may be derived from various adenovirus serotypes, for example, from great ape adenovirus serotypes GAd20, GAd19, GAd21, GAd25, GAd26, GAd27, GAd28, GAd29, GAd30, GAd31, ChAd3, ChAd4, ChAd5, ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd55, ChAd63, ChAd73, ChAd82, ChAd83, ChAd146, ChAd147, PanAd1, PanAd2, or PanAd3.

[0348] Adenoviruses are known in the art. The sequences of most of the human and non-human adenoviruses are known, and for others can be obtained using routine procedures. An exemplary

genome sequence of Ad26 is found in GenBank Accession number EF153474 and in SEQ ID NO: 1 of Int. Pat. Publ. No. WO2007/104792. An exemplary genome sequence of Ad35 is found in Fig. 6 of Int. Pat. Publ. No. WO2000/70071. Ad26 is described, for example, in Int. Pat. Publ. No. WO2007/104792. Ad35 is described, for example, in U.S. Pat. No. 7,270,811 and Int. Pat. Publ. No. WO2000/70071. ChAd3, ChAd4, ChAd5, ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd63, and ChAd82 are described in WO2005/071093. PanAd1, PanAd2, PanAd3, ChAd55, ChAd73, ChAd83, ChAd146, and ChAd147 are described in Int. Pat. Publ. No. WO2010/086189.

[0349] Adenoviruses are engineered to comprise at least one functional deletion or a complete removal of a gene product that is essential for viral replication, such as one or more of the adenoviral regions E1, E2, and E4, therefore rendering the adenovirus to be incapable of replication. The deletion of the E1 region may comprise deletion of EIA, EIB 55K, or EIB 21K, or any combination thereof. Replication deficient adenoviruses are propagated by providing the proteins encoded by the deleted region(s) in trans by the producer cell by utilizing helper plasmids or engineering the produce cell to express the required proteins. Adenoviruses may also have a deletion in the E3 region, which is dispensable for replication, and hence such a deletion does not have to be complemented. The adenovirus may comprise a functional deletion or a complete removal of the E1 region and at least part of the E3 region. The adenovirus may further comprise a functional deletion or a complete removal of the E4 region and/or the E2 region. Suitable producer cells that can be utilized are human retina cells immortalized by E1, e.g. 911 or PER.C6 cells (see, e.g., U.S. Pat. No. 5,994,128), E1-transformed amniocytes (See, e.g., EP 1230354), E1-transformed A549 cells (see e.g. Int. Pat. Publ. No. WO1998/39411, U.S. Pat. No. 5,891,690). Ad26 comprising a functional E1 coding region that is sufficient for viral replication, a deletion in the E3 coding region, and a deletion in the E4 coding region may be used, provided that E4 open reading frame 6/7 is not deleted (see e.g. U.S. Pat. No. 9,750,801)

[0350] In some embodiments, the adenovirus is a human adenovirus (Ad). In some embodiments, the Ad is derived from Ad5. In some embodiments, the Ad is derived from Ad11. In some embodiments, the Ad is derived from Ad26. In some embodiments, the Ad is derived from Ad34. In some embodiments, the Ad is derived from Ad35. In some embodiments, the Ad is derived from Ad48. In some embodiments, the Ad is derived from Ad49. In some embodiments, the Ad is derived from Ad50.

[0351] In some embodiments, the adenovirus is a great ape adenovirus (GAd). In some embodiments, the GAd is derived from GAd20. In some embodiments, the GAd is derived from GAd19. In some embodiments, the GAd is derived from GAd21. In some embodiments, the GAd is derived from GAd25. In some embodiments, the GAd is derived from GAd26. In some embodiments, the GAd is derived from GAd27. In some embodiments, the GAd is derived from GAd28. In some embodiments, the GAd is derived from GAd29. In some embodiments, the GAd is derived from GAd30. In some embodiments, the GAd is derived from GAd31. In some embodiments, the GAd is derived from ChAd4.

In some embodiments, the GAd is derived from ChAd5. In some embodiments, the GAd is derived from ChAd6. In some embodiments, the GAd is derived from ChAd7. In some embodiments, the GAd is derived from ChAd8. In some embodiments, the GAd is derived from ChAd9. In some embodiments, the GAd is derived from ChAd20. In some embodiments, the GAd is derived from ChAd22. In some embodiments, the GAd is derived from ChAd24. In some embodiments, the GAd is derived from ChAd26. In some embodiments, the GAd is derived from ChAd30. In some embodiments, the GAd is derived from ChAd31. In some embodiments, the GAd is derived from ChAd32. In some embodiments, the GAd is derived from ChAd33. In some embodiments, the GAd is derived from ChAd37. In some embodiments, the GAd is derived from ChAd38. In some embodiments, the GAd is derived from ChAd44. In some embodiments, the GAd is derived from ChAd55. In some embodiments, the GAd is derived from ChAd63. In some embodiments, the GAd is derived from ChAd68. In some embodiments, the GAd is derived from ChAd73. In some embodiments, the GAd is derived from ChAd82. In some embodiments, the GAd is derived from ChAd83.

[0352] GAd19-21 and GAd25-31 are described in Int. Pat. Publ. No. WO2019/008111 and represent strains with high immunogenicity and no pre-existing immunity in the general human population. The polynucleotide sequence of GAd20 genome is shown in SEQ ID NO: 622 as disclosed in WO2019/008111.

[0353] The disclosed polynucleotides may be inserted into a site or region (insertion region) in the virus that does not affect virus viability of the resultant recombinant virus. The polynucleotides may be inserted into the deleted E1 region in parallel (transcribed 5′ to 3′) or anti-parallel (transcribed in a 3′ to 5′ direction relative to the vector backbone) orientation. In addition, appropriate transcriptional regulatory elements that are capable of directing expression of the polypeptides in the mammalian host cells that the virus is being prepared for use may be operatively linked to the polynucleotides. "Operatively linked" sequences include both expression control sequences that are contiguous with the nucleic acid sequences that they regulate and regulatory sequences that act in trans, or at a distance to control the regulated nucleic acid sequence.

[0354] Recombinant adenoviral particles may be prepared and propagated according to any conventional technique in the field of the art (e.g., Int. Pat. Publ. No. WO1996/17070) using a complementation cell line or a helper virus, which supplies in trans the missing viral genes necessary for viral replication. The cell lines 293 (Graham *et al.*, 1977, J. Gen. Virol. 36: 59-72), PER.C6 (see e.g. U.S. Pat. No. 5,994,128), E1 A549 and 911 are commonly used to complement El deletions. Other cell lines have been engineered to complement defective vectors (Yeh, *et al.*, 1996, J. Virol. 70: 559-565; Kroughak and Graham, 1995, Human Gene Ther. 6: 1575-1586; Wang, *et al.*, 1995, Gene Ther. 2: 775-783; Lusky, *et al.*, 1998, J. Virol. 72: 2022-203; EP 919627 and Int. Pat. Publ. No. WO1997/04119). The adenoviral particles may be recovered from the culture supernatant but also from the cells after lysis and optionally further purified according to standard techniques (e.g., chromatography, ultracentrifugation, as described in Int. Pat. Publ. No. WO1996/27677, Int. Pat. Publ. No. WO1998/00524, Int. Pat. Publ. No.

WO1998/26048 and Int. Pat. Publ. No. WO2000/50573). The construction and methods for propagating adenoviruses are also described in for example, U.S. Pat. Nos. 5,559,099, 5,837,511, 5,846,782, 5,851,806, 5,994,106, 5,994,128, 5,965,541, 5,981,225, 6,040,174, 6,020,191, and 6,113,913.

[0355] Poxvirus (*Poxviridae*) may be derived from smallpox virus (variola), vaccinia virus, cowpox virus, or monkeypox virus. Exemplary vaccinia viruses are the Copenhagen vaccinia virus (W), New York Attenuated Vaccinia Virus (NYVAC), ALVAC, TROVAC, and Modified Vaccinia Ankara (MVA).

[0356] MVA originates from the dermal vaccinia strain Ankara (Chorioallantois vaccinia Ankara (CVA) virus) that was maintained in the Vaccination Institute, Ankara, Turkey for many years and used as the basis for vaccination of humans. However, due to the often severe post-vaccinal complications associated with vaccinia viruses (VACV), there were several attempts to generate a more attenuated, safer smallpox vaccine. MVA has been generated by 516 serial passages on chicken embryo fibroblasts of the CVA virus (see Meyer *et al.*, *J. Gen. Virol.*, 72: 1031-1038 (1991) and U.S. Pat. No. 10,035,832). As a consequence of these long-term passages the resulting MVA virus deleted about 31 kilobases of its genomic sequence and, therefore, was described as highly host cell restricted to avian cells (Meyer, H. *et al.*, Mapping of deletions in the genome of the highly attenuated vaccinia virus MVA and their influence on virulence, J. Gen. Virol. 72, 1031-1038, 1991; Meisinger-Henschel *et al.*, Genomic sequence of chorioallantois vaccinia virus Ankara, the ancestor of modified vaccinia virus Ankara, J. Gen. Virol. 88, 3249-3259, 2007). Comparison of the MVA genome to its parent, CVA, revealed 6 major deletions of genomic DNA (deletion I, II, III, IV, V, and VI), totaling 31,000 basepairs. (Meyer *et al.*, J. Gen. Virol. 72:1031-8 (1991)). It was shown in a variety of animal models that the resulting MVA was significantly avirulent (Mayr, A. & Danner, K. Vaccination against pox diseases under immunosuppressive conditions, Dev. Biol. Stand. 41: 225-34, 1978). Being that many passages were used to attenuate MVA, there are a number of different strains or isolates, depending on the passage number in CEF cells, such as MVA 476 MG/14/78, MVA-571, MVA-572, MVA-574, MVA-575, and MVA-BN. MVA 476 MG/14/78 is described, for example, in Int. Pat. Publ. No. WO2019/115816A1. MVA-572 strain was deposited at the European Collection of Animal Cell Cultures ("ECACC"), Health Protection Agency, Microbiology Services, Porton Down, Salisbury SP4 0JG, United Kingdom ("UK"), under the deposit number ECACC 94012707 on Jan. 27, 1994. MVA-575 strain was deposited at the ECACC under deposit number ECACC 00120707 on Dec. 7, 2000; MVA-Bavarian Nordic ("MVA-BN") strain was deposited at the ECACC under deposit number V00080038 on Aug. 30, 2000. The genome sequences of MVA-BN and MVA-572 are available at GenBank (Accession numbers DQ983238 and DQ983237, respectively). The genome sequences of other MVA strains can be obtained using standard sequencing methods.

[0357] The disclosed viruses may be derived from any MVA strain or further derivatives of the MVA strain. A further exemplary MVA strain is deposit VR-1508, deposited at the American Type Culture collection (ATCC), Manassas, Va. 20108, USA.

[0358] "Derivatives" of MVA refer to viruses exhibiting essentially the same characteristics as the parent MVA, but exhibiting differences in one or more parts of their genomes. In some embodiments, the MVA is derived from MVA 476 MG/14/78. In some embodiments, the MVA is derived from MVA-571. In some embodiments, the MVA is derived from MVA-572. In some embodiments, the MVA is derived from MVA-574. In some embodiments, the MVA is derived from MVA-575. In some embodiments, the MVA is derived from MVA-BN.

[0359] The disclosed polynucleotides may be inserted into a site or region (insertion region) in the MVA virus that does not affect viability of the resultant recombinant virus. Such regions can be readily identified by testing segments of virus DNA for regions that allow recombinant formation without seriously affecting viability of the recombinant virus. The thymidine kinase (TK) gene is an insertion region that may be used and is present in many viruses, such as in all examined poxvirus genomes. Additionally, MVA contains 6 natural deletion sites, each of which may be used as insertion sites (e.g. deletion I, II, III, IV, V, and VI; see e.g. U.S. Pat. No. 5,185,146 and U.S. Pat. No. 6.440,442). One or more intergenic regions (IGR) of the MVA may also be used as an insertion site, such as IGRs IGR07/08, IGR 44/45, IGR 64/65, IGR 88/89, IGR 136/137, and IGR 148/149 (see e.g. U.S. Pat. Publ. No. 2018/0064803). Additional suitable insertion sites are described in Int. Pat. Publ. No. WO2005/048957.

[0360] Recombinant poxviral particles such as MVA can be prepared as described in the art (Piccini, *et al.*, 1987, Methods of Enzymology 153: 545-563; U.S. Pat. No. 4,769,330; U.S. Pat. No. 4,772,848; U.S. Pat. No. 4,603,112; U.S. Pat. No. 5,100,587; and U.S. Pat. No. 5,179,993). In an exemplary method, the DNA sequence to be inserted into the virus can be placed into an *E. coli* plasmid construct into which DNA homologous to a section of DNA of the MVA has been inserted. Separately, the DNA sequence to be inserted can be ligated to a promoter. The promoter-gene linkage can be positioned in the plasmid construct so that the promoter-gene linkage is flanked on both ends by DNA homologous to a DNA sequence flanking a region of MVA DNA containing a non-essential locus. The resulting plasmid construct can be amplified by propagation within *E. coli* bacteria and isolated. The isolated plasmid containing the DNA gene sequence to be inserted can be transfected into a cell culture, e.g., of chicken embryo fibroblasts (CEFs), at the same time the culture is infected with MVA. Recombination between homologous MVA DNA in the plasmid and the viral genome, respectively, can generate an MVA modified by the presence of foreign DNA sequences. MVA particles may be recovered from the culture supernatant or from the cultured cells after a lysis step (e.g., chemical lysis, freezing/thawing, osmotic shock, sonication and the like). Consecutive rounds of plaque purification can be used to remove contaminating wild type virus. Viral particles can then be purified using the techniques known in the art (e.g., chromatographic methods or ultracentrifugation on cesium chloride or sucrose gradients).

[0361] Other viruses include those derived from human adeno-associated viruses, such as AAV-2 (adeno-associated virus type 2). An attractive feature of AAV is that they do not express any viral genes. The only viral DNA sequences included in the AAV are the 145 bp inverted terminal repeats

(ITR). Thus, as in immunization with naked DNA, the only gene expressed is that of the antigen, or antigen chimera. Additionally, AAVs are known to transduce both dividing and non-dividing cells, such as human peripheral blood monocyte-derived dendritic cells, with persistent transgene expression, and with the possibility of oral and intranasal delivery for generation of mucosal immunity. Moreover, the amount of DNA required appears to be much less by several orders of magnitude, with maximum responses at doses of 10^{10} to 10^{11} particles or copies of DNA in contrast to naked DNA doses of 50 μg or about 10^{15} copies. AAVs are packaged by co-transfection of a suitable cell line (e.g., human 293 cells) with the DNA contained in the AAV ITR chimeric protein encoding constructs and an AAV helper plasmid ACG2 containing the AAV coding region (AAV rep and cap genes) without the ITRs. The cells are subsequently infected with the adenovirus. Viruses can be purified from cell lysates using methods known in the art (e.g., such as cesium chloride density gradient ultracentrifugation) and are validated to ensure that they are free of detectable replication-competent AAV or adenovirus (e.g., by a cytopathic effect bioassay).

[0362] Retroviruses may also be used. Retroviruses are a class of integrative viruses which replicate using a virus-encoded reverse transcriptase, to replicate the viral RNA genome into double stranded DNA which is integrated into chromosomal DNA of the infected cells (e.g., target cells). Such viruses include those derived from murine leukemia viruses, especially Moloney (Gilboa, *et al.*, 1988, Adv. Exp. Med. Biol. 241: 29) or Friend's FB29 strains (Int. Pat. Publ. No. WO1995/01447). Generally, a retrovirus is deleted of all or part of the viral genes gag, pol, and env and retains 5' and 3' LTRs and an encapsidation sequence. These elements may be modified to increase expression level or stability of the retrovirus. Such modifications include the replacement of the retroviral encapsidation sequence by one of a retrotransposon such as VL30 (see, e.g., U.S. Pat. No. 5,747,323). The disclosed polynucleotides may be inserted downstream of the encapsidation sequence, such as in opposite direction relative to the retroviral genome. Retroviral particles are prepared in the presence of a helper virus or in an appropriate complementation (packaging) cell line which contains integrated into its genome the retroviral genes for which the retrovirus is defective (e.g. gag/pol and env). Such cell lines are described in the prior art (Miller and Rosman, 1989, BioTechniques 7: 980; Danos and Mulligan, 1988, Proc. Natl. Acad. Sci. USA 85: 6460; Markowitz, *et al.*, 1988, Virol. 167: 400). The product of the env gene is responsible for the binding of the viral particle to the viral receptors present on the surface of the target cell and, therefore determines the host range of the retroviral particle. Packaging cell line, such as the PA317 cells (ATCC CRL 9078) or 293EI6 (W097/35996) containing an amphotropic envelope protein may therefore be used to allow infection of human and other species' target cells. The retroviral particles are recovered from the culture supernatant and may optionally be further purified according to standard techniques (e.g. chromatography, ultracentrifugation).

[0363] The prostate cancer vaccine can comprise one or more recombinant viruses derived from, for example, hAd5, hAd7, hAd11, hAd26, hAd34, hAd35, hAd48, hAd49, hAd50, GAd20, GAd19, GAd21, GAd25, GAd26, GAd27, GAd28, GAd29, GAd30, GAd31, ChAd3, ChAd4, ChAd5,

ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd55, ChAd63, ChAd73, ChAd82, ChAd83, ChAd146, ChAd147, PanAd1, PanAd2, PanAd3, Copenhagen vaccinia virus (W), New York Attenuated Vaccinia Virus (NYVAC), ALVAC, TROVAC, modified vaccinia ankara (MVA), and combinations thereof.

[0364] The prostate cancer vaccine can comprise one or more recombinant viruses derived from GAd20, wherein the recombinant virus comprises a polynucleotide encoding one or more polypeptides selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, and fragments of the preceding sequences. In some embodiments, the recombinant virus comprises a polynucleotide encoding one or more polypeptides selected from the group consisting of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, and fragments thereof.

[0365] The prostate cancer vaccine can comprise one or more recombinant viruses derived from GAd20, wherein the recombinant virus comprises one or more polynucleotides selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 380, 382, 384, 386, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516,

517, 519, 520, 521, 522, 523, 524, 525, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, and fragments of the preceding sequences.

[0366] In some embodiments, the prostate cancer vaccine comprise one or more recombinant viruses derived from GAd20, wherein the recombinant virus comprises:

a) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 276, 382, 334, 338, 270, 254, 310, 326, 272, 306, 252, 246, 262, 266, 318, 256, 278, 298, 286, 448, 450, 453, 455, 380, 344, 212, 350, 214, 216, 222, 220, 226, 346, 354, 236, 224, 168, 172, 20, 24, 178, and fragments thereof; or

b) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, and fragments thereof.

[0367] In some aspects, the vaccine comprises a recombinant virus derived from GAd20 comprising a polynucleotide encoding a polypeptide of SEQ ID NO: 541, 550, 554, 555, 556, 623, or 624.

[0368] In some embodiments, the prostate cancer vaccine can comprise one or more recombinant viruses derived from MVA, wherein the recombinant virus comprises a polynucleotide encoding one or more polypeptides selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, and fragments of the preceding sequences. In some embodiments, the recombinant virus can comprise a polynucleotide encoding one or more polypeptides selected from the group consisting of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, and fragments thereof.

[0369] In some embodiments, the prostate cancer vaccine can comprise one or more recombinant viruses derived from MVA, wherein the recombinant virus comprises one or more polynucleotides selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78,

80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 380, 382, 384, 386, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 519, 520, 521, 522, 523, 524, 525, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, and fragments of the preceding sequences.

[0370] In some embodiments, the prostate cancer vaccine can comprise one or more recombinant viruses derived from MVA, wherein the recombinant virus comprises:

a) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 276, 382, 334, 338, 270, 254, 310, 326, 272, 306, 252, 246, 262, 266, 318, 256, 278, 298, 286, 448, 450, 453, 455, 380, 344, 212, 350, 214, 216, 222, 220, 226, 346, 354, 236, 224, 168, 172, 20, 24, 178, and fragments thereof; or

b) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 500, 501, 461, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 477, 519, 520, 521, 522, 523, 524, 525, 485, 486, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, and fragments thereof.

[0371] In some aspects, the vaccine comprises a recombinant virus derived from MVA comprising a polynucleotide encoding a polypeptide of SEQ ID NO: 543, 552, 557, 558, 559, 625, or 626.

[0372] In some embodiments, the prostate cancer vaccine can comprise one or more recombinant viruses derived from hAd26, wherein the recombinant virus comprises a polynucleotide encoding one or more polypeptides selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400,

401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, and fragments of the preceding sequences. In some embodiments, the recombinant virus can comprise a polynucleotide encoding one or more polypeptides selected from the group consisting of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, and fragments thereof.

[0373] In some embodiments, the prostate cancer vaccine can comprise one or more recombinant viruses derived from hAd26, wherein the recombinant virus comprises one or more polynucleotides selected from the group consisting of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 380, 382, 384, 386, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 519, 520, 521, 522, 523, 524, 525, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, and fragments of the preceding sequences.

[0374] In some embodiments, the prostate cancer vaccine can comprise one or more recombinant viruses derived from hAd26, wherein the recombinant virus comprises:

a) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 276, 382, 334, 338, 270, 254, 310, 326, 272, 306, 252, 246, 262, 266, 318, 256, 278, 298, 286, 448, 450, 453, 455, 380, 344, 212, 350, 214, 216, 222, 220, 226, 346, 354, 236, 224, 168, 172, 20, 24, 178, and fragments thereof;

b) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, and fragments thereof; or

c) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 500, 501, 461, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 477, 519, 520, 521, 522, 523, 524, 525, 485, 486, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, and fragments thereof.

[0375] In some aspects, the vaccine comprises a recombinant virus derived from hAd26 comprising a polynucleotide encoding a polypeptide of SEQ ID NO: 541, 550, 554, 555, 556, 623, or 624. In some aspects, the vaccine comprises a recombinant virus derived from hAd26 comprising a polynucleotide encoding a polypeptide of SEQ ID NO: 543, 552, 557, 558, 559, 625, or 626.

[0376] The vaccine can comprise a polynucleotide encoding a polypeptide of SEQ ID NO: 541, wherein the vaccine is a recombinant virus derived from GAd20. The vaccine can comprise a polynucleotide encoding a polypeptide of SEQ ID NO: 550, wherein the vaccine is a recombinant virus derived from GAd20. The vaccine can comprise a polynucleotide encoding a polypeptide of SEQ ID NO: 554, wherein the vaccine is a recombinant virus derived from GAd20. The vaccine can comprise a polynucleotide encoding a polypeptide of SEQ ID NO: 555, wherein the vaccine is a recombinant virus derived from GAd20. The vaccine can comprise a polynucleotide encoding a polypeptide of SEQ ID NO: 556, wherein the vaccine is a recombinant virus derived from GAd20. The vaccine can comprise a polynucleotide encoding a polypeptide of SEQ ID NO: 623, wherein the vaccine is a recombinant virus derived from GAd20. The vaccine can comprise a polynucleotide encoding a polypeptide of SEQ ID NO: 624, wherein the vaccine is a recombinant virus derived from GAd20. The vaccine can comprise a polynucleotide sequence of SEQ ID NO: 713, wherein the vaccine is a recombinant virus derived from GAd20. The vaccine can comprise a polynucleotide of SEQ ID NOs: 542, wherein the vaccine is a recombinant virus derived from GAd20. The vaccine can comprise a polynucleotide of SEQ ID NOs: 551, wherein the vaccine is a recombinant virus derived from GAd20.

[0377] The vaccine can comprise a polynucleotide encoding a polypeptide of SEQ ID NO: 543, wherein the vaccine is a recombinant virus derived from MVA. The vaccine can comprise a polynucleotide encoding a polypeptide of SEQ ID NO: 552, wherein the vaccine is a recombinant virus derived from MVA. The vaccine can comprise a polynucleotide encoding a polypeptide of SEQ ID NO: 557, wherein the vaccine is a recombinant virus derived from MVA. The vaccine can comprise a polynucleotide encoding a polypeptide of SEQ ID NO: 558, wherein the vaccine is a recombinant virus derived from MVA. The vaccine can comprise a polynucleotide encoding a polypeptide of SEQ ID NO: 559, wherein the vaccine is a recombinant virus derived from MVA. The vaccine can comprise a polynucleotide encoding a polypeptide of SEQ ID NO: 625, wherein the vaccine is a recombinant virus derived from MVA. The vaccine can comprise a polynucleotide encoding a polypeptide of SEQ ID NO: 626, wherein the vaccine is a recombinant virus derived from MVA. The vaccine can comprise a polynucleotide of SEQ ID NOs: 544, wherein the vaccine is a recombinant virus derived from MVA. The vaccine can comprise a polynucleotide of SEQ ID NOs: 553, wherein the vaccine is a recombinant virus derived from MVA.

[0378] In some embodiments, the vaccine comprising a recombinant virus derived from GAd20 is administered as a prime. In some embodiments, the vaccine comprising a recombinant virus derived from MVA is administered as a boost.

[0379] In some embodiments, the vaccine comprising the polynucleotide sequence encoding a polypeptide of SEQ ID NOs: 541 or 550 is administered as a prime. In some embodiments, the vaccine comprising the polynucleotide sequence encoding a polypeptide of SEQ ID NOs 543 or 552 is administered as a boost.

[0380] The methods of treatment can comprise administering to the subject a therapeutically effective amount of a first vaccine comprising any of the Ad26 for priming the immune response and administering to the subject a therapeutically effective amount of a second vaccine comprising any of the MVA for boosting the immune response, thereby treating the prostate cancer in the subject.

[0381] The methods of treatment can comprise administering to the subject a therapeutically effective amount of a first vaccine comprising any of the GAd for priming the immune response and administering to the subject a therapeutically effective amount of a second vaccine comprising any of the MVA for boosting the immune response, thereby treating the prostate cancer in the subject.

[0382] The methods of treatment can comprise administering to the subject a therapeutically effective amount of a first vaccine comprising any of the GAd20 for priming the immune response and administering to the subject a therapeutically effective amount of a second vaccine comprising any of the MVA for boosting the immune response, thereby treating the prostate cancer in the subject.

[0383] The methods of treatment can comprise:

a) administering to the subject a therapeutically effective amount of a vaccine comprising a polynucleotide encoding one, two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, or 41 polypeptides selected from the group consisting of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23 and 177, and fragments thereof as a prime; and

b) administering to the subject a therapeutically effective amount of a vaccine comprising a polynucleotide encoding one, two, three, four, five, six, seven, eight, nine, ten, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, or 41 polypeptides selected from the group consisting of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23 and 177, and fragments thereof as a boost, thereby treating or preventing the prostate cancer in the subject.

[0384] The methods of treatment can comprise:

a) administering a first vaccine comprising a polynucleotide encoding a polypeptide comprising an amino acid sequence of SEQ ID NOs: 541, 550, 554, 555, 556, 623 or 624; and

b) a second vaccine comprising a polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NOs: 543, 552, 557, 558, 559, 625 or 626.

[0385] The methods of treatment can comprise administering:

a) a first vaccine comprising a first polynucleotide encoding a first polypeptide, wherein the first polypeptide comprises an amino acid sequence of SEQ ID NO: 541; and

b) a second vaccine comprising a second polynucleotide encoding a second polypeptide, wherein the second polypeptide comprises the amino acid sequence of SEQ ID NO: 543.

[0386] The methods of treatment can comprise administering:

a) a first vaccine comprising a first polynucleotide encoding a first polypeptide, wherein the first polypeptide comprises an amino acid sequence of SEQ ID NO: 550; and

b) a second vaccine comprising a second polynucleotide encoding a second polypeptide, wherein the second polypeptide comprises the amino acid sequence of SEQ ID NO: 552.

[0387] The methods of treatment can comprise administering:

a) a first vaccine comprising a first polynucleotide encoding a first polypeptide, wherein the first polypeptide comprises an amino acid sequence of SEQ ID NO: 541, wherein the first vaccine is a recombinant GAd20; and

b) a second vaccine comprising a second polynucleotide encoding a second polypeptide, wherein the second polypeptide comprises the amino acid sequence of SEQ ID NO: 543, wherein the second vaccine is a recombinant MVA.

[0388] The methods of treatment can comprise administering:

a) a first vaccine comprising a first polynucleotide encoding a first polypeptide, wherein the first polypeptide comprises an amino acid sequence of SEQ ID NO: 550, wherein the first vaccine is a recombinant GAd20; and

b) a second vaccine comprising a second polynucleotide encoding a second polypeptide, wherein the second polypeptide comprises the amino acid sequence of SEQ ID NO: 552, wherein the second vaccine is a recombinant MVA.

[0389] In some embodiments, the first vaccine is administered between about 1-16 weeks prior to administering the second vaccine. In some embodiments, the first vaccine is administered about 1 week prior to administering the second vaccine. In some embodiments, the first vaccine is administered about 2 weeks prior to administering the second vaccine. In some embodiments, the first vaccine is administered about 3 weeks prior to administering the second vaccine. In some embodiments, the first vaccine is administered about 4 weeks prior to administering the second vaccine. In some embodiments, the first vaccine is administered about 5 weeks prior to administering the second vaccine. In some embodiments, the first vaccine is administered about 6 weeks prior to administering the second vaccine. In some embodiments, the first vaccine is administered about 7 weeks prior to administering the second vaccine. In some embodiments, the first vaccine is administered about 8 weeks prior to administering the second vaccine. In some embodiments, the first vaccine is administered about 9 weeks prior to administering the second vaccine. In some embodiments, the first vaccine is administered about 10 weeks prior to administering the second vaccine. In some embodiments, the first vaccine is administered about

11 weeks prior to administering the second vaccine. In some embodiments, the first vaccine is administered about 12 weeks prior to administering the second vaccine. In some embodiments, the first vaccine is administered about 13 weeks prior to administering the second vaccine. In some embodiments, the first vaccine is administered about 14 weeks prior to administering the second vaccine. In some embodiments, the first vaccine is administered about 15 weeks prior to administering the second vaccine. In some embodiments, the first vaccine is administered about 16 weeks prior to administering the second vaccine.

[0390] The polynucleotides, polypeptides, or recombinant vaccines can be administered, for example, intramuscularly, subcutaneously, intravenously, cutaneously, intradermally, or nasally. Intramuscular administration of the vaccines can be achieved by using a needle. Alternatively, a needleless injection device (using, e.g., Biojector(TM)) or a freeze-dried powder containing the vaccine can be used.

[0391] For intravenous, cutaneous, or subcutaneous injection, or injection at the site of affliction, the vector may be the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, and Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives can be included, as required. A slow-release formulation may also be employed.

[0392] Typically, administration will have a prophylactic aim to generate an immune response against the prostate neoantigens before development of symptoms of prostate cancer.

[0393] The vaccines are administered to a subject, giving rise to an immune response in the subject. An amount of the vaccine to induce a detectable immune response is considered an "immunologically effective dose." The vaccines of the disclosure may induce a humoral as well as a cell-mediated immune response. In a typical embodiment the immune response is a protective immune response.

[0394] The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g., decisions on dosage etc., is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners.

[0395] In one exemplary regimen, the adenovirus is administered (e.g., intramuscularly) in a volume ranging between about 100 μL to about 10 ml containing concentrations of about 10^4 to 10^{12} virus particles/ml. The adenovirus may be administered in a volume ranging between 0.25 and 1.0 ml, such as in a volume of 0.5 ml. The adenovirus may be administered in an amount of about 10^9 to about 10^{12} viral particles (vp) to a human subject during one administration, more typically in an amount of about 10^{10} to about 10^{12} vp.

[0396] In one exemplary regimen, the MVA is administered (e.g., intramuscularly) in a volume ranging between about 100 µl to about 10 ml of saline solution containing a dose of about 1 x 10^7 $TCID_{50}$ to 1 x 10^9 $TCID_{50}$ (50% Tissue Culture Infective Dose) or Inf.U. (Infectious Unit). The MVA may be administered in a volume ranging between 0.25 and 1.0 ml.

[0397] Boosting compositions may be administered two or more times, weeks or months after administration of the priming composition, for example, about 1 week, or 2 weeks, or 3 weeks, or 4 weeks, or 6 weeks, or 8 weeks, or 12 weeks, or 16 weeks, or 20 weeks, or 24 weeks, or 28 weeks, or 32 weeks, or one to two years after administration of the priming composition. Additional boosting compositions may be administered 6 weeks to 5 years after the boosting step (b), such as 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 weeks, or 7, 8, 9, 10, 11, or 12 months, or 2, 3, 4, or 5 years, after the initial boosting inoculation. Optionally, the further boosting step (c) can be repeated one or more times as needed.

[0398] The preparation of vaccine compositions is well known. Vaccines may comprise or may be formulated into a pharmaceutical composition comprising the vaccine and a pharmaceutically acceptable excipient. "Pharmaceutically acceptable" refers to the excipient that at the dosages and concentrations employed, will not cause unwanted or harmful effects in the subjects to which they are administered and include carrier, buffers, stabilizers, or other materials well known to those skilled in the art. The precise nature of the carrier or other material may depend on the route of administration, e.g., intramuscular, subcutaneous, oral, intravenous, cutaneous, intramucosal (e.g., gut), intranasal, or intraperitoneal routes. Liquid carriers such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil may be included. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. Exemplary viral formulations are the Adenovirus World Standard (Hoganson *et al.*, 2002): 20 mM Tris pH 8, 25 mM NaCl, 2.5% glycerol; or 20 mM Tris, 2 mM $MgCl_2$, 25 mM NaCl, sucrose 10% w/v, polysorbate-80 0.02% w/v; or 10-25 mM citrate buffer pH 5.9-6.2, 4-6% (w/w) hydroxypropyl-beta-cyclodextrin (HBCD), 70-100 mM NaCl, 0.018-0.035% (w/w) polysorbate-80, and optionally 0.3-0.45% (w/w) ethanol. Many other buffers can be used, and examples of suitable formulations for the storage and for pharmaceutical administration of purified pharmaceutical preparations are known.

[0399] The vaccine may comprise one or more adjuvants. Suitable adjuvants include QS-21, Detox-PC, MPL-SE, MoGM-CSF, TiterMax-G, CRL-1005, GERBU, TERamide, PSC97B, Adjumer, PG-026, GSK-I, GcMAF, B-alethine, MPC-026, Adjuvax, CpG ODN, Betafectin, Alum, and MF59. Other adjuvants that may be used include lectins, growth factors, cytokines and lymphokines such as alpha-interferon, gamma interferon, platelet derived growth factor (PDGF), granulocyte-colony stimulating factor (gCSF), granulocyte macrophage colony stimulating factor (gMCSF), tumor necrosis factor (TNF), epidermal growth factor (EGF), IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12 or TLR agonists.

[0400] "Adjuvant" and "immune stimulant" are used interchangeably herein and are defined as one or more substances that cause stimulation of the immune system. In this context, an adjuvant is used to enhance an immune response to the vaccines described herein.

[0401] The disclosed methods can be used to treat any form of prostate cancer in a subject. The disclosed methods can treat, for example, a localized prostate adenocarcinoma, a relapsed prostate cancer, a refractory prostate cancer, a metastatic prostate cancer, a castration resistant prostate cancer, or any combination thereof. In some embodiments, the prostate cancer is an adenocarcinoma. In some embodiments, the prostate cancer is a metastatic prostate cancer. In some embodiments, the prostate cancer has metastasized to rectum, lymph node, or bone, or any combination thereof. In some embodiments, the prostate cancer is a relapsed or a refractory prostate cancer. In some embodiments, the prostate cancer is a castration resistant prostate cancer. In some embodiments, the prostate cancer is sensitive to an androgen deprivation therapy. In some embodiments, the prostate cancer is insensitive to the androgen deprivation therapy.

[0402] In some embodiments, the subject is treatment naïve. In some embodiments, the subject has received androgen deprivation therapy. In some embodiments, the subject has an elevated level of prostate specific antigen (PSA).

[0403] Androgen deprivation therapies include abiraterone, ketoconazole, enzalutamide, galeterone, ARN-509, and orteronel (TAK-700), or prostatectomy.

[0404] The methods of treatment can comprise administering any of the disclosed vaccines in combination with at least one additional cancer therapeutic agent for treating prostate cancer. The additional cancer therapeutic agent may be a chemotherapy, an androgen deprivation therapy, radiation therapy, targeted therapy, a checkpoint inhibitor, or any combination thereof. Any of the disclosed vaccines can also be used in combination with a surgery.

[0405] Exemplary chemotherapeutic agents include alkylating agents; nitrosoureas; antimetabolites; antitumor antibiotics; plant alkyloids; taxanes; hormonal agents; and miscellaneous agents, such as busulfan, carboplatin, chlorambucil, cisplatin, cyclophosphamide, dacarbazine, ifosfamide, mechlorethamine hydrochloride, melphalan, procarbazine, thiotepa, uracil mustard, 5-fluorouracil, 6-mercaptopurine, capecitabine, cytosine arabinoside, floxuridine, fludarabine, gemcitabine, methotrexate, thioguanine, dactinomycin, daunorubicin, doxorubicin, idarubicin, mitomycin-C, mitoxantrone, vinblastine, vincristine, vindesine, vinorelbine, paclitaxel, and docetaxel.

[0406] Exemplary androgen deprivation therapies include abiraterone acetate, ketoconazole, enzalutamide, galeterone, ARN-509 and orteronel (TAK-700) and surgical removal of the testicles.

[0407] Radiation therapy may be administered using various methods, including external-beam therapy, internal radiation therapy, implant radiation, stereotactic radiosurgery, systemic radiation therapy, radiotherapy and permanent or temporary interstitial brachytherapy. External-beam therapy involves three-dimensional, conformal radiation therapy where the field of radiation is designed, local radiation (e.g., radiation directed to a preselected target or organ), or focused radiation. Focused radiation

may be selected from stereotactic radiosurgery, fractionated stereotactic radiosurgery, or intensity-modulated radiation therapy. Focused radiation may have particle beam (proton), cobalt-60 (photon) linear accelerator (x-ray) as a radiation source (see e.g. WO 2012/177624). "Brachytherapy," refers to radiation therapy delivered by a spatially confined radioactive material inserted into the body at or near a tumor or other proliferative tissue disease site, and includes exposure to radioactive isotopes (e.g., At-211, I-131, I-125, Y-90, Re-186, Re-188, Sm-153, Bi-212, P-32, and radioactive isotopes of Lu). Suitable radiation sources for use as a cell conditioner include both solids and liquids. The radiation source can be a radionuclide, such as I-125, I-131, Yb-169, Ir-192 as a solid source, I-125 as a solid source, or other radionuclides that emit photons, beta particles, gamma radiation, or other therapeutic rays. The radioactive material may also be a fluid made from any solution of radionuclide(s), e.g., a solution of I-125 or I-131, or a radioactive fluid can be produced using a slurry of a suitable fluid containing small particles of solid radionuclides, such as Au-198, Y-90. The radionuclide(s) may be embodied in a gel or radioactive micro spheres.

[0408] Targeted therapies include anti-androgen therapies, inhibitors of angiogenesis such as bevacizumab, anti-PSA, or anti-PSMA antibodies or vaccines enhancing immune responses to PSA or PSMA.

[0409] Exemplary checkpoint inhibitors are antagonists of PD-1, PD-L1, PD-L2, VISTA, BTNL2, B7-H3, B7-H4, HVEM, HHLA2, CTLA-4, LAG-3, TIM-3, BTLA, CD160, CEACAM-1, LAIR1, TGFβ, IL-10, Siglec family protein, KIR, CD96, TIGIT, NKG2A, CD112, CD47, SIRPA or CD244. "Antagonist" refers to a molecule that, when bound to a cellular protein, suppresses at least one reaction or activity that is induced by a natural ligand of the protein. A molecule is an antagonist when the at least one reaction or activity is suppressed by at least about 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% more than the at least one reaction or activity suppressed in the absence of the antagonist (*e.g.,* negative control), or when the suppression is statistically significant when compared to the suppression in the absence of the antagonist. Antagonist may be an antibody, a soluble ligand, a small molecule, a DNA, or RNA such as siRNA. Exemplary antagonists of checkpoint inhibitors are described in U.S. Pat. Publ. No. 2017/0121409.

[0410] In some embodiments, one or more vaccines are administered in combination with a CTLA-4 antibody, a CTLA4 ligand, a PD-1 axis inhibitor, a PD-L1 axis inhibitor, a TLR agonist, a CD40 agonist, an OX40 agonist, hydroxyurea, ruxolitinib, fedratinib, a 41BB agonist, aa CD28 agonist, a STING antagonist, a RIG-1 antagonist, TCR-T therapy, CAR-T therapy, FLT3 ligand, aluminum sulfate, BTK inhibitor, CD38 antibody, CDK inhibitor, CD33 antibody, CD37 antibody, CD25 antibody, GM-CSF inhibitor, IL-2, IL-15, IL-7, CD3 redirection molecules, pomalimib, IFNγ, IFNα, TNFα, VEGF antibody, CD70 antibody, CD27 antibody, BCMA antibody or GPRC5D antibody, any combination thereof.

[0411] In some embodiments, the checkpoint inhibitor is ipilimumab, cetrelimab, pembrolizumab, nivolumab, sintilimab, cemiplimab, toripalimab, camrelizumab, tislelizumab, dostralimab, spartalizumab, prolgolimab, AK-105, HLX-10, balstilimab, MEDI-0680, HX-008, GLS-010, BI-754091, genolimzumab, AK-104, MGA-012, F-520, 609A, LY-3434172, AMG-404, SL-279252, SCT-I10A, RO-7121661, ICTCAR-014, MEDI-5752, CS-1003, XmAb-23104, Sym-021, LZM-009, hAB21, BAT-1306, MGD-019, JTX-4014, budigalimab, XmAb-20717, AK-103, MGD-013, IBI-318, sasanlimab, CC-90006, avelumab, atezolizumab, durvalumab, CS-1001, bintrafusp alpha, envafolimab, CX-072, GEN-1046, GS-4224, KL-A167, BGB-A333, SHR-1316, CBT-502, IL-103, KN-046, ZKAB-001, CA-170, TG_1501, LP-002, INCB-86550, ADG-104, SHR-1701, BCD-135, IMC-001, MSB-2311, FPT-155, FAZ-053, HLX-20, iodapolimab, FS-118, BMS-986189, AK-106, MCLA-145, IBI-318 or CK-301, or any combination thereof.

[0412] In some embodiments, one or more vaccines are administered in combination with ipilimumab, cetrelimab, pembrolizumab, nivolumab, sintilimab, cemiplimab, toripalimab, camrelizumab, tislelizumab, dostralimab, spartalizumab, prolgolimab, balstilimab, budigalimab, sasanlimab, avelumab, atezolizumab, durvalumab, envafolimab or iodapolimab, or any combination thereof.

Methods for monitoring responsiveness to a therapeutic agent

[0413] Also disclosed are methods for monitoring responsiveness of a subject having prostate cancer to a therapeutic agent, the method comprising:

(a) evaluating expression of one or more prostate cancer biomarkers, wherein the one or more prostate cancer biomarkers comprise: RCN1, STEAP1, PITX2, TIMP1, KLK4, KRT18, KLK3, ACPP, KLK2, TSPAN1, ATF3, SPDEF, NPY, SPINK1, HOXB13, FOXA1, KRT17, FOLH1, TNFRSF19, GREB1, KRT8, FLNC, GRHL2, RAB3B, JCHAIN, TMEFF2, AGR2, ACADL, AZGP1, MUC1, STEAP2, UGT2B17, METTL7A, HPN, NKX3-1, GNMT, ADAMTS15, HSD3B2, EPHA3, KCNN2, LGR5, IDO1, GPR39, C9orf152, MYBPC1, THBS2, ETV7, COL1A1, FGFR4, NR0B1, AR, ARv3, TMPRSS2:ERG, ROR1, FGF8, NKX2-2, EDIL3, RELN, FGF9, AKR1C4, CLUL1, KISS1R, CYP3A5, CYP17A1, SFRP4, HNF1A, CALCR, SYP, MSLN, or combinations thereof;

(b) administering a therapeutic agent to the subject; and

(c) evaluating the expression of the one or more prostate cancer biomarkers evaluated in step (a), wherein a decrease in the expression of the one or more prostate cancer biomarkers compared to the expression in step (a) is indicative of responsiveness to the therapeutic agent.

[0414] The sample from the subject from which the prostate cancer biomarkers is evaluated can comprise any biological sample known to contain or suspected of containing tumor material including, for example, a prostate cancer tissue sample or other types of materials containing cancer cells or biological derivatives from cancer cells (exosomes, apoptotic modies, circulating nucleic acids, etc.). In some embodiments, the sample is a plasma sample. In some aspects, the sample is from plasma exosomes. In some embodiments, the sample is a blood sample.

[0415] The one or more prostate cancer biomarkers can comprise: RCN1, STEAP1, PITX2, TIMP1, KLK4, KRT18, KLK3, ACPP, KLK2, TSPAN1, ATF3, SPDEF, NPY, SPINK1, HOXB13, FOXA1, KRT17, FOLH1, TNFRSF19, GREB1, KRT8, FLNC, GRHL2, RAB3B, JCHAIN, TMEFF2, AGR2, ACADL, AZGP1, MUC1, STEAP2, UGT2B17, METTL7A, HPN, NKX3-1, GNMT, ADAMTS15, HSD3B2, EPHA3, KCNN2, LGR5, IDO1, GPR39, C9orf152, MYBPC1, THBS2, ETV7, COL1A1, FGFR4, NR0B1, AR, ARv3, TMPRSS2:ERG, or combinations thereof. In some embodiments, the one or more prostate cancer biomarkers are from a plasma sample. In some aspects, the one or more prostate cancer biomarkers are from plasma exosomes.

[0416] The one or more prostate cancer biomarkers can comprise: HPN, ROR1, FLNC, GPR39, FGF8, NKX2-2, MUC1, NKX3-1, EDIL3, LGR5, FGFR4, STEAP1, ATF3, RELN, UGT2B17, KLK3, C9orf152, GNMT, METTL7A, FGF9, SPDEF, FOXA1, AKR1C4, GREB1, CLUL1, TMEFF2, HOXB13, KLK2, NPY, GRHL2, STEAP2, THBS2, KISS1R, KRT8, TNFRSF19, CYP3A5, KLK4, IDO1, FOLH1, NR0B1, EPHA3, CYP17A1, SFRP4, KRT18, TSPAN1, HNF1A, ADAMTS15, ACPP, CALCR, SYP, AZGP1, AR, ARv3, MSLN, TMPRSS2:ERG, and combinations thereof. In some embodiments, the one or more prostate cancer biomarkers are from a blood sample.

[0417] The presence of the one or more prostate cancer biomarkers can be evaluated by, for example, PCR, qPCR, various forms of nucleic acid sequencing (including but not limited to Illumina, Ion Torrent, Pacific Bioscience, Oxford Nanopore platforms), and various hybridization based approaches (including not limited to Affymetrix Gene Chip or Nanostring platforms). In some embodiments, the presence of the one or more prostate cancer biomarkers is evaluated by qPCR. In some aspects, the methods further comprise, prior to performing the qPCR, extracting RNA from the sample from the subject and synthesizing cDNA from the extracted RNA.

[0418] The expression of the one or more prostate cancer biomarkers detected in step (a) is the baseline expression of the cancer biomarker. Evaluating the expression of the one or more prostate cancer biomarkers after administering the therapeutic agent (step (c)) provides an indication of responsiveness/therapeutic efficacy. For example, a decrease in expression of the one or more prostate cancer biomarkers after administering the therapeutic agent compared to the expression prior to administering the therapeutic agent is indicative of responsiveness to the therapeutic agent.

[0419] Suitable therapeutic agents include any of the prostate cancer vaccines and additional agents disclosed above including, for example, surgery, chemotherapy, androgen deprivation therapy, radiation therapy, targeted therapy, checkpoint inhibitor, or any combination thereof

Methods for preparing a cDNA from a subject with prostate cancer useful for analyzing an expression of prostate cancer neoantigens

[0420] Also provided are methods for preparing a cDNA from a subject with prostate cancer useful for analyzing an expression of prostate cancer neoantigens, the method comprising:

(a) extracting RNA from a sample from the subject;

(b) producing amplified cDNA from the RNA extracted in step (a) by:

(i) reverse transcribing the extracted RNA to produce the cDNA, and

(ii) amplifying the cDNA; and

(c) analyzing the amplified cDNA produced in step (b) for one or more prostate cancer neoantigens, wherein the cDNA encodes an amino acid sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, fragments of the preceding sequences, or any combination thereof.

[0421] The sample from the subject can comprise any biological sample known to contain or suspected of containing tumor material including, for example, a prostate cancer tissue sample or other types of materials containing cancer cells or biological derivatives from cancer cells (exosomes, apoptotic modies, circulating nucleic acids, etc.). In some embodiments, the sample from the subject is a prostate cancer tissue sample.

[0422] In some embodiments, the cDNA encodes an amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, fragments of the preceding sequences, or any combination thereof.

[0423] In some embodiments, the cDNA encodes an amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, fragments of the preceding sequences, or any combination thereof.

[0424] In some embodiments, the cDNA encodes an amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, and 223.

Exemplary polypeptide and polynucleotide sequences for the prostate cancer vaccines

[0425] The prostate cancer vaccine can comprise two or more polypeptides selected from the group consisting of:

- AS18 comprising the amino acid sequence WKFEMSYTVGGPPPHVHARPRHWKTDR (SEQ ID NO: 275);
- P87 comprising the amino acid sequence YEAGMTLGGKILFFLFLLLPLSPFSLIF (SEQ ID NO: 381);
- AS55 comprising the amino acid sequence DGHSYTSKVNCLLLQDGFHGCVSITGAAGRRNLSIFLFLMLCKLEFHAC (SEQ ID NO: 333);
- AS57 comprising the amino acid sequence TGGKSTCSAPGPQSLPSTPFSTYPQWVILITEL (SEQ ID NO: 337);
- AS15 comprising the amino acid sequence VLRFLDLKVRYLHS (SEQ ID NO: 269);
- AS7 comprising the amino acid sequence DYWAQKEKGSSSFLRPSC (SEQ ID NO: 253);
- AS43 comprising the amino acid sequence VPFRELKNVSVLEGLRQGRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSI (SEQ ID NO: 309);
- AS51 comprising the amino acid sequence GMECTLGQVGAPSPRREEDGWRGGHSRFKADVPAPQGPCWGGQPGSAPSSAPPEQSLLD (SEQ ID NO: 325);
- AS16 comprising the amino acid sequence GNTTLQQLGEASQAPSGSLIPLRLPLLWEVRG (SEQ ID NO: 271);
- AS41 comprising the amino acid sequence EAFQRAAGEGGPGRGGARRGARVLQSPFCRAGAGEWLGHQSLR (SEQ ID NO: 305);
- AS6 comprising the amino acid sequence DYWAQKEKISIPRTHLC (SEQ ID NO: 251);
- AS3 comprising the amino acid sequence VAMMVPDRQVHYDFGL (SEQ ID NO: 245);
- AS11 comprising the amino acid sequence VPFRELKNQRTAQGAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAGRGPEAGG GVLQPQRPAPEKPGCPCRRGQPRLHTVKMWRA (SEQ ID NO: 261);
- AS13 comprising the amino acid sequence KRSFAVTERII (SEQ ID NO: 265);
- AS47 comprising the amino acid sequence FKKFDGPCGERGGGRTARALWARGDSVLTPALDPQTPVRAPSLTRAAAAV (SEQ ID NO: 317);
- AS8 comprising the amino acid sequence LVLGVLSGHSGSRL (SEQ ID NO: 255);
- AS19 comprising the amino acid sequence QWQHYHRSGEAAGTPLWRPTRN (SEQ ID NO: 277);

- AS37 comprising the amino acid sequence CHLFLQPQVGTPPPHTASARAPSGPPHPHESCPAGRRPARAAQTCARRQHGLPGCEEAGT ARVPSLHLHLHQAALGAGRGRGWGEACAQVPPSRG (SEQ ID NO: 297);
- AS23 comprising the amino acid sequence KIQNKNCPD (SEQ ID NO: 285);
- MS1 comprising the amino acid sequence HYKLIQQPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCSTNDICVTANFCISVTFLKP CFLLHEASASQ (SEQ ID NO: 437);
- MS3 comprising the amino acid sequence RTALTHNQDFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITPQLGGQSDSSQPLL TTGRPQGWQDQALRHTQQASPASCATITIPIHSAALGDHSGDPGPAWDTCPPLPLTTLIPR APPPYGDSTARSWPSRCGPLG (SEQ ID NO: 439);
- MS6 comprising the amino acid sequence YAYKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFLSRALRALHVLWNG FQLHCQ (SEQ ID NO: 442);
- MS8 comprising the amino acid sequence TMPAILKLQKNCLLSL (SEQ ID NO: 444); and
- P82 comprising the amino acid sequence YEAGMTLGEKFRVGNCKHLKMTRP (SEQ ID NO: 379),
- and fragments thereof.

[0426] In some embodiments, the prostate cancer vaccine can comprise one or more polypeptides selected from the group consisting of:

- P16 comprising the amino acid sequence GVPGDSTRRAVRRMNTF (SEQ ID NO: 343);
- FUS1 comprising the amino acid sequence CGASACDVSLIAMDSA (SEQ ID NO: 211);
- P22 comprising the amino acid sequence SLYHREKQLIAMDSAI (SEQ ID NO: 349);
- FUS2 comprising the amino acid sequence TEYNQKLQVNQFSESK (SEQ ID NO: 213);
- FUS3 comprising the amino acid sequence TEISCCTLSSEENEYLPRPEWQLQ (SEQ ID NO: 215);
- FUS6 comprising the amino acid sequence CEERGAAGSLISCE (SEQ ID NO: 221);
- FUS5 comprising the amino acid sequence NSKMALNSEALSVVSE (SEQ ID NO: 219);
- FUS8 comprising the amino acid sequence WGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLPLRTLAGCLARTAHLRPG AESLPQPQLHCT (SEQ ID NO: 225);
- FUS15 comprising the amino acid sequence HVVGYGHLDTSGSSSSSSWP (SEQ ID NO: 345);
- P35 comprising the amino acid sequence NSKMALNSLNSIDDAQLTRIAPPRSHCCFWEVNAP (SEQ ID NO: 353);
- FUS19 comprising the amino acid sequence KMHFSLKEHPPPPCPP (SEQ ID NO: 235); and

- FUS7 comprising the amino acid sequence LWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSARTCCGTKWPSSQEAALGLGSGLLR FSCGTAAIR (SEQ ID NO: 223),
- and fragments thereof.

[0427] The prostate cancer vaccine can comprise two or more polypeptides selected from the group consisting of:

- P16 comprising the amino acid sequence GVPGDSTRRAVRRMNTF (SEQ ID NO: 343);
- FUS1 comprising the amino acid sequence CGASACDVSLIAMDSA (SEQ ID NO: 211);
- P22 comprising the amino acid sequence SLYHREKQLIAMDSAI (SEQ ID NO: 349);
- FUS2 comprising the amino acid sequence TEYNQKLQVNQFSESK (SEQ ID NO: 213);
- FUS3 comprising the amino acid sequence TEISCCTLSSEENEYLPRPEWQLQ (SEQ ID NO: 215);
- FUS6 comprising the amino acid sequence CEERGAAGSLISCE (SEQ ID NO: 221);
- FUS5 comprising the amino acid sequence NSKMALNSEALSVVSE (SEQ ID NO: 219);
- FUS8 comprising the amino acid sequence WGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLPLRTLAGCLARTAHLRPG AESLPQPQLHCT (SEQ ID NO: 225);
- FUS15 comprising the amino acid sequence HVVGYGHLDTSGSSSSSSWP (SEQ ID NO: 345);
- P35 comprising the amino acid sequence NSKMALNSLNSIDDAQLTRIAPPRSHCCFWEVNAP (SEQ ID NO: 353);
- FUS19 comprising the amino acid sequence KMHFSLKEHPPPPCPP (SEQ ID NO: 235); and
- FUS7 comprising the amino acid sequence LWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSARTCCGTKWPSSQEAALGLGSGLLR FSCGTAAIR (SEQ ID NO: 223),
- and fragments thereof.

[0428] The prostate cancer vaccine can comprise one or more polypeptides selected from the group consisting of:

- AS18 comprising the amino acid sequence WKFEMSYTVGGPPPHVHARPRHWKTDR (SEQ ID NO: 275);
- P87 comprising the amino acid sequence YEAGMTLGGKILFFLFLLLPLSPFSLIF (SEQ ID NO: 381);
- AS55 comprising the amino acid sequence DGHSYTSKVNCLLLQDGFHGCVSITGAAGRRNLSIFLFLMLCKLEFHAC (SEQ ID NO: 333);
- AS57 comprising the amino acid sequence TGGKSTCSAPGPQSLPSTPFSTYPQWVILITEL (SEQ ID NO: 337);

- AS15 comprising the amino acid sequence VLRFLDLKVRYLHS (SEQ ID NO: 269);
- AS7 comprising the amino acid sequence DYWAQKEKGSSSFLRPSC (SEQ ID NO: 253);
- AS43 comprising the amino acid sequence VPFRELKNVSVLEGLRQGRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSI (SEQ ID NO: 309);
- AS51 comprising the amino acid sequence GMECTLGQVGAPSPRREEDGWRGGHSRFKADVPAPQGPCWGGQPGSAPSSAPPEQSLLD (SEQ ID NO: 325);
- AS16 comprising the amino acid sequence GNTTLQQLGEASQAPSGSLIPLRLPLLWEVRG (SEQ ID NO: 271);
- AS41 comprising the amino acid sequence EAFQRAAGEGGPGRGGARRGARVLQSPFCRAGAGEWLGHQSLR (SEQ ID NO: 305);
- AS6 comprising the amino acid sequence DYWAQKEKISIPRTHLC (SEQ ID NO: 251);
- AS3 comprising the amino acid sequence VAMMVPDRQVHYDFGL (SEQ ID NO: 245);
- AS11 comprising the amino acid sequence VPFRELKNQRTAQGAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAGRGPEAGG GVLQPQRPAPEKPGCPCRRGQPRLHTVKMWRA (SEQ ID NO: 261);
- AS13 comprising the amino acid sequence KRSFAVTERII (SEQ ID NO: 265);
- AS47 comprising the amino acid sequence FKKFDGPCGERGGGRTARALWARGDSVLTPALDPQTPVRAPSLTRAAAAV (SEQ ID NO: 317);
- AS8 comprising the amino acid sequence LVLGVLSGHSGSRL (SEQ ID NO: 255);
- AS19 comprising the amino acid sequence QWQHYHRSGEAAGTPLWRPTRN (SEQ ID NO: 277);
- AS37 comprising the amino acid sequence CHLFLQPQVGTPPPHTASARAPSGPPHPHESCPAGRRPARAAQTCARRQHGLPGCEEAGT ARVPSLHLHLHQAALGAGRGRGWGEACAQVPPSRG (SEQ ID NO: 297);
- AS23 comprising the amino acid sequence KIQNKNCPD (SEQ ID NO: 285);
- MS1 comprising the amino acid sequence HYKLIQQPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCSTNDICVTANFCISVTFLKP CFLLHEASASQ (SEQ ID NO: 437);
- MS3 comprising the amino acid sequence RTALTHNQDFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITPQLGGQSDSSQPLL TTGRPQGWQDQALRHTQQASPASCATITIPIHSAALGDHSGDPGPAWDTCPPLPLTTLIPR APPPYGDSTARSWPSRCGPLG (SEQ ID NO: 439);

- MS6 comprising the amino acid sequence YAYKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFLSRALRALHVLWNG FQLHCQ (SEQ ID NO: 442);
- MS8 comprising the amino acid sequence TMPAILKLQKNCLLSL (SEQ ID NO: 444); and
- P82 comprising the amino acid sequence YEAGMTLGEKFRVGNCKHLKMTRP (SEQ ID NO: 379),
- and fragments thereof.

[0429] In some embodiments, the prostate cancer vaccine can comprise one or more polypeptides selected from the group consisting of:

- M84 comprising the amino acid sequence IARELHQFAFDLLIKSH (SEQ ID NO: 167);
- M86 comprising the amino acid sequence QPDSFAALHSSLNELGE (SEQ ID NO: 171);
- M10 comprising the amino acid sequence FVQGKDWGLKKFIRRDF (SEQ ID NO: 19);
- M12 comprising the amino acid sequence FVQGKDWGVKKFIRRDF (SEQ ID NO: 23); and
- FR1 comprising the amino acid sequence QNLQNGGGSRSSATLPGRRRRRWLRRRRQPISVAPAGPPRRPNQKPNPPGGARCVIMRPT WPGTSAFT (SEQ ID NO: 177),
- and fragments thereof.

[0430] The prostate cancer vaccine can comprise one or more polynucleotides selected from the group consisting of:

- the polynucleotide sequence of TGGAAATTCGAGATGAGCTACACGGTGGGTGGCCCGCCTCCCCATGTTCATGCTAGA CCCAGGCATTGGAAAACTGATAGA (SEQ ID NO: 276) (encoding AS18);
- the polynucleotide sequence of TATGAAGCAGGGATGACTCTGGGAGGTAAGATACTTTTCTTTCTCTTCCTCCTCCTTC CTCTCTCCCCCTTCTCCCTCATTTTC (SEQ ID NO: 382) (encoding P87);
- the polynucleotide sequence of GATGGCCACTCCTACACATCCAAGGTGAATTGTTTACTCCTTCAAGATGGGTTCCATG GCTGTGTGAGCATCACCGGGGCAGCTGGAAGAAGAAACCTGAGCATCTTCCTGTTCT TGATGCTGTGCAAATTGGAGTTCCATGCTTGT (SEQ ID NO: 334) (encoding AS55);
- the polynucleotide sequence of ACAGGGGGCAAAAGCACCTGCTCGGCTCCTGGCCCTCAGTCTCTCCCCTCCACTCCA TTCTCCACCTACCCACAGTGGGTCATTCTGATCACCGAACTG (SEQ ID NO: 338) (encoding AS57);
- the polynucleotide sequence of GTGCTGCGCTTTCTGGACTTAAAGGTGAGATACCTGCACTCT (SEQ ID NO: 270) (encoding AS15);

- the polynucleotide sequence of GACTACTGGGCTCAAAAGGAGAAGGGATCATCTTCATTCCTGCGACCATCCTGT (SEQ ID NO: 254) (encoding AS7);
- the polynucleotide sequence of GTGCCCTTCCGGGAGCTCAAGAACGTGAGTGTCCTGGAGGGGCTCCGTCAAGGCCGG CTTGGGGGTCCCTGTTCATGTCACTGCCCAAGACCTTCCCAGGCCAGGCTCACGCCA GTGGATGTGGCAGGTCCCTTCTTGTGTCTGGGGGATCCTGGGCTGTTCCCCCCAGTCA AGAGCAGTATC (SEQ ID NO: 310) (encoding AS43);
- the polynucleotide sequence of GGCATGGAGTGCACCCTGGGGCAGGTGGGTGCCCCGTCCCCTCGGAGGGAGGAGGA CGGTTGGCGTGGGGGCCACAGCCGATTCAAGGCTGATGTACCAGCACCGCAGGGAC CCTGCTGGGGTGGCCAACCTGGCTCTGCCCCCTCCTCAGCTCCTCCTGAACAGTCATT ATTAGAT (SEQ ID NO: 326) (encoding AS51);
- the polynucleotide sequence of GGCAACACCACCCTCCAGCAGCTGGGTGAGGCCTCCCAGGCGCCCTCAGGCTCCCTC ATCCCTCTGAGGCTGCCTCTGCTCTGGGAAGTGAGGGGC ((SEQ ID NO: 272) (encoding AS16);
- the polynucleotide sequence of GAGGCCTTCCAGAGGGCCGCTGGTGAGGGCGGCCCGGGCCGCGGTGGGGCACGGCG CGGTGCCAGGGTGTTGCAGAGCCCCTTTTGCAGGGCAGGAGCTGGGGAGTGGTTAGG ACATCAGTCCCTCAGG (SEQ ID NO: 306) (encoding AS41);
- the polynucleotide sequence of GACTACTGGGCTCAAAAGGAGAAGATCAGCATCCCCAGAACACACCTGTGT (SEQ ID NO: 252) (encoding AS6);
- the polynucleotide sequence of GTTGCTATGATGGTTCCTGATAGACAGGTTCATTATGACTTTGGATTG (SEQ ID NO: 246) (encoding AS3);
- the polynucleotide sequence of GTGCCCTTCCGGGAGCTCAAGAACCAGAGAACAGCACAAGGGGCTCCTGGGATCCA CCACGCGGCTTCCCCCGTTGCTGCCAACCTCTGCGACCCGGCGAGACACGCACAGCA CACACGCATCCCCTGCGGCGCTGGCCAAGTGCGTGCTGGCCGAGGTCCCGAAGCAGG TGGTGGAGTACTACAGCCACAGAGGCCTGCCCCCGAGAAGCCTGGGTGTCCCTGCCG GAGAGGCCAGCCCAGGCTGCACACCGTGAAGATGTGGAGGGCG (SEQ ID NO: 262) (encoding AS11);
- the polynucleotide sequence of AAGAGAAGTTTTGCTGTCACGGAGAGGATCATC (SEQ ID NO: 266) (encoding AS13);

- the polynucleotide sequence of TTCAAGAAGTTCGACGGCCCTTGTGGTGAGCGCGGCGGCGGGCGCACGGCTCGAGCT CTGTGGGCGCGCGGCGACAGCGTCCTGACTCCTGCCCTCGACCCCCAGACCCCTGTC AGGGCGCCCTCCCTGACCCGAGCCGCAGCTGCCGTG (SEQ ID NO: 318) (encoding AS47);
- the polynucleotide sequence of CTTGTACTTGGTGTATTGAGCGGGCACAGTGGCTCACGCCTA (SEQ ID NO: 256) (encoding AS8);
- the polynucleotide sequence of CAGTGGCAGCACTACCACCGGTCAGGTGAGGCCGCAGGGACTCCCCTCTGGAGACCC ACAAGAAAC (SEQ ID NO: 278) (encoding AS19);
- the polynucleotide sequence of TGCCACCTCTTCCTGCAGCCCCAGGTTGGCACCCCCCCCCCCCACACTGCCAGTGCTC GAGCCCCCAGTGGTCCACCCCACCCTCATGAAAGTTGCCCTGCAGGGCGAAGACCTG CGAGAGCTGCGCAGACATGTGCACGCCGACAGCACGGACTTCCTGGCTGTGAAGAG GCTGGTACAGCGCGTGTTCCCAGCCTGCACCTGCACCTGCACCAGGCCGCCCTCGGA GCAGGAAGGGGCCGTGGGTGGGGAGAGGCCTGTGCCCAAGTACCCCCCTCAAGAGG C (SEQ ID NO: 298) (encoding AS37);
- the polynucleotide sequence of AAAATTCAGAATAAAAATTGTCCAGAC (SEQ ID NO: 286) (encoding AS23);
- the polynucleotide sequence of CACTACAAATTAATTCAACAACCCATATCCCTCTTCTCCATCACTGATAGGCTCCATA AGACGTTCAGTCAGCTGCCCTCGGTCCATCTCTGCTCAATCACCTTCCAGTGGGGACA CCCGCCCATTTTCTGCTCAACAAATGATATCTGTGTCACGGCCAACTTCTGCATCTCG GTCACATTCCTTAAACCGTGCTTCCTCCTACATGAGGCATCTGCCTCACAG (SEQ ID NO: 448) (encoding MS1);
- the polynucleotide sequence of AGGACCGCCCTGACACACAATCAGGACTTCTCTATCTACAGGCTCTGTTGCAAGAGG GGGTCCCTCTGCCACGCTTCCCAGGCCAGATCCCCGGCTTTCCCGAAGCCGGTCAGA CCTCTTCCTGCCCCCATCACCAGAATCACCCCCCAACTGGGGGGACAATCTGACTCG AGTCAACCCCTTCTCACTACGGGAAGACCTCAGGGGTGGCAAGATCAAGCTCTTAGA CACACCCAGCAAGCCAGTCCTGCCTCTTGTGCCACCATCACCATTCCCATCCACTCAG CTGCCCTTGGTGACCACTCCGGAGACCCTGGTCCAGCCTGGGACACCTGCCCGCCGC TGCCGCTCACTACCCTCATCCCCCGAGCTCCCCCGCCGTATGGAGACAGCACTGCCA GGTCCTGGCCCTCCCGCTGTGGGCCCCTCGGC (SEQ ID NO: 450) (encoding MS3);

- the polynucleotide sequence of TATGCTTACAAGGACTTTCTCTGGTGTTTTCCTTTTTCTTTAGTTTTTCTCCAAGAGAT TCAAATCTGCTGCCATGTTAGCTGTCTTTGCTGTATCTGCTGTAGTACACGAATATGC CTTGGCTGTTTGCTTGAGCTTTTTCTATCCCGTGCTCTTCGTGCTCTTCATGTTCTTTG GAATGGCTTTCAACTTCATTGTCAA (SEQ ID NO: 453) (encoding MS6);
- the polynucleotide sequence of ACCATGCCTGCTATTTTAAAGTTACAGAAGAATTGTCTTCTCTCCTTA (SEQ ID NO: 455) (encoding MS8); and
- the polynucleotide sequence of TATGAAGCAGGGATGACTCTGGGAGAAAAATTCCGGGTTGGCAATTGCAAGCATCTC AAAATGACCAGACCC (SEQ ID NO: 380) (encoding P82); and fragments thereof.

[0431] The prostate cancer vaccine can comprise one or more polynucleotides selected from the group consisting of:

- the polynucleotide sequence of GGAGTTCCAGGAGATTCAACCAGGAGAGCAGTGAGGAGAATGAATACCTTC (SEQ ID NO: 344) (encoding P16);
- the polynucleotide sequence of TGCGGGGCCTCTGCCTGTGATGTCTCCCTCATTGCTATGGACAGTGCT (SEQ ID NO: 212) (encoding FUS1);
- the polynucleotide sequence of TCCCTCTACCACCGGGAGAAGCAGCTCATTGCTATGGACAGTGCTATC (SEQ ID NO: 350) (encoding P22);
- the polynucleotide sequence of ACCGAATACAACCAGAAATTACAAGTGAATCAATTTAGTGAATCCAAA (SEQ ID NO: 214) (encoding FUS2);
- the polynucleotide sequence of ACAGAAATTTCATGTTGCACCCTGAGCAGTGAGGAGAATGAATACCTTCCAAGACCA GAGTGGCAGCTCCAG (SEQ ID NO: 216) (encoding FUS3);
- the polynucleotide sequence of TGTGAGGAGCGCGGCGCGGCAGGAAGCCTTATCAGTTGTGAG (SEQ ID NO: 222) (encoding FUS6);
- the polynucleotide sequence of AACAGCAAGATGGCTTTGAACTCAGAAGCCTTATCAGTTGTGAGTGAG (SEQ ID NO: 220) (encoding FUS5);
- the polynucleotide sequence of TGGGGGATGGAGTTGGCAGCGTCTCGGAGGTTCTCCTGGGACCACCACTCCGCCGGG

GGGCCGCCCAGAGTGCCAAGCGTCCGATCCGGCGCCGCCCAAGTGCAGCCCAAGGA CCCGCTCCCGCTCCGCACCCTGGCAGGCTGCCTAGCCAGGACTGCGCACCTGCGCCC TGGGGCGGAGTCCTTACCCCAACCCCAGCTTCACTGCACA (SEQ ID NO: 226) (encoding FUS8);

- the polynucleotide sequence of CACGTGGTGGGCTATGGCCACCTTGATACTTCCGGGTCATCCTCCTCCTCCTCCTGGC CC (SEQ ID NO: 346) (encoding FUS15);
- the polynucleotide sequence of AACAGCAAGATGGCTTTGAACTCATTAAACTCCATTGATGATGCACAGTTGACAAGA ATTGCCCCTCCAAGATCTCATTGCTGTTTCTGGGAAGTAAACGCTCCT (SEQ ID NO: 354) (encoding P35);
- the polynucleotide sequence of AAAATGCACTTCTCCCTCAAGGAGCACCCACCGCCCCCTTGCCCGCCT (SEQ ID NO: 236) (encoding FUS19); and
- the polynucleotide sequence of CTGTGGTTCCAGAGCAGTGAGCTGTCCCCGACGGGAGCGCCATGGCCCAGCCGCCGC CCGACGTGGAGGGGGACGACTGTCTCCCCGCGTACCGCCACCTCTTCTGCCCGGACC TGCTGCGGGACAAAGTGGCCTTCATCACAGGAGGCGGCTCTGGGATTGGGTTCCGGA TTGCTGAGATTTTCATGCGGCACGGCTGCCATACGG (SEQ ID NO: 224) (encoding FUS7),
- and fragments thereof.

[0432] The prostate cancer vaccine can comprise one or more polynucleotides selected from the group consisting of:

- the polynucleotide sequence of GGAGTTCCAGGAGATTCAACCAGGAGAGCAGTGAGGAGAATGAATACCTTC (SEQ ID NO: 344) (encoding P16);
- the polynucleotide sequence of TGCGGGGCCTCTGCCTGTGATGTCTCCCTCATTGCTATGGACAGTGCT (SEQ ID NO: 212) (encoding FUS1);
- the polynucleotide sequence of TCCCTCTACCACCGGGAGAAGCAGCTCATTGCTATGGACAGTGCTATC (SEQ ID NO: 350) (encoding P22);
- the polynucleotide sequence of ACCGAATACAACCAGAAATTACAAGTGAATCAATTTAGTGAATCCAAA (SEQ ID NO: 214) (encoding FUS2);

- the polynucleotide sequence of ACAGAAATTTCATGTTGCACCCTGAGCAGTGAGGAGAATGAATACCTTCCAAGACCA GAGTGGCAGCTCCAG (SEQ ID NO: 216) (encoding FUS3);
- the polynucleotide sequence of TGTGAGGAGCGCGGCGCGGCAGGAAGCCTTATCAGTTGTGAG (SEQ ID NO: 222) (encoding FUS6);
- the polynucleotide sequence of AACAGCAAGATGGCTTTGAACTCAGAAGCCTTATCAGTTGTGAGTGAG (SEQ ID NO: 220) (encoding FUS5);
- the polynucleotide sequence of TGGGGGATGGAGTTGGCAGCGTCTCGGAGGTTCTCCTGGGACCACCACTCCGCCGGG GGGCCGCCCAGAGTGCCAAGCGTCCGATCCGGCGCCGCCCAAGTGCAGCCCAAGGA CCCGCTCCCGCTCCGCACCCTGGCAGGCTGCCTAGCCAGGACTGCGCACCTGCGCCC TGGGGCGGAGTCCTTACCCCAACCCCAGCTTCACTGCACA (SEQ ID NO: 226) (encoding FUS8);
- the polynucleotide sequence of CACGTGGTGGGCTATGGCCACCTTGATACTTCCGGGTCATCCTCCTCCTCCTCCTGGC CC (SEQ ID NO: 346) (encoding FUS15);
- the polynucleotide sequence of AACAGCAAGATGGCTTTGAACTCATTAAACTCCATTGATGATGCACAGTTGACAAGA ATTGCCCCTCCAAGATCTCATTGCTGTTTCTGGGAAGTAAACGCTCCT (SEQ ID NO: 354) (encoding P35);
- the polynucleotide sequence of AAAATGCACTTCTCCCTCAAGGAGCACCCACCGCCCCCTTGCCCGCCT (SEQ ID NO: 236) (encoding FUS19); and
- the polynucleotide sequence of CTGTGGTTCCAGAGCAGTGAGCTGTCCCCGACGGGAGCGCCATGGCCCAGCCGCCGC CCGACGTGGAGGGGGACGACTGTCTCCCCGCGTACCGCCACCTCTTCTGCCCGGACC TGCTGCGGGACAAAGTGGCCTTCATCACAGGAGGCGGCTCTGGGATTGGGTTCCGGA TTGCTGAGATTTTCATGCGGCACGGCTGCCATACGG (SEQ ID NO: 224) (encoding FUS7).

EXAMPLES

[0433] The following examples are provided to further describe some of the embodiments disclosed herein. The examples are intended to illustrate, not to limit, the disclosed embodiments.

Example 1 General methods

Peptide synthesis

[0434] Peptides were synthesized by New England Peptide with purity >80%. The lyophilized peptides were solubilized in 100% DMSO.

In vitro immunogenicity assessment of neoantigens ("Patient PBMC restimulation assay")

[0435] PBMCs from human patients with metastatic castrate-resistant prostate cancer were thawed using media (RPMI 1640 supplemented with Glutamax, 10% HI FBS, and 1X Sodium Pyruvate). Cells were counted and plated in a 96 well round bottom microplate at a concentration of 250,000 viable cells per well. Lyophilized peptides were solubilized in 100% DMSO and diluted in media to 10 µg/mL. Neoantigen peptides were added in equal volume to PBMCs for a final concentration of 5 µg/mL. CEF Peptide Pool "Plus" (Cellular Technologies, Ltd.) was utilized as a positive control and DMSO at the same final concentration as the experimental peptides was utilized as a negative control. Human IL-15 (Peprotech) was added to all wells at final concentration of 10 ng/mL.

[0436] Plates were incubated at 37°C (5% CO_2) for a total of 13 days. Media was refreshed every 2 days with IL-15 (10 ng/mL final concentration) and IL-2 (R&D systems, 10 IU/mL final concentration). On day 12, PBMCs were re-stimulated with identical experimental peptides or controls, at same concentration as peptide stimulation on Day 1. After 1-hour incubation, protein Inhibitor Cocktail (eBioscience) was added to every well and plate was incubated overnight.

[0437] On day 13, cells were stained for intracellular flow cytometry analysis. The cells were washed with PBS and stained with Live/Dead Fixable Aqua Dead Cell stain (Thermo-Fisher). Following the live/dead stain, cells were blocked using Biotin-Free Fc Receptor Blocker (Accurate Chemical & Scientific Corp). Extracellular cellular flow panel (1 µL/antibody per well in 50 µL) consisted of CD3 PerCP-Cy5.5 (Biolegend), CD4 BV421 (Biolegend), and CD8 APC-Cy7 (Biolegend). After extracellular staining, cells were fixed using Foxp3/Transcription Factor Staining Buffer Set (eBioscience) and stained for intracellular proteins (1:50 dilution) using TNFα FITC (R&D Systems) and IFNγ BV785 (Biolegend). Cells were washed and resuspended in stain buffer and analyzed using a BD Celesta flow cytometer.

[0438] Flow cytometry cell staining analysis was completed using FlowJo v10. Cells were gated on live, singlet, CD3+ cells. The CD8+ T cells were analyzed for TNFα/IFNγ expression and the frequency of double positive TNFα/IFNγ CD8+ T cells was recorded. Responses were assessed to be positive when the frequency of double positive TNFα/IFNγ CD8+ T cells due to stimulation with an experimental peptide was increased greater than or equal to 2-fold over the DMSO only negative control for that patient. Peptides were analyzed in 1 to 7 patient samples.

In vitro immunogenicity assessment of neoantigens ("Exogenous autologous normal donor restimulation assay")

[0439] CD1c+ Dendritic Cells (DC) isolated from human normal PBMCs were thawed using media (IMDM (Gibco) supplemented with glutamine, HEPES, 5% human serum (Sigma), and 1X Pen-Strep). DC cells were resuspended in media supplemented with IL-4 (Peprotech, 80 ng/mL) and GM-CSF (Gibco, 80 ng/mL), plated in 6 well microplates, and rested overnight at 37° C (5% CO_2). The following day, DC cells were counted and plated in a 96 well round bottom microplate at a concentration of 30,000 viable cells per well. Lyophilized peptides (15-mer overlapping peptides) were solubilized in 100% DMSO and pooled by neoantigen to between 5 mg/mL and 20 mg/mL. Neoantigen peptides pools were added to DCs for a final concentration of 2.5 µg/mL to 10 µg/mL and rested for 2 hours at 37° C (5% CO_2). CEF Peptide Pool "Plus" (Cellular Technologies, Ltd.) was utilized as a positive control and DMSO at the same final concentration as the experimental peptides was utilized as a negative control. After 2 hours, DC cells were irradiated with 50 gray of ionizing radiation. Autologous CD3+ Pan-T cells isolated from human normal PBMCs were thawed using media. Following irradiation, autologous Pan-T cells were added to the irradiated DCs at 300,000 viable cells per well. Human IL-15 (Peprotech) was added to all wells at final concentration of 10 ng/mL.

[0440] Plates were incubated at 37°C (5% CO_2) for a total of 12 days. Media was refreshed every 2-3 days with IL-15 (10 ng/mL final concentration) and IL-2 (R&D systems, 10 IU/mL final concentration). On day 11 cells were re-stimulated with identical experimental peptide pools or controls, at same concentration as peptide stimulation on Day 1. Protein Inhibitor Cocktail (eBioscience) was added to every well and plate was incubated overnight at 37° C (5% CO_2).

[0441] On day 12, cells were stained for intracellular flow cytometry analysis. The cells were washed with PBS and stained with Live/Dead Fixable Aqua Dead Cell stain (Thermo-Fisher). Following the live/dead stain, cells were blocked using Biotin-Free Fc Receptor Blocker (Accurate Chemical & Scientific Corp). Extracellular cellular flow panel (1 µL/antibody per well in 50 µL) consisted of CD3 PerCP-Cy5.5 (Biolegend), CD4 BV421 (Biolegend), and CD8 APC-Cy7 (Biolegend). After extracellular staining, cells were fixed using Foxp3/Transcription Factor Staining Buffer Set (eBioscience) and stained for intracellular proteins (1:50 dilution) using TNFα FITC (R&D Systems) and IFNγ BV785 (Biolegend). Cells were washed and resuspended in stain buffer and analyzed using a BD Celesta flow cytometer.

[0442] Flow cytometry cell staining analysis was completed using FlowJo v10. Cells were gated on live, singlet, CD3+ cells. The CD8+ and CD4+ T cells were analyzed for TNFα/IFNγ expression and the frequency of double positive TNFα/IFNγ CD8+ and the frequency of double positive TNFα/IFNγ CD4+ T cells were recorded. Responses were assessed to be positive when the frequency of double positive TNFα/IFNγ CD8+ or TNFα/IFNγ CD4+ T cells due to stimulation with an experimental peptide pool was increased greater than or equal to 3-fold over the DMSO only negative control for that donor and at least 0.01%.

In vitro endogenous immunogenicity assessment of neoantigens ("Endogenous autologous normal donor restimulation assay")

[0443] CD1c+ Dendritic Cells (DC) isolated from human normal PBMCs were thawed using media (IMDM (Gibco) supplemented with glutamine, HEPES, 5% human serum (Sigma), and 1X Pen-Strep). DC cells were resuspended in media supplemented with IL-4 (Peprotech, 80 ng/mL) and GM-CSF (Gibco, 80 ng/mL), plated in 6 well microplates, and rested overnight at 37° C (5% CO_2). The following day, DC cells were counted and plated in a 96 well round bottom microplate at a concentration of 30,000 viable cells per well. Ad5 vectors (Vector Biolabs) were dilute in media to an MOI (Multiplicity Of Infection) of 5000 based on Plaque Forming Units. Ad5 vectors for the CEF pool and a "null" were used as controls. DCs were transduced with Ad5 vectors overnight at 37° C (5% CO_2). The following day, the Ad5 vectors were washed off the plate by three sequential centrifugation/aspiration steps using sterile Phosphate Buffered Saline. After the final wash, transduced DCs were resuspended in 100 µL media. Autologous CD3+ Pan-T cells isolated from human normal PBMCs were thawed using media. Pan-T cells were added to the irradiated DCs at 300,000 viable cells per well (100 µL/well). Human IL-15 (Peprotech) was added to all wells at final concentration of 10 ng/mL.

[0444] Plates were incubated at 37°C (5% CO_2) for a total of 12 days. Media was refreshed every 2-3 days with IL-15 (10 ng/mL final concentration) and IL-2 (R&D systems, 10 IU/mL final concentration). On day 11 lyophilized peptides (15-mer overlapping peptides) were solubilized in 100% DMSO and pooled by neoantigen to between 5 mg/mL and 20 mg/mL. Neoantigen peptides pools were added to cells for a final concentration of 2.5 µg/mL to 10 µg/mL. CEF Peptide Pool "Plus" (Cellular Technologies, Ltd.) was utilized as a positive control and DMSO at the same final concentration as the experimental peptides was utilized as a negative control. Protein Inhibitor Cocktail (eBioscience) was added to every well and plate was incubated overnight at 37° C (5% CO_2).

[0445] On day 12, cells were stained for intracellular flow cytometry analysis. The cells were washed with PBS and stained with Live/Dead Fixable Aqua Dead Cell stain (Thermo-Fisher). Following the live/dead stain, cells were blocked using Biotin-Free Fc Receptor Blocker (Accurate Chemical & Scientific Corp). Extracellular cellular flow panel (1 µL/antibody per well in 50 µL) consisted of CD3 PerCP-Cy5.5 (Biolegend), CD4 BV421 (Biolegend), and CD8 APC-Cy7 (Biolegend). After extracellular staining, cells were fixed using Foxp3/Transcription Factor Staining Buffer Set (eBioscience) and stained for intracellular proteins (1:50 dilution) using TNFα FITC (R&D Systems) and IFNγ BV785 (Biolegend). Cells were washed and resuspended in stain buffer and analyzed using a BD Celesta flow cytometer.

[0446] Flow cytometry cell staining analysis was completed using FlowJo v10. Cells were gated on live, singlet, CD3+ cells. The CD8+ and CD4+ T cells were analyzed for TNFα/IFNγ expression and the frequency of double positive TNFα/IFNγ CD8+ and the frequency of double positive TNFα/IFNγ CD4+ T cells were recorded. Responses were assessed to be positive when the frequency of double positive TNFα/IFNγ CD8+ or TNFα/IFNγ CD4+ T cells due to stimulation with an experimental peptide pool was increased greater than or equal to 3-fold over the DMSO only negative control for that donor and at least 0.01%.

In vitro binding of neoantigens to Class I MHC

[0447] The 9 mer peptides identified by bioinformatics analysis were analyzed for their binding propensities to 6 common HLA class I alleles (HLA-A*01:01, A*02:01, A*03:01, A*24:02, B*07:02, B*08:01). The principle of the method is briefly described below and consists of two parts, one involving exchange of peptide with a positive control induced by Ultraviolet (UV) radiation, and the second is an enzyme immunoassay to detect stable HLA-peptide and empty HLA complexes.

[0448] HLA-bound peptides are critical for the stability of the HLA complex. A conditional HLA class I complex was stabilized by an UV-labile peptide utilizing a different peptide (*Pos*) for each HLA (*Pos:* HLA-A*01:01: CTELKLSDY(SEQ ID NO: 409), HLA-A*02:01: NLVPMVATV (SEQ ID NO: 410), HLA-A*03:01: LIYRRRLMK (SEQ ID NO: 411), HLA-A*24:02: LYSACFWWL (SEQ ID NO: 412), HLA-B*07:02: NPKASLLSL (SEQ ID NO: 413), HLA-B*08:01: ELRSRYWAI (SEQ ID NO: 414), which could be cleaved by UV irradiation when bound to the HLA molecule. Upon cleavage, the resulting peptide fragments dissociated from the HLA class I complex since their length was insufficient to bind stably to HLA. Under the conditions in which peptide cleavage was performed (neutral pH, on melting ice), the peptide-free HLA complex remained stable. Thus, when cleavage was performed in the presence of another HLA class I peptide of choice, this reaction resulted in net exchange of the cleaved UV-labile peptide *Pos* with the chosen peptide (Rodenko, B et al. (2006) Nature Protocols 1: 1120-32, Toebes, M et al. (2006) Nat Med 12: 246-51, Bakker, AH et al. (2008) Proc Natl Acad Sci USA 105: 3825-30).

[0449] The exchange efficiency between the peptide of interest and *Pos* was analyzed using an HLA class I ELISA. The combined technologies allowed the identification of ligands for an HLA molecule of interest which are potentially immunogenic.

[0450] Exchange control peptide *Pos* was a high affinity binder to the relevant HLA class I allele while exchange control peptide Neg was a non-binder. The UV control represented UV-irradiation of conditional HLA class I complex in the absence of a rescue peptide. The binding of exchange control peptide *Neg* (HLA-A*01:01: NPKASLLSL (SEQ ID NO: 413), HLA-A*02-01: IVTDFSVIK (SEQ ID NO: 416), HLA-A*03:01: NPKASLLSL (SEQ ID NO: 413), HLA-A*24:02: NLVPMVATV (SEQ ID NO: 410), HLA-B*07:02: LIYRRRLMK (SEQ ID NO: 411), HLA-B*08:01: NLVPMVATV (SEQ ID NO: 410) and all experimental peptides were evaluated relative to that of exchange control peptide *Pos*. The absorption of the latter peptide was set at 100%. This procedure resulted in a range of different exchange percentages that reflected the affinities of the different experimental peptides for the HLA allele used.

[0451] The HLA class I ELISA is an enzyme immunoassay based on the detection of beta2-microglobulin (B2M) of (peptide-stabilized) HLA class I complexes. To this end streptavidin was bound onto polystyrene microtiter wells. After washing and blocking, HLA complex present in exchange reaction mixtures or ELISA controls was captured by the streptavidin on the microtiter plate via its biotinylated heavy chain. Non-bound material was removed by washing. Subsequently, horseradish

peroxidase (HRP)-conjugated antibody to human B2M was added. The HRP-conjugated antibody binds only to an intact HLA complex present in the microtiter well because unsuccessful peptide exchange results in disintegration of the original UV-sensitive HLA complex upon UV illumination. In the latter case B2M was removed during the washing step. After removal of non-bound HRP conjugate by washing, a substrate solution was added to the wells. A coloured product formed in proportion to the amount of intact HLA complex present in the samples. After the reaction was terminated by the addition of a stop solution, absorbance was measured in a microtiter plate reader. The absorbance was normalized to the absorbance of an exchange control peptide (represents 100%). Suboptimal HLA binding of peptides with a moderate to low affinity for HLA class I molecules can also be detected by this ELISA technique (Rodenko, B et al. (2006) Nature Protocols 1: 1120-32).

[0452] Peptides that had 10% or greater exchange efficiency in one of the 6 HLA alleles were considered for further immunogenicity testing and analysis.

Example 2. Identification of neoantigens by bioinformatics

[0453] A computational framework was developed to analyze various prostate cancer RNA-seq datasets by bioinformatics means to identify common prostate cancer neoantigens resulting from aberrant transcriptional programs such as gene fusion events, intron retention, alternatively spliced variants and aberrant expression of developmentally silenced genes.

[0454] The datasets queried were:

- The Genotype-Tissue Expression (GTEx) Consortium. This dataset encompasses 6137 RNA-seq datasets from 49 normal tissues and was used to annotate RNA features in normal tissues and assess frequency of potential prostate neoantigen candidates in normal tissue.
- The Cancer Genome Atlas Prostate Adenocarcinoma (TCGA PRAD) (Cancer Genome Atlas Research Network. Cell. 2015 Nov 5;163(4):1011-25. doi: 10.1016/j.cell.2015.10.025). This dataset encompasses RNA-seq datasets from 508 prostate cancer subjects and was used to identify neoantigen candidates in localized prostate adenocarcinoma.
- Stand Up To Cancer (SU2C) (Robinson D et al., Cell. 2015 May 21;161(5):1215-1228. doi: 10.1016/j.cell.2015.05.001). This dataset encompasses RNA-seq datasets from 43 mCRPC subjects.

[0455] Quality control (QC) of raw data was conducted prior to analysis. Sequencing reads were first trimmed to remove Illumina's adapter sequences and reads mapping to human tRNA and rRNA were removed from downstream analysis. Reads were also trimmed of bases with poor base quality score (<10, PHRED scale; indicating a base with a 1 in 10 probability of being incorrect) at either ends. PHRED quality score measures the quality of the identification of the bases generated by automated DNA sequencing instruments. Trimmed reads with less than 25 bps were removed from the datasets. Additionally, following QC steps were considered to remove poor quality reads: remove reads having maximal base quality PHRED score of <15, remove reads with average base quality PHRED score

of <10, remove reads having polyATCG rate >80%, remove RNA sequences in which one of the two reads failed.

[0456] Reads that passed the QC criteria were mapped to Human Genome Build 38 using ArrayStudio ((www_omicsoft_com/array-studio/) platform. Refseq gene model (release date June 6, 2017) was used for annotation of novel RNA features.

[0457] The results published here are in whole or part based upon data generated by The Cancer Genome Atlas managed by the NCI and NHGRI. Information about TCGA can be found at http://_cancergenme_nih_gov.

Identification of gene fusion events

[0458] FusionMap algorithm (Ge H et al., Bioinformatics. 2011 Jul 15; 27(14):1922-8. doi: 10.1093/bioinformatics/btr310. Epub 2011 May 18) was used to identify gene fusion events in the prostate cancer datasets described above. FusionMap detects fusion junctions based on seed reads which contain the fusion breakpoint position in the middle region of the reads. The algorithm dynamically creates a pseudo fusion transcript/sequence library based on the consensus of mapped fusion junctions from the seed reads. FusionMap then aligns unmapped possible fusion reads to the pseudo fusion reference to further identify rescued reads. The program reports a list of detected fusion junctions, statistics of supporting reads, fusion gene pairs, as well as genomic locations of breakpoints and junction sequences, which characterize fusion genes comprehensively at base-pair resolution.

[0459] Neoantigens originating from chimeric read-through fusions as shown in FIG. 1 and fusions resulting from chromosomal alterations as shown in FIG. 2 were identified using FusionMap. Neoantigens were classified as originating from gene fusion events when following criteria were met: fusion junction was supported by at least two seed reads with different mapping positions in the genome, at least 4 sequencing reads (seed and rescued reads) parsing the junction, and at least one junction spanning read. The prevalence of neoantigens were queried in tumor tissue and normal tissue using the datasets mentioned above. Neoantigens were identified as common when the prevalence was identified to be >10% in at least one disease cohort (TCGA and SU2C) and <2% in normal tissue (6137 RNA-seq datasets from 49 normal tissues). Gene fusion events with less than 10% prevalence in disease cohort were included if they generated long stretches of novel peptide sequences or were present in genes of interest.

Identification of splice variants

[0460] A custom bioinformatic software was developed to analyze paired-end RNA-seq data to identify potential neoantigens arising from alternative splicing events. Utilizing the developed process, splice variants with alternative 5' or 3' splice sites, retained introns, excluded exons, alternative terminations or insertion(s) of novel cassettes as show in in FIG. 3 were identified. The process identified splice variants that were not present in the RefSeq gene model through two main functionalities: 1)

Identification of novel junctions based on reads with gaps of 6 or more bp and sequences of at least 15 bp aligned on each side of the gap, henceforth referred to as split-mapped reads. Novel junctions were reported if they were represented by at least 5 split-mapped reads and one mate pair of reads flanking the junction on each end. 2) Identification of islands of aligned reads, henceforth referred to as coverage islands. Further details on parameters used for determining island boundaries are described below. FIG. 4 shows the cartoon of the approach.

[0461] In order to differentiate reads mapping to intronic regions due to true splicing variation as opposed to genomic DNA and/or pre-mRNA contamination, two parameters were developed to establish the distribution of contamination across 200 highly expressed housekeeping genes. The tail ends of these distributions were then used as cut-offs for discovery of novel splice variants where relevant.

1. Intron depth of coverage (IDC): 90th percentile depth of coverage for all housekeeping intronic bases. If the coverage of a particular region fell below this value, the first base where this occurred was defined as a coverage island boundary.
2. Intron/exon coverage ratio (IECR): 90th percentile of the ratio between median intron coverage and median coverage of the nearest upstream exon of all housekeeping gene introns.

[0462] All reported splice variants were required to meet the following criteria:

- Alternative 3'/5' splice site identification:
  - Novel splice site was supported by at least 5 split-mapped reads and one mate pair of reads flanking the junction
  - Intronic region resulting from using the splice site (if applicable) exceeded IECR and entire region exceeded IDC
- Novel cassette identification:
  - Two novel splice sites in an intronic region were supported by at least 5 split-mapped reads and one mate pair of reads flanking the junction
  - Region between the two splice sites exceeded IECR and entire region exceeded IDC
- Intron retention identification:
  - Intronic region exceeded IECR and entire region exceeded IDC
  - At least 5 reads span both intron-exon boundaries, with at least 15 bp aligned on each side of the boundaries
- Alternative termination identification:
  - 3' boundary defined as the edge of a coverage island that did not fall within 60 bp of the 3' end of a canonical exon
  - Any intronic regions between 5' end of a canonical exon and the 3' boundary exceeded IECR and entire region exceeded IDC
- Exon exclusion identification:

- Novel junction was supported by at least 5 split-mapped reads and one mate pair of reads flanking the junction where one or more canonical exons were skipped

[0463] Neoantigens derived from aberrant splicing events were identified as common when the incidence was identified to be about >10% in disease cohort (TCGA and SU2C datasets) and about <1% in normal tissue (GTEx Consortium dataset).

Identification of DNA mutations (point and frameshift) based neoantigens

[0464] The TCGA, SU2C and the integrated DFCI/Sloane Kettering datasets (Integrated DFCI/Sloane Kettering dataset (Armenia et al., Nat Genet. 2018 May;50(5):645-651. doi: 10.1038/s41588-018-0078-z. Epub 2018 Apr 2) as described above containing exome sequencing data from patients with prostate cancer were examined. Mutation calls published by the consortia that generated these datasets were downloaded, and gene mutations that were present in > 10% of the patient population or in genes known to be drivers of prostate cancer (such as AR) were identified. For each single point mutation chosen, a 17 mer peptide with the mutated amino acid at its center was identified for further validation studies.

Splicing isoform prediction

[0465] In certain cases, there were multiple reading frames and exons upstream of the identified splicing events that could impact the canonical peptide sequence preceding the neoepitope sequence. In these genes, it was determined which canonical exons neighbored each neoepitope feature based on the split-mapped reads present at the exon boundaries. The most highly expressed isoform with the highest average expression in the disease cohort with the highest prevalence of the event that could contain the predicted neoepitope was chosen for translation into the corresponding protein by choice of the open reading frame associated with the isoform. The neoepitope portion of the protein sequence was extracted, with an additional 8 amino acid residues upstream of the first altered amino acid included and used for subsequent validation studies. A similar procedure was followed to identify putative immunogenic antigens from DNA frameshift alterations. For both frameshift deletions and insertions, the resulting DNA sequence was translated into the corresponding protein by choice of the appropriate open reading frame, and the frameshift altered portion of the protein sequence was extracted, with an additional 8 amino acid residues upstream of the first altered amino acid included.

[0466] Table 1 shows the gene origin, the specific mutation, the amino acid sequences of identified neoantigens with single amino acid mutations (M) and frequency in patients. Each mutation is bolded in Table 1. Table 2 shows their corresponding polynucleotide sequences. The mutant sequences are capitalized in Table 2. Patient frequency (%) in Table 1 was obtained from Armenia *et al., Nat Genet* 50(5): 645-651, 2018.

Table 1.

Neoepitope ID	Gene	Mutation	Patient Frequency (%)	Amino acid sequence	SEQ ID NO:
M1	TP53	R248Q	1.0859	SSCMGGMN**Q**RPILTIIT	1
M2	TP53	R248W	0.0987	SSCMGGMN**W**RPILTIIT	3
M3	TP53	R273C	0.6910	LGRNSFEV**C**VCACPGRD	5
M4	TP53	R273L	0.3949	LGRNSFEV**L**VCACPGRD	7
M5	TP53	G245S	0.6910	MCNSSCMG**S**MNRRPILT	9
M6	TP53	Y220C	0.4936	FRHSVVVP**C**EPPEVGSD	11
M7	TP53	R282W	0.4936	VCACPGRD**W**RTEEENLR	13
M8	SPOP	F133C	0.5923	FVQGKDWG**C**KKFIRRDF	15
M9	SPOP	F133I	0.3949	FVQGKDWG**I**KKFIRRDF	17
M10	SPOP	F133L	1.1846	FVQGKDWG**L**KKFIRRDF	19
M11	SPOP	F133S	0.3949	FVQGKDWG**S**KKFIRRDF	21
M12	SPOP	F133V	0.9872	FVQGKDWG**V**KKFIRRDF	23
M13	SPOP	W131C	0.0987	YRFVQGKD**C**GFKKFIRR	25
M14	SPOP	W131G	1.2833	YRFVQGKD**G**GFKKFIRR	27
M15	SPOP	W131L	0.1974	YRFVQGKD**L**GFKKFIRR	29
M16	SPOP	W131R	0.1974	YRFVQGKD**R**GFKKFIRR	31
M17	SPOP	W131S	0.0987	YRFVQGKD**S**GFKKFIRR	33
M18	KMT2D	R5214H	0.1974	YPVGYEAT**H**IYWSLRTN	35
M19	FOXA1	R261C	0.1974	MFENGCYL**C**RQKRFKCE	37
M20	FOXA1	H247Q	0.1974	GKGSYWTL**Q**PDSGNMFE	39
M21	FOXA1	H247L	0.0987	GKGSYWTL**L**PDSGNMFE	41
M22	FOXA1	H247N	0.0987	GKGSYWTL**N**PDSGNMFE	43
M23	FOXA1	H247Y	0.0987	GKGSYWTL**Y**PDSGNMFE	45
M24	FOXA1	F266C	0.0987	CYLRRQKR**C**KCEKQPGA	47
M25	FOXA1	F266S	0.0987	CYLRRQKR**S**KCEKQPGA	49
M26	FOXA1	D226G	0.0987	IRHSLSFN**G**CFVKVARS	51
M27	FOXA1	D226N	0.1974	IRHSLSFN**N**CFVKVARS	53
M28	FOXA1	R219C	0.0987	QQRWQNSI**C**HSLSFNDC	55
M29	FOXA1	R219S	0.1974	QQRWQNSI**S**HSLSFNDC	57
M30	FOXA1	M253K	0.1974	TLHPDSGN**K**FENGCYLR	59
M31	FOXA1	M253R	0.0987	TLHPDSGN**R**FENGCYLR	61
M32	CDK12	R858W	0.1974	CHKKNFLH**W**DIKCSNIL	63
M33	PTEN	R130Q	0.2962	IHCKAGKG**Q**TGVMICAY	65
M34	PTEN	V119F	0.1974	WLSEDDNH**F**AAIHCKAG	67
M35	ATM	N2875S	0.0987	GLGDRHVQ**S**ILINEQSA	69
M36	ATM	N2875K	0.0987	GLGDRHVQ**K**ILINEQSA	71
M37	KDM6A	C1164S	0.0987	NINIGPGD**S**EWFVVPEG	73
M38	KDM6A	C1164Y	0.0987	NINIGPGD**Y**EWFVVPEG	75
M39	PIK3CA	H1047R	0.4936	FMKQMNDA**R**HGGWTTKM	77
M40	PIK3CA	E545K	0.2962	RDPLSEIT**K**QEKDFLWS	79
M41	PIK3CA	E545G	0.0987	RDPLSEIT**G**QEKDFLWS	81

M42	PIK3CA	E545A	0.0987	RDPLSEIT**A**QEKDFLWS	83
M43	CTNNB1	T41A	0.4936	SGIHSGAT**A**TAPSLSGK	85
M44	CTNNB1	D32A	0.0987	HWQQQSYL**A**SGIHSGAT	87
M45	CTNNB1	D32H	0.0987	HWQQQSYL**H**SGIHSGAT	89
M46	CTNNB1	D32V	0.0987	HWQQQSYL**V**SGIHSGAT	91
M47	CTNNB1	D32Y	0.1974	HWQQQSYL**Y**SGIHSGAT	93
M48	CTNNB1	S37A	0.0987	SYLDSGIH**A**GATTTAPS	95
M49	CTNNB1	S37C	0.0987	SYLDSGIH**C**GATTTAPS	97
M50	CTNNB1	S37F	0.0987	SYLDSGIH**F**GATTTAPS	99
M51	CTNNB1	S37Y	0.0987	SYLDSGIH**Y**GATTTAPS	101
M52	CTNNB1	S45C	0.0987	SGATTTAP**C**LSGKGNPE	103
M53	CTNNB1	S45F	0.0987	SGATTTAP**F**LSGKGNPE	105
M54	CTNNB1	S45P	0.0987	SGATTTAP**P**LSGKGNPE	107
M55	COL5A1	T348K	0.1974	YVPSEDYY**K**PSPYDDLT	109
M56	TAF1L	A869T	0.1974	IRKRLKLC**T**DFKRTGMD	111
M57	MED12	L1224F	0.7897	VDGAVFAV**F**KAVFVLGD	113
M58	MED12	V1223G	0.0987	IVDGAVFA**G**LKAVFVLG	115
M59	MED12	V1223L	0.0987	IVDGAVFA**L**LKAVFVLG	117
M60	MGA	R2435W	0.1974	THTANERR**W**RGEMRDLF	119
M61	ARID1A	P1756R	0.1974	GRFSKVSS**R**APMEGGEE	121
M62	CUL3	M299R	0.4936	GKTEDLGC**R**YKLFSRVP	123
M63	USP7	Q4H	0.4936	MNH**H**QQQQQQKA	125
M64	SF3B1	K700E	0.1974	HGLVDEQQ**E**VRTISALA	127
M65	U2AF1	S34F	0.2962	VCRHGDRC**F**RLHNKPTF	129
M66	CDC27	Y73C	0.1974	SCTTPQCK**C**LLAKCCVD	131
M67	CDC27	N260H	0.1974	SILSKQVQ**H**KPKTGRSL	133
M68	BRAF	G469A	0.2962	QRIGSGSF**A**TVYKGKWH	135
M69	BRAF	K601E	0.1974	GDFGLATV**E**SRWSGSHQ	137
M70	RAG1	R112C	0.0987	QANLRHLC**C**ICGNSFRA	139
M71	RAG1	R112H	0.1974	QANLRHLC**H**ICGNSFRA	141
M72	CNOT3	E20K	0.3949	DRCLKKVS**K**GVEQFEDI	143
M73	CNOT3	E70K	0.2962	IKTWVASN**K**IKDKRQLI	145
M74	PIK3CB	E1051K	0.2962	QKFDEALR**K**SWTTKVNW	147
M75	IDH1	R132C	0.1974	WVKPIIIG**C**HAYGDQYR	149
M76	IDH1	R132G	0.0987	WVKPIIIG**G**HAYGDQYR	151
M77	IDH1	R132H	0.4936	WVKPIIIG**H**HAYGDQYR	153
M78	KRAS	G12D	0.1974	YKLVVVGA**D**GVGKSALT	155
M79	KRAS	G12R	0.2962	YKLVVVGA**R**GVGKSALT	157
M80	KRAS	Q61K	0.2962	LDILDTAG**K**EEYSAMRD	159
M81	KRAS	Q61L	0.0987	LDILDTAG**L**EEYSAMRD	161
M82	KRAS	Q61R	0.0987	LDILDTAG**R**EEYSAMRD	163
M83	AKT1	E17K	0.4936	EGWLHKRG**K**YIKTWRPR	165
M84	AR	T878A	1.2833	IARELHQFAFDLLIKSH	167
M85	AR	T878G	0.0987	IARELHQF**G**FDLLIKSH	169
M86	AR	L702H	1.0859	QPDSFAALHSSLNELGE	171

M87	AR	W742L	0.1974	QMAVIQYSLMGLMVFAM	173
M88	AR	W742F	0.0987	QMAVIQYSFMGLMVFAM	175

Table 2.

Neoepitope ID	Polynucleotide sequence	SEQ ID NO:
M1	agttcctgcatgggcggcatgaaccAgaggcccatcctcaccatcatcaca	2
M2	agttcctgcatgggcggcatgaaTTggaggcccatcctcaccatcatcaca	4
M3	ctgggacggaacagctttgaggtgTgtgtttgtgcctgtcctgggagagac	6
M4	ctgggacggaacagctttgaggtgcTtgtttgtgcctgtcctgggagagac	8
M5	atgtgtaacagttcctgcatgggcAgcatgaaccggaggcccatcctcacc	10
M6	tttcgacatagtgtggtggtgccctGtgagccgcctgaggttggctctgac	12
M7	gtttgtgcctgtcctgggagagaTTggcgcacagaggaagagaatctccgc	14
M8	tttgtgcaaggcaaagactggggatGcaagaaattcatccgtagagatttt	16
M9	tttgtgcaaggcaaagactggggaAtcaagaaattcatccgtagagatttt	18
M10	tttgtgcaaggcaaagactggggattAaagaaattcatccgtagagatttt	20
M11	tttgtgcaaggcaaagactggggatCcaagaaattcatccgtagagatttt	22
M12	tttgtgcaaggcaaagactggggaGtcaagaaattcatccgtagagatttt	24
M13	tataggtttgtgcaaggcaaagactgTggattcaagaaattcatccgtaga	26
M14	tataggtttgtgcaaggcaaagacGggggattcaagaaattcatccgtaga	28
M15	tataggtttgtgcaaggcaaagactggggattcaagaaattcatccgtaga	30
M16	tataggtttgtgcaaggcaaagacCggggattcaagaaattcatccgtaga	32
M17	tataggtttgtgcaaggcaaagactCgggattcaagaaattcatccgtaga	34
M18	tatcccgtgggctacgaggccacgcAcatctattggagcctccgcaccaac	36
M19	atgttcgagaacggctgctacttgTgccgccagaagcgcttcaagtgcgag	38
M20	ggcaagggctcctactggacgctgcaGccggactccggcaacatgttcgag	40
M21	ggcaagggctcctactggacgctgcTcccggactccggcaacatgttcgag	42
M22	ggcaagggctcctactggacgctgAacccggactccggcaacatgttcgag	44
M23	ggcaagggctcctactggacgctgTacccggactccggcaacatgttcgag	46
M24	tgctacttgcgccgccagaagcgctGcaagtgcgagaagcagccgggggcc	48
M25	tgctacttgcgccgccagaagcgctCcaagtgcgagaagcagccgggggcc	50
M26	atccgccactcgctgtccttcaatgGctgcttcgtcaaggtggcacgctcc	52
M27	atccgccactcgctgtccttcaatAactgcttcgtcaaggtggcacgctcc	54
M28	cagcagcgctggcagaactccatcTgccactcgctgtccttcaatgactgc	56
M29	cagcagcgctggcagaactccatcAgccactcgctgtccttcaatgactgc	58
M30	acgctgcacccggactccggcaacaAgttcgagaacggctgctacttgcgc	60
M31	acgctgcacccggactccggcaacaGgttcgagaacggctgctacttgcgc	62
M32	tgtcacaaaaagaatttcctgcatTgggatattaagtgttctaacattttg	64
M33	attcactgtaaagctggaaagggacAaactggtgtaatgatatgtgcatat	66
M34	tggctaagtgaagatgacaatcatTttgcagcaattcactgtaaagctgga	68
M35	ggacttggtgatagacatgtacagaGtatcttgataaatgagcagtcagca	70
M36	ggacttggtgatagacatgtacagaaAatcttgataaatgagcagtcagca	72
M37	aacataaatattggcccaggtgactCtgaatggtttgttgttcctgaaggt	74
M38	aacataaatattggcccaggtgactAtgaatggtttgttgttcctgaaggt	76

M39	ttcatgaaacaaatgaatgatgcacGtcatggtggctggacaacaaaaatg	78
M40	cgagatcctctctctgaaatcactAagcaggagaaagattttctatggagt	80
M41	cgagatcctctctctgaaatcactgGgcaggagaaagattttctatggagt	82
M42	cgagatcctctctctgaaatcactgCgcaggagaaagattttctatggagt	84
M43	tctggaatccattctggtgccactGccacagctccttctctgagtggtaaa	86
M44	cactggcagcaacagtcttacctggCctctggaatccattctggtgccact	88
M45	cactggcagcaacagtcttacctgCactctggaatccattctggtgccact	90
M46	cactggcagcaacagtcttacctggTctctggaatccattctggtgccact	92
M47	cactggcagcaacagtcttacctgTactctggaatccattctggtgccact	94
M48	tcttacctggactctggaatccatGctggtgccactaccacagctccttct	96
M49	tcttacctggactctggaatccattGtggtgccactaccacagctccttct	98
M50	tcttacctggactctggaatccattTtggtgccactaccacagctccttct	100
M51	tcttacctggactctggaatccattAtggtgccactaccacagctccttct	102
M52	tctggtgccactaccacagctccttGtctgagtggtaaaggcaatcctgag	104
M53	tctggtgccactaccacagctccttTtctgagtggtaaaggcaatcctgag	106
M54	tctggtgccactaccacagctcctCctctgagtggtaaaggcaatcctgag	108
M55	tacgtgcccagtgaggactactacaAgccctcaccgtatgatgacctcacc	110
M56	atccggaagaggctaaagctctgcActgacttcaaacgcacagggatggat	112
M57	gtggatggagccgtgtttgctgttTtcaaggctgtgtttgtacttggggat	114
M58	atcgtggatggagccgtgtttgctgGtctcaaggctgtgtttgtacttggg	116
M59	atcgtggatggagccgtgtttgctCttctcaaggctgtgtttgtacttggg	118
M60	acacacactgccaatgagcggcggTggcgtggtgaaatgagggatctcttt	120
M61	gggaggttcagcaaggtgtctagtcGagctcccatggagggtggggaagaa	122
M62	ggaaagacagaagaccttggttgcaGgtacaagttatttagtcgtgtgcca	124
M63	atgaaccaccaCcagcagcagcagcagcagaaagcg	126
M64	catggtcttgtggatgagcagcagGaagttcggaccatcagtgctttggcc	128
M65	gcatgtcgtcatggagacaggtgctTtcggttgcacaataaaccgacgttt	130
M66	agttgtactacaccgcaatgcaaatGcctgcttgcaaaatgttgtgttgat	132
M67	tccatattatctaaacaggttcaaCataaaccaaaaactggtcgaagttta	134
M68	caaagaattggatctggatcatttgCaacagtctacaagggaaagtggcat	136
M69	ggtgattttggtctagctacagtgGaatctcgatggagtgggtcccatcag	138
M70	caagccaaccttcgacatctctgcTgcatctgtgggaattcttttagagct	140
M71	caagccaaccttcgacatctctgccAcatctgtgggaattcttttagagct	142
M72	gatcgctgcctcaagaaggtgtccAagggcgtggagcagtttgaagatatt	144
M73	atcaagacatgggtagcgtccaacAagatcaaggacaagaggcagcttata	146
M74	caaaaatttgatgaggcgctcaggAaaagctggactactaaagtgaactgg	148
M75	tgggtaaaacctatcatcataggtTgtcatgcttatggggatcaatacaga	150
M76	tgggtaaaacctatcatcataggtGgtcatgcttatggggatcaatacaga	152
M77	tgggtaaaacctatcatcataggtcAtcatgcttatggggatcaatacaga	154
M78	tataagctggtggtggtgggcgccgAcggtgtgggcaagagtgcgctgacc	156
M79	tataagctggtggtggtgggcgccCgcggtgtgggcaagagtgcgctgacc	158
M80	ttggacatcctggataccgccggAAaggaggagtacagcgccatgcgggac	160
M81	ttggacatcctggataccgccggccTggaggagtacagcgccatgcgggac	162
M82	ttggacatcctggataccgccggccGggaggagtacagcgccatgcgggac	164
M83	gagggttggctgcacaaacgagggAagtacatcaagacctggcggccacgc	166

M84	attgcgagagagctgcatcagttcGcttttgacctgctaatcaagtcacac	168
M85	attgcgagagagctgcatcagttcGGttttgacctgctaatcaagtcacac	170
M86	cagcccgactcctttgcagccttgcActctagcctcaatgaactgggagag	172
M87	cagatggctgtcattcagtactcctTgatggggctcatggtgtttgccatg	174
M88	cagatggctgtcattcagtactcctTTatggggctcatggtgtttgccatg	176

[0467] Table 3 shows the gene origin, the specific frameshift mutation (FR), the amino acid sequences of the identified neoantigens that arose from frameshift events and frequency of the mutation in patients. The wild-type sequence is bolded in Table 3, followed by the novel sequence due to frameshift. Table 4 shows their corresponding polynucleotide sequences. The mutant sequences are capitalized in Table 4. Patient frequency (%) in Table 3 was obtained from Armenia *et al., Nat Genet* 50(5): 645-651, 2018.

Table 3.

Neoepitope ID	Gene	Frameshift	Patient Frequency (%)	Amino acid sequence	SEQ ID NO:
FR1	ZFHX3	E763Sfs*61	0.2962	**QNLQNGGG**SRSSATLPGRRRRRWLRRRRQPISVAPAGPPRRPNQKPNPPGGARCVIMRPTWPGTSAFT	177
FR2	ZFHX3	E763Gfs*26	0.0987	**QNLQNGGG**GAGLQPHCRGGGGGGGCGGGGSQYQ	179
FR3	APC	T1556Nfs*3	0.3949	**NQEKEAEK**NY	181
FR4	SPEN	A2105Lfs*33	0.1974	**DAAVSPRG**LQHRQGRGNLGWWQSPLRKVRVPKRRMVYHPS	183
FR5	BRCA2	T3085Nfs*26	0.1974	**FVVSVVKK**NRTCPFRLFVRRMLQFTGNKVLDRP	185
FR6	BRCA2	K2674Rfs*2	0.1974	**RSRRSAIK**R	187
FR7	ARID4A	S1067Rfs*16	0.2962	**SIIVQERE**RAERRVRRGQVMEIVD	189
FR8	SMARCAD1	N770Kfs*28	0.1974	**NNLVTEKK**HRNVQCHDAVEENGQSSFITSPILHS	191
FR9	RNF43	G659Vfs*41	0.3949	**HPQRKRRG**VPPSPPLALGPRMQLCTQLARFFPITPPVWHILGPQRHTP	193
FR10	AXIN2	G665Afs*24	0.1974	**ASRHHLWG**ATAGTPAPPPVPTCSPRTLRCLP	195
FR11	ERF	L525Sfs*6	0.2962	**GPGEAGGP**SPQGG	197
FR12	ERF	G299Efs*12	0.2962	**GGGPSGSG**EAPTSPSALRT	199

FR13	CHD3	R599Vfs*16	0.3949	**GNPDVPPP**VLFKADQSESSLSSG	201
FR14	KMT2C	S143Vfs*3	0.2962	**AFCYCGEK**VP	203
FR15	FOXA1	M253_N256del	0.0987	**TLHPDSGN**GCYLRRQK	205
FR16	FOXA1	F254_N256delinsY	0.2962	**LHPDSGNM**YGCYLRRQ	207
FR17	FOXA1	F254_G257delinsC	0.0987	**LHPDSGNM**CCYLRRQKR	209

Table 4.

Neoantigen ID	Polynucleotide sequence	SEQ ID NO:
FR1	Cagaacctgcagaatggaggggggagcaggtcttcagccacactgccggggcggcggcggcggcggtggctgcggcggcggcggcagccaatatcagtagctcctgcggggcccctcgccgaccaaaccaaaaaccaaacccacctggcggtgcgaggtgtgtgattatgagaccaacgtggccaggaacctccgcattcaca	178
FR2	cagaacctgcagaatggaggggggGgagcaggtcttcagccacactgccggggcggcggcggcggcggtggctgcggcggcggcggcagccaatatcagtag	180
FR3	aaccaagagaaagaggcagAaaaaaactattga	182
FR4	gatgctgctgtcagtcccaggggctgcagcacaggcaggggagagggaatctggggtggtggcagtctcccctgagaaaagtgagagtccccaaaaggaggatggtttatcatcccagttga	184
FR5	tttgtcgtttctgttgtgaAaaaaaacaggacttgccccttcgtctatttgtcagacgaatgttacaatttactggcaataaagttttggatagaccttaa	186
FR6	agaagcagaagatcggctataaaaagataatg	188
FR7	agtataattgtacaAGagagagagagagcagagagaagggtcagaagaggccaagtgatggaaatagtggattaa	190
FR8	aataacttggtcacagAaaaaaaacacagaaatgtgcaatgtcatgatgcagttgaggaaaatggccaatcatcctttattacatcgccaatattacacagctgaaa	192
FR9	cacccacagaggaaaaggcgggggtccctccgagcccacccctggctctcggccccaggatgcaactgtgcacccagcttgccagatttttccccattacacccccagtgtggcatatccttggtccccagaggcacacccccttgatc	194
FR10	gccagccggcaccatctgtgggggggcaacagcgggcacccccgcaccacccccccgtgcccacctgttcacccaggaccctgcgatgcctccccctgacc	196
FR11	gggcctggggaggctgggggcccctcaccccaaggcgggtgagc	198
FR12	ggcgggggcccagcggctcaggggaggctcccacttctccttcagccctgaggacatgaaa	200
FR13	ggaaatccagatgtcccacccccgtcctcttcaaggcagatcagagcgagagttctttgtcaagtgggtag	202
FR14	gctttttgttactgtggggaaaaagttccttagga	204
FR15	acgctgcacccggactccggcaacggctgctacttgcgccgccagaagcg	206
FR16	acgctgcacccggactccggcaacatgtacggctgctacttgcgccgccagaa	208

FR17	ctgcacccggactccggcaacatgtgctgctacttgcgccgccagaagcgc	210

[0468] Table 5 shows the gene origin and amino acid sequences of the identified neoantigens that arose from gene fusion (FUS) events. Table 6 shows their corresponding polynucleotide sequences. Table 7 shows the prevalence of the FUS neoantigens in analyzed databases.

Table 5.

Neoantigen ID	Fusion Gene	Amino acid sequence	SEQ ID NO:
FUS1	SLC45A3->ELK4	CGASACDVSLIAMDSA	211
FUS2	ARHGEF38->ARHGEF38-IT1	TEYNQKLQVNQFSESK	213
FUS3	MSMB->NCOA4	TEISCCTLSSEENEYLPRPEWQLQ	215
FUS4	LIPE->CNFN	GLVSFGEHFCLPCALC	217
FUS5	TMPRSS2->ERG	NSKMALNSEALSVVSE	219
FUS6	TMPRSS2->ERG	CEERGAAGSLISCE	221
FUS7	NME4->DECR2	LWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSARTCCGTKWPSSQEAALGLGSGLLRFSCGTAAIR	223
FUS8	INCA1->CAMTA2	WGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLPLRTLAGCLARTAHLRPGAESLPQPQLHCT	225
FUS9	AP5S1->MAVS	KEQILAVASLVSSQSIHPSWGQSPLSRI	227
FUS10	DIP2A->DIP2A-IT1	LELELSEGVCFRLR	229
FUS11	MBTPS2->YY2	QQLRIFCAAMASNEDFS	231
FUS15	D2HGDH->GAL3ST2	HVVGYGHLDTSGSSSSSSWP	345
FUS18	OPN3->CHML	DGFSGSLFAVVTRRCYFLKWRTIFPQSLMWL	233
FUS19	GTF2F1->PSPN	KMHFSLKEHPPPPCPP	235

FUS23	NUDT14->JAG2	DLRRVATYCAPLPSSWRPGTGTTIPPRMRSC	237
FUS24	DMPK->SIX5	LQERMELLACGAERGAGGWGGGGGGGGG DRRGGGGSAPALADFAGGRG	239

Table 6.

Neoantigen ID	Polynucleotide sequence	SEQ ID NO:
FUS1	TGCGGGGCCTCTGCCTGTGATGTCTCCCTCATTGCTA TGGACAGTGCT	212
FUS2	ACCGAATACAACCAGAAATTACAAGTGAATCAATTT AGTGAATCCAAA	214
FUS3	ACAGAAATTTCATGTTGCACCCTGAGCAGTGAGGAG AATGAATACCTTCCAAGACCAGAGTGGCAGCTCCAG	216
FUS4	GGGCTGGTGTCCTTCGGGGAGCACTTTTGTCTGCCCT GCGCCCTCTGCCA	218
FUS5	AACAGCAAGATGGCTTTGAACTCAGAAGCCTTATCA GTTGTGAGTGAG	220
FUS6	TGTGAGGAGCGCGGCGCGGCAGGAAGCCTTATCAGT TGTGAG	222
FUS7	CTGTGGTTCCAGAGCAGTGAGCTGTCCCCGACGGGA GCGCCATGGCCCAGCCGCCGCCCGACGTGGAGGGGG ACGACTGTCTCCCCGCGTACCGCCACCTCTTCTGCCC GGACCTGCTGCGGGACAAAGTGGCCTTCATCACAGG AGGCGGCTCTGGGATTGGGTTCCGGATTGCTGAGAT TTTCATGCGGCACGGCTGCCATACGG	224
FUS8	TGGGGGATGGAGTTGGCAGCGTCTCGGAGGTTCTCC TGGGACCACCACTCCGCCGGGGGGCCGCCCAGAGTG CCAAGCGTCCGATCCGGCGCCGCCCAAGTGCAGCCC AAGGACCCGCTCCCGCTCCGCACCCTGGCAGGCTGC CTAGCCAGGACTGCGCACCTGCGCCCTGGGGCGGAG TCCTTACCCCAACCCCAGCTTCACTGCACA	226
FUS9	AAGGAACAGATTTTAGCTGTGGCCAGTCTCGTTTCCT CTCAGTCCATCCACCCTTCATGGGGCCAGAGCCCTCT CTCCAGAATC	228
FUS10	CTGGAGCTGGAGCTGTCGGAAGGAGTCTGCTTCAGA TTAAGA	230
FUS11	CAGCAGCTAAGGATATTTTGTGCAGCCATGGCCTCC AACGAAGATTTCTCCA	232
FUS15	CACGTGGTGGGCTATGGCCACCTTGATACTTCCGGGT CATCCTCCTCCTCCTCCTGGCCC	346
FUS18	GACGGGTTTAGCGGCAGCCTCTTCGCAGTTGTCACC AGACGCTGTTACTTCCTAAAATGGCGGACAATCTTCC CACAGAGTTTGATGTGGTTA	234

FUS19	AAAATGCACTTCTCCCTCAAGGAGCACCCACCGCCC CCTTGCCCGCCT	236
FUS23	GATCTGCGCCGGGTCGCCACATACTGCGCTCCTTTAC CCTCATCGTGGAGGCCTGGGACTGGGACAACGATAC CACCCCGAATGAGGAGCTGC	238
FUS24	TTGCAGGAGCGGATGGAGTTGCTTGCCTGCGGAGCC GAGCGCGGGGCCGGCGGCTGGGGGGGAGGCGGTGG CGGCGGCGGCGGCGACCGAAGAGGAGGAGGAGGAA GCGCGCCAGCTCTTGCAGACTTTGCAGGCGGCCGAG GG	240

Table 7.

Neoantigen ID	TCGA (%)	SU2C (%)
FUS1	30.51	23.26
FUS2	63.58	46.51
FUS3	35.04	23.26
FUS4	12.20	11.63
FUS5	12.40	18.60
FUS6	21.46	32.56
FUS7	3.35	16.28
FUS8	1.18	32.56
FUS9	N.O.	18.60
FUS10	N.O.	13.95
FUS11	1.57	13.95
FUS15	0.39	9.30
FUS18	0.39	9.30
FUS19	8.86	30.23
FUS23	N.O.	9.30
FUS24	N.O.	9.30
N.O. not observed		

[0469] Table 8 shows the gene origin and amino acid sequences of the identified neoantigens that arose from alternative splicing (AS) events. Table 9 shows their corresponding polynucleotide sequences. Table 10 shows the prevalence of the AS neoantigens in analyzed databases.

Table 8.

Neoepitope ID	Gene	Amino acid sequence	SEQ ID NO:
AS1	ABCC4	**LTFLDFIQ**VTLRVMSGSQMENGSSYFFK PFSWGLGVGLSAWLCVMLT	241
AS2	SLC30A4	**FMIGELVG**ELCCQLTFRLPFLESLCQAV VTQALRFNPSFQEVCIYQDTDLM	243
AS3	DNAH8	**VAMMVPDR**QVHYDFGL	245
AS4	NCAPD3	**WCPLDLRL**GSTGCLTCRHHQTSHE	247
AS5	DHDH	**VVGRRHET**APQPLLVPDRAGGEGGA	249
AS6	ACSM1	**DYWAQKEK**ISIPRTHLC	251
AS7	ACSM1	**DYWAQKEK**GSSSFLRPSC	253
AS8	CACNA1D	**LVLGVLSG**HSGSRL	255
AS9	CACNA1D	**PVPTATPG**VRSVTSPQGLGLFLKFI	257
AS10	CHRNA5	**KENDVREV**CDVYLQMQIFFHFKFRSYF H	259
AS11, AS33	CPNE7	**VPFRELKN**QRTAQGAPGIHHAASPVAA NLCDPARHAQHTRIPCGAGQVRAGRGP EAGGGVLQPQRPAPEKPGCPCRRGQPRL HTVKMWRA	261
AS12	EVPL	**FARKMLEK**VHRQHLQLSHNSQE	263
AS13	GRIN3A	**KRSFAVTE**RII	265
AS14	IQCG	**MFLRKEQQ**VGPHSFSML	267
AS15	LRRC45	**VLRFLDLK**VRYLHS	269
AS16	LRRC45	**GNTTLQQL**GEASQAPSGSLIPLRLPLLW EVRG	271
AS17	MPHOSPH 9	**GLNLNTDR**PGGYSYSIWWKNNAKNR	273
AS18	NWD1	**WKFEMSYT**VGGPPPHVHARPRHWKTD R	275
AS19	NWD1	**QWQHYHRS**GEAAGTPLWRPTRN	277
AS20	PFKFB4	**KVLNEIDA**VVTVPPSLSTSQIPQGCCIIL	279
AS21	RECQL4	**ANLKGTLQ**VRSGQAVSPR	281
AS22	TONSL	**LQAAASGQ**GKQGVPCPWGCCAYAESP RALISGDAPSQVEREVPGPCLNTHSLSHR SPQLPGLPHPKQPSV	283
AS23	ZNF614	**KIQNKNCPD**	285

AS32	TONSL	**GEVELSEG**GEGQRHLAFPWACSGPGWR GVCCAAVEPA	287
AS63	TDRD1	**IEMKKLLKS**	289
AS34	LRRC45	**KMRAIQAE**GGHGQACCGGAWGWAPG DGGPQGMLTHTLPTLGFQSAWTWRRED ADRAWRTPKACASRRWSI	291
AS35	AMACR	**LLEPFRRG**EPGPRGLLSGSSRGGEGPGR SIEAAPATPLPCCRKNPCRPQPSRFLPPRV LLVIILPKLDCPKLGF	293
AS36	CCNF	**PSGRRTKR**LVTLRSGCAIQCWHPRAGP VPSALPHTERPPRLVRGAADPRTVTLGR SPAVMPRAPA	295
AS37	RECQL4	**CHLFLQPQ**VGTPPPHTASARAPSGPPHP HESCPAGRRPARAAQTCARRQHGLPGC EEAGTARVPSLHLHLHQAALGAGRGRG WGEACAQVPPSRG	297
AS38	LRRC45	**KELKLEQQ**VGGQGLRGVGQGVRGGFV TLTTHTPFPSQ**EAAERESK**	299
AS39	CCNF	**GEISQEEV**PPSRHLGVSWGAGVWAGLT LGASAPPNSSFPSGAELQPVVCCIRSDTR QPRPPDFPQHRGDPRLPQLSLGAENQTV SYPAFWLRHTMLASSCRPSSLSASSHRE APKACQGSSRSQDSDPGTEPCSHASGPC VTSTVSSPGLLPQRLLPLALTGLPVEEDG FEHAGA	301
AS40	LRRC45	**DCMLSEEG**GQARRGGSLCSLAAHTIAS AARGRFLSRLSNFCAVVKASRGAPSCTW E	303
AS41	RHPN1	**EAFQRAAG**EGGPGRGGARRGARVLQSP FCRAGAGEWLGHQSLR	305
AS42	SLC39A4	**PEPRRLSP**GEPRGRPRKGWGIWGLCGA RVGPKAWR	307
AS43	CPNE7	**VPFRELKN**VSVLEGLRQGRLGGPCSCH CPRPSQARLTPVDVAGPFLCLGDPGLFPP VKSSI	309
AS44	FASN	**FVSLTAIQ**MASSATPWGRWPVATPTAA CPRRRPSSLPTGGDSASKKPISRRAPWQP WACPGRSVNSAAPRAWCPPATTPRTQSP SRDLRPRCLSSWSS	311
AS45	RBM47	**PVAIKPGT**GPPNNSSIHGGSKRSENSYCR DLRGQLRAICCSSYSHDRHTTEERGSRG RHVWRIRRLHTSGLPCCCHSGPHPRRLP DILRLVTSTKTDHTNTTEGTLDYL	313
AS46	SERINC5	**KWNKNWTA**TLGALTIRGHKLLCHLPHL LSSVQQTCRSSSR	315
AS47	AGRN	**FKKFDGPC**GERGGGRTARALWARGDS VLTPALDPQTPVRAPSLTRAAAAV	317

AS48	SYT17	**ENASLVFT**GSNSPIPACELSSHPAHGISP WIPSPGNEHFHGIKKQVKAIKVE	319
AS49	PDF	**RLTQRLVQ**GWTPMENRWCGRRAGGQP ASSSTRWTTCRAACLLTKWTAGRSQTSI G	321
AS50	LRIF1	**ENSGNASR**WLHVPSSSDDWLGWKKSSA ITSNS	323
AS51	CPNE7	**GMECTLGQ**VGAPSPRREEDGWRGGHS RFKADVPAPQGPCWGGQPGSAPSSAPPE QSLLD	325
AS52	ILDR1	**KGSVERRS**VSLGHPAEGWAWAERSLQP GMTTANTGCLSFHHRGCLLPVLPKLHCG LGGLPLVRAKEIKRVQRAGESSLPVKGL LTVASAVIAVLWGRPSEVTGENEAQHD	327
AS53	PEX10	**FGLTTLAG**RSSHGTSGLRAATHTKSGD GGQGAARQCEKLLELARATRPWGRSTS ASSRWTHRGYMCPPRCAVACW	329
AS54	ABCC4	**IIDSDKIM**AVCMGCLLTRHVQCQAMEM QQ	331
AS55	SPOCK1	**DGHSYTSK**VNCLLLQDGFHGCVSITGA AGRRNLSIFLFL**MLCKLEFHAC**	333
AS56	TM9SF3	**LLNAEDYR**CAIHSKEIYLLSPSPHQAMD KFSLCCINCNLCLHVFLLLLFFQNKDVW LISNIILLWIYGGI	335
AS57	KLK3	**TGGKSTCS**APGPQSLPSTPFSTYPQ**WVI LITEL**	337
AS58	CREB3L1	**VETLENAN**SFSSGIQPLLCSLIGLENPT	339
AS59	ACSL3	**AGAGTISE**GSVLHGQRLECDARRFFGCG TTILAEWEHH	341
AS55.1	SPOCK1	**DGHSYTSK**VNCLLLQDGFHGCVSITGA AGRRNLSIFLFLMLCKLEFHA	385

Table 9.

Neoepitope ID	Polynucleotide sequence	SEQ ID NO:
AS1	CTGACGTTTTTAGATTTCATCCAGGTAACGTTGAGAGT AATGTCAGGATCTCAAATGGAAAACGGAAGTTCCTAT TTTTTCAAGCCCTTTTCATGGGGTCTGGGGGTGGGACT CTCGGCCTGGCTGTGTGTAATGTTAACT	242
AS2	TTCATGATTGGAGAACTTGTAGGTGAGTTGTGTTGCCA ACTCACTTTCCGTTTACCTTTCCTCGAGAGTCTTTGTCA AGCTGTAGTTACACAGGCTTTGAGGTTTAACCCATCTT TTCAGGAAGTTTGTATTTATCAAGACACTGATCTCATG	244
AS3	GTTGCTATGATGGTTCCTGATAGACAGGTTCATTATGA CTTTGGATTG	246
AS4	TGGTGTCCGCTGGATCTTAGACTCGGTTCCACTGGATG TCTCACATGCAGACATCATCAAACGTCACATGAG	248

AS5	GTCGTGGGAAGGCGTCATGAAACAGCTCCTCAACCCC TGCTGGTGCCCGACCGAGCTGGTGGTGAAGGGGGAGC A	250
AS6	GACTACTGGGCTCAAAAGGAGAAGATCAGCATCCCCA GAACACACCTGTGT	252
AS7	GACTACTGGGCTCAAAAGGAGAAGGGATCATCTTCAT TCCTGCGACCATCCTGT	254
AS8	CTTGTACTTGGTGTATTGAGCGGGCACAGTGGCTCACG CCTA	256
AS9	CCTGTCCCAACTGCTACACCTGGGGTAAGATCAGTGAC TAGTCCCCAGGGGCTGGGCCTTTTCCTTAAGTTTATT	258
AS10	AAGGAAAATGATGTCCGTGAGGTCTGTGATGTGTATTT ACAAATGCAGATCTTCTTCCATTTTAAGTTCAGAAGTT ACTTTCAT	260
AS11, AS33	GTGCCCTTCCGGGAGCTCAAGAACCAGAGAACAGCAC AAGGGGCTCCTGGGATCCACCACGCGGCTTCCCCCGTT GCTGCCAACCTCTGCGACCCGGCGAGACACGCACAGC ACACACGCATCCCCTGCGGCGCTGGCCAAGTGCGTGC TGGCCGAGGTCCCGAAGCAGGTGGTGGAGTACTACAG CCACAGAGGCCTGCCCCCGAGAAGCCTGGGTGTCCCT GCCGGAGAGGCCAGCCCAGGCTGCACACCGTGAAGAT GTGGAGGGCG	262
AS12	TTTGCTAGAAAAATGCTGGAGAAGGTACACAGACAAC ACCTACAGCTTTCCCACAATAGCCAGGAA	264
AS13	AAGAGAAGTTTTGCTGTCACGGAGAGGATCATC	266
AS14	ATGTTCCTTAGAAAGGAGCAGCAGGTGGGTCCCCACA GCTTTTCTATGCTT	268
AS15	GTGCTGCGCTTTCTGGACTTAAAGGTGAGATACCTGCA CTCT	270
AS16	GGCAACACCACCCTCCAGCAGCTGGGTGAGGCCTCCC AGGCGCCCTCAGGCTCCCTCATCCCTCTGAGGCTGCCT CTGCTCTGGGAAGTGAGGGGC	272
AS17	GGACTGAACTTAAATACTGATAGACCAGGTGGTTACA GCTATTCAATTTGGTGGAAAAACAATGCCAAGAACAG A	274
AS18	TGGAAATTCGAGATGAGCTACACGGTGGGTGGCCCGC CTCCCCATGTTCATGCTAGACCCAGGCATTGGAAAACT GATAGA	276
AS19	CAGTGGCAGCACTACCACCGGTCAGGTGAGGCCGCAG GGACTCCCCTCTGGAGACCCACAAGAAAC	278
AS20	AAGGTCCTCAACGAGATCGATGCGGTAGTTACCGTCC CTCCCTCCCTGTCTACCTCCCAGATACCGCAGGGCTGC TGCATCATATTG	280
AS21	GCCAATCTGAAAGGCACCCTGCAGGTGAGGAGTGGGC AGGCAGTGAGTCCACGC	282
AS22	CTCCAGGCGGCTGCCTCGGGCCAAGGCAAGCAGGGCG TCCCTTGTCCCTGGGGTTGCTGTGCCTACGCTGAGAGT CCCCGGGCCCTGATTTCGGGAGATGCTCCATCACAGGT GGAGCGGGAGGTGCCGGGCCCCTGCCTCAACACGCAT TCTCTCTCCCACAGATCCCCACAGCTCCCAGGCCTTCC ACACCCCAAGCAGCCTTCTGTT	284

AS23	AAAATTCAGAATAAAAATTGTCCAGAC	286
AS32	GGCGAGGTGGAGCTCTCAGAGGGCGGTGAGGGCCAGC GGCACCTTGCATTTCCCTGGGCCTGCTCTGGGCCGGGC TGGAGAGGGGTGTGCTGTGCTGCTGTGGAGCCTGCT	288
AS63	ATTGAAATGAAAAAACTGTTAAAAAGT	290
AS34	AAGATGCGGGCCATCCAGGCCGAGGGTGGGCACGGGC AGGCCTGCTGTGGAGGGGCCTGGGGATGGGCACCGGG GGACGGGGGCCCCCAGGGGATGCTCACGCATACTCTG CCCACCCTGGGCTTCCAGAGCGCCTGGACATGGAGAA GAGAAGATGCAGACAGAGCCTGGAGGACTCCGAAAG CCTGCGCATCAAGGAGGTGGAGCATA	292
AS35	CTGCTGGAGCCCTTCCGCCGCGGTGAGCCCGGGCCCC GCGGGCTGCTCTCGGGAAGTTCCCGCGGAGGGGAGGG GCCTGGCCGTTCGATCGAGGCTGCACCCGCCACACCTT TGCCCTGTTGCCGCAAGAACCCTTGTCGGCCCCAGCCT TCCAGATTTTTGCCTCCTAGGGTATTGTTAGTGATCATT CTTCCCAAACTGGATTGTCCAAAACTTGGGTTC	294
AS36	CCCTCGGGGCGGAGAACCAAACGGTTAGTTACCCTGC GTTCTGGCTGCGCCATACAATGCTGGCATCCTCGTGCC GGCCCAGTTCCCTCAGCGCTTCCTCACACAGAGAGGCC CCCAAGGCTTGTCAGGGGAGCAGCAGATCCCAGGACA GTGACCCTGGGACGGAGCCCTGCAGTCATGCCTCGGG CCCCTGCG	296
AS37	TGCCACCTCTTCCTGCAGCCCCAGGTTGGCACCCCCCC CCCCCACACTGCCAGTGCTCGAGCCCCCAGTGGTCCAC CCCACCCTCATGAAAGTTGCCCTGCAGGGCGAAGACC TGCGAGAGCTGCGCAGACATGTGCACGCCGACAGCAC GGACTTCCTGGCTGTGAAGAGGCTGGTACAGCGCGTG TTCCCAGCCTGCACCTGCACCTGCACCAGGCCGCCCTC GGAGCAGGAAGGGGCCGTGGGTGGGGAGAGGCCTGT GCCCAAGTACCCCCCTCAAGAGGC	298
AS38	AAGGAGCTCAAGCTGGAGCAGCAGGTGGGTGGGCAG GGCTTGAGAGGGGTGGGCCAAGGGGTGCGTGGCGGCT TCGTGACCCTCACTACCCATACCCCGTTCCCCTCCCAG GAAGCTGCAGAGCGGGAGTCTAAA	300
AS39	GGAGAAATCAGCCAGGAAGAGGTGCCTCCCTCCCGCC ACCTGGGCGTCTCATGGGGTGCTGGGGTGTGGGCGGG CCTCACCCTCGGGGCCTCTGCACCCCCTAACTCTAGCT TCCCCTCAGGTGCTGAGCTACAGCCAGTTGTGTGCTGC ATTAGGAGTGACACAAGACAGCCCCGACCCCCCGACT TTCCTCAGCACAGGGGAGATCCACGCCTTCCTCAGCTC TCCCTCGGGGCGGAGAACCAAACGGTTAGTTACCCTG CGTTCTGGCTGCGCCATACAATGCTGGCATCCTCGTGC CGGCCCAGTTCCCTCAGCGCTTCCTCACACAGAGAGGC CCCCAAGGCTTGTCAGGGGAGCAGCAGATCCCAGGAC AGTGACCCTGGGACGGAGCCCTGCAGTCATGCCTCGG GCCCCTGCGTAACCTCCACTGTCTCCAGCCCAGGTCTC CTTCCTCAGAGGCTATTGCCTCTCGCTCTGACTGGGCT CCCTGTGGAGGAAGATGGTTTCGAGCACGCGGGAGCC	302
AS40	GACTGCATGCTCAGCGAGGAAGGTGGGCAGGCGCGGC GGGGTGGATCCCTCTGCTCCTTAGCTGCCCACACCATT	304

	GCCTCGGCAGCCCGAGGTCGCTTCCTCTCCAGGCTCTC CAATTTCTGTGCCGTAGTTAAAGCGAGCAGGGGCGCC CCTTCCTGCACCTGGGAG	
AS41	GAGGCCTTCCAGAGGGCCGCTGGTGAGGGCGGCCCGG GCCGCGGTGGGGCACGGCGCGGTGCCAGGGTGTTGCA GAGCCCCTTTTGCAGGGCAGGAGCTGGGGAGTGGTTA GGACATCAGTCCCTCAGG	306
AS42	CCTGAGCCCAGGAGACTGAGCCCAGGTGAGCCCAGGG GGCGACCCCGGAAGGGCTGGGGGATCTGGGGTTTGTG TGGAGCGCGGGTGGGGCCCAAGGCTTGGCGG	308
AS43	GTGCCCTTCCGGGAGCTCAAGAACGTGAGTGTCCTGG AGGGGCTCCGTCAAGGCCGGCTTGGGGGTCCCTGTTC ATGTCACTGCCCAAGACCTTCCCAGGCCAGGCTCACGC CAGTGGATGTGGCAGGTCCCTTCTTGTGTCTGGGGGAT CCTGGGCTGTTCCCCCCAGTCAAGAGCAGTATC	310
AS44	TTTGTGAGCCTGACTGCCATCCAGATGGCATCGTCGGC CACTCCCTGGGGGAGGTGGCCTGTGGCTACGCCGACG GCTGCCTGTCCCAGGAGGAGGCCGTCCTCGCTGCCTAC TGGAGGGGACAGTGCATCAAAGAAGCCCATCTCCCGC CGGGCGCCATGGCAGCCGTGGGCTTGTCCTGGGAGGA GTGTAAACAGCGCTGCCCCCCGGGCGTGGTGCCCGCC TGCCACAACTCCAAGGACACAGTCACCATCTCGGGAC CTCAGGCCCCGGTGTTTGAGTTCGTGGAGCAGC	312
AS45	CCAGTTGCCATTAAACCTGGTACAGGGCCGCCCAATA ACTCCAGTATACACGGTGGCTCCAAACGTTCAGAGAA TTCCTACTGCCGGGATCTACGGGGCCAGTTACGTGCCA TTTGCTGCTCCAGCTACAGCCACGATCGCCACACTACA GAAGAACGCGGCAGCCGCGGCCGCCATGTATGGAGGA TACGCAGGCTACATACCTCAGGCCTTCCCTGCTGCTGC CATTCAGGTCCCCATCCCCGACGTCTACCAGACATACT GAGGCTGGTGACCAGCACGAAGACAGACCACACAAAC ACCACTGAAGGAACGCTTGACTATTTA	314
AS46	AAGTGGAACAAGAACTGGACAGCCACACTCGGGGCTC TTACAATCAGGGGTCATAAGCTGCTATGTCACCTACCT CACCTTCTCAGCTCTGTCCAGCAAACCTGCAGAAGTAG TTCTAGA	316
AS47	TTCAAGAAGTTCGACGGCCCTTGTGGTGAGCGCGGCG GCGGGCGCACGGCTCGAGCTCTGTGGGCGCGCGGCGA CAGCGTCCTGACTCCTGCCCTCGACCCCCAGACCCCTG TCAGGGCGCCCTCCCTGACCCGAGCCGCAGCTGCCGT G	318
AS48	GAAAATGCCAGCCTAGTGTTTACAGGATCCAACAGCC CCATACCAGCCTGCGAACTGAGTAGTCACCCAGCTCAT GGTATCAGTCCTTGGATACCCTCACCTGGAAATGAACA TTTCCATGGCATAAAGAAGCAAGTAAAGGCAATAAAA GTAGAA	320
AS49	CGGCTGACGCAACGGCTGGTCCAGGGCTGGACCCCAA TGGAGAACAGGTGGTGTGGCAGGCGAGCGGGTGGGCA GCCCGCATCATCCAGCACGAGATGGACCACCTGCAGG GCTGCCTGTTTATTGACAAAATGGACAGCAGGACGTTC ACAAACGTCTATTGGA	322
AS50	GAAAATTCAGGCAACGCCTCGCGTTGGCTGCATGTAC CAAGTAGTTCAGACGATTGGCTCGGATGGAAAAAATC TTCTGCAATTACTTCCAATTCC	324

AS51	GGCATGGAGTGCACCCTGGGGCAGGTGGGTGCCCCGT CCCCTCGGAGGGAGGAGGACGGTTGGCGTGGGGGCCA CAGCCGATTCAAGGCTGATGTACCAGCACCGCAGGGA CCCTGCTGGGGTGGCCAACCTGGCTCTGCCCCCTCCTC AGCTCCTCCTGAACAGTCATTATTAGAT	326
AS52	AAAGGGAGTGTGGAGAGGCGCTCGGTGAGCCTGGGGC ATCCTGCTGAGGGTTGGGCATGGGCAGAGAGGAGCCT CCAGCCAGGCATGACCACAGCCAACACAGGCTGCCTC TCATTCCACCACAGAGGGTGCCTCCTCCCTGTTTTGCC CAAATTACACTGTGGGCTAGGTGGACTACCTCTTGTCA GAGCTAAAGAAATCAAGCGAGTGCAGAGGGCAGGGG AGAGTTCGCTGCCTGTGAAGGGCCTTCTCACCGTCGCT TCGGCTGTCATCGCAGTCCTGTGGGGTAGGCCAAGCG AGGTCACAGGAGAAAATGAGGCTCAGCATGAT	328
AS53	TTTGGCCTCACCACACTTGCAGGTAGAAGCTCCCACGG GACCTCAGGACTGAGGGCAGCCACACACACCAAGTCT GGGGACGGTGGCCAGGGGGCTGCCAGGCAGTGTGAGA AGCTCCTGGAGCTGGCCCGGGCTACCAGACCCTGGGG GAGGAGTACGTCAGCATCATCCAGGTGGACCCATCGC GGATACATGTGCCCTCCTCGCTGCGCCGTGGCGTGCTG G	330
AS54	ATTATTGACAGCGACAAGATAATGGCAGTGTGCATGG GGTGCCTGCTCACACGTCATGTGCAATGCCAGGCCATG GAGATGCAACAG	332
AS55	GATGGCCACTCCTACACATCCAAGGTGAATTGTTTACT CCTTCAAGATGGGTTCCATGGCTGTGTGAGCATCACCG GGGCAGCTGGAAGAAGAAACCTGAGCATCTTCCTGTT CTTGATGCTGTGCAAATTGGAGTTCCATGCTTGT	334
AS56	CTACTAAATGCAGAAGATTACCGGTGTGCCATTCATTC AAAAGAGATTTATCTTCTTTCCCCCTCCCCCCACCAGG CAATGGACAAGTTTTCTCTCTGCTGCATCAACTGCAAT CTATGTTTACATGTATTCCTTTTACTACTATTTTTTCAA AACAAAGATGTATGGCTTATTTCAAACATCATTTTACT TTGGATATATGGCGGTATT	336
AS57	ACAGGGGGCAAAAGCACCTGCTCGGCTCCTGGCCCTC AGTCTCTCCCCTCCACTCCATTCTCCACCTACCCACAG TGGGTCATTCTGATCACCGAACTG	338
AS58	GTGGAGACCCTGGAGAATGCCAACAGCTTCTCCAGCG GGATCCAGCCACTCCTCTGTTCCCTGATTGGCCTGGAG AATCCCACC	340
AS59	GCTGGGGCTGGAACAATTTCCGAAGGTAGTGTTCTCCA TGGTCAGAGGCTGGAGTGTGATGCCAGACGTTTTTTTG GGTGTGGGACTACAATACTGGCAGAGTGGGAGCACCA T	342
AS55.1	GATGGCCACTCCTACACATCCAAGGTGAATTGTTTACT CCTTCAAGATGGGTTCCATGGCTGTGTGAGCATCACCG GGGCAGCTGGAAGAAGAAACCTGAGCATCTTCCTGTT CTTGATGCTGTGCAAATTGGAGTTCCATGCT	386

Table 10.

Neoepitope ID	TCGA (%)	SU2C (%)
AS1	28.5	2.3

AS2	18.5	N.O.
AS3	10.4	25.6
AS4	27.4	41.9
AS5	18.7	9.3
AS6	5.1	16.3
AS7	5.1	16.3
AS8	N.O.	14.0
AS9	1.2	18.6
AS10	8.9	27.9
AS11	1.2	48.8
AS12	0.4	34.9
AS13	5.7	32.6
AS14	N.O.	30.2
AS15	4.5	46.5
AS16	0.6	18.6
AS17	N.O.	37.2
AS18	12.6	20.9
AS19	12.6	20.9
AS20	0.2	16.3
AS21	N.O.	11.6
AS22	0.2	20.9
AS23	3.1	18.6
AS32	57.1	N.O.
AS33	47.6	N.O.
AS34	N.O.	42.9
AS35	N.O.	42.9
AS36	N.O.	40.5
AS37	N.O.	38.1
AS38	N.O.	35.7
AS39	N.O.	33.3
AS40	N.O.	33.3
AS41	N.O.	33.3
AS42	N.O.	33.3
AS43	N.O.	31
AS44	N.O.	28.6
AS45	N.O.	26.2
AS46	N.O.	26.2

AS47	N.O.	23.8
AS48	N.O.	23.8
AS49	N.O.	23.8
AS50	N.O.	23.8
AS51	N.O.	23.8
AS52	15.9	38.1
AS53	16	9.5
AS54	11.9	N.O.
AS55	12.1	N.O.
AS56	14.7	N.O.
AS57	14.9	N.O.
AS58	16.6	N.O.
AS59	17.6	N.O.
AS63	18.0	
N.O. not observed		

Example 3: Identification of additional neoantigens using bioinformatics

[0470] Additional neoantigen sequences were identified by further queries as described in Example 2. Table 11 shows the amino acid sequences of the additional neoantigens. Table 12 shows the corresponding polynucleotide sequences.

Table 11.

Neoantigen ID	Gene(s)	Amino acid sequence	SEQ ID NO:
P16	MSMB-NCOA4	GVPGD**STRRAVRRM**NTF	343
P17	MSMB-NOCA4	GVPGDSTR**RAVRRMNTF**	343
P19	TMEM222-LOC644961	WTP**IPVLTRWPL**PHPPPWRRATSCRMARSSPSATSGSSVRRRCSSLPSWVWNLAASTRPRSTPS	347
P22	SLC45A3-ELK4	SLYHREK**QLIAMDSAI**	349
P27	FAM126B-ORC2	LHPQRETFTPRWSGANYWKLA**FPVGAEGTF**PAAATQRGVVRPA	351
P35	TMPRSS2-ERG	**NSKMALNS**LNSIDDAQLTRIAPPRSHCCFWEVNAP	353
P37	TSTD1-F11R	MAG**GVLRRLLCR**EPDRDGDKGASREETVVPLHIGDPVVLPGIGQCYSALF	355
P46	TP53 (R213D)	DDRNTFDIVWW**CPMSRLRLAL**TVPPSTTTTCVTVPAWAA	357
P48	AR.p.H875Y	VQPIAREL**YQFTFDLLI**	359
P50	AR (W742C)	QMAV**IQYSCMGLM**VFAM	361
P56	SPOP (F102C)	PKSEV**RAKCKFSIL**NAK	363
P58	AR (Q903H)	MMAEIISV**HVPKILSGK**	365
P59	FOXA1 (F254V)	LHPDSG**NMVENGCYL**RR	367
P60	FOXA1.p.F266L	C**YLRRQKRLK**CEKQPGA	369

P61	FOXA1.p.R261G	MFENG**CYLGRQKRF**KCE	371
P73	TP53 (G266E)	DSSGN**LLERNSFEV**RV	373
P76	AR-V3	VFFKRAAEGFFRMNKLKESSDTNPKP**Y CMAAPMGL**TENNRNRKKSYRETNLK AVSWPLNHT	375
P77	AR-V3	V**FFKRAAEGF**FRMNKLKESSDTNPKP YCMAAPMGLTENNRNRKKSYRETNL KAVSWPLNHT	375
P82	AR-V7	YEAGMTLGEKF**RVGNCKHLK**MTRP	379
P87	AR-Intron	YEAGMTLGGKILF**FLFLLLPLS**PFSLIF	381
P97	FOXRED2-TXN2	GYLRMQG**LMAQRLLLR**	383
P98	TP53 (R213D)	DDRNTFD**IVWWCPMSR**LRLALTVPPS TTTTCVTVPAWAA	357

Table 12.

Neoantigen ID	Gene(s)	Nucleotide sequence	SEQ ID NO:
P16	MSMB-NCOA4	GGAGTTCCAGGAGATTCAACCAGGA GAGCAGTGAGGAGAATGAATACCTT C	344
P17	MSMB-NOCA4	GGAGTTCCAGGAGATTCAACCAGGA GAGCAGTGAGGAGAATGAATACCTT C	344
P19	TMEM222-LOC644961	TGGACGCCCATCCCGGTGCTCACGA GATGGCCACTACCACATCCTCCTCCC TGGAGAAGAGCTACAAGCTGCCGGA TGGCCAGGTCATCACCATCAGCAAC AAGCGGTTCCAGTGTCCGGAGGCGC TGTTCCAGCCTTCCTTCCTGGGTATG GAATCTTGCGGCATCCACGAGACCA CGTTCAACTCCATCATGAA	348
P22	SLC45A3-ELK4	TCCCTCTACCACCGGGAGAAGCAGC TCATTGCTATGGACAGTGCTATC	350
P27	FAM126B-ORC2	CTTCATCCTCAGAGGGAAACATTCAC TCCCCGGTGGTCGGGCGCGAATTACT GGAAATTGGCTTTTCCCGTTGGGGCC GAAGGTACCTTCCCTGCGGCGGCGA CTCAGCGGGGTGTCGTTCGGCCGGC GTG	352
P35	TMPRSS2-ERG	AACAGCAAGATGGCTTTGAACTCATT AAACTCCATTGATGATGCACAGTTGA CAAGAATTGCCCCTCCAAGATCTCAT TGCTGTTTCTGGGAAGTAAACGCTCC T	354
P37	TSTD1-F11R	ATGGCTGGAGGAGTCCTTCGGCGGC TGTTGTGTCGGGAGCCTGATCGCGAT GGGGACAAAGGCGCAAGTCGAGAGG AAACTGTTGTGCCTCTTCATATTGGC GATCCTGTTGTGCTCCCTGGCATTGG GCAGTGTTACAGTGCACTCTTCT	356
P46	TP53 (R213D)	GATGACAGAAACACTTTCGACATAG TGTGGTGGTGCCCTATGAGCCGCCTG	358

		AGGTTGGCTCTGACTGTACCACCATC CACTACAACTACATGTGTAACAGTTC CTGCATGGGCGGCATGA	
P48	AR.p.H875Y	GTGCAGCCTATTGCGAGAGAGCTGC ATCAGTTCACTTTTGACCTGCTAATC	360
P50	AR (W742C)	CAGATGGCTGTCATTCAGTACTCCTG CATGGGGCTCATGGTGTTTGCCATG	362
P56	SPOP (F102C)	CAAAGAGTGAAGTTCGGGCAAAATT CAAATGCTCCATCCTGAATGCCAAG	364
P58	AR (Q903H)	ATGATGGCAGAGATCATCTCTGTGCA CGTGCCCAAGATCCTTTCTGGGAAA	366
P59	FOXA1 (F254V)	CTGCACCCGGACTCCGGCAACATGG TCGAGAACGGCTGCTACTTGCGCCGC	368
P60	FOXA1.p.F266L	TGCTACTTGCGCCGCCAGAAGCGCTT GAAGTGCGAGAAGCAGCCGGGGGCC	370
P61	FOXA1.p.R261G	ATGTTCGAGAACGGCTGCTACTTGGG CCGCCAGAAGCGCTTCAAGTGCGAG	372
P73	TP53 (G266E)	GACTCCAGTGGTAATCTACTGGAAC GGAACAGCTTTGAGGTGCGTGTT	374
P76	AR-V3	GTCTTCTTCAAAAGAGCCGCTGAAG GATTTTTCAGAATGAACAAATTAAA AGAATCATCAGACACTAACCCCAAG CCATACTGCATGGCAGCACCAATGG GACTGACAGAAAACAACAGAAATAG GAAGAAATCCTACAGAGAAACAAAC TTGAAAGCTGTCTCATGGCCTTTGAA TCATACT	376
P77	AR-V3	GTCTTCTTCAAAAGAGCCGCTGAAG GATTTTTCAGAATGAACAAATTAAA AGAATCATCAGACACTAACCCCAAG CCATACTGCATGGCAGCACCAATGG GACTGACAGAAAACAACAGAAATAG GAAGAAATCCTACAGAGAAACAAAC TTGAAAGCTGTCTCATGGCCTTTGAA TCATACT	376
P82	AR-V7	TATGAAGCAGGGATGACTCTGGGAG AAAAATTCCGGGTTGGCAATTGCAA GCATCTCAAAATGACCAGACCC	380
P87	AR-Intron	TATGAAGCAGGGATGACTCTGGGAG GTAAGATACTTTTCTTTCTCTTCCTCC TCCTTCCTCTCTCCCCCTTCTCCCTCA TTTTC	382
P97	FOXRED2-TXN2	GGGTACCTGAGGATGCAGGGACTCA TGGCTCAGCGACTTCTTCTGAGG	384
P98	TP53 (R213D)	GATGACAGAAACACTTTCGACATAG TGTGGTGGTGCCCTATGAGCCGCCTG AGGTTGGCTCTGACTGTACCACCATC	358

		CACTACAACTACATGTGTAACAGTTC CTGCATGGGCGGCATGA	

Example 4: HLA binding predictions

[0471] The amino acid sequences of the neoantigens identified using the various approaches as described in Example 3 were split into all possible unique, contiguous 9 mer amino acid fragments and HLA binding predictions to six common HLA alleles (HLA-A*01:01, HLA-A*02:01, HLA-A*03:01, HLA-A*24:02, HLA-B*07:02, HLA-B*08:01) were performed for each of these 9mers using netMHCpan4.0. Several 9 mer fragments were selected for further analysis based on ranking by likelihood of binding to one or more of the tested HLA alleles and their prevalence in prostate cancer patients.

[0472] Table 13 shows the amino acid sequences of select 9 mer fragments and their neoantigen origin. Table 14 shows the prevalence of neoantigens in the analyzed cohorts.

Table 13.

Neoantigen ID	Gene(s)	Type	Amino acid sequence or 9-mer	SEQ ID NO: of the 9mer
P16	MSMB-NCOA4	Fusion	STRRAVRRM	387
P17	MSMB-NOCA4	Fusion	RAVRRMNTF	388
P19	TMEM222-LOC644961	Fusion	IPVLTRWPL	389
P22	SLC45A3-ELK4	Fusion	QLIAMDSAI	390
P27	FAM126B-ORC2	Fusion	FPVGAEGTF	391
P35	TMPRSS2-ERG	Fusion	NSKMALNSL	392
P37	TSTD1-F11R	Fusion	GVLRRLLCR	393
P46	TP53 (R213D)	Frameshift Mutation	CPMSRLRLA	394
P48	AR.p.H875Y	Missense Mutation	YQFTFDLLI	395
P50	AR (W742C)	Missense Mutation	IQYSCMGLM	396
P56	SPOP (F102C)	Missense Mutation	RAKCKFSIL	397
P58	AR (Q903H)	Missense Mutation	HVPKILSGK	398
P59	FOXA1 (F254V)	Missense Mutation	NMVENGCYL	399
P60	FOXA1.p.F266L	Missense Mutation	YLRRQKRLK	400

P61	FOXA1.p.R261G	Missense Mutation	CYLGRQKRF	401
P73	TP53 (G266E)	Missense Mutation	LLERNSFEV	402
P76	AR-V3	Splice Variant	YCMAAPMGL	403
P77	AR-V3	Splice Variant	FFKRAAEGF	404
P82	AR-V7	Splice Variant	RVGNCKHLK	405
P87	AR-Intron	Splice Variant	FLFLLLPLS	406
P97	FOXRED2-TXN2	Fusion	LMAQRLLLR	407
P98	TP53 (R213D)	Frameshift Mutation	IVWWCPMSR	408

Table 14.

Neoantigen ID	Gene	Prevalence	
		TCGA	SU2C
P16	MSMB-NCOA4	27.16%	23.25%
P17	MSMB-NOCA4	27.16%	23.25%
P19	TMEM222-LOC644961	N.O.	13.95%
P22	SLC45A3-ELK4	17.71%	13.95%
P27	FAM126B-ORC2	5.11%	18.60%
P35	TMPRSS2-ERG	2.75%	11.62%
P37	TSTD1-F11R	16.33%	9.30%
P46	TP53 (R213D)	N.O.	1%
P48	AR.p.H875Y	N.O.	1%
P50	AR (W742C)	N.O.	1.25%
P56	SPOP (F102C)	0.40%	2.00%
P58	AR (Q903H)	N.O.	1.00%
P59	FOXA1 (F254V)	0.20%	1.00%
P60	FOXA1.p.F266L	0.20%	1%
P61	FOXA1.p.R261G	0.20%	1%
P73	TP53 (G266E)	N.O.	1%
P76	AR-V3	Present	Present
P77	AR-V3	Present	Present
P82	AR-V7	Present	Present
P87	AR-Intron	Present	Present
P97	FOXRED2-TXN2	3.74%	11.62%
P98	TP53 (R213D)	0.00%	1.00%
N.O: not observed; Present: AR splice variants were expressed at variable levels and hence prevalence was not determined			

Example 5: Immunogenicity assessment of neoantigens

[0473] The 9 mer fragments shown in Table 13 were assessed for their ability to activate T cells using the Patient PBMC restimulation assay described in Example 1 using TNFα and IFNγ production by $CD8^+$ T cells as a readout. Self-antigens shown in Table 15 were also used in the assays. FIG. 5A, FIG. 5B, FIG. 5C, FIG. 5D and FIG. 5E show flow cytometry dot plots depicting $TNF\alpha^+IFN\gamma^+CD8^+$ T cell frequencies in PBMC samples after no stimulation (DMSO negative control) (FIG. 5A), after stimulation with CEF peptide (positive control (FIG. 5B), after stimulation with P16 (FIG. 5C), after stimulation with P98 (FIG. 5D), and after stimulation with P3 (FIG. 5E). Table 16 shows the maximum frequency of $TNF\alpha^+IFN\gamma^+CD8^+$ T cells and maximum fold change over negative control for each peptide analyzed, indicating the highest frequency of $TNF\alpha^+IFN\gamma^+CD8^+$ T cells and resulting fold change across the PBMC donors evaluated for the peptide. All neoantigens evaluated were found to stimulate $CD8^+$ T cells. FIG. 6 shows the number of prostate cancer patients whose PBMC samples demonstrated a positive immune response to the specified neoantigens. PBMCs from ten patients were evaluated.

Table 15.

Peptide ID	Gene name	Amino Acid Sequence of the 9-mer	SEQ ID NO:
P3	ERG	KLSRALRYY	421
P6	FOLH1	MVFELANSI	422
P7	ERG	ILFQNIDGK	423
P9	FOLH1	KIVIARYGK	424
P92	ERG	FLLELLSDS	425

Table 16.

Peptide Name	Maximum fold change over negative control	Maximum frequency of $TNF\alpha^+IFN\gamma^+CD8^+$ T cells (Percent)	Immunogenic
Negative control	n/a	0.011 - 0.8 (depending on patient)	n/a
P16	65.82	4.620	Yes
P17	2.17	0.130	Yes
P19	5.00	0.480	Yes

Peptide Name	Maximum fold change over negative control	Maximum frequency of $TNF\alpha^{+}IFN\gamma^{+}CD8^{+}$ T cells (Percent)	Immunogenic
P22	5.00	0.120	Yes
P27	3.43	0.430	Yes
P35	2.67	0.064	Yes
P37	3.13	1.160	Yes
P46	2.33	0.140	Yes
P48	2.33	0.220	Yes
P50	5.14	0.190	Yes
P56	11.57	1.620	Yes
P58	19.18	5.370	Yes
P59	10.75	3.010	Yes
P60	2.08	0.340	Yes
P61	2.27	0.084	Yes
P73	2.97	0.110	Yes
P76	2.30	0.170	Yes
P77	3.24	0.160	Yes
P82	3.46	0.970	Yes
P87	3.24	0.120	Yes
P97	4.55	0.160	Yes
P98	14.93	1.000	Yes
Maximum frequency refers to the greatest frequency of $TNF\alpha^{+}IFN\gamma^{+}CD8^{+}$ T cells among all tested PBMC donors			

Example 6: Binding of neoantigens to HLA

[0474] Binding of select neoepitopes to HLA-A*01:01, HLA-A*02:01, HLA-A*03:01, HLA-A*24:02, HLA-B*07:02 and HLA-B*08:01 was evaluated using the assay described in Example 1. The results of binding the various neoantigens to HLA is shown in Table 17. Each HLA allele tested had a corresponding positive control (*Pos*) and a negative control (Neg) peptide against which the peptide of interest was exchanged. An exchange rate of 100% with *Pos*, thus means that the peptide of interest has the same binding affinity to the HLA allele as the positive control peptide. As a criterion for further evaluation, peptides with an exchange rate of at least 10% with the corresponding *Pos* peptide for at least one of the 6 HLA alleles that we considered for further evaluation. The exchange rates with the allele specific Pos peptides, of the 24 neoantigens so identified are summarized below in Table 17. Higher percentages correspond to stronger binding to the HLA allele.

Table 17.

Peptide Name	HLA-A*01:01	HLA-A*02:01	HLA-A*03:01	HLA-A*24:02	HLA-B*07:02	HLA-B*08:01
P16	5.5	10.4	6.1	0.8	13.7	6.2
P17	4.9	9.3	3.8	0.1	38.7	9
P19	5.2	8.2	4.1	0.1	91.3	14.5
P22	4.4	46.9	4.8	0	8.3	3.9
P27	4.8	10	4.5	0.7	7.8	6.4
P35	2.5	12.5	4.2	0.2	12.1	13.7
P37	1.4	12.5	6.4	-0.1	9.4	3.3
P46	2.4	14.4	10.3	-0.4	64.8	14.5
P48	4.3	99.3	4.4	0.1	12.8	8.9
P50	2.3	8.8	5.5	0.1	10.4	4.7
P56	2.6	8.4	6.8	0.5	82.5	38
P58	2.5	11.8	32.5	0.2	9.7	5.3
P59	2.2	11.9	4.5	-0.5	7.5	5.5
P60	3.1	7.9	36.6	0.9	6.1	10.2
P61	1.7	5.8	2.1	90.3	9.6	3.6
P73	2.1	89.2	3.5	0.5	8.7	2.6
P76	0.1	85.9	6	-0.5	91.5	8.5
P77	1.9	9.6	2.8	1.7	14.2	3.4
P82	1.5	6.3	58.1	-0.1	12.9	1.4
P87	1.1	64	2.4	0.2	5	3.1
P97	2.5	4.6	39	0.1	7	2.7
P98	2.5	7.9	51.1	-0.1	6.7	2.4

Example 7: MHC I-peptide complex profiling of prostate cancer tissues identified unique MHC I-presented peptides in prostate cancer

[0475] MHC I-peptide complexes were isolated from samples of 11 human prostate cancer and peptides presented by MHC I were identified using unbiased mass spectrometry. At collection, the subjects were diagnosed with grade 7 adenocarcinoma or stromal sarcoma with two subject having invasive adenocarcinoma.

[0476] Frozen human prostate cancer tissues with HLA-A*02:01, HLA-A*03:03, HLA-B*27:0 and HLA-B*08:01 haplotypes were mechanically disrupted in non-ionic detergent including protease inhibitors and processed. A pan-MHC allele monoclonal antibody was used to immunopurify MHC I-peptide complexes from the samples. After acid elution, recovery of the MHC I-peptide complexes was assessed by ELISA and recovered peptides desalted and subjected to LC-MS/MS analyses.

[0477] The raw LC-MS/MS data files from prostate tumors were analyzed to search against the neoantigen database that was created from corresponding RNAseq data obtained from the 11 human

prostate cancer samples. These peptides had a theoretical mass for parent ions (MS1) and a list of theoretical fragment ions (MS2). A list of MS1 ions that had triggered MS2 scans were searched against the theoretical list of peptides and matched by mass. All theoretical peptides within a set MS1 ppm mass accuracy (5 ppm) then had their in silico MS2 spectrum compared to the empirical MS2 for that parent ion (peptide spectral matches or PSMs). A score was computed based on how closely the empirical spectrum matched the theoretical spectrum. Each LC-MS/MS run (one file per tumor sample) produced thousands of PSMs. However, the vast majority of these peptides were canonical sequences that were found in the human reference database (Swissprot). These were filtered out and peptides of interest (putative neoantigens) were compiled. From this list, peptides that had sufficient evidence for being positive were selected.

[0478] Table 18 shows the amino acid sequences of the peptides identified in complex with MHC I using LC-MS/MS and the gene origin of the peptides.

[0479] Table 19 shows the amino acid sequences of the corresponding longer neoantigens of the peptides identified in complex with MHC I using LC/MS/MS.

[0480] Table 20 shows the polynucleotide sequences encoding the corresponding longer neoantigens.

[0481] The MHC I complexed peptides described herein confirmed the expression, processing, and presentation of immunogenic epitopes specific to prostate cancer aberrant gene alterations. Evaluation of RNAseq databases mapped the identified MHC I complexed peptides within longer aberrant transcripts present in prostate cancer. Hence, these data identified prostate cancer neoantigens that contained at least one MHC class I epitope that is immunologically relevant and capable of eliciting an adaptive T cell response.

Table 18.

Neoantigen ID	Amino acid sequence	SEQ ID NO:	Gene	type
MS1	VTFLKPCFLL	426	TTLL7	Alternative 5' SS
MS2	TDIVKQSV	427	CHD7	Alternative Last Exon
MS3	SPAFPKPVRP	428	TESK1	Alternative Last Exon
MS4	SYFSLTNIFNFV	429	PPIP5K2	Alternative Last Exon
MS5	EFSPETCAFRLS	430	SRPK2	Alternative Last Exon
MS6	FLSRALRAL	431	SOAT1	Alternative 5' SS
MS7	KKDLELIL	432	PDE4D	Alternative Last Exon
MS8	KLQKNCLL	433	ZYG11A	Exon Skip
MS9	SALSGNSWV	434	SYNE2	Alternative Last Exon

MS10	TVRAILL	435	USP21	Intron Retention
MS11	GSLHFHEVLK	436	TDG	Novel Cassette

Table 19.

Neoantigen ID	**Gene ID**	**Peptide Sequence**	**Peptide SEQ ID**
MS1	TTLL7	**HYKLIQQP**ISLFSITDRLHKTFSQLPSVHLC SITFQWGHPPIFCSTNDICVTANFCISVTFLK PCFLLHEASASQ	437
MS2	CHD7	**WTDIVKQS**VSTNCISIKKGSYTKLFSLVFLI FCWPLIIQL	438
MS3	TESK1	**RTALTHNQ**DFSIYRLCCKRGSLCHASQARS PAFPKPVRPLPAPITRITPQLGGQSDSSQPLL TTGRPQGWQDQALRHTQQASPASCATITIPI HSAALGDHSGDPGPAWDTCPPLPLTTLIPR APPPYGDSTARSWPSRCGPLG	439
MS4	PPIP5K2	**LRYGALCN**VSRISYFSLTNIFNFVIKSLTAIF TVKF	440
MS5	SRPK2	**RKERNIRK**SESTLRLSPFPTPAPSGAPAAAQ GKVVRVPGPAGGLVPRDAGARLLPPAGGP GGGAAAGEGRAGRGRFPSITEPRPRDLPPR VATGRRAGGRRKGAGQGVRTRPLPASWPG GRGPFRKGPRRLPLGSGPPAAGVQRLRCSH LSRGPRRRRGRVCGRACVSPPLPPRPPPVGL SAENLSWLSSGLPRACSWREFSPETCAFRLS GLDSKLSARVERDLGALRAPGSRAAQGGG RVRGSRSEWKTRPWRPPPAWPLTRAGGPL PKNPFLESCSETAQRRRVFSFSTPLS	441
MS6	SOAT1	**YAYKDFLW**CFPFSLVFLQEIQICCHVSCLC CICCSTRICLGCLLELFLSRALRALHVLWNG FQLHCQ	442
MS7	PDE4D	**SINKATIT**GKKDLELILHVSRKKPFLPRVNI TPTPISCCNLKMLKKFFLLYIIISIIDLTNCLS CYLEHFYRFTFFTDVHYF	443
MS8	ZYG11A	**TMPAILKL**QKNCLLSL	444
MS9	SYNE2	**PYYSALSG**NSWVPSTLESDPFGYVFSPLAT RPALNDQESILWPTLTSVVSCALSCPSLNLP ENWLTLITGGMKGGKKMKFTFRH	445
MS10	USP21	**GLRNLGNT**VRAILLSFLSKRNVKWCWGW GKPTSLGKACGRRALKLF	446
MS11	TDG	**MEAENAGS**LHFHEVLKMGHVKF	447

Table 20.

Neoantigen ID	Gene ID	Polynucleotide sequence	DNA SEQ ID NO:
MS1	TTLL7	**CACTACAAATTAATTCAACAACCCA**TATCCCTCTTCTCCATCACTGATAGGCTCCATAAGACGTTCAGTCAGCTGCCCTCGGTCCATCTCTGCTCAATCACCTTCCAGTGGGGACACCCGCCCATTTTCTGCTCAACAAATGATATCTGTGTCACGGCCAACTTCTGCATCTCGGTCACATTCCTTAAACCGTGCTTCCTCCTACATGAGGCATCTGCCTCACAG	448
MS2	CHD7	**TGGACTGATATAGTTAAGCAGTCT**GTAAGTACAAACTGCATTTCTATCAAGAAAGGTAGCTATACAAAACTGTTTTCCTTAGTCTTTCTTATTTTCTGTTGGCCATTAATTATTCAGTTG	449
MS3	TESK1	**AGGACCGCCCTGACACACAATCAGG**ACTTCTCTATCTACAGGCTCTGTTGCAAGAGGGGGTCCCTCTGCCACGCTTCCCAGGCCAGATCCCCGGCTTTCCCGAAGCCGGTCAGACCTCTTCCTGCCCCCATCACCAGAATCACCCCCCAACTGGGGGGACAATCTGACTCGAGTCAACCCCTTCTCACTACGGGAAGACCTCAGGGGTGGCAAGATCAAGCTCTTAGACACACCCAGCAAGCCAGTCCTGCCTCTTGTGCCACCATCACCATTCCCATCCACTCAGCTGCCCTTGGTGACCACTCCGGAGACCCTGGTCCAGCCTGGGACACCTGCCCGCCGCTGCCGCTCACTACCCTCATCCCCCGAGCTCCCCCGCCGTATGGAGACAGCACTGCCAGGTCCTGGCCCTCCCGCTGTGGGCCCCTCGGC	450
MS4	PPIP5K2	**CTTCGCTATGGTGCCTTATGCAAT**GTAAGTAGAATAAGTTATTTCAGTCTAACAAATATATTTAATTTTGTAATTAAATCACTAACTGCTATTTTTACTGTGAAATTT	451

MS5	SRPK2	**CGAAAAGAGAGAAACATCCGAAAAA**GTGAGTCC ACGCTGCGCCTGTCCCCGTTCCCCACCCCCGCCCCG TCGGGGGCGCCCGCGGCCGCGCAGGGGAAAGTTGT CCGGGTCCCCGGGCCGGCGGGCGGGCTGGTCCCCC GGGACGCTGGCGCTCGGCTCCTGCCCCCGGCGGGC GGCCCGGGGGGAGGGGCGGCGGCGGGGGAGGGGC GCGCGGGCCGCGGCCGGTTCCCTAGCATCACGGAG CCTCGACCCCGCGACCTCCCGCCCCGGGTCGCCAC CGGCCGGCGGGCGGGAGGCCGGCGGAAAGGCGCC GGGCAGGGCGTGCGCACCCGTCCCTTGCCCGCGAG CTGGCCCGGGGGTCGCGGCCCTTTCCGGAAGGGGC CCCGGCGTCTGCCGCTGGGCTCCGGCCCGCCCGCT GCGGGAGTGCAGCGGCTGCGTTGCTCCCACCTGAG CCGCGGGCCGAGGAGGCGGAGGGGCCGAGTGTGC GGGAGGGCGTGTGTCTCGCCTCCCCTTCCTCCCCGG CCCCCGCCTGTCGGCCTTTCTGCTGAGAACCTAAGC TGGTTGTCAAGTGGTTTGCCTCGGGCGTGTTCCTGG CGCGAGTTCAGCCCCGAGACCTGTGCGTTTCGGCT CTCGGGTTTGGATTCGAAACTTTCCGCTCGGGTTGA GCGTGACTTGGGTGCGCTGCGGGCGCCGGGGTCGC GGGCTGCGCAGGGCGGTGGGCGTGTGCGCGGGAG CCGGTCGGAGTGGAAAACGCGCCCGTGGCGGCCAC CTCCAGCCTGGCCGCTCACCCGAGCAGGGGGGCCG CTGCCCAAGAACCCTTTCCTGGAGAGCTGCTCCGA GACCGCACAGCGCCGCCGCGTCTTCTCCTTTTCCAC TCCTCTCTCC	452
MS6	SOAT 1	**TATGCTTACAAGGACTTTCTCTGG**TGTTTTCCTTT TTCTTTAGTTTTTCTCCAAGAGATTCAAATCTGCTG CCATGTTAGCTGTCTTTGCTGTATCTGCTGTAGTAC ACGAATATGCCTTGGCTGTTTGCTTGAGCTTTTTCT ATCCCGTGCTCTTCGTGCTCTTCATGTTCTTTGGAA TGGCTTTCAACTTCATTGTCAA	453
MS7	PDE4 D	**TCCATCAACAAAGCCACCATAACAG**GTAAGAAA GATCTGGAGCTTATTCTTCATGTGTCTAGGAAGAA ACCATTTCTGCCAAGAGTCAATATAACACCAACAC CAATTTCATGCTGCAATTTGAAAATGTTAAAGAAA TTCTTTCTTCTCTACATTATCATTTCTATCATTGATC TCACAAATTGTCTAAGCTGTTATTTGGAACATTTTT ACCGATTTACGTTTTTTACTGATGTACATTATTTT	454
MS8	ZYG11 A	**ACCATGCCTGCTATTTTAAAG**TTACAGAAGAATT GTCTTCTCTCCTTA	455
MS9	SYNE 2	**CCATACTACAGCGCACTGTCAG**GTAACAGCTGGG TTCCCAGCACCCTGGAAAGTGACCCGTTTGGCTAT GTTTTTAGCCCCTTAGCAACACGGCCAGCTCTCAAT GACCAAGAGTCCATCTTGTGGCCGACCCTGACTTCT GTGGTTTCCTGTGCTCTATCCTGCCCATCTCTTAAC TTACCTGAGAATTGGCTCACTCTCATCACAGGTGG AATGAAAGGGGGAAAAAAAATGAAATTCACATTC AGACAC	456

MS10	USP21	**GGCCTTCGAAACCTGGGAAACACG**GTGAGAGCT ATTCTCCTATCTTTCCTCTCTAAAAGGAATGTGAAA TGGTGCTGGGGGTGGGGAAAACCCACGAGCTTGGG GAAGGCATGTGGAAGGAGAGCTCTGAAGCTCTTC	457
MS11	TDG	**ATGGAAGCGGAGAACGCGGGCAGT**TTGCATTTTC ATGAAGTGCTCAAAATGGGACATGTGAAATTC	458

Example 8 Expression profiling of prostate neoantigens in tumor and normal tissues

[0482] The identified prostate neoantigens were profiled for their expression in about 90 FFPE tissue samples from prostate cancer (adenocarcinoma, clinical stages II-IV, Gleason score 8-9, subjects were treatment naïve or treated with CASODEX® (bicalutamide), LUPRON DEPOT® (leuprolide acetate for depot suspension) or FIRMAGON® (degarelix)) and a panel of normal tissues including liver, kidney, pancreas, ovary, prostate, mammary gland, colon, stomach, skeletal muscle and lung, in PBMCs obtained from healthy subjects and in prostate cancer cell lines including DU145-1, MDA-MB-436-1, LREX-1, 22RV1-1, H660-1. And other tissue cell lines including NCI-H2106-1, L-363-1, HCI-N87-1, OCI-AML5-1, MDA-PCa-2b-1 and GDM-1-1. Total RNA was extracted from formalin fixed paraffin embedded tissue samples using CELLDATA's RNAstorm-RNA isolation kit following kit protocol. RNA from cultured cell lines and PBMCs were isolated using Qiagen RNA isolation kits using standard methods. 200ng of Total RNA from FFPE samples was used to prepare cDNA using High-capacity cDNA reverse transcription kit (ABI) and standard protocols. 37.5ng cDNA was preamplified with gene markers in 15µl preamplification mix using TaqMan preamplification kit (ThermoFisher Scientific) and standard protocols. To test gene expression of the identified neoantigens in the various samples, primers spanning the breakpoint sequences were designed for each of the identified prostate neoantigens and expression was assessed using Fluidigm Biomark™ HD. Percent (%) of expression positive FFPE prostate cancer samples were recorded for each neoantigen with relative average CT calculated in the prostate cancer samples. The results of the expression profiling of select neoantigens is shown in Table 21. The prevalence of each neoantigen in TCGA, SU2C and GTEx database is shown in Table 22.

Table 21.

Neoantigen ID	Amino acid SEQ ID NO:	Polynucleotide SEQ ID NO:	qPCR		
			% Positive FFPE	Relative Average Ct	Normal Tissue Expression
AS18	275	276	95.6	6.3	Ovary, Prostate
P87	381	382	85.6	8.3	Ovary, Prostate
AS55	333	334	83.3	8.2	Prostate
AS57	337	338	83.3	7.9	Prostate

AS15	269	270	68.9	11.4	Ovary, Mammary Gland
AS7	253	254	57.8	11.0	None
AS43	309	310	52.2	11.2	Mammary Gland
AS51	325	326	47.8	10.5	Ovary
AS16	271	272	47.8	10.8	Ovary
AS41	305	306	45.6	11.6	Ovary
AS6	251	252	33.3	10.0	None
AS3	245	246	26.7	10.8	None
AS11	261	262	25.6	12.1	None
AS13	265	266	21.1	11.1	None
AS47	317	318	16.7	12.3	Ovary
AS8	255	256	13.3	12.5	None
AS19	277	278	95.6	6.3	Ovary, Prostate
AS37	297	298	0.0	N/A	None
AS23	285	286	22.0	13.0	Ovary, Prostate, Mammary Gland
MS1	437	448	N/A	N/A	None
MS3	439	450	N/A	N/A	None
MS6	442	453	N/A	N/A	None
MS8	444	455	N/A	N/A	None
P82	379	380	37.0	11	
P16	343	344	76	9	Prostate
FUS1	211	212	72	9	Prostate
P22	349	350	70	9	Prostate
FUS2	213	214	55	11	Mammary Gland
FUS3	215	216	43	11	Prostate
FUS6	221	222	19	11	None
FUS5	219	220	14	7	None
FUS8	225	226	11	14	None
FUS15	345	346	8	13	None
P35	353	354	5	13	None

FUS19	235	236	4	13	None
FUS7	223	224	0	N/A	None
M84	167	168	N/A	N/A	N/A
M86	171	172	N/A	N/A	N/A
M10	19	20	N/A	N/A	N/A
M12	23	24	N/A	N/A	N/A
FR1	177	178	N/A	N/A	N/A

Table 22.

Neoantigen ID	Amino acid SEQ ID NO:	Frozen prostate cancer tissues* (n=11)	TCGA %	SU2C %	GTEx %
AS18	275	54.5	12.6	20.9	0.03
P87	381	0.0	0	20.9	0
AS55	333	18.2	12.1	0	0
AS57	337	36.4	14.9	0	0
AS15	269	36.4	4.5	46.5	0
AS7	253	27.3	5.1	16.3	0
AS43	309	0.0	0	31	0.4
AS51	325	9.1	0	23.8	0.52
AS16	271	45.5	0.6	18.6	0
AS41	305	0.0	0	33.3	0.16
AS6	251	27.3	5.1	16.3	0
AS3	245	27.3	10.4	25.6	0.03
AS11	261	9.1	1.2	48.8	0.07
AS13	265	27.3	5.7	32.6	0
AS47	317	0.0	0	23.8	0.05
AS8	255	0.0	0	14	0
AS19	277	54.5	12.6	20.9	0.03
AS37	297	0.0	0	38.1	0
AS23	285	45.5	3.1	18.6	0.09
MS1	437	18.2	0	0	0
MS3	439	9.1	0.197	0	0.13
MS6	442	9.1	0.197	0	0
MS8	444	18.2	0	0	0.016
P82	379		Varied Expression		

P16	343		27.17	2.33	0.02
FUS1	211		17.72	13.95	#N/A
P22	349		30.51	23.26	0.03
FUS2	213		63.58	46.51	1.78
FUS3	215		35.04	23.26	0.02
FUS6	221		21.46	32.56	#N/A
FUS5	219		12.40	18.60	#N/A
FUS8	225		1.18	32.56	0.54
FUS15	345		0.39	9.30	0.11
P35	353		1.38	6.98	#N/A
FUS19	235		8.86	30.23	1.04
FUS7	223		3.35	16.28	0.51
M84	167			1.28	
M86	171			1.09	
M10	19			1.18	
M12	23			0.99	
FR1	177			0.30	

Example 10 Generation of viral vectors encoding the identified neoantigens

[0483] The identified neoantigens were validated and prioritized for their inclusion into a universal prostate cancer vaccine. 41 of the identified neoantigens were selected to be included into the expression cassettes based on their expression across prostate cancer samples, low expression in normal tissues, binding to HLA, and immunogenicity. The selected 41 neoantigens are shown in Table 21 and Table 22 and include:

- AS18 (WKFEMSYTVGGPPPHVHARPRHWKTDR; SEQ ID NO: 275),
- P87 (YEAGMTLGGKILFFLFLLLPLSPFSLIF; SEQ ID NO: 381),
- AS55 (DGHSYTSKVNCLLLQDGFHGCVSITGAAGRRNLSIFLFLMLCKLEFHAC; SEQ ID NO: 333),
- AS57 (TGGKSTCSAPGPQSLPSTPFSTYPQWVILITEL; SEQ ID NO: 337),
- AS15 (VLRFLDLKVRYLHS; SEQ ID NO: 269),
- AS7 (DYWAQKEKGSSSFLRPSC; SEQ ID NO: 253),
- AS43 (VPFRELKNVSVLEGLRQGRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSI; SEQ ID NO: 309),
- AS51 (GMECTLGQVGAPSPRREEDGWRGGHSRFKADVPAPQGPCWGGQPGSAPSSAPPEQSLLD; SEQ ID NO: 325),

- AS16 (GNTTLQQLGEASQAPSGSLIPLRLPLLWEVRG; SEQ ID NO: 271),
- AS41 (EAFQRAAGEGGPGRGGARRGARVLQSPFCRAGAGEWLGHQSLR; SEQ ID NO: 305),
- AS6 (DYWAQKEKISIPRTHLC (SEQ ID NO: 251),
- AS3 (VAMMVPDRQVHYDFGL (SEQ ID NO: 245),
- AS11 (VPFRELKNQRTAQGAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAGRGPEAGG GVLQPQRPAPEKPGCPCRRGQPRLHTVKMWRA; SEQ ID NO: 261),
- AS13 (KRSFAVTERII; SEQ ID NO: 265),
- AS47 (FKKFDGPCGERGGGRTARALWARGDSVLTPALDPQTPVRAPSLTRAAAAV; SEQ ID NO: 317),
- AS8 (LVLGVLSGHSGSRL; SEQ ID NO: 255),
- AS19 (QWQHYHRSGEAAGTPLWRPTRN; SEQ ID NO: 277),
- AS37 (CHLFLQPQVGTPPPHTASARAPSGPPHPHESCPAGRRPARAAQTCARRQHGLPGCEEAG TARVPSLHLHLHQAALGAGRGRGWGEACAQVPPSRG; SEQ ID NO: 297),
- AS23 (KIQNKNCPD; SEQ ID NO: 285),
- MS1 (HYKLIQQPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCSTNDICVTANFCISVTFLK PCFLLHEASASQ; SEQ ID NO: 437),
- MS3 (RTALTHNQDFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITPQLGGQSDSSQPL LTTGRPQGWQDQALRHTQQASPASCATITIPIHSAALGDHSGDPGPAWDTCPPLPLTTLIP RAPPPYGDSTARSWPSRCGPLG; SEQ ID NO: 439),
- MS6 (YAYKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFLSRALRALHVLWNG FQLHCQ; SEQ ID NO: 442),
- MS8 (TMPAILKLQKNCLLSL; SEQ ID NO: 444),
- P82 (YEAGMTLGEKFRVGNCKHLKMTRP; SEQ ID NO: 379).
- P16 (GVPGDSTRRAVRRMNTF; SEQ ID NO: 343),
- FUS1 (CGASACDVSLIAMDSA; SEQ ID NO: 211),
- P22 (SLYHREKQLIAMDSAI; SEQ ID NO: 349),
- FUS2 (TEYNQKLQVNQFSESK; SEQ ID NO: 213),
- FUS3 (TEISCCTLSSEENEYLPRPEWQLQ; SEQ ID NO: 215),
- FUS6 (CEERGAAGSLISCE; SEQ ID NO: 221),
- FUS5 (NSKMALNSEALSVVSE; SEQ ID NO: 219),

- FUS8 (WGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLPLRTLAGCLARTAHLRPG AESLPQPQLHCT; SEQ ID NO: 225),
- FUS15 (HVVGYGHLDTSGSSSSSSWP; SEQ ID NO: 345),
- P35 (NSKMALNSLNSIDDAQLTRIAPPRSHCCFWEVNAP; SEQ ID NO: 353),
- FUS19 (KMHFSLKEHPPPPCPP; SEQ ID NO: 235), and
- FUS7 (LWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSARTCCGTKWPSSQEAALGLGSGLL RFSCGTAAIR; SEQ ID NO: 223),
- M84 (IARELHQFAFDLLIKSH; SEQ ID NO: 167),
- M86 (QPDSFAALHSSLNELGE; SEQ ID NO: 171),
- M10 (FVQGKDWGLKKFIRRDF; SEQ ID NO: 19),
- M12 (FVQGKDWGVKKFIRRDF; SEQ ID NO: 23), and
- FR1 (QNLQNGGGSRSSATLPGRRRRRWLRRRRQPISVAPAGPPRRPNQKPNPPGGARCVIMRP TWPGTSAFT; SEQ ID NO: 177).

[0484] Expression cassettes were designed for cloning into viral backbones Modified Vaccinia Ankara (MVA) and Great Ape Adenovirus 20 (GAd20) by joining the 41 neoantigen sequences one after the other without any linker. Each neoantigen sequences was codon-optimized for expression in either MVA or GAd20. The optimized polynucleotide sequences are shown in Table 23 for GAd20 and Table 24 for MVA expression.

Table 23.

Neoantigen ID	Gene ID	Amino acid SEQ ID NO:	Codon-optimized polynucleotide for GAd20 expression; SEQ ID NO:	Codon-optimized polynucleotide sequence for GAd20 expression
AS18	NWD1	275	459	TGGAAGTTCGAGATGAGCTACACCGTCGGC GGACCTCCACCTCATGTTCATGCCAGACCTC GGCACTGGAAAACCGACAGA
P87	AR-Intron	381	460	TATGAGGCCGGCATGACACTCGGCGGCAAG ATCCTGTTCTTCCTGTTCCTGCTGCTCCCTCT GAGCCCCTTCAGCCTGATCTTC
AS55	SPOC	333	461	GATGGCCACAGCTACACCAGCAAAGTGAAC TGCCTCCTGCTGCAGGATGGCTTCCACGGCT GTGTGTCTATTACTGGCGCCGCTGGCAGAC GGAACCTGAGCATCTTTCTGTTTCTGATGCT GTGCAAGCTCGAGTTCCACGCCTGC

AS57	KLK3	337	462	ACAGGCGGCAAGTCCACATGTTCTGCCCCT GGACCTCAGAGCCTGCCTAGCACACCCTTC AGCACATACCCTCAGTGGGTCATCCTGATC ACCGAACTC
AS15	LRRC45	269	463	GTGCTGAGATTCCTGGACCTGAAAGTGCGC TACCTGCACAGC
AS7	ACSM1	253	464	GACTATTGGGCTCAAAAAGAGAAGGGCAGC AGCAGCTTCCTGCGGCCTAGCTGT
AS43	CPNE7	309	465	GTCCCCTTCAGAGAGCTGAAGAACGTTTCC GTGCTGGAAGGCCTGAGACAGGGCAGACTT GGCGGCCCTTGTAGCTGTCACTGCCCCAGA CCTAGTCAGGCCAGACTGACACCTGTGGAT GTGGCCGGACCTTTCCTGTGTCTGGGAGATC CTGGCCTGTTTCCACCTGTGAAGTCCAGCAT C
AS51	CPNE7	325	466	GGCATGGAATGCACCCTGGGACAAGTGGGA GCCCCATCTCCTAGAAGAGAAGAGGATGGC TGGCGCGGAGGCCACTCTAGATTCAAAGCT GATGTGCCCGCTCCTCAGGGCCCTTGTTGGG GAGGACAACCTGGATCTGCCCCATCTTCTGC CCCACCTGAACAGTCCCTGCTGGAT
AS16	LRRC45	271	467	GGCAACACAACCCTCCAGCAACTGGGAGAA GCCTCTCAGGCTCCTAGCGGCTCTCTGATCC CTCTCAGACTGCCTCTCCTGTGGGAAGTTCG GGGC
AS41	RHPN1	305	468	GAGGCTTTCCAAAGAGCTGCTGGCGAAGGC GGACCTGGTAGAGGTGGTGCTAGAAGAGGT GCTAGGGTGCTGCAGAGCCCATTCTGTAGA GCAGGCGCAGGCGAATGGCTGGGCCATCAG AGTCTGAGA
AS6	ACSM1	251	469	GATTATTGGGCCCAGAAAGAAAAGATCAGC ATCCCCAGAACACACCTGTGC
AS3	DNAH8	245	470	GTGGCCATGATGGTGCCCGATAGACAGGTC CACTACGACTTTGGACTG
AS11	CPNE7	261	471	GTGCCCTTCCGGGAACTGAAGAACCAGAGA ACAGCTCAGGGCGCTCCTGGAATCCACCAT GCTGCTTCTCCAGTGGCCGCCAACCTGTGTG ATCCTGCCAGACATGCCCAGCACACCAGGA TTCCTTGTGGCGCTGGACAAGTGCGCGCTG GAAGAGGACCTGAAGCAGGCGGAGGTGTTC TGCAACCTCAAAGACCCGCTCCTGAGAAGC CTGGCTGCCCTTGCAGAAGAGGACAGCCTA GACTGCACACCGTGAAAATGTGGCGAGCC
AS13	GRIN3A	265	472	AAGAGAAGCTTTGCCGTGACCGAGCGGATC ATC
AS47	AGRN	317	473	TTCAAGAAGTTCGACGGCCCTTGCGGAGAA AGAGGCGGAGGCAGAACAGCTAGAGCCCTT TGGGCTAGAGGCGACAGCGTTCTGACACCA GCTCTGGACCCTCAGACACCTGTTAGGGCC CCTAGCCTGACAAGAGCTGCCGCCGCTGTG
AS8	CACNA1D	255	474	CTGGTGCTGGGAGTGCTGTCTGGACACTCTG GCAGCAGACTG

AS19	NWD1	277	475	CAGTGGCAGCACTATCACAGATCTGGCGAA GCCGCCGGAACACCCCTTTGGAGGCCAACA AGAAAC
AS37	RECQL4	297	476	TGCCACTTGTTTCTGCAGCCCCAAGTGGGCA CACCTCCTCCACATACAGCCTCTGCTAGAGC ACCTAGCGGCCCTCCACATCCTCACGAATCT TGTCCTGCCGGAAGAAGGCCTGCCAGAGCC GCTCAAACATGTGCCAGACGACAGCACGGA CTGCCTGGATGTGAAGAGGCTGGAACAGCC AGAGTGCCTAGCCTGCACCTCCATCTGCATC AGGCTGCTCTTGGAGCCGGAAGAGGTAGAG GATGGGGCGAAGCTTGTGCTCAGGTGCCAC CTTCTAGAGGC
AS23	ZNF614	285	477	AAGATCCAGAACAAGAACTGCCCCGAC
MS1	TTLL7	437	478	CACTACAAGCTGATCCAGCAGCCAATCAGC CTGTTCAGCATCACCGACCGGCTGCACAAG ACATTCAGCCAGCTGCCAAGCGTGCACCTG TGCTCCATCACCTTCCAGTGGGGACACCCTC CTATCTTTTGCTCCACCAACGACATCTGCGT GACCGCCAACTTCTGTATCAGCGTGACCTTC CTGAAGCCTTGCTTTCTGCTGCACGAGGCCA GCGCCTCTCAG
MS3	TESK1	439	479	CGAACCGCTCTGACACACAACCAGGACTTC AGCATCTACAGACTGTGTTGCAAGCGGGGC TCCCTGTGCCATGCAAGCCAAGCTAGAAGC CCCGCCTTTCCTAAACCTGTGCGACCTCTGC CAGCTCCAATCACCAGAATTACCCCTCAGCT CGGCGGCCAGAGCGATTCATCTCAACCTCT GCTGACCACCGGCAGACCTCAAGGCTGGCA AGACCAAGCTCTGAGACACACCCAGCAGGC TAGCCCTGCCTCTTGTGCCACCATCACAATC CCCATCCACTCTGCCGCTCTGGGCGATCATT CTGGCGATCCTGGACCAGCCTGGGACACAT GTCCTCCACTGCCACTCACAACACTGATCCC TAGGGCTCCTCCACCTTACGGCGATTCTACC GCTAGAAGCTGGCCCAGCAGATGTGGACCA CTCGGA
MS6	SOAT1	442	480	TACGCCTACAAGGACTTCCTGTGGTGCTTCC CCTTCTCTCTGGTGTTCCTGCAAGAGATCCA GATCTGCTGTCATGTGTCCTGCCTGTGCTGC ATCTGCTGTAGCACCAGAATCTGCCTGGGCT GTCTGCTGGAACTGTTCCTGAGCAGAGCCCT GAGAGCACTGCACGTGCTGTGGAACGGATT CCAGCTGCACTGCCAG
MS8	ZYG11A	444	481	ACAATGCCCGCCATCCTGAAGCTGCAGAAG AATTGCCTCCTAAGCCTG
P82	AR-V7	379	482	TACGAAGCCGGGATGACCCTGGGCGAGAAG TTCAGAGTGGGCAACTGCAAGCACCTGAAG ATGACCCGGCCT
P16	MSMB-NCOA4-1	343	483	GGCGTGCCAGGCGATAGCACTCGGAGAGCC GTCAGACGGATGAACACCTTT

FUS1	SLC45A3 -> ELK4 - 1	211	484	TGTGGCGCCTCTGCCTGTGACGTGTCCCTGA TCGCTATGGACTCCGCC
P22	SLC45A3 -ELK4 - 2	349	485	AGCCTGTACCACCGGGAAAAGCAGCTCATT GCCATGGACAGCGCCATC
FUS2	ARHGEF 38-> ARHGEF 38-IT1	213	486	ACCGAGTACAACCAGAAACTGCAAGTGAAC CAGTTCAGCGAGAGCAAG
FUS3	MSMB-> NCOA4- 2	215	487	ACCGAGATCAGCTGCTGCACCCTGAGCAGC GAGGAAAACGAGTACCTGCCTAGACCTGAG TGGCAGCTGCAG
FUS6	TMPRSS 2-> ERG	221	488	TGCGAAGAGAGAGGCGCCGCAGGATCTCTG ATCTCCTGCGAA
FUS5	TMPRSS 2-> ERG	219	489	AACAGCAAGATGGCCCTGAATAGCGAGGCC CTGTCTGTGGTGTCTGAA
FUS8	INCA1-> CAMTA 2	225	490	TGGGGCATGGAACTGGCCGCCAGCAGAAGA TTCAGCTGGGATCATCATAGCGCAGGCGGC CCACCTAGAGTGCCATCTGTTAGAAGCGGA GCTGCCCAGGTGCAGCCTAAAGATCCTCTG CCACTGAGAACACTGGCCGGCTGCCTTGCT AGAACAGCCCATCTTAGACCTGGCGCCGAG TCTCTGCCTCAGCCACAACTGCACTGTACC
FUS15	D2HGD H-> GAL3ST 2	345	491	CATGTCGTCGGCTACGGCCACCTGGATACA AGCGGAAGCAGCTCTAGCTCCAGCTGGCCT
P35	TMPRSS 2-ERG	353	492	AACTCAAAAATGGCTCTGAACAGCCTGAAC TCCATCGACGACGCCCAGCTGACAAGAATC GCCCCTCCTAGATCTCACTGCTGCTTTTGGG AAGTGAACGCCCCA
FUS19	GTF2F1- >PSPN	235	493	AAGATGCACTTTAGCCTGAAAGAACACCCT CCACCACCTTGTCCTCCA
FUS7	NME4- >DECR2	223	494	CTGTGGTTCCAGTCCAGCGAGCTGTCTCCTA CTGGTGCCCCTTGGCCATCTAGACGCCCTAC TTGGAGAGGCACCACCGTGTCACCAAGAAC CGCCACAAGCAGCGCCAGAACCTGTTGTGG CACAAAGTGGCCCTCCAGCCAAGAAGCCGC TCTCGGACTTGGAAGCGGACTGCTGAGGTT CTCTTGTGGAACCGCCGCCATTCGG
M84	AR- T878A	167	495	ATCGCTAGAGAGCTGCACCAGTTCGCCTTC GACCTGCTGATCAAGAGCCAC
M86	AR- L702H	171	496	CAGCCTGATTCTTTTGCCGCACTGCACAGCT CCCTGAACGAGCTGGGAGAG
M10	SPOP- F133L	19	497	TTCGTGCAAGGCAAGGATTGGGGCCTCAAA AAGTTTATCCGCAGAGACTTC
M12	SPOP- W133V	23	498	TTTGTGCAGGGCAAAGACTGGGGCGTGAAG AAGTTCATCCGGCGGGACTTC

FR1	ZFHX3	177	499	CAGAACCTGCAGAACGGCGGAGGCTCTAGA AGCTCTGCTACACTTCCTGGCAGGCGGCGG AGAAGATGGCTGAGAAGAAGGCGGCAGCC TATCTCTGTGGCTCCTGCTGGACCTCCTAGA CGGCCCAACCAGAAGCCTAATCCTCCTGGC GGAGCCAGATGCGTGATCATGAGGCCTACA TGGCCTGGCACCAGCGCCTTCACC

Table 24.

Neoantigen ID	Gene ID	Amino acid SEQ ID NO:	Codon-optimized polynucleotide for MVA expression; SEQ ID NO:	Codon-optimized polynucleotide sequence for MVA expression
AS18	NWD1	275	500	TGGAAGTTCGAGATGAGCTACACCGTTGG CGGCCCTCCACCACATGTTCACGCCAGAC CTAGACACTGGAAAACCGACAGA
P87	AR-Intron	381	501	TACGAGGCCGGCATGACACTCGGAGGCAA GATCCTGTTCTTCCTGTTCCTGCTGCTCCCT CTGAGCCCCTTCAGCCTGATCTTT
AS55	SPOC	333	461	GATGGCCACAGCTACACCAGCAAAGTGAA CTGCCTCCTGCTGCAGGATGGCTTCCACGG CTGTGTGTCTATTACTGGCGCCGCTGGCAG ACGGAACCTGAGCATCTTTCTGTTTCTGAT GCTGTGCAAGCTCGAGTTCCACGCCTGC
AS57	KLK3	337	503	ACAGGCGGCAAGAGCACATGTTCTGCCCC TGGACCTCAGTCTCTGCCCAGCACACCCTT CAGCACATACCCTCAGTGGGTCATCCTGA TCACCGAGCTG
AS15	LRRC45	269	504	GTGCTGCGGTTCCTGGATCTCAAAGTGCG CTACCTGCACAGC
AS7	ACSM1	253	505	GATTATTGGGCCCAGAAAGAAAAGGGCAG CAGCAGCTTCCTGCGGCCTAGCTGT
AS43	CPNE7	309	506	GTGCCCTTCCGGGAACTGAAGAACGTGTC CGTTCTGGAAGGCCTGAGGCAGGGCAGAC TTGGCGGACCTTGTAGCTGCCACTGTCCTA GACCAAGCCAGGCCAGACTGACCCCTGTG GATGTGGCTGGCCCATTTCTGTGTCTGGGC GACCCTGGACTGTTCCCTCCAGTGAAGTCT AGCATC
AS51	CPNE7	325	507	GGCATGGAATGTACACTGGGCCAAGTGGG AGCCCCATCTCCTAGAAGAGAAGAGGATG GCTGGCGCGGAGGCCACTCTAGATTCAAA GCTGATGTGCCCGCTCCTCAGGGCCCTTGT TGGGGAGGACAACCTGGATCTGCCCCATC TTCTGCCCCACCTGAACAGAGCCTGCTGG AT
AS16	LRRC45	271	508	GGCAACACCACACTGCAACAGCTGGGAGA AGCCTCTCAGGCCCCAAGCGGTTCTCTGAT

				CCCTCTCAGACTGCCCCTCCTGTGGGAAGT GCGGGGC
AS41	RHPN1	305	509	GAGGCTTTCCAGAGAGCAGCTGGCGAAGG CGGACCTGGCAGAGGTGGTGCTAGAAGAG GTGCTAGAGTGCTGCAGAGCCCATTCTGT AGAGCTGGCGCTGGCGAATGGCTGGGCCA CCAATCTCTTAGA
AS6	ACSM1	251	510	GACTATTGGGCTCAAAAAGAGAAGATCAG CATCCCCAGAACACACCTGTGC
AS3	DNAH8	245	511	GTGGCCATGATGGTGCCCGACAGACAGGT GCACTACGACTTCGGCCTG
AS11	CPNE7	261	512	GTGCCCTTCAGAGAGCTGAAAAACCAGAG AACAGCCCAGGGCGCTCCTGGAATCCATC ATGCTGCTTCTCCAGTGGCCGCCAATCTGT GCGATCCTGCCAGACATGCCCAGCATACC AGGATTCCTTGTGGCGCTGGACAAGTGCG CGCTGGAAGAGGACCTGAAGCTGGTGGCG GAGTTCTGCAGCCTCAAAGACCTGCTCCT GAGAAGCCTGGCTGCCCCTGTAGAAGAGG ACAGCCTAGACTGCACACCGTGAAGATGT GGCGGGCC
AS13	GRIN3A	265	513	AAGAGAAGCTTCGCCGTGACCGAGCGGAT CATC
AS47	AGRN	317	514	TTTAAGAAGTTTGACGGCCCCTGCGGCGA GAGAGGCGGAGGAAGAACTGCAAGAGCC CTTTGGGCCAGAGGCGACTCTGTTCTGAC ACCAGCTCTGGACCCTCAGACACCTGTTA GGGCCCCTAGCCTGACAAGAGCTGCCGCT GCTGTT
AS8	CACNA1D	255	515	CTGGTGCTGGGCGTGCTGTCTGGCCACTCT GGAAGCAGACTG
AS19	NWD1	277	516	CAATGGCAGCACTACCACAGATCTGGCGA AGCCGCTGGAACCCCACTTTGGAGGCCTA CCAGAAAC
AS37	RECQL4	297	517	TGCCACTTGTTTCTCCAGCCACAAGTGGGC ACCCCTCCACCTCATACAGCCTCTGCTAGA GCACCTAGCGGCCCACCTCATCCTCACGA ATCTTGTCCTGCCGGAAGAAGGCCTGCCA GAGCCGCTCAAACATGTGCCAGACGACAG CACGGACTGCCCGGATGTGAAGAAGCCGG AACAGCCAGAGTGCCTAGCCTGCACCTTC ATCTGCATCAGGCCGCTCTTGGAGCCGGA AGAGGTAGAGGATGGGGAGAAGCTTGTGC CCAGGTGCCACCTTCTAGAGGC
AS23	ZNF614	285	477	AAGATCCAGAACAAGAACTGCCCCGAC
MS1	TTLL7	437	519	CACTACAAGCTGATCCAGCAGCCAATCAG CCTGTTCTCCATCACCGACCGGCTGCACAA GACATTCAGCCAGCTGCCTTCCGTGCATCT GTGCAGCATCACCTTCCAGTGGGGACACC CTCCTATCTTTTGCTCCACCAACGACATCT GCGTGACCGCCAACTTCTGTATCAGCGTG

				ACCTTCCTGAAGCCTTGCTTTCTGCTGCAC GAGGCCTCCGCCAGCCAG
MS3	TESK1	439	520	CGGACCGCTCTGACCCACAACCAGGACTT CAGCATCTACCGGCTGTGCTGCAAGAGGG GCTCTCTGTGTCATGCTAGCCAGGCTAGA AGCCCCGCCTTTCCTAAGCCTGTCAGACCT CTGCCTGCTCCTATCACCAGAATCACCCCT CAGCTCGGCGGCCAGTCTGATTCATCTCA GCCACTGCTGACCACCGGCAGACCTCAAG GATGGCAAGACCAGGCTCTGAGACACACA CAGCAGGCTAGCCCAGCCTCTTGCGCCAC CATCACAATACCAATACATTCTGCCGCTCT GGGCGATCACAGCGGAGATCCTGGACCTG CCTGGGATACTTGTCCTCCTCTGCCCCTAA CTACACTGATCCCTAGGGCTCCTCCACCTT ACGGCGATAGCACAGCCAGATCCTGGCCT AGCAGATGTGGCCCTCTGGGC
MS6	SOAT1	442	521	TACGCCTACAAGGACTTCCTGTGGTGCTTC CCCTTCTCTCTGGTGTTCCTGCAAGAAATC CAGATCTGCTGTCACGTGTCCTGCCTGTGC TGTATCTGCTGTAGCACCCGGATCTGTCTG GGCTGTCTGCTGGAACTGTTCCTGAGCAG AGCCCTGAGAGCACTGCACGTGCTGTGGA ACGGATTCCAGCTGCACTGCCAG
MS8	ZYG11A	444	522	ACCATGCCTGCCATTCTGAAGCTGCAGAA GAATTGTCTTCTAAGCCTG
P82	AR-V7	379	523	TATGAGGCTGGAATGACCCTGGGCGAGAA GTTCAGAGTGGGCAACTGCAAGCACCTGA AGATGACCCGGCCT
P16	MSMB-NCOA4-1	343	524	GGAGTGCCTGGCGATTCTACTAGAAGGGC CGTGCGGCGGATGAACACCTTT
FUS1	SLC45A3-> ELK4 - 1	211	525	TGTGGCGCATCTGCCTGCGACGTGTCCCTG ATCGCTATGGATAGCGCC
P22	SLC45A3-ELK4 - 2	349	485	AGCCTGTACCACCGGGAAAAGCAGCTCAT TGCCATGGACAGCGCCATC
FUS2	ARHGEF38-> ARHGEF38-IT1	213	486	ACCGAGTACAACCAGAAACTGCAAGTGAA CCAGTTCAGCGAGAGCAAG
FUS3	MSMB-> NCOA4-2	215	528	ACCGAGATCAGCTGCTGCACCCTGAGCAG CGAGGAAAACGAGTACCTGCCTAGACCTG AATGGCAGCTGCAG
FUS6	TMPRSS2 -> ERG	221	529	TGCGAGGAAAGAGGCGCAGCCGGATCTCT GATCTCTTGCGAG
FUS5	TMPRSS2 -> ERG	219	530	AACAGCAAGATGGCCCTGAATAGCGAGGC CCTGTCTGTGGTGTCCGAG
FUS8	INCA1-> CAMTA2	225	531	TGGGGAATGGAACTGGCCGCTAGCAGGCG GTTTAGCTGGGATCATCATTCTGCCGGCGG

				ACCTCCAAGAGTGCCAAGCGTTAGAAGCG GAGCAGCCCAGGTCCAGCCTAAAGATCCA CTGCCACTGAGAACACTGGCCGGCTGCCT TGCCAGAACAGCTCATCTTAGACCTGGCG CCGAAAGCCTGCCTCAACCTCAGCTGCAT TGCACA
FUS15	D2HGDH->GAL3ST2	345	532	CACGTTGTCGGCTATGGCCACCTGGATAC AAGCGGCTCCTCTAGCAGTAGCTCCTGGC CT
P35	TMPRSS2-ERG	353	533	AATTCTAAGATGGCTCTCAACAGCCTGAA CTCCATCGACGACGCCCAGCTGACAAGAA TCGCCCCTCCAAGAAGCCACTGTTGCTTTT GGGAAGTGAACGCCCCT
FUS19	GTF2F1->PSPN	235	534	AAGATGCACTTCTCACTGAAAGAGCACCC GCCACCGCCGTGCCCACCG
FUS7	NME4->DECR2	223	535	CTGTGGTTCCAGTCCAGCGAACTGTCTCCT ACTGGCGCTCCATGGCCAAGCAGAAGGCC TACTTGGAGAGGCACCACCGTGTCTCCAA GAACCGCTACAAGCAGCGCCAGAACCTGT TGCGGCACAAAATGGCCCTCCAGCCAAGA AGCTGCCCTCGGACTTGGAAGCGGACTGC TGAGATTCAGCTGTGGCACAGCCGCCATC AGA
M84	AR-T878A	167	536	ATCGCCAGAGAACTGCACCAGTTCGCCTT CGACCTGCTGATCAAGAGCCAC
M86	AR-L702H	171	537	CAGCCTGACAGCTTTGCTGCCCTGCATAGC TCCCTGAATGAGCTGGGCGAA
M10	SPOP-F133L	19	538	TTTGTGCAGGGTAAAGATTGGGGCCTCAA AAAGTTTATCAGACGGGACTTC
M12	SPOP-W133V	23	539	TTCGTGCAGGGCAAAGACTGGGGCGTGAA GAAGTTCATCCGGCGGGACTTT
FR1	ZFHX3	177	540	CAGAACCTGCAGAACGGCGGAGGCTCTAG AAGCTCTGCTACACTTCCTGGCAGGCGGC GGAGAAGATGGCTGAGAAGAAGGCGGCA GCCTATCTCTGTGGCTCCTGCTGGACCTCC TAGACGGCCCAACCAGAAGCCTAATCCTC CTGGCGGAGCCAGATGCGTGATCATGAGG CCTACATGGCCTGGCACCAGCGCCTTTACC

Synthetic gene design

[0485] The 41 neoantigen amino acidic sequences were joined head to tail. The order of the neoantigens sequences was determined according to a strategy that minimized the formation of predicted junctional epitopes that may be generated by the juxtaposition of two adjacent neoantigen peptides.

[0486] To this purpose, custom tools were developed to split the 41 neoantigens into 4 smaller lists (sublists) of similar cumulative length and to generate, for each sublist, 2 million scrambled layouts of the synthetic gene with a different neoantigen order. The tool proceeded iteratively. At each loop a

scrambled layout was generated and compared to the layouts already generated. If the number of predicted junctional epitopes in the new layout was lower than the number of the previously best layout, the new layout was considered as the best. Each scrambled layout was analyzed estimating the number of potential junctional epitopes predicted to bind one out of a subset of 9 class I HLA haplotypes with an IC_{50} <=1500nM (considering only 9mer epitopes predicted by the IEDB_recommended method included in the IEDB 2.17 software). The 9 class I HLA haplotypes cumulatively cover 82% of the world population as estimated by analyzing haplotypes annotated for subjects in the 1000 genomes project. Scrambled layouts with neoantigens that formed predicted junctional epitopes with the N-terminal T-cell enhancer or the C-terminal TAG sequence were excluded. As an additional constraint, in each layout junctions that contained a 9mer peptide that matched a protein annotated in the human wildtype proteome were also excluded.

[0487] The best layouts obtained after scrambling 2 million times each of the 4 sublists were then joined to generate an overall layout comprising all 41 neoantigens. Out of all possible combinations of the best 4 layouts the one with the minimal number of predicted epitopes formed by the newly formed junctions was selected.

[0488] The whole procedure described was applied two times independently to generate two artificial genes to be encoded alternatively by the GAd20 or MVA vector. For the MVA vector the scrambled layouts were designed with the additional constraint of avoiding the junctions with predicted junctional epitopes that were already present in the layout selected for the Adenoviral transgene.

[0489] Amino acid sequences of the optimized layout for the GAd20 is shown in SEQ ID NO: 541 and for MVA SEQ ID NO: 543. Neoantigens in the GAd20 insert of SEQ ID NO: 541 were in the following order: FR1-AS13-AS7-AS6-AS8-P87-FUS3-AS43-AS57-AS51-AS18-AS55-AS23-AS47-MS1-AS37-AS15-AS19-AS11-AS3-P16-P82-FUS5-FUS1-M12-MS6-FUS2-P22-FUS6-MS8-MS3-AS16-M86-M84-M10-FUS8-FUS7-FUS19-AS41-FUS15-P35. Neoantigens in the MVA insert of SEQ DID NO: 543 were in the following order: FR1-AS51-AS6-AS18-AS7-AS43-FUS3-P87-AS8-AS13-AS57-AS55-AS19-AS3-AS23-AS15-AS11-AS37-MS1-AS47-P16-FUS1-FUS6-P22-M12-MS8-FUS5-P82-FUS2-MS3-MS6-AS16-P35-M10-AS41-FUS8-M84-FUS19-FUS15-M86-FUS7.

[0490] Five additional alternative optimized layouts of scrambled neoantigens were assessed for each vector. The five alternative layouts had the same number of predicted junctional epitopes compared to SEQ ID NO: 541 and SEQ ID NO: 543. The five alternative layouts for Gad20 are shown in SEQ ID NO: 554, SEQ ID NO; 555, SEQ ID NO: 556, SEQ ID NO: 623 and SEQ ID NO: 624. The five alternative layouts for MVA are shown in SEQ ID NO: 557, SEQ ID NO: 558, SEQ ID NO: 559, SEQ ID NO: 625 and SEQ ID NO: 626. The neoantigens in the alternative optimized layouts were in the following order:

- SEQ ID NO: 554: FR1-AS13-AS8-P87-FUS3-AS43-AS57-AS51-AS7-AS6-AS18-P16-P82-FUS5-FUS1-M12-MS6-FUS2-P22-FUS6-MS8-MS3-AS55-AS23-AS47-MS1-AS37-AS15-AS19-AS11-AS3-AS16-M86-M84-M10-FUS8-FUS7-FUS19-AS41-FUS15-P35

- SEQ ID NO: 555: FR1-AS13-FUS3-P87-AS7-AS43-AS57-AS51-AS6-AS8-AS18-AS55-AS23-AS47-MS1-AS37-AS15-AS19-AS11-AS3-P16-P82-FUS5-FUS1-M12-MS6-FUS2-P22-FUS6-MS8-MS3-AS16-M86-M84-M10-FUS8-FUS7-FUS19-AS41-FUS15-P35
- SEQ ID NO: 556: FR1-AS13-AS7-AS43-AS8-P87-FUS3-AS57-AS51-AS6-AS18-AS55-AS23-AS47-MS1-AS37-AS15-AS19-AS11-AS3-P16-P82-FUS5-FUS1-M12-MS6-FUS2-P22-FUS6-MS8-MS3-AS16-M86-M84-M10-FUS8-FUS7-FUS19-AS41-FUS15-P35
- SEQ ID NO: 623: P16-P82-FUS5-FUS1-M12-MS6-FUS2-P22-FUS6-MS8-MS3-AS16-M86-M84-M10-FUS8-FUS7-FUS19-AS41-FUS15-P35-AS55-AS23-AS47-MS1-AS37-AS15-AS19-AS11-AS3-FR1-AS13-AS8-P87-FUS3-AS43-AS57-AS51-AS7-AS6-AS18
- SEQ ID NO: 624: AS16-M86-M84-M10-FUS8-FUS7-FUS19-AS41-FUS15-P35-P16-P82-FUS5-FUS1-M12-MS6-FUS2-P22-FUS6-MS8-MS3-AS55-AS23-AS47-MS1-AS37-AS15-AS19-AS11-AS3-FR1-AS13-FUS3-P87-AS7-AS43-AS57-AS51-AS6-AS8-AS18
- SEQ ID NO: 557: FR1-AS51-AS6-AS18-AS7-AS43-FUS3-P87-AS8-AS13-AS57-AS55-AS37-MS1-AS3-AS23-AS15-AS11-AS19-AS47-P16-FUS1-FUS6-P22-M12-MS8-FUS5-P82-FUS2-MS3-MS6-AS16-P35-M10-AS41-FUS8-M84-FUS19-FUS15-M86-FUS7
- SEQ ID NO: 558: AS55-AS19-AS3-AS15-AS23-AS11-AS37-MS1-AS47-FR1-AS51-AS6-AS18-AS7-AS43-FUS3-P87-AS8-AS13-AS57-P16-FUS1-FUS6-P22-M12-MS8-FUS5-P82-FUS2-MS3-MS6-AS16-P35-M10-AS41-FUS8-M84-FUS19-FUS15-M86-FUS7
- SEQ ID NO: 559: AS16-P35-M10-AS41-FUS8-M84-FUS19-FUS15-M86-FUS7-AS55-AS19-AS3-AS23-AS15-AS11-AS37-MS1-AS47-P16-FUS1-FUS2-P82-MS8-FUS5-FUS6-P22-M12-MS3-MS6-FR1-AS51-AS6-AS18-AS7-AS43-FUS3-P87-AS8-AS13-AS57
- SEQ ID NO: 625: AS16-P35-M10-AS41-FUS8-M84-FUS19-FUS15-M86-FUS7-FR1-AS51-AS6-AS18-AS7-AS43-FUS3-P87-AS8-AS13-AS57-AS55-AS19-AS3-AS15-AS23-AS11-AS37-MS1-AS47-P16-FUS1-FUS6-P22-M12-MS8-FUS5-P82-FUS2-MS3-MS6
- SEQ ID NO: 626: AS55-AS11-AS19-AS23-AS3-AS15-AS37-MS1-AS47-FR1-AS51-AS6-AS18-AS7-AS43-FUS3-P87-AS8-AS13-AS57-AS16-P35-M10-AS41-FUS8-M84-FUS19-FUS15-M86-FUS7-P16-FUS1-FUS6-P22-M12-P82-MS8-FUS5-FUS2-MS3-MS6

Insertion of T-cell enhancer and TAG sequences

[0491] A small peptide fragment with length of 28aa from the mandarin fish invariant chain (MGQKEQIHTLQKNSERMSKQLTRSSQAV; SEQ ID NO: 549) was placed at the N-terminus of each transgene encoding the 41 neoantigens. Preclinical data has shown this sequence to increase the immunological response of the viral vector. A small segment of 7 amino acids (TAG sequence; seq: SHHHHHH; SEQ ID NO: 627) was added at the C-terminus of the transgene for the purpose of monitoring the expression of the encoded transgene.

[0492] Amino acid sequences of the optimized layout for the GAd20 that includes the TCE sequence and omits the tag sequence are shown in SEQ ID NO: 550 and for MVA SEQ ID NO: 551.

Conversion into nucleotide sequence and optimization to remove predicted miRNA binding sites.

[0493] The conversion from amino acid sequence into nucleotide sequence was performed using codon optimizing according to the human codon usage applying additional constraints to avoid as much as possible the following features:

- internal TATA-boxes, chi-sites and ribosomal entry sites
- AT-rich or GC-rich sequence stretches
- RNA instability motifs
- repeat sequences and RNA secondary structures
- (cryptic) splice donor and acceptor sites in higher eukaryotes
- TTTTTnT termination motifs for the MVA vector

[0494] EcoR1, BamH1 restriction sites and a KOZAK sequence were then added upstream the optimized nucleotide sequence. 2 STOP codons followed by Asc1 and Not1 restriction sites were added downstream the optimized nucleotide sequence.

[0495] The optimized nucleotide sequence of each transgene was then further analyzed with the PITA and miranda software to detect predicted miRNA target sites that might downregulate the expression of the synthetic transgene. 9 miRNA binding sites detected by both methods were removed by modifying the nucleotide sequence of the regions that are predicted to bind the miRNA "seed" by introducing synonymous changes in the corresponding codons. The synthesis of GAd20 and MVA transgenes, was performed using standard methods.

[0496] The codon optimized polynucleotide sequence encoding the GAd20 neoantigen layout of SEQ ID NO: 541 is shown in SEQ ID NO: 542.

[0497] The codon optimized polynucleotide sequence encoding the MVA (neoMVA) neoantigen layout of SEQ ID NO: 543 is shown in SEQ ID NO: 544.

[0498] The codon optimized polynucleotide sequence encoding the GAd20 neoantigen layout including the TCE sequence and excluding the TAG sequence of SEQ ID NO: 550 is shown in SEQ ID NO: 551.

[0499] The codon optimized polynucleotide sequence encoding the MVA neoantigen layout including the TCE sequence and excluding the TAG sequence of SEQ ID NO: 552 is shown in SEQ ID NO: 553.

[0500] Kozak sequence: CGCGACTTCGCCGCC; SEQ ID NO: 545

[0501] Polynucleotide encoding the TCE: ATGGGCCAGAAAGAGCAGATCCACACACTGCAGAAAAACAGCGAGCGGATGAGCAAGCAG CTGACCAGATCTTCTCAGGCCGTG; SEQ ID NO: 546

[0502] Polynucleotide encoding the serine-histidine tag: AGCCATCACCATCACCACCAT; SEQ ID NO: 547

[0503] Two stop codons (TAGTAA)

Polypeptide sequence of the TCE: MGQKEQIHTLQKNSERMSKQLTRSSQAV; SEQ ID NO: 549

GAd20 virus production

[0504] The GAd20 transgene was subcloned into a shuttle plasmid between CMV promoter with two TetO repeats and a BGH polyA via ECOR1-NOT1 restriction sites.

[0505] The resulting expression cassette was transferred into the GAd20 genome by homologous recombination in suitable *E. coli* strains, transformed with the CMV-transgene-BGH DNA fragment and with a construct carrying the GAd20 genome.

[0506] Recombination involved CMV and BGH as homology arms, that were already present in the GAd20 construct in place of the E1 deletion (insertion site of the transgene). Recombinant GAd20 vectors were then rescued by transfection of the E1 complementing, TetR expressing M9 cells and amplified by subsequent re-infection of fresh M9 cells.

[0507] CMV promoter with TetO sites: SEQ ID NO: 628

Ccattgcatacgttgtatccatatcataatatgtacatttatattggctcatgtccaacattaccgccatgttgacattgattattgactagttattaatagtaatca
attacggggtcattagttcatagcccatatatggagttccgcgttacataacttacggtaaatggcccgcctggctgaccgcccaacgacccccgcccatt
gacgtcaataatgacgtatgttcccatagtaacgccaatagggactttccattgacgtcaatgggtggagtatttacggtaaactgcccacttggcagtacat
caagtgtatcatatgccaagtacgccccctattgacgtcaatgacggtaaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctactt
ggcagtacatctacgtattagtcatcgctattaccatggtgatgcggttttggcagtacatcaatgggcgtggatagcggtttgactcacggggatttccaag
tctccaccccattgacgtcaatgggagtttgttttggcaccaaaatcaacgggactttccaaaatgtcgtaacaactccgccccattgacgcaaatgggcgg
taggcgtgtacggtgggaggtctatataagcagagctctccctatcagtgatagagatctccctatcagtgatagagatcgtcgacgagctcgtttagtgaa
ccgtcagatcgcctggagacgccatccacgctgttttgacctccatagaagacaccgggaccgatccagcctccgcggccgggaacggtgcattggaa
cgcggattccccgtgccaagagtga

[0508] BGH polyA SEQ ID NO: 629

ctgtgccttctagttgccagccatctgttgtttgcccctcccccgtgccttccttgaccctggaaggtgccactcccactgtcctttcctaataaaatgaggaa
attgcatcgcattgtctgagtaggtgtcattctattctgggggggtggggtggggcaggacagcaaggggaggattgggaagacaatagcaggcatgct
ggggatgcggtgggctctatggcc

MVA virus production

[0509] The MVA transgene was subcloned into the p94 shuttle plasmid via BAMH1-ASC1 restriction sites, under the control of the vaccinia P7.5 early/late promoter (SEQ ID NO: 630), between sequences homologous to the deletion III locus of MVA (FlankIII-1 and -2 regions). An additional expression cassette for eGFP protein, flanked by a repeated sequence named "Z", was present in the p94 shuttle plasmid, between Flank III regions.

[0510] The parental MVA vector used for recombinant vaccine viruses' generation carried the HcRed1-1 fluorescent protein transgene at the Deletion III locus and was indicated as MVA-RED 476 MG.

[0511] Recombinant MVA, with transgene insertion at the Deletion III locus, were generated by two events of *in vivo* recombination in Chicken embryo fibroblasts (CEF) cells. The first recombination event occurred in cells infected with MVA-RED 476 MG and transfected with the p94 shuttle plasmid, and resulted in replacement of the HcRed protein gene with the transgene/eGFP cassette. Infected cells containing MVA-Green intermediate were isolated by FACS sorting of green cells. The intermediate recombinant MVA resulting from first recombination carried both the transgene and the eGFP cassette but was unstable due to the presence of repeated Z regions. Thus, a spontaneous second recombination event occurred involving Z regions and removed the eGFP cassette. The resulting recombinant MVA was colorless and carried the transgene cassette at the Deletion III locus (insertion site) of MVA-RED 476 MG. This was isolated by FACS sorting of colorless infected cells and amplified by re-infection of fresh CEF cells. The obtained lysate was used to infect Age1 cells to produce the research batch.

[0512] P7.5 early/late promoter SEQ ID NO: 630

GATCACTAATTCCAAACCCACCCGCTTTTTATAGTAAGTTTTTCACCCATAAATAATAAATAC
AATAATTAATTTCTCGTAAAAGTAGAAAATATATTCTAATTTATTGCACGGTAAGGAAGTAG
AATCATAAAGAACAGTGACGGATC

[0513] neoGAd20 protein SEQ ID NO: 541 (no TCE, no HIS tag)

QNLQNGGGSRSSATLPGRRRRRWLRRRRQPISVAPAGPPRRPNQKPNPPGGARCVIMRPTWPGT
SAFTKRSFAVTERIIDYWAQKEKGSSSFLRPSCDYWAQKEKISIPRTHLCLVLGVLSGHSGSRLYE
AGMTLGGKILFFLFLLLPLSPFSLIFTEISCCTLSSEENEYLPRPEWQLQVPFRELKNVSVLEGLRQ
GRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSITGGKSTCSAPGPQSLPSTPFSTY
PQWVILITELGMECTLGQVGAPSPRREEDGWRGGHSRFKADVPAPQGPCWGGQPGSAPSSAPPE
QSLLDWKFEMSYTVGGPPPHVHARPRHWKTDRDGHSYTSKVNCLLLQDGFHGCVSITGAAGRR
NLSIFLFLMLCKLEFHACKIQNKNCPDFKKFDGPCGERGGGRTARALWARGDSVLTPALDPQTP
VRAPSLTRAAAAVHYKLIQQPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCSTNDICVTANF
CISVTFLKPCFLLHEASASQCHLFLQPQVGTPPPHTASARAPSGPPHPHESCPAGRRPARAAQTCA
RRQHGLPGCEEAGTARVPSLHLHLHQAALGAGRGRGWGEACAQVPPSRGVLRFLDLKVRYLHS
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PCGAGQVRAGRGPEAGGGVLQPQRPAPEKPGCPCRRGQPRLHTVKMWRAVAMMVPDRQVHY
DFGLGVPGDSTRRAVRRMNTFYEAGMTLGEKFRVGNCKHLKMTRPNSKMALNSEALSVVSEC
GASACDVSLIAMDSAFVQGKDWGVKKFIRRDFYAYKDFLWCFPFSLVFLQEIQICCHVSCLCCIC
CSTRICLGCLLELFLSRALRALHVLWNGFQLHCQTEYNQKLQVNQFSESKSLYHREKQLIAMDS
AICEERGAAGSLISCETMPAILKLQKNCLLSLRTALTHNQDFSIYRLCCKRGSLCHASQARSPAFP
KPVRPLPAPITRITPQLGGQSDSSQPLLTTGRPQGWQDQALRHTQQASPASCATITIPIHSAALGDH
SGDPGPAWDTCPPLPLTTLIPRAPPPYGDSTARSWPSRCGPLGGNTTLQQLGEASQAPSGSLIPLR
LPLLWEVRGQPDSFAALHSSLNELGEIARELHQFAFDLLIKSHFVQGKDWGLKKFIRRDFWGME
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CTLWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSARTCCGTKWPSSQEAALGLGSGLLRFSC
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[0514] neoGAd20 polynucleotide SEQ ID NO: 542 (no TCE, no HIS tag)

CAGAACCTGCAGAACGGCGGAGGCTCTAGAAGCTCTGCTACACTTCCTGGCAGGCGGCGGA
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GGCTCAAAAAGAGAAGGGCAGCAGCAGCTTCCTGCGGCCTAGCTGTGATTATTGGGCCCAG
AAAGAAAAGATCAGCATCCCCAGAACACACCTGTGCCTGGTGCTGGGAGTGCTGTCTGGAC
ACTCTGGCAGCAGACTGTATGAGGCCGGCATGACACTCGGCGGCAAGATCCTGTTCTTCCTG
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AGCTGAAGAACGTTTCCGTGCTGGAAGGCCTGAGACAGGGCAGACTTGGCGGCCCTTGTAG
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GGTCATCCTGATCACCGAACTCGGCATGGAATGCACCCTGGGACAAGTGGGAGCCCCATCTC
CTAGAAGAGAAGAGGATGGCTGGCGCGGAGGCCACTCTAGATTCAAAGCTGATGTGCCCGC
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CATGCCAGACCTCGGCACTGGAAAACCGACAGAGATGGCCACAGCTACACCAGCAAAGTGA
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AGCTGCCCAGGTGCAGCCTAAAGATCCTCTGCCACTGAGAACACTGGCCGGCTGCCTTGCTA
GAACAGCCCATCTTAGACCTGGCGCCGAGTCTCTGCCTCAGCCACAACTGCACTGTACCCTG
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AGAGTCTGAGACATGTCGTCGGCTACGGCCACCTGGATACAAGCGGAAGCAGCTCTAGCTC
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CAAGAATCGCCCCTCCTAGATCTCACTGCTGCTTTTGGGAAGTGAACGCCCCA

[0515] neoMVA protein SEQ ID NO: 543 (no TCE, no HIS Tag)

QNLQNGGGSRSSATLPGRRRRRWLRRRRQPISVAPAGPPRRPNQKPNPPGGARCVIMRPTWPGT
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FRELKNVSVLEGLRQGRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSITEISCCTL
SSEENEYLPRPEWQLQYEAGMTLGGKILFFLFLLLPLSPFSLIFLVLGVLSGHSGSRLKRSFAVTER
IITGGKSTCSAPGPQSLPSTPFSTYPQWVILITELDGHSYTSKVNCLLLQDGFHGCVSITGAAGRRN
LSIFLFLMLCKLEFHACQWQHYHRSGEAAGTPLWRPTRNVAMMVPDRQVHYDFGLKIQNKNCP
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SGPPHPHESCPAGRRPARAAQTCARRQHGLPGCEEAGTARVPSLHLHLHQAALGAGRGRGWGE
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VTFLKPCFLLHEASASQFKKFDGPCGERGGGRTARALWARGDSVLTPALDPQTPVRAPSLTRAA
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SCLCCICCSTRICLGCLLELFLSRALRALHVLWNGFQLHCQGNTTLQQLGEASQAPSGSLIPLRLP
LLWEVRGNSKMALNSLNSIDDAQLTRIAPPRSHCCFWEVNAPFVQGKDWGLKKFIRRDFEAFQR
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[0516] neoMVA polynucleotide SEQ ID NO: 544

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CCTGGCACCAGCGCCTTTACCGGCATGGAATGTACACTGGGCCAAGTGGGAGCCCCATCTCC
TAGAAGAGAAGAGGATGGCTGGCGCGGAGGCCACTCTAGATTCAAAGCTGATGTGCCCGCT
CCTCAGGGCCCTTGTTGGGGAGGACAACCTGGATCTGCCCCATCTTCTGCCCCACCTGAACA
GAGCCTGCTGGATGACTATTGGGCTCAAAAAGAGAAGATCAGCATCCCCAGAACACACCTG
TGCTGGAAGTTCGAGATGAGCTACACCGTTGGCGGCCCTCCACCACATGTTCACGCCAGACC
TAGACACTGGAAAACCGACAGAGATTATTGGGCCCAGAAAGAAAAGGGCAGCAGCAGCTTC
CTGCGGCCTAGCTGTGTGCCCTTCCGGGAACTGAAGAACGTGTCCGTTCTGGAAGGCCTGAG
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CTACTGGCGCTCCATGGCCAAGCAGAAGGCCTACTTGGAGAGGCACCACCGTGTCTCCAAG
AACCGCTACAAGCAGCGCCAGAACCTGTTGCGGCACAAAATGGCCCTCCAGCCAAGAAGCT
GCCCTCGGACTTGGAAGCGGACTGCTGAGATTCAGCTGTGGCACAGCCGCCATCAGA

[0517] neoGAd20 expression cassette protein SEQ ID NO: 550

MGQKEQIHTLQKNSERMSKQLTRSSQAVQNLQNGGGSRSSATLPGRRRRRWLRRRRQPISVAPA
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QKEKISIPRTHLCLVLGVLSGHSGSRLYEAGMTLGGKILFFLFLLLPLSPFSLIFTEISCCTLSSEENE
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[0518] neoGAd20 expression cassette polynucleotide SEQ ID NO: 551

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CCGCTGTGCACTACAAGCTGATCCAGCAGCCAATCAGCCTGTTCAGCATCACCGACCGGCTG
CACAAGACATTCAGCCAGCTGCCAAGCGTGCACCTGTGCTCCATCACCTTCCAGTGGGGACA
CCCTCCTATCTTTTGCTCCACCAACGACATCTGCGTGACCGCCAACTTCTGTATCAGCGTGAC
CTTCCTGAAGCCTTGCTTTCTGCTGCACGAGGCCAGCGCCTCTCAGTGCCACTTGTTTCTGCA
GCCCCAAGTGGGCACACCTCCTCCACATACAGCCTCTGCTAGAGCACCTAGCGGCCCTCCAC
ATCCTCACGAATCTTGTCCTGCCGGAAGAAGGCCTGCCAGAGCCGCTCAAACATGTGCCAGA
CGACAGCACGGACTGCCTGGATGTGAAGAGGCTGGAACAGCCAGAGTGCCTAGCCTGCACC
TCCATCTGCATCAGGCTGCTCTTGGAGCCGGAAGAGGTAGAGGATGGGGCGAAGCTTGTGCT
CAGGTGCCACCTTCTAGAGGCGTGCTGAGATTCCTGGACCTGAAAGTGCGCTACCTGCACAG
CCAGTGGCAGCACTATCACAGATCTGGCGAAGCCGCCGGAACACCCCTTTGGAGGCCAACA
AGAAACGTGCCCTTCCGGGAACTGAAGAACCAGAGAACAGCTCAGGGCGCTCCTGGAATCC
ACCATGCTGCTTCTCCAGTGGCCGCCAACCTGTGTGATCCTGCCAGACATGCCCAGCACACC
AGGATTCCTTGTGGCGCTGGACAAGTGCGCGCTGGAAGAGGACCTGAAGCAGGCGGAGGTG
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AGACTGCACACCGTGAAAATGTGGCGAGCCGTGGCCATGATGGTGCCCGATAGACAGGTCC
ACTACGACTTTGGACTGGGCGTGCCAGGCGATAGCACTCGGAGAGCCGTCAGACGGATGAA
CACCTTTTACGAAGCCGGGATGACCCTGGGCGAGAAGTTCAGAGTGGGCAACTGCAAGCAC
CTGAAGATGACCCGGCCTAACAGCAAGATGGCCCTGAATAGCGAGGCCCTGTCTGTGGTGTC
TGAATGTGGCGCCTCTGCCTGTGACGTGTCCCTGATCGCTATGGACTCCGCCTTTGTGCAGGG
CAAAGACTGGGGCGTGAAGAAGTTCATCCGGCGGGACTTCTACGCCTACAAGGACTTCCTGT
GGTGCTTCCCCTTCTCTCTGGTGTTCCTGCAAGAGATCCAGATCTGCTGTCATGTGTCCTGCC
TGTGCTGCATCTGCTGTAGCACCAGAATCTGCCTGGGCTGTCTGCTGGAACTGTTCCTGAGC
AGAGCCCTGAGAGCACTGCACGTGCTGTGGAACGGATTCCAGCTGCACTGCCAGACCGAGT
ACAACCAGAAACTGCAAGTGAACCAGTTCAGCGAGAGCAAGAGCCTGTACCACCGGGAAAA
GCAGCTCATTGCCATGGACAGCGCCATCTGCGAAGAGAGAGGCGCCGCAGGATCTCTGATC
TCCTGCGAAACAATGCCCGCCATCCTGAAGCTGCAGAAGAATTGCCTCCTAAGCCTGCGAAC
CGCTCTGACACACAACCAGGACTTCAGCATCTACAGACTGTGTTGCAAGCGGGGCTCCCTGT
GCCATGCAAGCCAAGCTAGAAGCCCCGCCTTTCCTAAACCTGTGCGACCTCTGCCAGCTCCA
ATCACCAGAATTACCCCTCAGCTCGGCGGCCAGAGCGATTCATCTCAACCTCTGCTGACCAC
CGGCAGACCTCAAGGCTGGCAAGACCAAGCTCTGAGACACACCCAGCAGGCTAGCCCTGCC
TCTTGTGCCACCATCACAATCCCCATCCACTCTGCCGCTCTGGGCGATCATTCTGGCGATCCT
GGACCAGCCTGGGACACATGTCCTCCACTGCCACTCACAACACTGATCCCTAGGGCTCCTCC
ACCTTACGGCGATTCTACCGCTAGAAGCTGGCCCAGCAGATGTGGACCACTCGGAGGCAAC
ACAACCCTCCAGCAACTGGGAGAAGCCTCTCAGGCTCCTAGCGGCTCTCTGATCCCTCTCAG
ACTGCCTCTCCTGTGGGAAGTTCGGGGCCAGCCTGATTCTTTTGCCGCACTGCACAGCTCCCT
GAACGAGCTGGGAGAGATCGCTAGAGAGCTGCACCAGTTCGCCTTCGACCTGCTGATCAAG
AGCCACTTCGTGCAAGGCAAGGATTGGGGCCTCAAAAAGTTTATCCGCAGAGACTTCTGGG
GCATGGAACTGGCCGCCAGCAGAAGATTCAGCTGGGATCATCATAGCGCAGGCGGCCCACC
TAGAGTGCCATCTGTTAGAAGCGGAGCTGCCCAGGTGCAGCCTAAAGATCCTCTGCCACTGA
GAACACTGGCCGGCTGCCTTGCTAGAACAGCCCATCTTAGACCTGGCGCCGAGTCTCTGCCT
CAGCCACAACTGCACTGTACCCTGTGGTTCCAGTCCAGCGAGCTGTCTCCTACTGGTGCCCCT
TGGCCATCTAGACGCCCTACTTGGAGAGGCACCACCGTGTCACCAAGAACCGCCACAAGCA
GCGCCAGAACCTGTTGTGGCACAAAGTGGCCCTCCAGCCAAGAAGCCGCTCTCGGACTTGG
AAGCGGACTGCTGAGGTTCTCTTGTGGAACCGCCGCCATTCGGAAGATGCACTTTAGCCTGA
AAGAACACCCTCCACCACCTTGTCCTCCAGAGGCTTTCCAAAGAGCTGCTGGCGAAGGCGGA

CCTGGTAGAGGTGGTGCTAGAAGAGGTGCTAGGGTGCTGCAGAGCCCATTCTGTAGAGCAG
GCGCAGGCGAATGGCTGGGCCATCAGAGTCTGAGACATGTCGTCGGCTACGGCCACCTGGA
TACAAGCGGAAGCAGCTCTAGCTCCAGCTGGCCTAACTCAAAAATGGCTCTGAACAGCCTG
AACTCCATCGACGACGCCCAGCTGACAAGAATCGCCCCTCCTAGATCTCACTGCTGCTTTTG
GGAAGTGAACGCCCCAAGCCATCACCATCACCACCATTAGTAAAGGCGCGCCTAGCGGCCG
Cgatctgctgtgccttctagttgccagccatctgttgtttgcccctccccgtgccttccttgaccctggaaggtgccactcccactgtcctttcctaataaaat
gaggaaattgcatcgcattgtctgagtaggtgtcattctattctgggggggtggggtggggcaggacagcaagggggaggattgggaagacaatagcag
gcatgctggggatgcggtgggctctatggcc

[0519] neoMVA expression cassette protein SEQ ID NO: 552

MGQKEQIHTLQKNSERMSKQLTRSSQAVQNLQNGGGSRSSATLPGRRRRRWLRRRRQPISVAPA
GPPRRPNQKPNPPGGARCVIMRPTWPGTSAFTGMECTLGQVGAPSPRREEDGWRGGHSRFKAD
VPAPQGPCWGGQPGSAPSSAPPEQSLLDDYWAQKEKISIPRTHLCWKFEMSYTVGGPPPHVHAR
PRHWKTDRDYWAQKEKGSSSFLRPSCVPFRELKNVSVLEGLRQGRLGGPCSCHCPRPSQARLTP
VDVAGPFLCLGDPGLFPPVKSSITEISCCTLSSEENEYLPRPEWQLQYEAGMTLGGKILFFLFLLLP
LSPFSLIFLVLGVLSGHSGSRLKRSFAVTERIITGGKSTCSAPGPQSLPSTPFSTYPQWVILITELDGH
SYTSKVNCLLLQDGFHGCVSITGAAGRRNLSIFLFLMLCKLEFHACQWQHYHRSGEAAGTPLWR
PTRNVAMMVPDRQVHYDFGLKIQNKNCPDVLRFLDLKVRYLHSVPFRELKNQRTAQGAPGIHH
AASPVAANLCDPARHAQHTRIPCGAGQVRAGRGPEAGGGVLQPQRPAPEKPGCPCRRGQPRLH
TVKMWRACHLFLQPQVGTPPPHTASARAPSGPPHPHESCPAGRRPARAAQTCARRQHGLPGCEE
AGTARVPSLHLHLHQAALGAGRGRGWGEACAQVPPSRGHYKLIQQPISLFSITDRLHKTFSQLPS
VHLCSITFQWGHPPIFCSTNDICVTANFCISVTFLKPCFLLHEASASQFKKFDGPCGERGGGRTAR
ALWARGDSVLTPALDPQTPVRAPSLTRAAAAVGVPGDSTRRAVRRMNTFCGASACDVSLIAMD
SACEERGAAGSLISCESLYHREKQLIAMDSAIFVQGKDWGVKKFIRRDFTMPAILKLQKNCLLSL
NSKMALNSEALSVVSEYEAGMTLGEKFRVGNCKHLKMTRPTEYNQKLQVNQFSESKRTALTHN
QDFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITPQLGGQSDSSQPLLTTGRPQGWQDQ
ALRHTQQASPASCATITIPIHSAALGDHSGDPGPAWDTCPPLPLTTLIPRAPPPYGDSTARSWPSRC
GPLGYAYKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFLSRALRALHVLWNGF
QLHCQGNTTLQQLGEASQAPSGSLIPLRLPLLWEVRGNSKMALNSLNSIDDAQLTRIAPPRSHCC
FWEVNAPFVQGKDWGLKKFIRRDFEAFQRAAGEGGPGRGGARRGARVLQSPFCRAGAGEWLG
HQSLRWGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLPLRTLAGCLARTAHLRPG
AESLPQPQLHCTIARELHQFAFDLLIKSHKMHFSLKEHPPPPCPPHVVGYGHLDTSGSSSSSSWPQ
PDSFAALHSSLNELGELWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSARTCCGTKWPSSQE
AALGLGSGLLRFSCGTAAIR

[0520] neoMVA expression cassette polynucleotide SEQ ID NO: 553

gatcactaattccaaacccacccgctttttatagtaagtttttcacccataaataataaatacaataattaatttctcgtaaaagtagaaaatatattctaatttattg
cacggtaaggaagtagaatcataaagaacagtgacGGATCCCGCGACTTCGCCGCCATGGGCCAGAAAGAGCAG
ATCCACACACTGCAGAAAAACAGCGAGCGGATGAGCAAGCAGCTGACCAGATCTTCTCAGG
CCGTGCAGAACCTGCAGAACGGCGGAGGCTCTAGAAGCTCTGCTACACTTCCTGGCAGGCG
GCGGAGAAGATGGCTGAGAAGAAGGCGGCAGCCTATCTCTGTGGCTCCTGCTGGACCTCCT
AGACGGCCCAACCAGAAGCCTAATCCTCCTGGCGGAGCCAGATGCGTGATCATGAGGCCTA
CATGGCCTGGCACCAGCGCCTTTACCGGCATGGAATGTACACTGGGCCAAGTGGGAGCCCC
ATCTCCTAGAAGAGAAGAGGATGGCTGGCGCGGAGGCCACTCTAGATTCAAAGCTGATGTG
CCCGCTCCTCAGGGCCCTTGTTGGGGAGGACAACCTGGATCTGCCCCATCTTCTGCCCCACCT
GAACAGAGCCTGCTGGATGACTATTGGGCTCAAAAAGAGAAGATCAGCATCCCCAGAACAC
ACCTGTGCTGGAAGTTCGAGATGAGCTACACCGTTGGCGGCCCTCCACCACATGTTCACGCC
AGACCTAGACACTGGAAAACCGACAGAGATTATTGGGCCCAGAAAGAAAAGGGCAGCAGC
AGCTTCCTGCGGCCTAGCTGTGTGCCCTTCCGGGAACTGAAGAACGTGTCCGTTCTGGAAGG
CCTGAGGCAGGGCAGACTTGGCGGACCTTGTAGCTGCCACTGTCCTAGACCAAGCCAGGCC
AGACTGACCCCTGTGGATGTGGCTGGCCCATTTCTGTGTCTGGGCGACCCTGGACTGTTCCCT
CCAGTGAAGTCTAGCATCACCGAGATCAGCTGCTGCACCCTGAGCAGCGAGGAAAACGAGT
ACCTGCCTAGACCTGAATGGCAGCTGCAGTACGAGGCCGGCATGACACTCGGAGGCAAGAT
CCTGTTCTTCCTGTTCCTGCTGCTCCCTCTGAGCCCCTTCAGCCTGATCTTTCTGGTGCTGGGC

GTGCTGTCTGGCCACTCTGGAAGCAGACTGAAGAGAAGCTTCGCCGTGACCGAGCGGATCA
TCACAGGCGGCAAGAGCACATGTTCTGCCCCTGGACCTCAGTCTCTGCCCAGCACACCCTTC
AGCACATACCCTCAGTGGGTCATCCTGATCACCGAGCTGGATGGCCACAGCTACACCAGCAA
AGTGAACTGCCTCCTGCTGCAGGATGGCTTCCACGGCTGTGTGTCTATTACTGGCGCCGCTG
GCAGACGGAACCTGAGCATCTTTCTGTTTCTGATGCTGTGCAAGCTCGAGTTCCACGCCTGC
CAATGGCAGCACTACCACAGATCTGGCGAAGCCGCTGGAACCCCACTTTGGAGGCCTACCA
GAAACGTGGCCATGATGGTGCCCGACAGACAGGTGCACTACGACTTCGGCCTGAAGATCCA
GAACAAGAACTGCCCCGACGTGCTGCGGTTCCTGGATCTCAAAGTGCGCTACCTGCACAGCG
TGCCCTTCAGAGAGCTGAAAAACCAGAGAACAGCCCAGGGCGCTCCTGGAATCCATCATGC
TGCTTCTCCAGTGGCCGCCAATCTGTGCGATCCTGCCAGACATGCCCAGCATACCAGGATTC
CTTGTGGCGCTGGACAAGTGCGCGCTGGAAGAGGACCTGAAGCTGGTGGCGGAGTTCTGCA
GCCTCAAAGACCTGCTCCTGAGAAGCCTGGCTGCCCCTGTAGAAGAGGACAGCCTAGACTG
CACACCGTGAAGATGTGGCGGGCCTGCCACTTGTTTCTCCAGCCACAAGTGGGCACCCCTCC
ACCTCATACAGCCTCTGCTAGAGCACCTAGCGGCCCACCTCATCCTCACGAATCTTGTCCTGC
CGGAAGAAGGCCTGCCAGAGCCGCTCAAACATGTGCCAGACGACAGCACGGACTGCCCGGA
TGTGAAGAAGCCGGAACAGCCAGAGTGCCTAGCCTGCACCTTCATCTGCATCAGGCCGCTCT
TGGAGCCGGAAGAGGTAGAGGATGGGGAGAAGCTTGTGCCCAGGTGCCACCTTCTAGAGGC
CACTACAAGCTGATCCAGCAGCCAATCAGCCTGTTCTCCATCACCGACCGGCTGCACAAGAC
ATTCAGCCAGCTGCCTTCCGTGCATCTGTGCAGCATCACCTTCCAGTGGGGACACCCTCCTAT
CTTTTGCTCCACCAACGACATCTGCGTGACCGCCAACTTCTGTATCAGCGTGACCTTCCTGAA
GCCTTGCTTTCTGCTGCACGAGGCCTCCGCCAGCCAGTTTAAGAAGTTTGACGGCCCCTGCG
GCGAGAGAGGCGGAGGAAGAACTGCAAGAGCCCTTTGGGCCAGAGGCGACTCTGTTCTGAC
ACCAGCTCTGGACCCTCAGACACCTGTTAGGGCCCCTAGCCTGACAAGAGCTGCCGCTGCTG
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TCTGCCTGCGACGTGTCCCTGATCGCTATGGATAGCGCCTGCGAGGAAAGAGGCGCAGCCG
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GCCATCTTCGTGCAGGGCAAAGACTGGGGCGTGAAGAAGTTCATCCGGCGGGACTTTACCAT
GCCTGCCATTCTGAAGCTGCAGAAGAATTGTCTTCTAAGCCTGAACAGCAAGATGGCCCTGA
ATAGCGAGGCCCTGTCTGTGGTGTCCGAGTATGAGGCTGGAATGACCCTGGGCGAGAAGTTC
AGAGTGGGCAACTGCAAGCACCTGAAGATGACCCGGCCTACCGAGTACAACCAGAAACTGC
AAGTGAACCAGTTCAGCGAGAGCAAGCGGACCGCTCTGACCCACAACCAGGACTTCAGCAT
CTACCGGCTGTGCTGCAAGAGGGGCTCTCTGTGTCATGCTAGCCAGGCTAGAAGCCCCGCCT
TTCCTAAGCCTGTCAGACCTCTGCCTGCTCCTATCACCAGAATCACCCCTCAGCTCGGCGGCC
AGTCTGATTCATCTCAGCCACTGCTGACCACCGGCAGACCTCAAGGATGGCAAGACCAGGCT
CTGAGACACACACAGCAGGCTAGCCCAGCCTCTTGCGCCACCATCACAATACCAATACATTC
TGCCGCTCTGGGCGATCACAGCGGAGATCCTGGACCTGCCTGGGATACTTGTCCTCCTCTGC
CCCTAACTACACTGATCCCTAGGGCTCCTCCACCTTACGGCGATAGCACAGCCAGATCCTGG
CCTAGCAGATGTGGCCCTCTGGGCTACGCCTACAAGGACTTCCTGTGGTGCTTCCCCTTCTCT
CTGGTGTTCCTGCAAGAAATCCAGATCTGCTGTCACGTGTCCTGCCTGTGCTGTATCTGCTGT
AGCACCCGGATCTGTCTGGGCTGTCTGCTGGAACTGTTCCTGAGCAGAGCCCTGAGAGCACT
GCACGTGCTGTGGAACGGATTCCAGCTGCACTGCCAGGGCAACACCACACTGCAACAGCTG
GGAGAAGCCTCTCAGGCCCCAAGCGGTTCTCTGATCCCTCTCAGACTGCCCCTCCTGTGGGA
AGTGCGGGGCAATTCTAAGATGGCTCTCAACAGCCTGAACTCCATCGACGACGCCCAGCTGA
CAAGAATCGCCCCTCCAAGAAGCCACTGTTGCTTTTGGGAAGTGAACGCCCCTTTTGTGCAG
GGTAAAGATTGGGGCCTCAAAAAGTTTATCAGACGGGACTTCGAGGCTTTCCAGAGAGCAG
CTGGCGAAGGCGGACCTGGCAGAGGTGGTGCTAGAAGAGGTGCTAGAGTGCTGCAGAGCCC
ATTCTGTAGAGCTGGCGCTGGCGAATGGCTGGGCCACCAATCTCTTAGATGGGGAATGGAAC
TGGCCGCTAGCAGGCGGTTTAGCTGGGATCATCATTCTGCCGGCGGACCTCCAAGAGTGCCA
AGCGTTAGAAGCGGAGCAGCCCAGGTCCAGCCTAAAGATCCACTGCCACTGAGAACACTGG
CCGGCTGCCTTGCCAGAACAGCTCATCTTAGACCTGGCGCCGAAAGCCTGCCTCAACCTCAG
CTGCATTGCACAATCGCCAGAGAACTGCACCAGTTCGCCTTCGACCTGCTGATCAAGAGCCA
CAAGATGCACTTCTCACTGAAAGAGCACCCGCCACCGCCGTGCCCACCGCACGTTGTCGGCT
ATGGCCACCTGGATACAAGCGGCTCCTCTAGCAGTAGCTCCTGGCCTCAGCCTGACAGCTTT
GCTGCCCTGCATAGCTCCCTGAATGAGCTGGGCGAACTGTGGTTCCAGTCCAGCGAACTGTC
TCCTACTGGCGCTCCATGGCCAAGCAGAAGGCCTACTTGGAGAGGCACCACCGTGTCTCCAA

GAACCGCTACAAGCAGCGCCAGAACCTGTTGCGGCACAAAATGGCCCTCCAGCCAAGAAGC
TGCCCTCGGACTTGGAAGCGGACTGCTGAGATTCAGCTGTGGCACAGCCGCCATCAGAAGCC
ATCACCATCACCACCATTAGTAAAGGCGCGCC

[0521] The amino acid sequence of additional five neoantigen layouts for GAd20 expression are shown in SEQ ID NOs: 554, 555, 556, 623 and 624.

[0522] SEQ ID NO: 554

MGQKEQIHTLQKNSERMSKQLTRSSQAVQNLQNGGGSRSSATLPGRRRRRWLRRRRQPISVAPA
GPPRRPNQKPNPPGGARCVIMRPTWPGTSAFTKRSFAVTERIILVLGVLSGHSGSRLYEAGMTLG
GKILFFLFLLLPLSPFSLIFTEISCCTLSSEENEYLPRPEWQLQVPFRELKNVSVLEGLRQGRLGGPC
SCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSITGGKSTCSAPGPQSLPSTPFSTYPQWVILIT
ELGMECTLGQVGAPSPRREEDGWRGGHSRFKADVPAPQGPCWGGQPGSAPSSAPPEQSLLDDY
WAQKEKGSSSFLRPSCDYWAQKEKISIPRTHLCWKFEMSYTVGGPPPHVHARPRHWKTDRGVP
GDSTRRAVRRMNTFYEAGMTLGEKFRVGNCKHLKMTRPNSKMALNSEALSVVSECGASACDV
SLIAMDSAFVQGKDWGVKKFIRRDFYAYKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLG
CLLELFLSRALRALHVLWNGFQLHCQTEYNQKLQVNQFSESKSLYHREKQLIAMDSAICEERGA
AGSLISCETMPAILKLQKNCLLSLRTALTHNQDFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPA
PITRITPQLGGQSDSSQPLLTTGRPQGWQDQALRHTQQASPASCATITIPIHSAALGDHSGDPGPA
WDTCPPLPLTTLIPRAPPPYGDSTARSWPSRCGPLGDGHSYTSKVNCLLLQDGFHGCVSITGAAG
RRNLSIFLFLMLCKLEFHACKIQNKNCPDFKKFDGPCGERGGGRTARALWARGDSVLTPALDPQ
TPVRAPSLTRAAAAVHYKLIQQPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCSTNDICVTA
NFCISVTFLKPCFLLHEASASQCHLFLQPQVGTPPPHTASARAPSGPPHPHESCPAGRRPARAAQT
CARRQHGLPGCEEAGTARVPSLHLHLHQAALGAGRGRGWGEACAQVPPSRGVLRFLDLKVRYL
HSQWQHYHRSGEAAGTPLWRPTRNVPFRELKNQRTAQGAPGIHHAASPVAANLCDPARHAQHT
RIPCGAGQVRAGRGPEAGGGVLQPQRPAPEKPGCPCRRGQPRLHTVKMWRAVAMMVPDRQVH
YDFGLGNTTLQQLGEASQAPSGSLIPLRLPLLWEVRGQPDSFAALHSSLNELGEIARELHQFAFDL
LIKSHFVQGKDWGLKKFIRRDFWGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLP
LRTLAGCLARTAHLRPGAESLPQPQLHCTLWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSA
RTCCGTKWPSSQEAALGLGSGLLRFSCGTAAIRKMHFSLKEHPPPPCPPEAFQRAAGEGGPGRGG
ARRGARVLQSPFCRAGAGEWLGHQSLRHVVGYGHLDTSGSSSSSSWPNSKMALNSLNSIDDAQ
LTRIAPPRSHCCFWEVNAP

[0523] SEQ ID NO: 555

MGQKEQIHTLQKNSERMSKQLTRSSQAVQNLQNGGGSRSSATLPGRRRRRWLRRRRQPISVAPA
GPPRRPNQKPNPPGGARCVIMRPTWPGTSAFTKRSFAVTERIITEISCCTLSSEENEYLPRPEWQLQ
YEAGMTLGGKILFFLFLLLPLSPFSLIFDYWAQKEKGSSSFLRPSCVPFRELKNVSVLEGLRQGRL
GGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSITGGKSTCSAPGPQSLPSTPFSTYPQ
WVILITELGMECTLGQVGAPSPRREEDGWRGGHSRFKADVPAPQGPCWGGQPGSAPSSAPPEQS
LLDDYWAQKEKISIPRTHLCLVLGVLSGHSGSRLWKFEMSYTVGGPPPHVHARPRHWKTDRDG
HSYTSKVNCLLLQDGFHGCVSITGAAGRRNLSIFLFLMLCKLEFHACKIQNKNCPDFKKFDGPCG
ERGGGRTARALWARGDSVLTPALDPQTPVRAPSLTRAAAAVHYKLIQQPISLFSITDRLHKTFSQ
LPSVHLCSITFQWGHPPIFCSTNDICVTANFCISVTFLKPCFLLHEASASQCHLFLQPQVGTPPPHT
ASARAPSGPPHPHESCPAGRRPARAAQTCARRQHGLPGCEEAGTARVPSLHLHLHQAALGAGRG
RGWGEACAQVPPSRGVLRFLDLKVRYLHSQWQHYHRSGEAAGTPLWRPTRNVPFRELKNQRT
AQGAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAGRGPEAGGGVLQPQRPAPEKPGCP
CRRGQPRLHTVKMWRAVAMMVPDRQVHYDFGLGVPGDSTRRAVRRMNTFYEAGMTLGEKFR
VGNCKHLKMTRPNSKMALNSEALSVVSECGASACDVSLIAMDSAFVQGKDWGVKKFIRRDFYA
YKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFLSRALRALHVLWNGFQLHCQT
EYNQKLQVNQFSESKSLYHREKQLIAMDSAICEERGAAGSLISCETMPAILKLQKNCLLSLRTALT
HNQDFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITPQLGGQSDSSQPLLTTGRPQGWQ
DQALRHTQQASPASCATITIPIHSAALGDHSGDPGPAWDTCPPLPLTTLIPRAPPPYGDSTARSWPS
RCGPLGGNTTLQQLGEASQAPSGSLIPLRLPLLWEVRGQPDSFAALHSSLNELGEIARELHQFAFD
LLIKSHFVQGKDWGLKKFIRRDFWGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPL
PLRTLAGCLARTAHLRPGAESLPQPQLHCTLWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSS

ARTCCGTKWPSSQEAALGLGSGLLRFSCGTAAIRKMHFSLKEHPPPPCPPEAFQRAAGEGGPGRG
GARRGARVLQSPFCRAGAGEWLGHQSLRHVVGYGHLDTSGSSSSSSWPNSKMALNSLNSIDDA
QLTRIAPPRSHCCFWEVNAP

[0524] SEQ ID NO: 556

MGQKEQIHTLQKNSERMSKQLTRSSQAVQNLQNGGGSRSSATLPGRRRRRWLRRRRQPISVAPA
GPPRRPNQKPNPPGGARCVIMRPTWPGTSAFTKRSFAVTERIIDYWAQKEKGSSSFLRPSCVPFRE
LKNVSVLEGLRQGRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSILVLGVLSGH
SGSRLYEAGMTLGGKILFFLFLLLPLSPFSLIFTEISCCTLSSEENEYLPRPEWQLQTGGKSTCSAPG
PQSLPSTPFSTYPQWVILITELGMECTLGQVGAPSPRREEDGWRGGHSRFKADVPAPQGPCWGG
QPGSAPSSAPPEQSLLDDYWAQKEKISIPRTHLCWKFEMSYTVGGPPPHVHARPRHWKTDRDGH
SYTSKVNCLLLQDGFHGCVSITGAAGRRNLSIFLFLMLCKLEFHACKIQNKNCPDFKKFDGPCGE
RGGGRTARALWARGDSVLTPALDPQTPVRAPSLTRAAAAVHYKLIQQPISLFSITDRLHKTFSQL
PSVHLCSITFQWGHPPIFCSTNDICVTANFCISVTFLKPCFLLHEASASQCHLFLQPQVGTPPPHTAS
ARAPSGPPHPHESCPAGRRPARAAQTCARRQHGLPGCEEAGTARVPSLHLHLHQAALGAGRGR
GWGEACAQVPPSRGVLRFLDLKVRYLHSQWQHYHRSGEAAGTPLWRPTRNVPFRELKNQRTA
QGAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAGRGPEAGGGVLQPQRPAPEKPGCPCR
RGQPRLHTVKMWRAVAMMVPDRQVHYDFGLGVPGDSTRRAVRRMNTFYEAGMTLGEKFRVG
NCKHLKMTRPNSKMALNSEALSVVSECGASACDVSLIAMDSAFVQGKDWGVKKFIRRDFYAYK
DFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFLSRALRALHVLWNGFQLHCQTEY
NQKLQVNQFSESKSLYHREKQLIAMDSAICEERGAAGSLISCETMPAILKLQKNCLLSLRTALTH
NQDFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITPQLGGQSDSSQPLLTTGRPQGWQD
QALRHTQQASPASCATITIPIHSAALGDHSGDPGPAWDTCPPLPLTTLIPRAPPPYGDSTARSWPSR
CGPLGGNTTLQQLGEASQAPSGSLIPLRLPLLWEVRGQPDSFAALHSSLNELGEIARELHQFAFDL
LIKSHFVQGKDWGLKKFIRRDFWGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLP
LRTLAGCLARTAHLRPGAESLPQPQLHCTLWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSA
RTCCGTKWPSSQEAALGLGSGLLRFSCGTAAIRKMHFSLKEHPPPPCPPEAFQRAAGEGGPGRGG
ARRGARVLQSPFCRAGAGEWLGHQSLRHVVGYGHLDTSGSSSSSSWPNSKMALNSLNSIDDAQ
LTRIAPPRSHCCFWEVNAP

[0525] SEQ ID NO: 623

MGQKEQIHTLQKNSERMSKQLTRSSQAVGVPGDSTRRAVRRMNTFYEAGMTLGEKFRVGNCK
HLKMTRPNSKMALNSEALSVVSECGASACDVSLIAMDSAFVQGKDWGVKKFIRRDFYAYKDFL
WCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFLSRALRALHVLWNGFQLHCQTEYNQK
LQVNQFSESKSLYHREKQLIAMDSAICEERGAAGSLISCETMPAILKLQKNCLLSLRTALTHNQDF
SIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITPQLGGQSDSSQPLLTTGRPQGWQDQALR
HTQQASPASCATITIPIHSAALGDHSGDPGPAWDTCPPLPLTTLIPRAPPPYGDSTARSWPSRCGPL
GGNTTLQQLGEASQAPSGSLIPLRLPLLWEVRGQPDSFAALHSSLNELGEIARELHQFAFDLLIKS
HFVQGKDWGLKKFIRRDFWGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLPLRTL
AGCLARTAHLRPGAESLPQPQLHCTLWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSARTCC
GTKWPSSQEAALGLGSGLLRFSCGTAAIRKMHFSLKEHPPPPCPPEAFQRAAGEGGPGRGGARR
GARVLQSPFCRAGAGEWLGHQSLRHVVGYGHLDTSGSSSSSSWPNSKMALNSLNSIDDAQLTRI
APPRSHCCFWEVNAPDGHSYTSKVNCLLLQDGFHGCVSITGAAGRRNLSIFLFLMLCKLEFHAC
KIQNKNCPDFKKFDGPCGERGGGRTARALWARGDSVLTPALDPQTPVRAPSLTRAAAAVHYKLI
QQPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCSTNDICVTANFCISVTFLKPCFLLHEASAS
QCHLFLQPQVGTPPPHTASARAPSGPPHPHESCPAGRRPARAAQTCARRQHGLPGCEEAGTARV
PSLHLHLHQAALGAGRGRGWGEACAQVPPSRGVLRFLDLKVRYLHSQWQHYHRSGEAAGTPL
WRPTRNVPFRELKNQRTAQGAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAGRGPEAG
GGVLQPQRPAPEKPGCPCRRGQPRLHTVKMWRAVAMMVPDRQVHYDFGLQNLQNGGGSRSSA
TLPGRRRRRWLRRRRQPISVAPAGPPRRPNQKPNPPGGARCVIMRPTWPGTSAFTKRSFAVTERII
LVLGVLSGHSGSRLYEAGMTLGGKILFFLFLLLPLSPFSLIFTEISCCTLSSEENEYLPRPEWQLQVP
FRELKNVSVLEGLRQGRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSITGGKSTC
SAPGPQSLPSTPFSTYPQWVILITELGMECTLGQVGAPSPRREEDGWRGGHSRFKADVPAPQGPC
WGGQPGSAPSSAPPEQSLLDDYWAQKEKGSSSFLRPSCDYWAQKEKISIPRTHLCWKFEMSYTV
GGPPPHVHARPRHWKTDR

[0526] SEQ ID NO: 624

MGQKEQIHTLQKNSERMSKQLTRSSQAVGNTTLQQLGEASQAPSGSLIPLRLPLLWEVRGQPDSF
AALHSSLNELGEIARELHQFAFDLLIKSHFVQGKDWGLKKFIRRDFWGMELAASRRFSWDHHSA
GGPPRVPSVRSGAAQVQPKDPLPLRTLAGCLARTAHLRPGAESLPQPQLHCTLWFQSSELSPTGA
PWPSRRPTWRGTTVSPRTATSSARTCCGTKWPSSQEAALGLGSGLLRFSCGTAAIRKMHFSLKEH
PPPPCPPEAFQRAAGEGGPGRGGARRGARVLQSPFCRAGAGEWLGHQSLRHVVGYGHLDTSGS
SSSSSWPNSKMALNSLNSIDDAQLTRIAPPRSHCCFWEVNAPGVPGDSTRRAVRRMNTFYEAGM
TLGEKFRVGNCKHLKMTRPNSKMALNSEALSVVSECGASACDVSLIAMDSAFVQGKDWGVKK
FIRRDFYAYKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFLSRALRALHVLWNG
FQLHCQTEYNQKLQVNQFSESKSLYHREKQLIAMDSAICEERGAAGSLISCETMPAILKLQKNCL
LSLRTALTHNQDFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITPQLGGQSDSSQPLLTT
GRPQGWQDQALRHTQQASPASCATITIPIHSAALGDHSGDPGPAWDTCPPLPLTTLIPRAPPPYGD
STARSWPSRCGPLGDGHSYTSKVNCLLLQDGFHGCVSITGAAGRRNLSIFLFLMLCKLEFHACKI
QNKNCPDFKKFDGPCGERGGGRTARALWARGDSVLTPALDPQTPVRAPSLTRAAAAVHYKLIQ
QPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCSTNDICVTANFCISVTFLKPCFLLHEASASQ
CHLFLQPQVGTPPPHTASARAPSGPPHPHESCPAGRRPARAAQTCARRQHGLPGCEEAGTARVPS
LHLHLHQAALGAGRGRGWGEACAQVPPSRGVLRFLDLKVRYLHSQWQHYHRSGEAAGTPLWR
PTRNVPFRELKNQRTAQGAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAGRGPEAGGG
VLQPQRPAPEKPGCPCRRGQPRLHTVKMWRAVAMMVPDRQVHYDFGLQNLQNGGGSRSSATL
PGRRRRRWLRRRRQPISVAPAGPPRRPNQKPNPPGGARCVIMRPTWPGTSAFTKRSFAVTERIITE
ISCCTLSSEENEYLPRPEWQLQYEAGMTLGGKILFFLFLLLPLSPFSLIFDYWAQKEKGSSSFLRPS
CVPFRELKNVSVLEGLRQGRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSITGG
KSTCSAPGPQSLPSTPFSTYPQWVILITELGMECTLGQVGAPSPRREEDGWRGGHSRFKADVPAP
QGPCWGGQPGSAPSSAPPEQSLLDDYWAQKEKISIPRTHLCLVLGVLSGHSGSRLWKFEMSYTV
GGPPPHVHARPRHWKTDR

[0527] The amino acid sequence of additional five neoantigen layouts for MVA expression are shown in SEQ ID NOs: 557, 558, 559, 625 and 626.

[0528] SEQ ID NO: 557

MGQKEQIHTLQKNSERMSKQLTRSSQAVQNLQNGGGSRSSATLPGRRRRRWLRRRRQPISVAPA
GPPRRPNQKPNPPGGARCVIMRPTWPGTSAFTGMECTLGQVGAPSPRREEDGWRGGHSRFKAD
VPAPQGPCWGGQPGSAPSSAPPEQSLLDDYWAQKEKISIPRTHLCWKFEMSYTVGGPPPHVHAR
PRHWKTDRDYWAQKEKGSSSFLRPSCVPFRELKNVSVLEGLRQGRLGGPCSCHCPRPSQARLTP
VDVAGPFLCLGDPGLFPPVKSSITEISCCTLSSEENEYLPRPEWQLQYEAGMTLGGKILFFLFLLLP
LSPFSLIFLVLGVLSGHSGSRLKRSFAVTERIITGGKSTCSAPGPQSLPSTPFSTYPQWVILITELDGH
SYTSKVNCLLLQDGFHGCVSITGAAGRRNLSIFLFLMLCKLEFHACCHLFLQPQVGTPPPHTASA
RAPSGPPHPHESCPAGRRPARAAQTCARRQHGLPGCEEAGTARVPSLHLHLHQAALGAGRGRG
WGEACAQVPPSRGHYKLIQQPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCSTNDICVTAN
FCISVTFLKPCFLLHEASASQVAMMVPDRQVHYDFGLKIQNKNCPDVLRFLDLKVRYLHSVPFR
ELKNQRTAQGAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAGRGPEAGGGVLQPQRPA
PEKPGCPCRRGQPRLHTVKMWRAQWQHYHRSGEAAGTPLWRPTRNFKKFDGPCGERGGGRTA
RALWARGDSVLTPALDPQTPVRAPSLTRAAAAVGVPGDSTRRAVRRMNTFCGASACDVSLIAM
DSACEERGAAGSLISCESLYHREKQLIAMDSAIFVQGKDWGVKKFIRRDFTMPAILKLQKNCLLS
LNSKMALNSEALSVVSEYEAGMTLGEKFRVGNCKHLKMTRPTEYNQKLQVNQFSESKRTALTH
NQDFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITPQLGGQSDSSQPLLTTGRPQGWQD
QALRHTQQASPASCATITIPIHSAALGDHSGDPGPAWDTCPPLPLTTLIPRAPPPYGDSTARSWPSR
CGPLGYAYKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFLSRALRALHVLWNG
FQLHCQGNTTLQQLGEASQAPSGSLIPLRLPLLWEVRGNSKMALNSLNSIDDAQLTRIAPPRSHC
CFWEVNAPFVQGKDWGLKKFIRRDFEAFQRAAGEGGPGRGGARRGARVLQSPFCRAGAGEWL
GHQSLRWGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLPLRTLAGCLARTAHLRP
GAESLPQPQLHCTIARELHQFAFDLLIKSHKMHFSLKEHPPPPCPPHVVGYGHLDTSGSSSSSSWP
QPDSFAALHSSLNELGELWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSARTCCGTKWPSSQ
EAALGLGSGLLRFSCGTAAIR

[0529] SEQ ID NO: 558

MGQKEQIHTLQKNSERMSKQLTRSSQAVDGHSYTSKVNCLLLQDGFHGCVSITGAAGRRNLSIF
LFLMLCKLEFHACQWQHYHRSGEAAGTPLWRPTRNVAMMVPDRQVHYDFGLVLRFLDLKVRY
LHSKIQNKNCPDVPFRELKNQRTAQGAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAGR
GPEAGGGVLQPQRPAPEKPGCPCRRGQPRLHTVKMWRACHLFLQPQVGTPPPHTASARAPSGPP
HPHESCPAGRRPARAAQTCARRQHGLPGCEEAGTARVPSLHLHLHQAALGAGRGRGWGEACA
QVPPSRGHYKLIQQPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCSTNDICVTANFCISVTFL
KPCFLLHEASASQFKKFDGPCGERGGGRTARALWARGDSVLTPALDPQTPVRAPSLTRAAAAVQ
NLQNGGGSRSSATLPGRRRRRWLRRRRQPISVAPAGPPRRPNQKPNPPGGARCVIMRPTWPGTS
AFTGMECTLGQVGAPSPRREEDGWRGGHSRFKADVPAPQGPCWGGQPGSAPSSAPPEQSLLDD
YWAQKEKISIPRTHLCWKFEMSYTVGGPPPHVHARPRHWKTDRDYWAQKEKGSSSFLRPSCVP
FRELKNVSVLEGLRQGRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSITEISCCTL
SSEENEYLPRPEWQLQYEAGMTLGGKILFFLFLLLPLSPFSLIFLVLGVLSGHSGSRLKRSFAVTER
IITGGKSTCSAPGPQSLPSTPFSTYPQWVILITELGVPGDSTRRAVRRMNTFCGASACDVSLIAMDS
ACEERGAAGSLISCESLYHREKQLIAMDSAIFVQGKDWGVKKFIRRDFTMPAILKLQKNCLLSLN
SKMALNSEALSVVSEYEAGMTLGEKFRVGNCKHLKMTRPTEYNQKLQVNQFSESKRTALTHNQ
DFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITPQLGGQSDSSQPLLTTGRPQGWQDQA
LRHTQQASPASCATITIPIHSAALGDHSGDPGPAWDTCPPLPLTTLIPRAPPPYGDSTARSWPSRCG
PLGYAYKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFLSRALRALHVLWNGFQ
LHCQGNTTLQQLGEASQAPSGSLIPLRLPLLWEVRGNSKMALNSLNSIDDAQLTRIAPPRSHCCF
WEVNAPFVQGKDWGLKKFIRRDFEAFQRAAGEGGPGRGGARRGARVLQSPFCRAGAGEWLGH
QSLRWGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLPLRTLAGCLARTAHLRPGA
ESLPQPQLHCTIARELHQFAFDLLIKSHKMHFSLKEHPPPPCPPHVVGYGHLDTSGSSSSSSWPQP
DSFAALHSSLNELGELWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSARTCCGTKWPSSQEA
ALGLGSGLLRFSCGTAAIR

[0530] SEQ ID NO: 559

MGQKEQIHTLQKNSERMSKQLTRSSQAVGNTTLQQLGEASQAPSGSLIPLRLPLLWEVRGNSKM
ALNSLNSIDDAQLTRIAPPRSHCCFWEVNAPFVQGKDWGLKKFIRRDFEAFQRAAGEGGPGRGG
ARRGARVLQSPFCRAGAGEWLGHQSLRWGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQ
PKDPLPLRTLAGCLARTAHLRPGAESLPQPQLHCTIARELHQFAFDLLIKSHKMHFSLKEHPPPPC
PPHVVGYGHLDTSGSSSSSSWPQPDSFAALHSSLNELGELWFQSSELSPTGAPWPSRRPTWRGTT
VSPRTATSSARTCCGTKWPSSQEAALGLGSGLLRFSCGTAAIRDGHSYTSKVNCLLLQDGFHGC
VSITGAAGRRNLSIFLFLMLCKLEFHACQWQHYHRSGEAAGTPLWRPTRNVAMMVPDRQVHYD
FGLKIQNKNCPDVLRFLDLKVRYLHSVPFRELKNQRTAQGAPGIHHAASPVAANLCDPARHAQH
TRIPCGAGQVRAGRGPEAGGGVLQPQRPAPEKPGCPCRRGQPRLHTVKMWRACHLFLQPQVGT
PPPHTASARAPSGPPHPHESCPAGRRPARAAQTCARRQHGLPGCEEAGTARVPSLHLHLHQAAL
GAGRGRGWGEACAQVPPSRGHYKLIQQPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCST
NDICVTANFCISVTFLKPCFLLHEASASQFKKFDGPCGERGGGRTARALWARGDSVLTPALDPQT
PVRAPSLTRAAAAVGVPGDSTRRAVRRMNTFCGASACDVSLIAMDSATEYNQKLQVNQFSESK
YEAGMTLGEKFRVGNCKHLKMTRPTMPAILKLQKNCLLSLNSKMALNSEALSVVSECEERGAA
GSLISCESLYHREKQLIAMDSAIFVQGKDWGVKKFIRRDFRTALTHNQDFSIYRLCCKRGSLCHA
SQARSPAFPKPVRPLPAPITRITPQLGGQSDSSQPLLTTGRPQGWQDQALRHTQQASPASCATITIP
IHSAALGDHSGDPGPAWDTCPPLPLTTLIPRAPPPYGDSTARSWPSRCGPLGYAYKDFLWCFPFSL
VFLQEIQICCHVSCLCCICCSTRICLGCLLELFLSRALRALHVLWNGFQLHCQQNLQNGGGSRSSA
TLPGRRRRRWLRRRRQPISVAPAGPPRRPNQKPNPPGGARCVIMRPTWPGTSAFTGMECTLGQV
GAPSPRREEDGWRGGHSRFKADVPAPQGPCWGGQPGSAPSSAPPEQSLLDDYWAQKEKISIPRT
HLCWKFEMSYTVGGPPPHVHARPRHWKTDRDYWAQKEKGSSSFLRPSCVPFRELKNVSVLEGL
RQGRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSITEISCCTLSSEENEYLPRPEW
QLQYEAGMTLGGKILFFLFLLLPLSPFSLIFLVLGVLSGHSGSRLKRSFAVTERIITGGKSTCSAPGP
QSLPSTPFSTYPQWVILITEL

[0531] SEQ ID NO: 625

MGQKEQIHTLQKNSERMSKQLTRSSQAVGNTTLQQLGEASQAPSGSLIPLRLPLLWEVRGNSKM
ALNSLNSIDDAQLTRIAPPRSHCCFWEVNAPFVQGKDWGLKKFIRRDFEAFQRAAGEGGPGRGG
ARRGARVLQSPFCRAGAGEWLGHQSLRWGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQ
PKDPLPLRTLAGCLARTAHLRPGAESLPQPQLHCTIARELHQFAFDLLIKSHKMHFSLKEHPPPPC
PPHVVGYGHLDTSGSSSSSSWPQPDSFAALHSSLNELGELWFQSSELSPTGAPWPSRRPTWRGTT
VSPRTATSSARTCCGTKWPSSQEAALGLGSGLLRFSCGTAAIRQNLQNGGGSRSSATLPGRRRRR
WLRRRRQPISVAPAGPPRRPNQKPNPPGGARCVIMRPTWPGTSAFTGMECTLGQVGAPSPRREE
DGWRGGHSRFKADVPAPQGPCWGGQPGSAPSSAPPEQSLLDDYWAQKEKISIPRTHLCWKFEM
SYTVGGPPPHVHARPRHWKTDRDYWAQKEKGSSSFLRPSCVPFRELKNVSVLEGLRQGRLGGP
CSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSITEISCCTLSSEENEYLPRPEWQLQYEAG
MTLGGKILFFLFLLLPLSPFSLIFLVLGVLSGHSGSRLKRSFAVTERIITGGKSTCSAPGPQSLPSTPF
STYPQWVILITELDGHSYTSKVNCLLLQDGFHGCVSITGAAGRRNLSIFLFLMLCKLEFHACQWQ
HYHRSGEAAGTPLWRPTRNVAMMVPDRQVHYDFGLVLRFLDLKVRYLHSKIQNKNCPDVPFRE
LKNQRTAQGAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAGRGPEAGGGVLQPQRPAP
EKPGCPCRRGQPRLHTVKMWRACHLFLQPQVGTPPPHTASARAPSGPPHPHESCPAGRRPARAA
QTCARRQHGLPGCEEAGTARVPSLHLHLHQAALGAGRGRGWGEACAQVPPSRGHYKLIQQPISL
FSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCSTNDICVTANFCISVTFLKPCFLLHEASASQFKKF
DGPCGERGGGRTARALWARGDSVLTPALDPQTPVRAPSLTRAAAAVGVPGDSTRRAVRRMNTF
CGASACDVSLIAMDSACEERGAAGSLISCESLYHREKQLIAMDSAIFVQGKDWGVKKFIRRDFT
MPAILKLQKNCLLSLNSKMALNSEALSVVSEYEAGMTLGEKFRVGNCKHLKMTRPTEYNQKLQ
VNQFSESKRTALTHNQDFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITPQLGGQSDSSQ
PLLTTGRPQGWQDQALRHTQQASPASCATITIPIHSAALGDHSGDPGPAWDTCPPLPLTTLIPRAP
PPYGDSTARSWPSRCGPLGYAYKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFL
SRALRALHVLWNGFQLHCQ

[0532] SEQ ID NO: 626

MGQKEQIHTLQKNSERMSKQLTRSSQAVDGHSYTSKVNCLLLQDGFHGCVSITGAAGRRNLSIF
LFLMLCKLEFHACVPFRELKNQRTAQGAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAG
RGPEAGGGVLQPQRPAPEKPGCPCRRGQPRLHTVKMWRAQWQHYHRSGEAAGTPLWRPTRNK
IQNKNCPDVAMMVPDRQVHYDFGLVLRFLDLKVRYLHSCHLFLQPQVGTPPPHTASARAPSGPP
HPHESCPAGRRPARAAQTCARRQHGLPGCEEAGTARVPSLHLHLHQAALGAGRGRGWGEACA
QVPPSRGHYKLIQQPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCSTNDICVTANFCISVTFL
KPCFLLHEASASQFKKFDGPCGERGGGRTARALWARGDSVLTPALDPQTPVRAPSLTRAAAAVQ
NLQNGGGSRSSATLPGRRRRRWLRRRRQPISVAPAGPPRRPNQKPNPPGGARCVIMRPTWPGTS
AFTGMECTLGQVGAPSPRREEDGWRGGHSRFKADVPAPQGPCWGGQPGSAPSSAPPEQSLLDD
YWAQKEKISIPRTHLCWKFEMSYTVGGPPPHVHARPRHWKTDRDYWAQKEKGSSSFLRPSCVP
FRELKNVSVLEGLRQGRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSITEISCCTL
SSEENEYLPRPEWQLQYEAGMTLGGKILFFLFLLLPLSPFSLIFLVLGVLSGHSGSRLKRSFAVTER
IITGGKSTCSAPGPQSLPSTPFSTYPQWVILITELGNTTLQQLGEASQAPSGSLIPLRLPLLWEVRGN
SKMALNSLNSIDDAQLTRIAPPRSHCCFWEVNAPFVQGKDWGLKKFIRRDFEAFQRAAGEGGPG
RGGARRGARVLQSPFCRAGAGEWLGHQSLRWGMELAASRRFSWDHHSAGGPPRVPSVRSGAA
QVQPKDPLPLRTLAGCLARTAHLRPGAESLPQPQLHCTIARELHQFAFDLLIKSHKMHFSLKEHPP
PPCPPHVVGYGHLDTSGSSSSSSWPQPDSFAALHSSLNELGELWFQSSELSPTGAPWPSRRPTWR
GTTVSPRTATSSARTCCGTKWPSSQEAALGLGSGLLRFSCGTAAIRGVPGDSTRRAVRRMNTFC
GASACDVSLIAMDSACEERGAAGSLISCESLYHREKQLIAMDSAIFVQGKDWGVKKFIRRDFYEA
GMTLGEKFRVGNCKHLKMTRPTMPAILKLQKNCLLSLNSKMALNSEALSVVSETEYNQKLQVN
QFSESKRTALTHNQDFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITPQLGGQSDSSQPL
LTTGRPQGWQDQALRHTQQASPASCATITIPIHSAALGDHSGDPGPAWDTCPPLPLTTLIPRAPPP
YGDSTARSWPSRCGPLGYAYKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFLSR
ALRALHVLWNGFQLHCQ

[0533] SEQ ID NO: 713 The polynucleotide sequence of the full GAd20 incorporating the GAd20 expression cassette

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ccgccccgcctcgccccgcccacctcattatcatattggcctcaatccaaaataaggtatattattgatgatg

Example 11 Neoantigens incorporated into NeoGAd20 and NeoMVA are immunogenic *in vitro*

[0534] Overlapping 15-mer peptides were designed to span each neoantigen incorporated into NeoGAd20 and NeoMVA to assess their ability to activate T cells using the exogenous autologous normal donor restimulation assay described in Example 1 as pools using TNFα and IFNγ production by $CD8^+$ and $CD4^+$ T cells as a readout. Table 25 shows the maximum frequency of $TNF\alpha^+IFN\gamma^+CD8^+$ and $TNF\alpha^+IFN\gamma^+CD4^+$ T cells and maximum fold change over negative control for the pool of peptides analyzed, indicating the highest frequency of $TNF\alpha^+IFN\gamma^+CD8^+$ and $TNF\alpha^+IFN\gamma^+CD4^+$ T cells and resulting fold change across the normal donors evaluated for the peptide. Table 26 shows the peptide sequences used. FIG. 7 shows the number of patients with a positive CD8+ response for each tested peptide pool for select neoantigens. FIG. 8 shows the number of patients with a positive CD4+ response for each tested peptide pool for select neoantigens.

Table 25.

Neoantigen ID (Alternative name)	Maximum Fold Change Over Background CD8+ cells	Maximum Frequency of TNFα/IFNγ Double Positive CD8+ T cells	Maximum Fold Change Over Background CD4+ cells	Maximum Frequency of TNFα/IFNγ Double Positive CD4+ T cells
AS18 (C2-NO1)	9.17	0.110	7.51	0.053
P87 (C2-NO4)	9.20	0.460	7.86	0.110
AS55 (C2-NO5)	1354.17	32.500	19.38	0.310
AS57 (C2-NO6)	4.67	0.056	11.00	0.110
AS15 (C2-NO11)	4.36	0.061	9.17	0.110
AS7 (C2-NO13)	52.60	2.630	2.50	0.045
AS43 (C2-NO15)	28.75	0.460	5.67	0.040
AS51 (C2-NO17)	33.13	0.530	8.75	0.140
AS16 (C2-NO19)	24.17	0.290	7.78	0.140
AS41 (C2-NO20)	190.60	9.530	2.20	0.022
AS6 (C2-NO22)	6.50	0.078	31.67	0.380
AS3 (C2-NO23)	7.92	0.095	3.86	0.054
AS11	5.11	0.07	2.98	0.03
AS13	1.67	0.10	1.96	0.05
AS47 (C2-NO30)	4.80	0.240	2.58	0.031
AS8 (C2-NO33)	54.55	0.600	4.08	0.049
AS19	5.87	0.63	2.59	0.1
AS37	19.47	0.74	7.51	0.04
AS23	3.24	0.07	5.08	0.01
MS1	5.56	0.1	50.19	0.39
MS3	36.15	0.47	13.61	0.09
MS6	4.17	0.08	111.97	0.87
MS8	4.44	0.14	15.60	0.40
P82	2.92	0.09	4.44	0.17
P16 (C2-NO8)	2.44	0.039	2.44	0.039
FUS1 (C2-NO9)	1.94	0.031	8.33	0.100
P22 (C2-NO10)	1.56	0.025	18.16	0.075
FUS2 (C2-NO14)	3.13	0.05	2.14	0.03
FUS3 (C2-NO21)	3.94	0.063	2.75	0.033
FUS6 (C2-NO28)	3.50	0.056	2.27	0.016
FUS5 (C2-NO32)	32.50	0.390	3.43	0.048
FUS8	1.89	0.08	7.15	0.04
FUS15 (C2-NO35)	1.75	0.028	2.79	0.039
P35	14.44	0.26	3.47	0.03
FUS19(C2-NO37)	1.88	0.030	3.15	0.013
FUS7	8.89	0.16	36.13	0.04
M84	1.39	0.03	35.84	0.31
M86	4.22	0.08	6.18	0.05
M10	1.89	0.09	14.14	0.1

M12	6.67	0.12	8.94	0.05
FR1	7.92	0.38	4.89	0.04

Table 26.

Neoantigen ID (alternative name)	Overlapping Peptide Sequences* (SEQ ID NO:)
AS18 (C2-NO1)	WKFEMSYTVGGPPPH (560) VGGPPPHVHARPRHW (561) PPHVHARPRHWKTD (562)
P87 (C2-NO4)	YEAGMTLGGKILFFL (563) GKILFFLFLLLPLSP (564) FFLFLLLPLSPFSLIF (565)
AS55 (C2-NO5)	DGHSYTSKVNCLLLQ (566) VNCLLLQDGFHGCVS (567) GFHGCVSITGAAGRR (568) TGAAGRRNLSIFLFL (569) LSIFLFLMLCKLEFH (570) LFLMLCKLEFHAC (571)
AS57 (C2-NO6)	TGGKSTCSAPGPQSL (572) APGPQSLPSTPFSTY (573) STPFSTYPQWVILIT (574)
AS15 (C2-NO11)	VLRFLDLKVRYLHS (269)
AS7 (C2-NO13)	DYWAQKEKGSSSFLR (576) QKEKGSSSFLRPSC (577)
AS43 (C2-NO15)	VPFRELKNVSVLEGL (578) VSVLEGLRQGRLGGP (579) QGRLGGPCSCHCPRP (580) SCHCPRPSQARLTPV (581) QARLTPVDVAGPFLC (582) VAGPFLCLGDPGLFP (583) GDPGLFPPVKSSI (584)
AS51 (C2-NO17)	GMECTLGQVGAPSPR (585) VGAPSPRREEDGWRG (586) EEDGWRGGHSRFKAD (587) HSRFKADVPAPQGPC (588) PAPQGPCWGGQPGSA (589) GGQPGSAPSSAPPEQ (590) GSAPSSAPPEQSLLD (591)
AS16 (C2-NO19)	GNTTLQQLGEASQAP (592) GEASQAPSGSLIPLR (593) GSLIPLRLPLLWEVRG (594)
AS41 (C2-NO20)	EAFQRAAGEGGPGRG (595) EGGPGRGGARRGARV (596) ARRGARVLQSPFCRA (597) QSPFCRAGAGEWLGH (598) CRAGAGEWLGHQSLR (599)
AS6 (C2-NO22)	DYWAQKEKISIPRTH (600) QKEKISIPRTHLC (601)
AS3 (C2-NO23)	VAMMVPDRQVHYDFG (602) VPDRQVHYDFGLR (603)

AS11	VPFRELKNQRTAQGA (631) QRTAQGAPGIHHAAS (632) GIHHAASPVAANLCD (633) VAANLCDPARHAQHT (634) ARHAQHTRIPCGAGQ (635) IPCGAGQVRAGRGPE (636) RAGRGPEAGGGVLQP (637) GGGVLQPQRPAPEKP (638) RPAPEKPGCPCRRGQ (639) CPCRRGQPRLHTVKM (640) RGQPRLHTVKMWRA (641)
AS13	KRSFAVTERII (265)
AS47 (C2-NO30)	FKKFDGPCGERGGGR (604) GERGGGRTARALWAR (605) ARALWARGDSVLTPA (606) DSVLTPALDPQTPVR (607) DPQTPVRAPSLTRAA (608) PVRAPSLTRAAAAV (609)
AS8 (C2-NO33)	LVLGVLSGHSGSRL (255)
AS19	QWQHYHRSGEAAGTP (710) GEAAGTPLWRPTRN (711)
AS37	CHLFLQPQVGTPPPH (642) VGTPPPHTASARAPS (643) ASARAPSGPPHPHES (644) PPHPHESCPAGRRPA (645) PAGRRPARAAQTCAR (646) AAQTCARRQHGLPGC (647) QHGLPGCEEAGTARV (648) EAGTARVPSLHLHLH (649) SLHLHLHQAALGAGR (650) AALGAGRGRGWGEAC (651) RGWGEACAQVPPSRG (652)
AS23	KIQNKNCPD (285)
MS1	HYKLIQQPISLFSIT (653) ISLFSITDRLHKTFS (654) RLHKTFSQLPSVHLC (655) LPSVHLCSITFQWGH (656) ITFQWGHPPIFCSTN (657) PIFCSTNDICVTANF (658) ICVTANFCISVTFLK (659) ISVTFLKPCFLLHEA (660) CFLLHEASASQ (661)
MS3	RTALTHNQDFSIYRL (662) DFSIYRLCCKRGSLC (663) CKRGSLCHASQARSP (664) ASQARSPAFPKPVRP (665) FPKPVRPLPAPITRI (666) PAPITRITPQLGGQS (667) PQLGGQSDSSQPLLT (668) SSQPLLTTGRPQGWQ (669) GRPQGWQDQALRHTQ (670) QALRHTQQASPASCA (671) ASPASCATITIPIHS (672) ITIPIHSAALGDHSG (673)

	ALGDHSGDPGPAWDT (674) PGPAWDTCPPLPLTT (675) PPLPLTTLIPRAPPP (676) IPRAPPPYGDSTARS (677) GDSTARSWPSRCGPLG (678)
MS6	YAYKDFLWCFPFSLV (679) CFPFSLVFLQEIQIC (680) LQEIQICCHVSCLCC (681) HVSCLCCICCSTRIC (682) CCSTRICLGCLLELF (683) GCLLELFLSRALRAL (684) SRALRALHVLWNGFQ (685) VLWNGFQLHCQ (686)
MS8	TMPAILKLQKNCLLSL (444)
P82	YEAGMTLGEKFRVGN (687) EKFRVGNCKHLKMTRP (688)
P16 (C2-NO8)	GVPGDSTRRAVRRMN (611) DSTRRAVRRMNTF (612)
FUS1 (C2-NO9)	CGASACDVSLIAMDSA (211)
P22 (C2-NO10)	SLYHREKQLIAMDSAI (349)
FUS2	TEYNQKLQVNQFSESK (712)
FUS3 (C2-NO21)	TEISCCTLSSEENEY (615) SSEENEYLPRPEWQLQ (616)
FUS6 (C2-NO28)	CEERGAAGSLISCE (221)
FUS5 (C2-NO32)	NSKMALNSEALSVVSE (219)
FUS8	WGMELAASRRFSWDH (689) RRFSWDHHSAGGPPR (690) SAGGPPRVPSVRSGA (691) PSVRSGAAQVQPKDP (692) QVQPKDPLPLRTLAG (693) PLRTLAGCLARTAHL (694) LARTAHLRPGAESLP (695) PGAESLPQPQLHCT (696)
FUS15 (C2-NO35)	HVVGYGHLDTSGSSS (619) YGHLDTSGSSSSSSWP (620)
P35	NSKMALNSLNSIDDA (697) LNSIDDAQLTRIAPP (698) LTRIAPPRSHCCFWE (699) APPRSHCCFWEVNAP (700)
FUS19 (C2-NO37)	KMHFSLKEHPPPPCPP (235)
FUS7	LWFQSSELSPTGAPW (701) SPTGAPWPSRRPTWR (702) SRRPTWRGTTVSPRT (703) TTVSPRTATSSARTC (704) TSSARTCCGTKWPSS (705) GTKWPSSQEAALGLG (706) EAALGLGSGLLRFSC(707) GLLRFSCGTAAIR (708)
M84	IARELHQFAFDLLIKSH (167)

M86	QPDSFAALHSSLNELGE (171)
M10	FVQGKDWGLKKFIRRDF (19)
M12	FVQGKDWGVKKFIRRDF (23)
FR1	QNLQNGGGSRSSATL (709) SRSSATLPGRRRRRW (575) GRRRRRWLRRRRQPI (610) RRRRQPISVAPAGPP (613) VAPAGPPRRPNQKPN (614) RPNQKPNPPGGARCV (617) PGGARCVIMRPTWPG (618) MRPTWPGTSAFT (621)

Example 12 Neoantigens incorporated into NeoGAd20 and NeoMVA are immunogenic when expressed endogenously *in vitro*

[0535] For three of the neoantigens, an Ad5 vector was designed to transduce normal Dendritic cells with the neoantigens. This assay assessed the ability of the endogenously expressed and presented neoantigens to activate autologous T cells following overlapping 15-mer peptide pools restimulation using the endogenous autologous normal donor restimulation assay described in Example 1 utilizing TNFα and IFNγ production by $CD8^+$ and $CD4^+$ T cells as a readout. Table 27 shows the maximum frequency of $TNF\alpha^+IFN\gamma^+CD8^+$ and $TNF\alpha^+IFN\gamma^+CD4^+$ T cells and maximum fold change over negative control for the pool of peptides analyzed, indicating the highest frequency of $TNF\alpha^+IFN\gamma^+CD8^+$ and $TNF\alpha^+IFN\gamma^+CD4^+$ T cells and resulting fold change across the normal donors evaluated for the peptide. Sixteen donors were used to assess endogenous immunogenicity.

Table 27.

Neoantigen ID	Overlapping Peptide Sequences* (SEQ ID NO:)	Maximum Fold Change Over Background CD8+ cells	Maximum Frequency of TNFα/IFNγ Double Positive CD8+ T cells	Maximum Fold Change Over Background CD4+ cells	Maximum Frequency of TNFα/IFNγ Double Positive CD4+ T cells
AS18 (C2-NO1)	WKFEMSYTVGGPPPH (560)	4.09	0.36	1.90	0.046
	VGGPPPHVHARPRHW (561)				
	PPHVHARPRHWKTD (562)				
P87 (C2-NO4)	YEAGMTLGGKILFFL (563)	2.47	0.39	2.41	0.079
	GKILFFLFLLLPLSP (564)				
	FFLFLLLPLSPFSLIF (565)				
AS55 (C2-NO5)	DGHSYTSKVNCLLLQ (566)	213.88	2.05	3.50	0.063
	VNCLLLQDGFHGCVS (567)				
	GFHGCVSITGAAGRR (568)				
	TGAAGRRNLSIFLFL (569)				
	LSIFLFLMLCKLEFH (570)				
	LFLMLCKLEFHAC (571)				

*All generated peptides had NH2 group at N-terminus and -OH group at C-terminus

Example 13: Neoantigens are immunogenic in vitro

[0536] Immunogenicity of various additional identified neoantigens was assessed. Overlapping 15-mer peptides were designed to span each neoantigen similarly to what was done in Example 11 to assess their ability to activate T cells using the exogenous autologous normal donor restimulation assay described in Example 1 as pools using TNFα and IFNγ production by $CD8^+$ and $CD4^+$ T cells as a readout. Table 28 shows the maximum frequency of $TNF\alpha^+IFN\gamma^+CD8^+$ and $TNF\alpha^+IFN\gamma^+CD4^+$ T cells and maximum fold change over negative control for the pool of peptides analyzed, indicating the highest frequency of $TNF\alpha^+IFN\gamma^+CD8^+$ and $TNF\alpha^+IFN\gamma^+CD4^+$ T cells and resulting fold change across the normal donors evaluated for the peptide. Table 29 shows the amino acid sequences of the peptides used in the assays for each neoantigen.

Table 28.

Neoantigen ID (Alternative name)	Max.Fold Change over background CD8+ TNFαINFγ double positive DP	Max. Frequency of CD8+ TNFαINFγ DP	Max.Fold Change over background CD4+ TNFαINFγ double positive DP	Max. Frequency of CD4+ TNFαINFγ DP
AS1 (Misc1-NO12)	8.15	0.11	3.57	0.02
AS2 (Misc1-NO13)	5.06	0.09	110.68	0.86
AS4 (Misc1-NO14)	5.4	0.17	37.38	1.43
AS5 (Misc1-NO15)	2.13	0.16	8.71	0.1
AS9 (Misc1-NO16)	3.81	0.12	4.18	0.16
AS10 (Misc1-NO17)	4.33	0.08	3.97	0.23
AS12 (Misc1-NO18)	6.03	0.19	7.32	0.28
AS14 (Misc1-NO19)	3.81	0.12	1.49	0.06
AS17 (Misc1-NO20)	2.38	0.07	3.18	0.01
AS20 (Misc1-NO21)	3.81	0.12	1.36	0.05
AS21 (Misc1-NO22)	3.81	0.12	1.7	0.07
AS22 (Misc1-NO23)	2.61	0.1	7.86	0.11
AS32 (Misc1-NO24)	3.81	0.12	2.04	0.08
AS34 (Misc1-NO26)	16.25	0.26	9.48	0.01
AS35 (Misc1-NO27)	11.32	0.43	60.93	0.23
AS36 (Misc2-NO1)	1544.74	58.7	129.8	0.49
AS40 (Misc2-NO3)	178.52	2.41	15.34	0.89
AS42 (Misc2-NO4)	4.65	0.69	24.58	0.59
AS44 (Misc2-NO5)	4.72	0.09	293.94	1.48
AS45 (Misc2-NO6)	4.96	0.07	78.51	0.61
AS46 (Misc2-NO7)	11.6	0.87	157.98	0.29
AS48 (Misc2-NO8)	9.21	0.29	13.45	0.13

AS49 (Misc2-NO9)	8.67	0.65	184.87	0.14
AS50 (Misc2-NO10)	1.6	0.12	17.22	0.07
AS52 (Misc2-NO11)	6.85	0.1	184.87	0.63
AS53 (Misc2-NO12)	4.02	0.35	113.91	0.43
AS54	88.15	8.33	17.87	0.18
AS55.1 (Misc2-NO14)	25.26	1.47	20.66	0.08
AS56	6.35	0.6	128.76	0.18
AS58 (Misc2-NO16)	4.54	0.6	6.27	0.24
AS59 (Misc2-NO17)	4.22	0.08	59.58	0.3
FUS9 (Misc1-NO2)	1.82	0.07	203.97	0.77
FUS10 (Misc1-NO3)	2.42	0.09	10.86	0.04
FUS11 (Misc1-NO4)	2.63	0.1	28.57	0.16
FUS18	42.33	0.91	31.76	0.04
FUS23 (Misc1-NO6)	11.05	0.62	12.77	0.12
FUS24 (Misc1-NO7)	8.53	0.64	4.4	0.05
MS2 (Excl-NO6)	36.92	0.48	15.12	0.1
MS4 (Excl-NO8)	24.13	0.76	367.35	2.43
MS5 (Excl-NO9)	32.89	1.95	126.05	0.2
MS7 (Excl-NO11)	6.67	0.12	10.52	0.09
MS9 (Excl-NO13)	1.38	0.1	455.63	1.72
MS10 (Excl-NO14)	3.42	0.13	74.17	0.28
MS11 (Excl-NO15)	3.81	0.12	3.4	0.13
P97 (Misc1-NO11)	7.47	1.11	3.14	0.12
P19 (Misc1-NO8)	4.76	0.15	6.67	0.16
P27 (Misc1-NO9)	7.87	0.59	45.38	0.05
P37 (Misc1-NO10)	2.05	0.13	22.78	0.09
P76, P77 (Misc2-NO18)	4.56	0.08	53.18	0.36

Table 29.

Neoantigen ID (Alternative name)	Peptide sequences
AS1 (Misc1-NO12)	LTFLDFIQVTLRVMS (SEQ ID NO: 377) VTLRVMSGSQMENGS (SEQ ID NO: 378) SQMENGSSYFFKPFS (SEQ ID NO: 415) YFFKPFSWGLGVGLS (SEQ ID NO: 417)
AS2 (Misc1-NO13)	FMIGELVGELCCQLT (SEQ ID NO: 418) ELCCQLTFRLPFLES (SEQ ID NO: 419) RLPFLESLCQAVVTQ (SEQ ID NO: 420) CQAVVTQALRFNPSF (SEQ ID NO: 502) LRFNPSFQEVCIYQD (SEQ ID NO: 518) EVCIYQDTDLM (SEQ ID NO: 526)
AS4 (Misc1-NO14)	WCPLDLRLGSTGCLT (SEQ ID NO: 527) GSTGCLTCRHHQTSHE (SEQ ID NO: 714)

AS5 (Misc1-NO15)	VVGRRHETAPQPLLV (SEQ ID NO: 715) APQPLLVPDRAGGEG (SEQ ID NO: 716) DRAGGEGGA (SEQ ID NO: 717)
AS9 (Misc1-NO16)	PVPTATPGVRSVTSP (SEQ ID NO: 718) VRSVTSPQGLGLFLK (SEQ ID NO: 719) GLGLFLKFI (SEQ ID NO: 720)
AS10 (Misc1-NO17)	KENDVREVCDVYLQM (SEQ ID NO: 721) CDVYLQMQIFFHFKF (SEQ ID NO: 722) IFFHFKFRSYFH (SEQ ID NO: 723)
AS12 (Misc1-NO18)	FARKMLEKVHRQHLQ (SEQ ID NO: 724) VHRQHLQLSHNSQE (SEQ ID NO: 725)
AS14 (Misc1-NO19)	MFLRKEQQVGPHSFS (SEQ ID NO: 726) VGPHSFSML (SEQ ID NO: 727)
AS17 (Misc1-NO20)	GLNLNTDRPGGYSYS (SEQ ID NO: 728) PGGYSYSIWWKNNAK (SEQ ID NO: 729) WWKNNAKNR (SEQ ID NO: 730)
AS20 (Misc1-NO21)	KVLNEIDAVVTVPPS (SEQ ID NO: 731) VVTVPPSLSTSQIPQ (SEQ ID NO: 732) STSQIPQGCCIIL (SEQ ID NO: 733)
AS21 (Misc1-NO22)	ANLKGTLQVRSGQAV (SEQ ID NO: 734) VRSGQAVSPR (SEQ ID NO: 735)
AS22 (Misc1-NO23)	LQAAASGQGKQGVPC (SEQ ID NO: 736) GKQGVPCPWGCCAYA (SEQ ID NO: 737) WGCCAYAESPRALIS (SEQ ID NO: 738) SPRALISGDAPSQVE (SEQ ID NO: 739) DAPSQVEREVPGPCL (SEQ ID NO: 740) EVPGPCLNTHSLSHR (SEQ ID NO: 741) THSLSHRSPQLPGLP (SEQ ID NO: 742) PQLPGLPHPKQPSV (SEQ ID NO: 743)
AS32 (Misc1-NO24)	GEVELSEGGEGQRHL (SEQ ID NO: 744) GEGQRHLAFPWACSG (SEQ ID NO: 745) FPWACSGPGWRGVCC (SEQ ID NO: 746) GWRGVCCAAVEPA (SEQ ID NO: 980)
AS34 (Misc1-NO26)	KMRAIQAEGGHGQAC (SEQ ID NO: 747) GGHGQACCGGAWGWA (SEQ ID NO: 748) GGAWGWAPGDGGPQG (SEQ ID NO: 749) GDGGPQGMLTHTLPT (SEQ ID NO: 750) LTHTLPTLGFQSAWT (SEQ ID NO: 751) GFQSAWTWRREDADR (SEQ ID NO: 752) RREDADRAWRTPKAC (SEQ ID NO: 753) WRTPKACASRRWSI (SEQ ID NO: 754)

AS35 (Misc1-NO27)	LLEPFRRGEPGPRGL (SEQ ID NO: 755) EPGPRGLLSGSSRGG (SEQ ID NO: 756) SGSSRGGEGPGRSIE (SEQ ID NO: 757) GPGRSIEAAPATPLP (SEQ ID NO: 758) APATPLPCCRKNPCR (SEQ ID NO: 759) CRKNPCRPQPSRFLP (SEQ ID NO: 760) QPSRFLPPRVLLVII (SEQ ID NO: 761) RVLLVIILPKLDCPK (SEQ ID NO: 762) PKLDCPKLGF (SEQ ID NO: 763)
AS36 (Misc2-NO1)	PSGRRTKRLVTLRSG (SEQ ID NO: 764) LVTLRSGCAIQCWHP (SEQ ID NO: 765) AIQCWHPRAGPVPSA (SEQ ID NO: 766) AGPVPSALPHTERPP (SEQ ID NO: 767) PHTERPPRLVRGAAD (SEQ ID NO: 768) LVRGAADPRTVTLGR (SEQ ID NO: 769) RTVTLGRSPAVMPRA (SEQ ID NO: 770) PAVMPRAPA (SEQ ID NO: 771)
AS40 (Misc2-NO3)	DCMLSEEGGQARRGG (SEQ ID NO: 772) GQARRGGSLCSLAAH (SEQ ID NO: 773) LCSLAAHTIASAARG (SEQ ID NO: 774) IASAARGRFLSRLSN (SEQ ID NO: 775) FLSRLSNFCAVVKAS (SEQ ID NO: 776) CAVVKASRGAPSCTWE (SEQ ID NO: 777)
AS42 (Misc2-NO4)	PEPRRLSPGEPRGRP (SEQ ID NO: 778) GEPRGRPRKGWGIWG (SEQ ID NO: 779) KGWGIWGLCGARVGP (SEQ ID NO: 780) CGARVGPKAWR (SEQ ID NO: 781)
AS44 (Misc2-NO5)	FVSLTAIQMASSATP (SEQ ID NO: 782) MASSATPWGRWPVAT (SEQ ID NO: 783) GRWPVATPTAACPRR (SEQ ID NO: 784) TAACPRRRPSSLPTG (SEQ ID NO: 785) PSSLPTGGDSASKKP (SEQ ID NO: 786) DSASKKPISRRAPWQ (SEQ ID NO: 787) SRRAPWQPWACPGRS (SEQ ID NO: 788) WACPGRSVNSAAPRA (SEQ ID NO: 789) NSAAPRAWCPPATTP (SEQ ID NO: 790) CPPATTPRTQSPSRD (SEQ ID NO: 791) TQSPSRDLRPRCLSS (SEQ ID NO: 792) RPRCLSSWSS (SEQ ID NO: 793)
AS45 (Misc2-NO6)	PVAIKPGTGPPNNSS (SEQ ID NO: 794) GPPNNSSIHGGSKRS (SEQ ID NO: 795) HGGSKRSENSYCRDL (SEQ ID NO: 796) NSYCRDLRGQLRAIC (SEQ ID NO: 797) GQLRAICCSSYSHDR (SEQ ID NO: 798) SSYSHDRHTTEERGS (SEQ ID NO: 799) TTEERGSRGRHVWRI (SEQ ID NO: 800) GRHVWRIRRLHTSGL (SEQ ID NO: 801) RLHTSGLPCCCHSGP (SEQ ID NO: 802) CCCHSGPHPRRLPDI (SEQ ID NO: 803) PRRLPDILRLVTSTK (SEQ ID NO: 804) RLVTSTKTDHTNTTE (SEQ ID NO: 805) DHTNTTEGTLDYL (SEQ ID NO: 806)

AS46 (Misc2-NO7)	KWNKNWTATLGALTI (SEQ ID NO: 807) TLGALTIRGHKLLCH (SEQ ID NO: 808) GHKLLCHLPHLLSSV (SEQ ID NO: 809) PHLLSSVQQTCRSSSR (SEQ ID NO: 810)
AS48 (Misc2-NO8)	ENASLVFTGSNSPIP (SEQ ID NO: 811) GSNSPIPACELSSHP (SEQ ID NO: 812) CELSSHPAHGISPWI (SEQ ID NO: 813) HGISPWIPSPGNEHF (SEQ ID NO: 814) SPGNEHFHGIKKQVK (SEQ ID NO: 815) GIKKQVKAIKVE (SEQ ID NO: 816)
AS49 (Misc2-NO9)	RLTQRLVQGWTPMEN (SEQ ID NO: 817) GWTPMENRWCGRRAG (SEQ ID NO: 818) WCGRRAGGQPASSST (SEQ ID NO: 819) QPASSSTRWTTCRAA (SEQ ID NO: 820) WTTCRAACLLTKWTA (SEQ ID NO: 821) LLTKWTAGRSQTSIG (SEQ ID NO: 822)
AS50 (Misc2-NO10)	ENSGNASRWLHVPSS (SEQ ID NO: 823) WLHVPSSSDDWLGWK (SEQ ID NO: 824) DDWLGWKKSSAITSNS (SEQ ID NO: 825)
AS52 (Misc2-NO11)	KGSVERRSVSLGHPA (SEQ ID NO: 826) VSLGHPAEGWAWAER (SEQ ID NO: 827) GWAWAERSLQPGMTT (SEQ ID NO: 828) LQPGMTTANTGCLSF (SEQ ID NO: 829) NTGCLSFHHRGCLLP (SEQ ID NO: 830) HRGCLLPVLPKLHCG (SEQ ID NO: 831) LPKLHCGLGGLPLVR (SEQ ID NO: 832) GGLPLVRAKEIKRVQ (SEQ ID NO: 833) KEIKRVQRAGESSLP (SEQ ID NO: 834) AGESSLPVKGLLTVA (SEQ ID NO: 835) KGLLTVASAVIAVLW (SEQ ID NO: 836) AVIAVLWGRPSEVTG (SEQ ID NO: 837) RPSEVTGENEAQHD (SEQ ID NO: 838)
AS53 (Misc2-NO12)	FGLTTLAGRSSHGTS (SEQ ID NO: 839) RSSHGTSGLRAATHT (SEQ ID NO: 840) LRAATHTKSGDGGQG (SEQ ID NO: 841) SGDGGQGAARQCEKL (SEQ ID NO: 842) ARQCEKLLELARATR (SEQ ID NO: 843) ELARATRPWGRSTSA (SEQ ID NO: 844) WGRSTSASSRWTHRG (SEQ ID NO: 845) SRWTHRGYMCPPRCA (SEQ ID NO: 846) MCPPRCAVACW (SEQ ID NO: 847)
AS54	IIDSDKIMAVCMGCL (SEQ ID NO: 848) DKIMAVCMGCLLTRH (SEQ ID NO: 849) AVCMGCLLTRHVQCQ (SEQ ID NO: 850) GCLLTRHVQCQAMEM (SEQ ID NO: 851) TRHVQCQAMEMQQ (SEQ ID NO: 852)
AS55.1 (Misc2-NO14)	DGHSYTSKVNCLLLQ (SEQ ID NO: 566) VNCLLLQDGFHGCVS (SEQ ID NO: 567) GFHGCVSITGAAGRR (SEQ ID NO: 568) TGAAGRRNLSIFLFL (SEQ ID NO: 569) LSIFLFLMLCKLEFHA (SEQ ID NO: 853)

AS56	LLNAEDYRCAIHSKE (SEQ ID NO: 854) CAIHSKEIYLLSPSP (SEQ ID NO: 855) YLLSPSPHQAMDKFS (SEQ ID NO: 856) QAMDKFSLCCINCNL (SEQ ID NO: 857) CCINCNLCLHVFLLL (SEQ ID NO: 858) LHVFLLLLFFQNKDV (SEQ ID NO: 859) FFQNKDVWLISNIIL (SEQ ID NO: 860) LISNIILLWIYGGI (SEQ ID NO: 861)
AS58 (Misc2-NO16)	VETLENANSFSSGIQ (SEQ ID NO: 862) SFSSGIQPLLCSLIG (SEQ ID NO: 863) LLCSLIGLENPT (SEQ ID NO: 864)
AS59 (Misc2-NO17)	AGAGTISEGSVLHGQ (SEQ ID NO: 865) GSVLHGQRLECDARR (SEQ ID NO: 866) LECDARRFFGCGTTI (SEQ ID NO: 867) FGCGTTILAEWEHH (SEQ ID NO: 868)
FUS9 (Misc1-NO2)	KEQILAVASLVSSQS (SEQ ID NO: 869) SLVSSQSIHPSWGQS (SEQ ID NO: 870) HPSWGQSPLSRI (SEQ ID NO: 871)
FUS10 (Misc1-NO3)	LELELSEGVCFRLR (SEQ ID NO: 229)
FUS11 (Misc1-NO4)	QQLRIFCAAMASNED (SEQ ID NO: 872) AMASNEDFS (SEQ ID NO: 873)
FUS18	DGFSGSLFAVVTRRC (SEQ ID NO: 874) AVVTRRCYFLKWRTI (SEQ ID NO: 875) FLKWRTIFPQSLMWL (SEQ ID NO: 876)
FUS23 (Misc1-NO6)	DLRRVATYCAPLPSS (SEQ ID NO: 877) CAPLPSSWRPGTGTT (SEQ ID NO: 878) RPGTGTTIPPRMRSC (SEQ ID NO: 879)
FUS24 (Misc1-NO7)	LQERMELLACGAERG (SEQ ID NO: 880) ACGAERGAGGWGGGG (SEQ ID NO: 881) GGWGGGGGGGGGDRR (SEQ ID NO: 882) GGGGDRRGGGGSAPA (SEQ ID NO: 883) GGGSAPALADFAGGRG (SEQ ID NO: 884)
MS2 (Excl-NO6)	WTDIVKQSVSTNCIS (SEQ ID NO: 885) VSTNCISIKKGSYTK (SEQ ID NO: 886) KKGSYTKLFSLVFLI (SEQ ID NO: 887) FSLVFLIFCWPLIIQL (SEQ ID NO: 888)
MS4 (Excl-NO8)	LRYGALCNVSRISYF (SEQ ID NO: 889) VSRISYFSLTNIFNF (SEQ ID NO: 890) LTNIFNFVIKSLTAI (SEQ ID NO: 891) IKSLTAIFTVKF (SEQ ID NO: 548)

MS5 (Excl-NO9)	RKERNIRKSESTLRL (SEQ ID NO: 892) SESTLRLSPFPTPAP (SEQ ID NO: 893) PFPTPAPSGAPAAAQ (SEQ ID NO: 894) GAPAAAQGKVVRVPG (SEQ ID NO: 895) KVVRVPGPAGGLVPR (SEQ ID NO: 896) AGGLVPRDAGARLLP (SEQ ID NO: 897) AGARLLPPAGGPGGG (SEQ ID NO: 898) AGGPGGGAAAGEGRA (SEQ ID NO: 899) AAGEGRAGRGRFPSI (SEQ ID NO: 900) RGRFPSITEPRPRDL (SEQ ID NO: 901) EPRPRDLPPRVATGR (SEQ ID NO: 902) PRVATGRRAGGRRKG (SEQ ID NO: 903) AGGRRKGAGQGVRTR (SEQ ID NO: 904) GQGVRTRPLPASWPG (SEQ ID NO: 905) LPASWPGGRGPFRKG (SEQ ID NO: 906) RGPFRKGPRRLPLGS (SEQ ID NO: 907) RRLPLGSGPPAAGVQ (SEQ ID NO: 908) PPAAGVQRLRCSHLS (SEQ ID NO: 909) LRCSHLSRGPRRRRG (SEQ ID NO: 910) GPRRRRGRVCGRACV (SEQ ID NO: 911) VCGRACVSPPLPPRP (SEQ ID NO: 912) PPLPPRPPPVGLSAE (SEQ ID NO: 913) PVGLSAENLSWLSSG (SEQ ID NO: 914) LSWLSSGLPRACSWR (SEQ ID NO: 915) PRACSWREFSPETCA (SEQ ID NO: 916) FSPETCAFRLSGLDS (SEQ ID NO: 917) RLSGLDSKLSARVER (SEQ ID NO: 918) LSARVERDLGALRAP (SEQ ID NO: 919) LGALRAPGSRAAQGG (SEQ ID NO: 920) SRAAQGGGRVRGSRS (SEQ ID NO: 921) RVRGSRSEWKTRPWR (SEQ ID NO: 922) WKTRPWRPPPAWPLT (SEQ ID NO: 923) PPAWPLTRAGGPLPK (SEQ ID NO: 924) AGGPLPKNPFLESCS (SEQ ID NO: 925) PFLESCSETAQRRRV (SEQ ID NO: 926) TAQRRRVFSFSTPLS (SEQ ID NO: 927)
MS7 (Excl-NO11)	SINKATITGKKDLEL (SEQ ID NO: 928) GKKDLELILHVSRKK (SEQ ID NO: 929) LHVSRKKPFLPRVNI (SEQ ID NO: 930) FLPRVNITPTPISCC (SEQ ID NO: 931) PTPISCCNLKMLKKF (SEQ ID NO: 932) LKMLKKFFLLYIIIS (SEQ ID NO: 933) LLYIIISIIDLTNCL (SEQ ID NO: 934) IDLTNCLSCYLEHFY (SEQ ID NO: 935) CYLEHFYRFTFFTDV (SEQ ID NO: 936) FTFFTDVHYF (SEQ ID NO: 937)
MS9 (Excl-NO13)	PYYSALSGNSWVPST (SEQ ID NO: 938) NSWVPSTLESDPFGY (SEQ ID NO: 939) ESDPFGYVFSPLATR (SEQ ID NO: 940) FSPLATRPALNDQES (SEQ ID NO: 941) ALNDQESILWPTLTS (SEQ ID NO: 942) LWPTLTSVVSCALSC (SEQ ID NO: 943) VSCALSCPSLNLPEN (SEQ ID NO: 944) SLNLPENWLTLITGG (SEQ ID NO: 945)

	LTLITGGMKGGKKMK (SEQ ID NO: 946) KGGKKMKFTFRH (SEQ ID NO: 947)
MS10 (Excl-NO14)	GLRNLGNTVRAILLS (SEQ ID NO: 948) VRAILLSFLSKRNVK (SEQ ID NO: 949) LSKRNVKWCWGWGKP (SEQ ID NO: 950) CWGWGKPTSLGKACG (SEQ ID NO: 951) SLGKACGRRALKLF (SEQ ID NO: 952)
MS11 (Excl-NO15)	MEAENAGSLHFHEVL (SEQ ID NO: 953) LHFHEVLKMGHVKF (SEQ ID NO: 954)
P97 (Misc1-NO11)	GYLRMQGLMAQRLLLR (SEQ ID NO: 383)
P19 (Misc1-NO8)	WTPIPVLTRWPLPHP (SEQ ID NO: 955) RWPLPHPPPWRRATS (SEQ ID NO: 956) PWRRATSCRMARSSP (SEQ ID NO: 957) RMARSSPSATSGSSV (SEQ ID NO: 958) ATSGSSVRRRCSSLP (SEQ ID NO: 959) RRCSSLPSWVWNLAA (SEQ ID NO: 960) WVWNLAASTRPRSTPS (SEQ ID NO: 961)
P27 (Misc1-NO9)	LHPQRETFTPRWSGA (SEQ ID NO: 962) TPRWSGANYWKLAFP (SEQ ID NO: 963) YWKLAFPVGAEGTFP (SEQ ID NO: 964) GAEGTFPAAATQRGV (SEQ ID NO: 965) AATQRGVVRPA (SEQ ID NO: 966)
P37 (Misc1-NO10)	MAGGVLRRLLCREPD (SEQ ID NO: 967) LLCREPDRDGDKGAS (SEQ ID NO: 968) DGDKGASREETVVPL (SEQ ID NO: 969) EETVVPLHIGDPVVL (SEQ ID NO: 970) IGDPVVLPGIGQCYS (SEQ ID NO: 971) GIGQCYSALF (SEQ ID NO: 972)
P76, P77 (Misc2-NO18)	VFFKRAAEGFFRMNK (SEQ ID NO: 973) GFFRMNKLKESSDTN (SEQ ID NO: 974) KESSDTNPKPYCMAA (SEQ ID NO: 975) KPYCMAAPMGLTENN (SEQ ID NO: 976) MGLTENNRNRKKSYR (SEQ ID NO: 977) NRKKSYRETNLKAVS (SEQ ID NO: 978) TNLKAVSWPLNHT (SEQ ID NO: 979)

Diagnostic assays

Step 1: Neo-antigen burden estimation

[0537] Samples for qPCR analysis - Radical prostatectomy specimens were collected from men diagnosed with prostate adenocarcinoma (PCa). Pathology was reviewed internally to select specimens with greater than or equal to 50% tumor content. Three to five, 5 micron rolls were cut for RNA extraction. Healthy Donor (HD) tissue RNA from ten different body organs (Takara Bio.) and peripheral blood mononuclear cells (PBMCs) from seven healthy donors were used as Normal controls.

[0538] RNA extraction, cDNA synthesis, and pre-amplification - RNA was extracted from paraffin rolls using RNAstorm™ kit (Cell Data Sciences) following manufacturer's instructions. Purified

RNA was eluted in 30 µl RNase-free water. RNA from PBMCs were extracted using Qiagen's RNeasy mini kit following kit protocol. cDNA was synthesized from 200 ng of PCa sample total RNA and 100 ng of HD tissue and PBMCs RNA using the High Capacity cDNA reverse transcription kit with RNase inhibitor from Applied Biosystems, Foster City, CA., following the manufacturer's protocol. HD tissue and PBMC cDNA samples were diluted 1:5 with nuclease free water. cDNA samples were then pre-amplified with selected gene panel (SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, and 223) for 14 cycles using Applied Biosystems TaqMan™ PreAmp MasterMix per manufacturers protocol. RPL19 gene was used as endogenous control while AR and ARv7 were used as controls for high and low expression of target genes.

[0539] Quantitative PCR using BioMark™ 96.96 Dynamic Arrays - Pre-amplified cDNA was diluted 1 to 20 with nuclease free water. Gene expression analysis of diluted preamplified product was performed on Fluidigm's BioMark™ Real-Time PCR system in a 96.96 chip format following the manufacturer's instructions (Fluidigm Corporation, San Francisco, CA). Each sample and TaqMan Assay were loaded on the 96.96 chip to give 4 replicate values per sample (2 sample loading + 2 TaqMan assay loading).

[0540] qPCR data analysis - Raw data were extracted using the BioMark™ real time qPCR analysis software with linear derivative for baseline correction and auto detector for Ct threshold settings. 999 values were converted to 40. Geometric mean Ct of four replicates for each sample were calculated. Ct values greater than 30 were considered as "no amplification." All samples included in the study were positive for endogenous control, RPL19. The average Ct for RPL19 between the three groups (normal tissue, PBMCs, and prostate adenoma cancer) were in the same range. Relative gene expression for each target and sample was calculated as Ct difference between Target gene and endogenous control gene RPL19. Target genes with gene expression only in PCa samples and not in any of the control HD tissue (except ovary, breast, and prostate) and PBMCs were considered "clean Targets" (i.e. having tumor specific expression) Percent expressed for clean targets were calculated based on number of samples with gene expression x 100/total number of samples tested.

[0541] Neo-antigen burden - Patient specific neoantigen burden are assessed at the time of patient enrollment into a vaccine clinical trial. Expression profiles generated from step 1 qPCR analysis are used to determine the expression of neoantigens in patient derived biopsies. Additionally, HLA typing is performed on patients to determine the expression of HLA class I antigens. A list of all potential 9 amino acid long peptides from expressed neoantigens are generated, which will be submitted to *in-silico* methods for HLA class I binding and proteasomal cleavage predictions, such as NetMHCpan 4.0 and NetChop 3.1, to estimate the number of immunogenic 9mer peptides (neoantigen burden) that are likely to be presented by the patient's HLA antigens. A cutoff for the neo-antigen burden is determined based on retrospective analysis of the relationship between patient neoantigen burden and vaccine response in clinical trials. This cutoff will further be used for future patient enrollment strategy. As an illustration, the

neoantigen burden for step 1 neoantigens for patients in the Stand Up to Cancer (SU2C) metastatic castration resistant prostate cancer cohort was estimated (data not shown).

[0542] The following steps were undertaken to estimate the neoantigen burden in the cohort: Neoantigen protein sequences were run through netChop 3.1 for predictions of cleavage sites using the "Cterm 3.0" method. Cleavage sites that had a predicted score of 0.5 or higher were regarded as legitimate cleavage sites. Peptides with 9 amino acid lengths were generated by choosing amino acid sequences of 9 amino acids upstream and ending at the cleavage site. The resulting peptides were then used to make binding predictions using netMHCpan4.0, and were selected to bind to a HLA allele if their binding rank score was <= 2.0. Finally personalized patient neoantigen burden was assessed by i) summing the total number of 9 amino acid peptides that were predicted to bind to a patients HLA Allele profile; and ii) identifying the total number of neo-orfs for a patient that had at least one predicted 9mer binder.

Step 2: Non-invasive monitoring of disease burden

[0543] Liquid biopsy Samples - Matched blood samples were collected from 20 Healthy Volunteers (HV) and 60 metastatic castrate resistant Prostate Cancer (mCRPC) patients through commercial vendors. Blood from each subject was collected and processed as follows:

- PAXgene blood: 2.5 ml blood was collected in PAXgene RNA tube and frozen at -80C freezer until ready for RNA extraction.
- Plasma EVs (exosomes): 10 ml blood collected in EDTA tube were processed for plasma within 2 hr of collection. The blood tube was spun for 10 min at 1500 x g using swinging bucket rotor. The upper layer of plasma was transferred to a new tube. The plasma tube was centrifuged for 10 min at 3,000 x g to remove additional cellular nucleic acids attached to cell debris. The clear supernatant was transferred to a new tube and frozen until ready for EVs enrichment and RNA extraction.

[0544] Gene marker selection - Differential gene expression analysis was performed between metastatic and primary prostate cancer tumor samples from multiple studies in Oncomine. Genes were hierarchically clustered into fifteen functional modules and those belonging to the androgen receptor signaling module were selected in the panel. Additionally, genes implicated in the prostate cancer progression and/or response to androgen deprivation therapy were included.

[0545] A total of 234 prostate cancer associated gene markers were evaluated. 92 gene markers with more than 30% specificity (100% specificity = no gene expression in healthy donor control samples) were selected for screening metastatic castrate resistant prostate cancer samples (mCRPC).

[0546] The lists of biomarkers were further filtered based on their discrepancy in the distinguishing between mCRPC and healthy volunteers. As a result, gene expression derived from 53 genes from plasma exosomes and 55 genes from PAXgene samples (based on their ROC cutoff of 0.55)

were used for classification between mCRPC and healthy volunteers using machine learning method. See Tables 33 and 34 below.

[0547] TaqMan gene expression assay for ARv7 (also referred to as AR-v3) gene was custom designed while best coverage was selected from Applied Biosystems for the panel of genes and endogenous control GAPDH. The neoantigen ID and primer and probe sequences are listed in Table 30.

Table 30. AR-V7 Primers and Probe

HG19_UID	geneID	ID*	Forward primer	Reverse Primer	MGB Probe
FUS_794998592_795015535(++)	ARHGEF38->ARHGEF38-IT1 (++)	FUS2	TGACAGGACCGAATACAACCAG	GGCAATGACACCCACTTCCT	ACAAGTGAATCAATTTAGTG
FUS_1720900661_1720922933(++)	MSMB->NCOA4 (--)_Gene 1	FUS3	CTCGGAGTGGCAGACTGACAA	CTCTGGTCTTGGAAGGTATTCATTC	GTTGCACCCTGAGCAG
FUS_2815919412_2818981741(++)	TMPRSS2-ERG(--)_Gene2	FUS6	AACGACTGGTCCTCACTCACAAC	CGGAGGTGAAAGCGGGT	AACTGATAAGGCTTCCTGC
FUS_2495768395_2495769535(++)	INCA1-CAMTA2 (--)	FUS8	GACGCTTGGCACTCTGGG	AACCCCCTTCACAATCCCTG	CCTCCGAGACGCTGC
FUS_2815947912_2818971910(++)	TMPRSS2-ERG (--)-Gene3	FUS31	GGAGGGGCAATTCTTGTCAAC	GCAGGTCATATTGAACATTCCAGATAC	CAATGGAGTTTAATGAGTTCAA
FUS_205623890_205659488(++)	SLC45A3->ELK4 (--)Design1	FUS1	GCTGAAGAAGGAACTGCCACA	ATCCTGCCCTACACACTGGC	TAGCAATGAGCTGCTTC
FUS_205623890_205661860(++)	SLC45A3->ELK4 (--) Design 2	FUS29	GCTGAAGAAGGAACTGCCACA	CTGCTCCCACCTCCACCC	CATAGCAATGAGGGAGACA
FUS_1720900661_1720923953(++)	MSMB->NCOA4 (++) Gene 2	FUS27	ACCTAATGAGGGAGTTCCAGGAG	TCTGGTCTTGGAAGGTATTCATTCT	CAACCAGGAGAGCAGTG
FUS_2815919412_2818971910(++)	TMP-ERG_4	FUS5	TCAAACAACGACTGGTCCTCACT	ATTCCAGATACCTATCATTACTCGATGC	CTGATAAGGCTTCTGAGTTC

FUS_2400843045_240084469 8(++)	NME4-DECR2 Gene 1	FUS7	CGCCAGCGACTCCG TG	AGGTCCGGGCAGAAGAGGT	CAGTGAGCTGTCCCC GA
FUS_490712493_490755544(++)	D2HGDH-GAL3ST2	FUS1 5	CGCACGCCAAGCAC G	TCCGAGTGCAGGAATCCG	CCACCTTGATACTTCC G
FUS_2660787519_266079218 8(++)	GTF2F1-PSPN	FUS1 9	ATGGACCAGACAGG GCTCG	GATCAACGACACAAAATGCAC TTCTC	GTGGGTGCTCCTTGA
CPNE7.89662239,CPNE7.896 62332	CPNE7-2332_Gene 1	AS11	ACAGTTCGTGCCCT TCCG	AGCAACGGGGGAAGCC	AAGAACCAGAGAACA GCA
RECQL4.145738522-145738600	RECQL-8600	AS37	CTGCCCACTGCCAC CTCTT	GCAGGGCAACTTTCATGAG G	CCCCAGGTTGGCACC
RHPN1.144461249-144461484	RHPN1-1484	AS41	CCGCCGCGCTATGG A	GCAAAAGGGGCTCTCTGCAAC	GAGGGCCGCTGGTGA G
CPNE7.89662012-89662891	CPNE7-2891	AS43	ACAGTTCGTGCCCT TCCG	ATGAACAGGGACCCCCCAAG	AGAACGTGAGTGTCC TGG
AGRN.976778-976857	AGRN-6857	AS47	CGCCCGCCAGGAGA AT	TCGAGCCGTGCGCC	CCCTTGTGGTGAGCG
CPNE7.89643947-APPEQSLLD*	CPNE7-SLLD	AS51	CTGGGGGGCATGGA GTG	CCTTGAATCGGCTGTGGC	TGGGGCAGGTGGGTG C
SPOCK1.13644361,SPOCK1 .13644359	SPOC-3559_Gene 1	AS64	TCAGCCATGGTCTG CGG	CACACAGCCATGGAACCCA	CCAAGGTGAATTGTTT ACTC
DNAH8.38866769-38866876-1	DNAH8-66876	AS3	GCGCCAGGAACTAC CAGAAA	GATCCAAGAGTCCTCAATAC AGACAG	TAGACAGGTTCATTAT GACTTT
ACSM1.20685193-20685403-1(double skip)_D2	ACSM-5403-Gene2	AS7	CAAGTTATGTACTG GACTACTGGGCTC	GGTCCAAGGTCTTTGACTTA ACACA	AGGAGAAGATCAGCA TCC
ACSM1.20685193-20685403-1(single skip)_D1	ACSM1-5403-Gene1	AS6	TTTGCAAGTTATGT ACTGGACTACTGG	GTAGTCGATAGAGAATGTCT TTGGCC	AGGAGAAGGGATCAT CTT
CACNA1D.53678994-LSGHSGSRL*-1	CACNA1D-GSRL	AS8	GTCAGTCTCGTCAT CTTTGGGTC	CTCCGAAAGTGCTGGGATTA TAG	TGAGCGGGCACAGTG
CPNE7.89595831,CPNE7.895 95924_D1	CPNE7-5924-Gene1	AS11	GGACATCGTACAGT TCGTGCC	CCGCGTGGTGGATCCC	CTCAAGAACCAGAGA ACA

GRIN3A.101609737-SFAVTERII*-1	GRIN3A-TERII	AS13	GGTGGTTCCCTGTGGCAAG	TGTCTGTCCTTAGGTCCTCCTCC	TGTCACGGAGAGGATCA
LRRC45.82024340-82024692-1	LRRC45-4692	AS15	TGCCAACACCGTGCTGC	TCCTCTTGCCCTTGGGTG	GGACTTAAAGGTGAGATAC
LRRC45.82025179-82025378-1	LRRC45-5378	AS16	CCTGAAGGGCAACACCACC	CAGTCCCCTAGCCCCTCACT	CAGCAGCTGGGTGAGG
ZNF614.52017576,ZNF614.52017697_D1	ZNF61-7697-Gene1	AS23	CCAGATGTACTCTCCAAGTTGGC	ATTGCACTCCAGCCTGGGT	GTCCAGACTGAGTCTC
TTLL7.83907686	TTLL7-686-B	CAS1	ATCTTAAACCCTCCAACCACTACAA	TTATGGAGCCTATCAGTGATGGAG	CAACAACCCATATCCCTC
TESK1.35608846-TATSHPRTV*	TESK-RTV-B	CAS2	TGAGCCAGCCCCACTCAC	CCTCTTGCAACAGAGCCTGTAGAT	ACACAATCAGGACTTCT
SOAT1.179350277	SOAT-277-B	CAS3	GGTGGTCCATGACTGGCTATATTA	TTTGAATCTCTTGGAGAAAAACTAAAGA	CTTTCTCTGGTGTTTTCCTT
ZYG11A.52863981-52864157	ZYG-157-B	CAS4	TTGTGAAGGAAGCCCTCCAC	GACAATTCTTCTGTAACTTTAAAATAGCAGG	GACATTTTCAATGGAGGTAAC
NWD1.16807586-RPRHWKTDR*-1	NWD1-KTDR	AS18	CAGGCACGCAAGTGGAAAT	CCTGGGTCTAGCATGAACATGG	AGCTACACGGTGGGTGG
AR.67686127-67689555-1	AR-9555	AS62	GTCTTCGGAAATGTTATGAAGCAG	AATGAGGGAGAAGGGGGAGAG	ACTCTGGGAGGTAAGATA
KLK3.51362735-SLVPWRGGV*	KLK3-RGGV	AS57	ATGCTGTGTGCTGGACGCT	GGTAGGTGGAGAATGGAGTGGAG	CACCTGCTCGGCTCCT
Androgen receptor variant 7	ARv7	AS61	GGAAATGTTATGAAGCAGGGATG	TTTGAGATGCTTGCAATTGCC	CTGGGAGAAAAATTCCGGGT
Housekeeping genes					
		AR	GCTTCTACCAGCTCACCAAGCT	GATTAGCAGGTCAAAAGTGAACTGAT	TGCAGCCTATTGCGAG
		RPL19	GCGGATTCTCATGGAACACA	GGTCAGCCAGGAGCTTCTTG	CCACAAGCTGAAGGC

ID* = neoantigen ID

[0548] RNA extraction, cDNA synthesis and pre-amplification - RNA was extracted using Qiagen kits (Qiagen, Gaithersburg, MD) as shown in Table 31 following manufacturers protocol.

[0549] **Table 31.** Qiagen kits

Sample type	**Qiagen kits**	**RNA elution volume (µl)**
PAXgene blood	PAXgene Blood RNA kit	80
Plasma EVs	exoRNeasy Serum/plasma maxi kit	14

[0550] cDNA was synthesized using 10 µl of total RNA from PAXgene blood and 12 µl from plasma EVs using High capacity cDNA reverse transcription Kit with RNase inhibitor from Applied Biosystems, Foster City, CA., following manufacturer's instructions. cDNA was pre-amplified with the selected gene panel for 14 cycles using Applied Biosystems TaqMan™ PreAmp MasterMix per manufacturers protocol. PAXgene preamplified cDNA was diluted 1 to 10 while the preamplified plasma EVs cDNA was diluted 1 to 1 with Nuclease free water (Integrated DNA Technologies, Coralville Iowa). Gene expression analysis of diluted preamplified product was performed on Fluidigm's BioMark Real-Time PCR system in a 96.96 chip format following Fluidigm's instructions (Fluidigm Corporation, San Francisco, CA). Each sample and TaqMan Assay were loaded on the 96.96 chip to give 4 replicate values per sample (2 sample loading + 2 TaqMan assay loading).

[0551] Data analysis - Briefly, raw data was extracted from BioMark system using Linear derivative for baseline correction and user detector for CT threshold method. Cycle Threshold (CT) values greater than 999 values were assigned a value of 40. Geometric mean of the 4 replicates was evaluated and an average CT of greater than or equal to 35 was considered to have no detectable expression. Each sample was considered evaluable only when the CT value of endogenous control gene GAPDH was less than 21. Using this threshold, two plasma EVs from the mCRPC cohort were excluded from further analysis. For each gene marker, we calculated the number of samples with detectable expression in mCRPC and HV cohorts.

Machine learning method for characterization of disease sample

[0552] Pre-processing of qPCR measurements of prostate cancer associated genes - In this assay, genes with no significantly detectable expression were assigned a PCR Ct value of 33 to 40, genes with high expression (e.g. house-keeping genes such as PTPRC and GAPDH) were assigned a Ct value of less than or equal to 17. Genes with a Ct value larger than 33 were then assigned a value of 33 and genes with a Ct value less than 17 were then assigned a value of 17. Genes with a Ct value of 17 were defined as the most highly expressed genes and genes with a Ct value of 33 were defined as the most lowly expressed genes. The measured qPCR Ct values were then are scaled between 0 and 1 using the following calculation:

[0553] A gene with PCR Ct value of 20 is subtracted from 33 (low expression cutoff) and divided by 17 (high expression cutoff) to arrive at a scaled value (e.g., (33-20)/17 = 0.765).

[0554] The scaled value represents the normalized Ct value. A normalized Ct value of 0 represents a lowly expressed gene, while a value of 1 represents a highly expressed gene. These normalized gene expression values were then used in the diagnostic models.

[0555] Use of receiver operating characteristic curve (ROC) to select the most informative prostate cancer associated genes - Area Under the Curve (AUC) of the ROC was calculated for each gene to assess its performance in distinguishing between mCRPC samples and healthy samples. The genes were then ranked by the AUC values. The genes with AUC values larger than 0.55 were selected as informative prostate cancer associated genes. See FIG. 10 and 12. Only those genes were included in the machine learning classification/diagnostic model.

[0556] Random forest based diagnostic model and evaluation of its performance - Random forest is a widely used machine learning method. This method was utilized as the classification/diagnostic algorithm based on the panel genes' Ct values. The mCRPC and healthy samples were randomly split into a training dataset and a test dataset, with a ratio of 0.7. In the training step, the algorithm learns the best classification tree structure based on known label of disease and healthy. In the test step, unseen dataset was used to classify the unknown label of the sample based on their normalized PCR Ct values. To achieve robust estimation, the training/test splitting was repeated 200 times, and for each run, the accuracy, sensitivity and specificity of the random forest model in the test dataset were calculated. Considering the following table, the accuracy represents (TP+TN)/(TP+FP+FN+TN), the sensitivity represents TP/(TP+FN), and the specificity represents TN/(TN+FP).

[0557] Table 32.

		Truth	
		mCRPC	Healthy
Test result	mCRPC	58 (TP)	1 (FP)
	Healthy	2 (FN)	19 (TN)

[0558] FIG. 11 and FIG. 13 illustrate the mean and standard deviation (error bar) of the accuracy, sensitivity, and specificity for the exosome samples (FIG. 11) and the PAXgene samples (FIG. 13). To evaluate the weights of the genes, all the samples, including mCRPC and healthy, were put in a random forest model to learn their relative importance. Tables 33 and 34 indicate the relative weights of the selected genes from exosomal samples (Table 33) and PAXgene samples (Table 34), where 100 is the most informative gene in this model.

Table 33. Relative weights (scaled) of genes based on Exosomal data

Biomarker	Weight	Biomarker	Weight	Biomarker	Weight
PITX2	100	COL1A1	3.404745	KLK2	0.41457
RCN1	89.12593	`AR full`	3.050954	GRHL2	0.347752
KRT17	27.51728	LGR5	2.514631	C9orf152	0.302417
ACPP	18.77256	AZGP1	2.323516	SPINK1	0.186328
ATF3	14.70256	NROB1	2.28231	EPHA3	0.186303
`NKX3-1`	10.86919	HPN	2.208863	TMEFF2	0.164948
`(CK18)Krt18`	10.64367	FOLH1	1.868003	GNMT	0.164439
TiMP1	9.184084	TNFRSF19	1.811444	`TMP:ERG`	0.148076
STEAP1	8.392075	TSPAN1	1.675128	AGR2	0.134924
UGT2B17	7.276335	`MUC-1`	1.650726	GPR39	0.093266
FLNC	7.005469	GREB1	1.459662	THBS2	0.093016
METTL7a	6.846778	STEAP2	1.13545	`AR v7`	0.092698
KRT8	6.659049	KCNN2	0.921032	FOXA1	0
IGJ	6.352049	RAB3B	0.887624	ACADL	0
KLK4	6.33976	`FGFR 4`	0.857734	ADAMS15	0
IDO1	6.203417	NPY	0.780801	HSD3B2	0
ETV7	3.742933	KLK3	0.716774	MYBPC1	0

Table 34. Relative weights (scaled) of genes based on PAXgene data

Biomarker	Weight	Biomarker	Weight	Biomarker	Weight
HPN	100	C9orf152	36.59141	IDO1	16.45665
GPR39	94.1898	SYP	36.456	THBS2	15.75712
ROR1	79.60198	GNMT	34.94274	KLK4	14.27767
FLNC	72.1005	`AR Full`	30.21543	ADAMS15	11.59232
`NKX3-1`	60.94338	EPHA3	29.92159	CYP3A5	10.69601
FGF8	59.7716	METTL7a	29.89579	FOLH1	10.25379
`MUC-1`	58.30615	STEAP1	29.55925	TMEFF2	7.989344
EDIL3	52.74437	KISS1R	29.35491	KLK2	7.960658
`NKX2-2`	49.4976	`(CACLR)CALCR`	29.30495	HOXB13	6.969058
ATF3	48.57946	KRT8	28.60391	`(CK18)Krt18`	6.759882
UGT2B17	44.08252	CYP17	28.09351	SFRP4	6.677861

`FGFR 4`	41.47322	SPDEF	26.60297	STEAP2	6.285243
GREB1	41.29564	NPY	26.55807	GRHL2	5.215167
LGR5	41.01954	MSLN	26.53083	ACPP	4.725696
FGF9	40.03938	`CLUL1(clusterin)`	26.28455	`AR v7`	3.702901
RELN	39.98147	TSPAN1	20.31485	NROB1	3.615673
TNFRSF19	39.75397	FOXA1	19.74772	`TMP:ERG`	1.599442

MC38 tumor clearance specific to the GA20 PCaNeoAg.

[0559] Mouse colon cancer cell line MC38 was engineered to stably express a protein comprised of 10 prostate neoantigens (Table 35). Multiple MC38 cell lines were identified with a range of protein expression (low, medium, and high) as confirmed by co-expressed mCherry signal. Mice (groups 1-4) were immunized with 1x 10^9 VP of GAd20-PCaNeoAg by intramuscular injection 14 days prior to MC38 cell line implantation. Groups 5-8 did not receive GAd20 immunization (Table 36). MC38 tumor volume (mm^3) was recorded through the study for each mouse. Animals immunized with GAd20-PCaNeoAg were shown to have significantly reduced tumor growth (group 3,4) or elimination of tumor (group 2) compared to animals that did not receive GAd20-PCaNeoAg immunization or animals implanted with the parental MC38 cell line that did not express the 10 Prostate Neo antigens. Overall these data indicate that immunization with GAd20-PCaNeoAg leads to MC38 tumor clearance only in tumors that expressing Prostate NeoAg.

Table 35

Gene ID	**GAd Position**	**Peptide Sequence**	**Lenti Position to minimize Junctional Immunogenicity**
SPOC	12	DGHSYTSKVNCLLLQDGFHGCVSITGA AGRRNLSIFLFLMLCKLEFHAC (SEQ ID NO: 333)	1
ZNF614	13	KIQNKNCPD (SEQ ID NO: 285)	2
AGRN	14	FKKFDGPCGERGGGRTARALWARGDS VLTPALDPQTPVRAPSLTRAAAAV (SEQ ID NO: 317)	9
TTLL7	15	HYKLIQQPISLFSITDRLHKTFSQLPSVH LCSITFQWGHPPIFCSTNDICVTANFCIS VTFLKPCFLLHEASASQ (SEQ ID NO: 437)	8

RECQL4	16	CHLFLQPQVGTPPPHTASARAPSGPPHP HESCPAGRRPARAAQTCARRQHGLPGC EEAGTARVPSLHLHLHQAALGAGRGR GWGEACAQVPPSRG (SEQ ID NO: 297)	3
LRRC45	17	VLRFLDLKVRYLHS (SEQ ID NO: 269)	4
NWD1	18	QWQHYHRSGEAAGTPLWRPTRN (SEQ ID NO: 277)	5
CPNE7	19	VPFRELKNQRTAQGAPGIHHAASPVAA NLCDPARHAQHTRIPCGAGQVRAGRGP EAGGGVLQPQRPAPEKPGCPCRRGQPR LHTVKMWRA (SEQ ID NO: 261)	6
DNAH8	20	VAMMVPDRQVHYDFGL (SEQ ID NO: 245)	7
MSMB-NCOA4-1	21	GVPGDSTRRAVRRMNTF (SEQ ID NO: 343)	10

[0560] Subpool 2 NetMHC 4.0 predictions for C57BL/6 MHC Binding:

H-2Kb: 5 predicted strong binding peptides; 11 predicted weak binding peptides

H-2Db: 1 predicted strong binding peptide; 20 predicted weak binding peptides

Table 36

Group	**# mice**	**GAd vaccine**	***MC38 cell line implant**
1. Prophylactic GAd vaccine + MC38-5Ag Parental	15	Day 1: 1e9 vp	Day 15: 5e5 MC38-5Ag parental
2. Prophylactic GAd vaccine + MC38-5Ag ProsNeo Low	15	Day 1: 1e9 vp	Day 15: 5e5 MC38-5Ag ProsNeo Low
3. Prophylactic GAd vaccine + MC38-5Ag ProsNeo Med	15	Day 1: 1e9 vp	Day 15: 5e5 MC38-5Ag ProsNeo Med
4. Prophylactic GAd vaccine + MC38-5Ag ProsNeo High	15	Day 1: 1e9 vp	Day 15: 5e5 MC38-5Ag ProsNeo High
5. MC38-5Ag Parental	15	n/a	Day 15: 5e5 MC38-5Ag parental
6. MC38-5Ag ProsNeo Low	15	n/a	Day 15: 5e5 MC38-5Ag ProsNeo Low
7. MC38-5Ag ProsNeo Med	15	n/a	Day 15: 5e5 MC38-5Ag ProsNeo Med
8. MC38-5Ag ProsNeo High	15	n/a	Day 15: 5e5 MC38-5Ag ProsNeo High

[0561] SEQ ID NO: 622

CATCATCAATAATATACCTTATTTTGGATTGAGGCCAATATGATAATGAGGTGGGCGGGGCGAGGCGGGGCGGTGACGTAGGACGCGCGAGTAGGGTTGGGAGGTGTGG
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TTTTTCTGGGCTCCTCTACATTGGCCACTGTGTCCCACATCGAGGTAGACTGCCTCACGCCCTTCACAGTCTACCTGCTTTTCGGCTTTGTCATCTGCACCTTTGTCTGCAGCG
TTATCACTGTAGTGATCTGCTTCATACAGTGCATCGACTACATCTGTGTGCGGGTGGCCTACTTTAGACACCACCCCCAGTATCGCAACAGGGACATAGCGGCTCTCCTAAG
ACTTGTTTAAATCATGGCCAAATTACCTGTGATTATCTGCTGCGTCCTAGCCGCGATTGGGACTCAACCTAATACCACCACCAGCGCTCCCAGAAAGAGA
CATGTATCCTGCAGCTTCAAGCGTCCCTGGAATATACCCCAATGCTTTACTGATGAACCTGAAATCTCTTTGGCTTGGTACTTCAGCGTCACCGCCCTTCTCATCTTCTGCAG

TACGGTTATTGCTCTTGCCATCTACCCTTCCCTTAACCTGGGCTGGAATGCTGTCTCAACTCTATGGAATATCCCACCTTCCCAGAACCAGACCTGCCAGAACCTGGTTGTTCTAA
ACGCGTTTCCTCCTCCTCCTCCAGTTCAAAATCAGTTTCGCCCTCCCGTCCCCTACGCCCACACTGAGGTCAGCTACTTTAATCTCAACAGGCGGAGATGACTGAAAACCTAGACCTAG
AAATGGACGGTCTCTGCAGCGAGCAACGCACACTAGAGAGGCGCCGGCAAAAAGCAGAGCTCGAGCGTCTTAAACAAGAGCTCCAAGACGCCGTGGCCATACACCAGTGC
AAAAAAAGGGCTCTTCTGTCTGGTAAAACAGGCCACGCTCACCTATGAAAAAACAGGTGACACCCACCGCCTAGGATACAAGCTGCCCACACAGCGCCAAAAGTTTGCCCTT
ATGATAGGTGAACAACCCATCACCGTCACCCAGCACTCCGTGGAGACAGAAGGCTGCATTCATGCTCCCTGCAGGGGCGCTGACTGCCTCTACACCTTGATCAAAACCCTC
TGCGGTCTCAGAGACCTTATCCCTTTCAATTGATCATACTGTAATCAATAAAAAATCACTTACTTGAAATCTGATAGCAAGACTCTGTCCAATTTTTTCAGCAAACACTTCCT
TCCCCTCCTCCCAACTCTGGTACTCTAGGCGCCTCCTAGCTGCAAACTTCCTCACACGTCTGAAGGGAATGTCAGATTCCTCCTCCTGTCCCTCCGCACCCACGATCTTCATG
TTGTTACAGATGAAACGCGCGCGAGATCGTCTGACGAGACCTTCAACCCCGTGTACCCCTACGATACCGAGATCGCTCCGACTTCTGTCCCTTTCCTTCACCCCTCCCTTTGTATC
ATCCGCAGGAATGCAAGAAAATCCAGCTGGGGTGCTGTCCCTGCACCTGTCAGAGCCCCTTACCACCCACAATGGGGCCTGACTCTAAAAAATGGGGGGCGGCCTGACCCT
GGACAAGGAAGGGAATCTCACTTCCCAAAACATCACCAGTGTCGATCCCCCTCTCAAAAAAGCAAGAACAACATCAGCCTTCAGACCGCCGCACCCCTCGCCGTCAGCTC
CGGGGCCCTAACCCTTTTTGCCACTCCCCCCCTAGCGGTCAGTCTCAGGCCCCTCTTACTTTGGAAGACTCAAACTAACTCTGGCCACC
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AACAATGACAGCCTAGGACTAGACATGCAAGCGCCCATCAGCTCTCGAGATGGAAAACTGGCTCTAACAGTGGCGGCCCCCCTAACTGTGGCCGAGGGTATCAATGCTTTG
GCAGTAGCCACAGGTAATGGTATTGGACTAAATGAAACCAACACACACCTGCAGGCAAAACTGGTCGCGCCCCTAGGCTTTGATACCAACGGCAACATTAAGCTAAGCGT
CGCAGGAGGCATGAGGCTAAACAATAACACACTGATACTAGATGTAAACTACCCATTTGAGGCTCAAGGCCAACTGAGAGTGGGCTCGGGCCCACTATATGTAG
ATTCTAGTAGTCATAACCTAACCATTAGATGCCTTAGGGGATTGTATGTAACATCTTCTAACAACCAAAACGGTCTAGAGGCCAACATTAAACTAACAAAAGGCCTTGTGT
ATGACGGAAATGCCATAGCAGTTAATGTTGGCAAAGGGCTGGAATACAGCCCTACTGGCACAACAGAAAAACCTATACAGACTAAAATAGGTCTAGGCATGGAGTATGAC
ACTGAGGGAGCCATGATGACAAAACTAGGCTCTGGACTAAGCTTTGACAATTCAGGAGCCATTGTGGTGGGAAACACAAAATGATGACAGGCTTACTTTGTGGACCACACC
GGACCCATCGCCCAACTGTCAGATTTACTCTGAAAAAGATGCTAAACTAACCTTGGTACTGACTAAATGTGGCAGTCAGGTTGTAGGCACAGTATCTATTGCCGCTCTTTAAA
GGTAGCCTTGTGCCAATCACTAGTGCAATCAGTGTGGTTCAGATATACCTAAAGGTTTGATGAAAATGGGGGTGCTGATGAGTAACTCTTCACTTAATGGCGAATACTGGAATT
TTAGAAACGGAGACTCAACTAATGGCACACCATATACAAACGCAGTGGGTTTTATGCCTAATCTACTGGCCTATCCTAAAGGTCAAACTACAACTGCAAAAAGTAACATTG
TCAGCCAGGTCTACATGAACGGGGACGATACTAAACCCATGACATTTACAACTTCAATGGCCTTAGTGAAACAGGGGATACCCCTGTCAGTAAATATTCCATGACAT
TCTCATGGAGGTGGCCAAATGGAAGCTACATAGGGCACAATTTTGTAACAAACTCCTTTACTTTCTCCTACATCGCCCAAGAATAAAGAAAGCACAGAGATGCTTGTTTTTG
ATTTCAAAATTGTGTGCTTTTATTTATTTTTCAAGCTTACAGTAGTCATTAGAATAGAGCTTAATTAAACTGCATGAGAACCCTTCCACATAGCTTAAATTATCA
CCAGTGCAAATGGAAAAAAAATCAACATACCTTTTTATCCAGATATCAAAGAACTCTAGTGGTCAGTTTTCCCCCACCCTCCCAGCTCACAGAATACACAGTCCTTTCCCCCC
GGCTGGCTTTAAACAACACTATCTCATTGGTAACAGACATATTTTTAGGTGTAATAATCCACACGGTCTCTTGGCGGGCCAAACGCTGGTCTGTGATGTTAATAAACTCCCC
AGGCAGCTCTTTCAAGTTCACGTCGCTGTCCAACTGCTGAAGCGCTCCGGGCTCCGACTGCGCCTCTAGCGGAGGCAACGGCAGCACCCGATCCTTGATCTATAAAGGAGT
AGAGTCATAAATCCCCCATAAGAATAGGGCGGTGATGCAGCAACAAGGCGCGCAGCAACTCCTGCCGCCGCCTCTCCGTACGACAGGAATGCAACGGGGTGGTGGTCTCCT
CCGCGATAATCCGCACCGCTCGCATCCTCGTCCTCCGGGCACAGCAGCGCATCCTGATCTCACTGAGATCGGCGCAGTAAGTGCAGCACACACCAAGATGT
TATTTAAGATCCCACAGTGCAAAGCACTGTACCCAAAGCTCATGGCGGAAGGACAGCCCCCACGTGACCATCGTACCAGGTAAATCAAATGACGACCTCTCA
TAAACACGCTGGACATATACATCACCTCCTTGGGCATGAGCTGATTCACCACACAGGCATCGCTGATTAATTAAAGACCCCTCGAGCACACCATCCTGAACCA
GGAAGCCAGCACCTGACCCCCCGCCAGGCACTGCAGTGGCAGTGAAGACTCCAGCGCTCGTAGCCGTGAACCATAGAGGCTGGTCATTATAT
CCACATTGGCACAACACAGACACACTTTCATACACTTTTTCATGATTAGCAGCTCCTCTCTAGTCAAGACCATATCCCAAGGAATCACCCACTCTTGAATCAAGGTAAATCC
CACACAGCAGGGCAGGCCTCTCACATAACTCACGTTATGCATGAGTGTCGCAATCTGGAAATACCGGATGATCTTCCATCACCGAAGCCCGGGTCTCCGTCTCAAA
GGGAGGTAAACGGTCCCTCGTGTAGGGACAGTGGCGGGATAATCGAGATCGTGTTGAACGTAGAGTCATGCCAAAGGGAACAGCGGACGTACTCATATTTCCTCCAGCAG
AACCAAGTGCGCGCGTGGCAGCTATCCCTGCGTCTTCTGTCTCGCCGCCTGCCCCGCTCGGTGTAGTTGTTGTAATACAGCCACTCCCTCAGACCGTCAAGGCGCTCCCTGG
CGTCCGGATCTATAACAACACCGTCCTGCAGCGCCGCCTGATGACATCCACCACGTAGAGTATGCCAAGCCCAGCCACGAAATGCCACTCACTTTGACAGCGAGAGATAG
GAGGAGCGGGAAGAGATGGAAGAACCATGATAGTAAAAGAACTTTTATTCCAATCGATCCTCTACAATGTCAAAGTGTAGATCTATCAGATGGCACTGGTCTCCTCCGCCTG
AGTCGATCAAAAATAACAGCTAAACCACAAACAACACGATTGGTCAAATGCTGCACAAGGGCTTGCAGCATAAAATCGCCTCGAAAGTCCACCGCAAGCATAACATCAAA
GCCACCGCCCCTATCATGATCTATGATAAAAACCCCACAGCTATCCACCAGACCCATATAGTTTTCATCTCTCCATCGTGAAAAAATATTTACAAGCTCCTCCTTTAAATCAC
CTCCAACCAATTCAAAAAGTTGAGCCAGACCGCCTCCACCTTCATTTTCAGCATGCGCATCATGATTGCAAAAATTCAGGCTCCTCAGACACCTGTATAAGATTGAGAAGC

GGAACGTTAACATCAATGTTTCGCTCGCGAAGATCGCGCCTCAGTGCAAGCATGATATAATCCCACAGGTCGGAGCGGATCAGCGAGGACATCTCCCCGCCAGGAACCAAC
TCAACGGAGCCTATGCTGATTATAATACGCATATTCGGGGCTATGCTAACCAGCACGGCCCCCAAATAGGCGTACTGCATAGGCGGCGACACAAAAAGTGAACAGTTTGGGTT
AAAAAAATCAGGCAAACACTCGCGCAAAAAAGCAAGAACATCATAACCATGCTCATGCAAATAGATGCAAGTAAGCTCAGGAACGAACCACACAGAAAAAATGCACACAATTTTTCT
CTCAAACATGACTGCGAGCCCTGCAAAAAATAAAAAAGAAACATTTACACAAGAGTAGCCTGTCTTACAATGGGATAGACTACTCTAACCAACATAAGACGGGCCACCGACA
TCGCCCGCGTGGCCATAAAAAAAAAATTATCCGTGTGATTAAAAAGAAGCACAGATAGCTGGCCAGTCATATCCGGAGTCATCACGTGCGAACCCGTGTAGACCCCCGGGTTG
GACACATCGGCCAAACAAAGAAAGCGGCCAATGTATCCCGGAGGAATGATAACACTAAGACGAAGATACAACAGAATAACCCCATGGGGGGCGAATAACAAAGTTAGTAG
GTGAATAAAAAACGATAAACACCCGAAACTCCCCTCCTGCGTAGGCAAAATAGCGCCCTCCCCTTCCAAAACAACATACACAGCGCTTCCACAGCAGCCATGACAAAAGACTCA
AAACACTCAAAAGACTCAGTCTTACCAGGAAAAATAAAAGCACTCTCACAGCACCAGCACTAATCAGAGTGTGAAGAGGGCCAAGTGCCGAACGAGTATATATAGGAATTA
AAAATGACGTAAATGTGTAAAGGTCAAAAAACGCCCAGAAAAATACACAGACCAAGCCCGCGAAACCAAAACCCCTCCCCTTGTCACCGCCCACAACTTACATAATCACAA
CTTCCGCTTTCCCACGATACGTCACTTCCTCAAAAATAGCAAACTACATTTCCCACATGTACAAACCAAAACACCCCTCCCCTTGTCACCGCCCACAACTTACATAATCACAA
ACGTCAAAGCCTACGTCACCCGCCCCGCCTCGCCCCGCCCACCTCATTATCATATTGGCCTCAATCCAAAATAAGGTATATTATTGATGATG

EMBODIMENTS

[0562] The following list of embodiments is intended to complement, rather than displace or supersede, the previous descriptions.

Embodiment 1. A method of diagnosing a subject with prostate cancer, the method comprising:

evaluating the presence of one or more prostate cancer neoantigens in a sample from the subject, the one or more prostate cancer neoantigens comprising the amino acid sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, fragments of the preceding sequences, or any combination thereof,

wherein the presence of the one or more prostate cancer neoantigens is indicative of prostate cancer in the subject.

Embodiment 2. The method of embodiment 1, wherein the one or more prostate cancer neoantigens comprise the amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, fragments of the preceding sequences, or any combination thereof.

Embodiment 3. The method of embodiment 1 or 2, wherein the one or more prostate cancer neoantigens comprise the amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, fragments of the preceding sequences, or any combination thereof.

Embodiment 4. The method of embodiment 3, wherein the method comprises evaluating the presence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, and 223.

Embodiment 5. The method of any one of the previous embodiments, wherein the presence of the one or more prostate cancer neoantigens is evaluated by qPCR.

Embodiment 6. The method of embodiment 5, further comprising, prior to performing the qPCR, extracting RNA from the sample from the subject and synthesizing cDNA from the extracted RNA.

Embodiment 7. The method of embodiment 6, wherein the RNA is produced from a DNA sequence comprising SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 380, 382, 384, 386, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 519, 520, 521, 522, 523, 524, 525, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, fragments of the preceding sequences, or any combination thereof.

Embodiment 8. The method of any one of the previous embodiments, wherein the sample comprises a prostate cancer tissue sample.

Embodiment 9. The method of any one of the previous embodiments, wherein the prostate cancer is a localized prostate adenocarcinoma, a relapsed prostate cancer, a refractory

prostate cancer, a metastatic prostate cancer, a castration resistant prostate cancer, or any combination thereof.

Embodiment 10. The method of any one of the previous embodiments, wherein the subject is treatment naïve.

Embodiment 11. The method of any one of embodiments 1-9, wherein the subject has received androgen deprivation therapy.

Embodiment 12. The method of any one of the previous embodiments, wherein the subject has an elevated level of prostate specific antigen (PSA).

Embodiment 13. A method of treating prostate cancer in a subject, the method comprising:

a) evaluating the presence of one or more prostate cancer neoantigens in a sample from the subject, the one or more prostate cancer neoantigens comprising the amino acid sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, fragments of the preceding sequences, or any combination thereof; and
b) administering a therapeutically effective amount of a prostate cancer vaccine to the subject to thereby treat the prostate cancer.

Embodiment 14. The method of embodiment 13, wherein the one or more prostate cancer neoantigens comprise the amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269,

253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, fragments of the preceding sequences, or any combination thereof.

Embodiment 15. The method of embodiment 13 or 14, wherein the one or more prostate cancer neoantigens comprise the amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, fragments of the preceding sequences, or any combination thereof.

Embodiment 16. The method of embodiment 15, wherein the method comprises evaluating the presence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, and 223.

Embodiment 17. The method of any one of embodiments 13-16, wherein the presence of the one or more prostate cancer neoantigens is evaluated by qPCR.

Embodiment 18. The method of embodiment 17, further comprising, prior to performing the qPCR, extracting RNA from the sample from the subject and synthesizing cDNA from the extracted RNA.

Embodiment 19. The method of any one of embodiments 13-18, wherein the sample comprises a prostate cancer tissue sample.

Embodiment 20. The method of any one of embodiments 13-19, wherein the prostate cancer vaccine comprises a polynucleotide encoding one or more polypeptides selected from the group consisting of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, and fragments thereof.

Embodiment 21. The method of embodiment 20, wherein the polynucleotide comprises:

a) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 276, 382, 334, 338, 270, 254, 310, 326, 272, 306, 252, 246, 262, 266, 318, 256, 278, 298, 286, 448, 450, 453, 455, 380, 344, 212, 350, 214, 216, 222, 220, 226, 346, 354, 236, 224, 168, 172, 20, 24, 178, and fragments thereof;

b) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, and fragments thereof; or

c) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 500, 501, 461, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 477, 519, 520, 521, 522, 523, 524, 525, 485, 486, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, and fragments thereof.

Embodiment 22. The method of any one of embodiments 13-21, wherein the polynucleotide encodes a polypeptide comprising the amino acid sequence of SEQ ID NOs: 541, 550, 554, 555, 556, 623, 624, 543, 552, 557, 558, 559, 625, or 626.

Embodiment 23. The method of embodiment 22, wherein the polynucleotide comprises a polynucleotide sequence of SEQ ID NOs: 542, 551, 544, or 553.

Embodiment 24. The method of any one of embodiments 13-23, wherein the polynucleotide is DNA or RNA.

Embodiment 25. The method of embodiment 24, wherein RNA is mRNA or self-replicating RNA.

Embodiment 26. The method of embodiment 13-25, wherein the vaccine is a recombinant virus.

Embodiment 27. The method of embodiment 26, wherein the recombinant virus is derived from an adenovirus (Ad), a poxvirus, an adeno-associated virus (AAV), or a retrovirus.

Embodiment 28. The method of embodiment 27, wherein the recombinant virus is derived from hAd5, hAd7, hAd11, hAd26, hAd34, hAd35, hAd48, hAd49, hAd50, GAd20, GAd19, GAd21, GAd25, GAd26, GAd27, GAd28, GAd29, GAd30, GAd31, ChAd3, ChAd4, ChAd5, ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd55, ChAd63, ChAd73, ChAd82, ChAd83, ChAd146, ChAd147, PanAd1, PanAd2, PanAd3, Copenhagen vaccinia virus (W), New York Attenuated Vaccinia Virus (NYVAC), ALVAC, TROVAC, or modified vaccinia ankara (MVA).

Embodiment 29. The method of embodiment 28, wherein the recombinant virus is derived from GAd20.

Embodiment 30. The method of embodiment 28, wherein the recombinant virus is derived from MVA.

Embodiment 31. The method of embodiment 28, wherein the recombinant virus is derived from hAd26.

Embodiment 32. The method of embodiment 29 or 31, wherein the polynucleotide encodes a polypeptide of SEQ ID NOs: 541, 550, 554, 555, 556, 623, or 624.

Embodiment 33. The method of embodiment 30 or 31, wherein the polynucleotide encodes a polypeptide of SEQ ID NOs: 543, 552, 557, 558, 559, 625, or 626.

Embodiment 34. The method of any one of embodiments 13-33, wherein the prostate cancer is a localized prostate adenocarcinoma, a relapsed prostate cancer, a refractory prostate cancer, a metastatic prostate cancer or a castration resistant prostate cancer, or any combination thereof.

Embodiment 35. The method of any one of embodiments 13-34, wherein the subject is treatment naïve.

Embodiment 36. The method of any one of embodiments 13-34, wherein the subject has received androgen deprivation therapy.

Embodiment 37. The method of any one of embodiments 13-36, wherein the subject has an elevated level of prostate specific antigen (PSA).

Embodiment 38. A method of treating prostate cancer in a subject, the method comprising:

a) evaluating the presence of one or more prostate cancer neoantigens in a sample from the subject, the one or more prostate cancer neoantigens comprising the amino acid sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77,

79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, fragments of the preceding sequences, or any combination thereof; and;

b) evaluating expression of one or more prostate cancer biomarkers in a sample from the subject, wherein the one or more prostate cancer biomarkers comprise: RCN1, STEAP1, PITX2, TIMP1, KLK4, KRT18, KLK3, ACPP, KLK2, TSPAN1, ATF3, SPDEF, NPY, SPINK1, HOXB13, FOXA1, KRT17, FOLH1, TNFRSF19, GREB1, KRT8, FLNC, GRHL2, RAB3B, JCHAIN, TMEFF2, AGR2, ACADL, AZGP1, MUC1, STEAP2, UGT2B17, METTL7A, HPN, NKX3-1, GNMT, ADAMTS15, HSD3B2, EPHA3, KCNN2, LGR5, IDO1, GPR39, C9orf152, MYBPC1, THBS2, ETV7, COL1A1, FGFR4, NR0B1, AR, ARv3, TMPRSS2:ERG, ROR1, FGF8, NKX2-2, EDIL3, RELN, FGF9, AKR1C4, CLUL1, KISS1R, CYP3A5, CYP17A1, SFRP4, HNF1A, CALCR, SYP, MSLN, or any combination thereof; and

c) administering a therapeutically effective amount of a prostate cancer vaccine to the subject.

Embodiment 39. The method of embodiment 38, further comprising, after administering the therapeutically effective amount of the prostate cancer vaccine, evaluating expression of the one or more prostate cancer biomarkers evaluated in step b), wherein a decrease in expression compared to the expression in step b) is indicative of responsiveness to the prostate cancer vaccine.

Embodiment 40. The method of embodiment 38 or 39, wherein the one or more prostate cancer neoantigens comprise the amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379,

343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, fragments of the preceding sequences, or any combination thereof.

Embodiment 41. The method of any one of embodiments 38-40, wherein the one or more prostate cancer neoantigens comprise the amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, fragments of the preceding sequences, or any combination thereof.

Embodiment 42. The method of embodiment 41, wherein the method comprises evaluating the presence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, and 223.

Embodiment 43. The method of any one of embodiments 38-42, wherein the presence of the one or more prostate cancer neoantigens is evaluated by qPCR.

Embodiment 44. The method of embodiment 43, further comprising, prior to performing the qPCR, extracting RNA from the sample from the subject and synthesizing cDNA from the extracted RNA.

Embodiment 45. The method of any one of embodiments 38-44, wherein the one or more neoantigens are from a prostate cancer tissue sample.

Embodiment 46. The method of any one of embodiments 38-45, wherein the one or more prostate cancer biomarkers comprise: RCN1, STEAP1, PITX2, TIMP1, KLK4, KRT18, KLK3, ACPP, KLK2, TSPAN1, ATF3, SPDEF, NPY, SPINK1, HOXB13, FOXA1, KRT17, FOLH1, TNFRSF19, GREB1, KRT8, FLNC, GRHL2, RAB3B, JCHAIN, TMEFF2, AGR2, ACADL, AZGP1, MUC1, STEAP2, UGT2B17, METTL7A, HPN, NKX3-1, GNMT, ADAMTS15, HSD3B2, EPHA3, KCNN2, LGR5, IDO1, GPR39, C9orf152, MYBPC1, THBS2, ETV7, COL1A1, FGFR4, NR0B1, AR, ARv3, TMPRSS2:ERG, or combinations thereof.

Embodiment 47. The method of any one of embodiments 38-45, wherein the one or more prostate cancer biomarkers comprise: HPN, ROR1, FLNC, GPR39, FGF8, NKX2-2, MUC1, NKX3-1, EDIL3, LGR5, FGFR4, STEAP1, ATF3, RELN, UGT2B17, KLK3, C9orf152, GNMT, METTL7A, FGF9, SPDEF, FOXA1, AKR1C4, GREB1, CLUL1, TMEFF2, HOXB13,

KLK2, NPY, GRHL2, STEAP2, THBS2, KISS1R, KRT8, TNFRSF19, CYP3A5, KLK4, IDO1, FOLH1, NR0B1, EPHA3, CYP17A1, SFRP4, KRT18, TSPAN1, HNF1A, ADAMTS15, ACPP, CALCR, SYP, AZGP1, AR, ARv3, MSLN, TMPRSS2:ERG, and combinations thereof.

Embodiment 48. The method of embodiment 46, wherein the one or more prostate cancer biomarkers are from a plasma sample.

Embodiment 49. The method of embodiment 47, wherein the one or more prostate cancer biomarkers are from a blood sample.

Embodiment 50. The method of any one of embodiments 38-49, wherein the expression of the one or more prostate cancer biomarkers is evaluated by qPCR.

Embodiment 51. The method of embodiment 50, further comprising, prior to performing the qPCR, extracting RNA from the sample from the subject and synthesizing cDNA from the extracted RNA.

Embodiment 52. The method of any one of embodiments 38-51, wherein the prostate cancer vaccine comprises a polynucleotide encoding one or more polypeptides selected from the group consisting of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, and fragments thereof.

Embodiment 53. The method of embodiment 52, wherein the polynucleotide comprises:

a) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 276, 382, 334, 338, 270, 254, 310, 326, 272, 306, 252, 246, 262, 266, 318, 256, 278, 298, 286, 448, 450, 453, 455, 380, 344, 212, 350, 214, 216, 222, 220, 226, 346, 354, 236, 224, 168, 172, 20, 24, 178, and fragments thereof;
b) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, and fragments thereof; or
c) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 500, 501, 461, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 477, 519, 520, 521, 522, 523, 524, 525, 485, 486, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, and fragments thereof.

Embodiment 54. The method of embodiment 52 or 53, wherein the polypeptide comprises the amino acid sequence of SEQ ID NOs: 541, 550, 554, 555, 556, 623, 624, 543, 552, 557, 558, 559, 625, or 626.

Embodiment 55. The method of embodiment 54, wherein the polynucleotide comprises a polynucleotide sequence of SEQ ID NOs: 542, 551, 544 or 553.

Embodiment 56. The method of any one of embodiments 52-55, wherein the polynucleotide is DNA or RNA.

Embodiment 57. The method of embodiment 56, wherein RNA is mRNA or self-replicating RNA.

Embodiment 58. The method of any one of embodiments 38-57, wherein the vaccine is a recombinant virus.

Embodiment 59. The method of embodiment 58, wherein the recombinant virus is derived from an adenovirus (Ad), a poxvirus, an adeno-associated virus (AAV), or a retrovirus.

Embodiment 60. The method of embodiment 59, wherein the recombinant virus is derived from hAd5, hAd7, hAd11, hAd26, hAd34, hAd35, hAd48, hAd49, hAd50, GAd20, GAd19, GAd21, GAd25, GAd26, GAd27, GAd28, GAd29, GAd30, GAd31, ChAd3, ChAd4, ChAd5, ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd55, ChAd63, ChAd73, ChAd82, ChAd83, ChAd146, ChAd147, PanAd1, PanAd2, PanAd3, Copenhagen vaccinia virus (W), New York Attenuated Vaccinia Virus (NYVAC), ALVAC, TROVAC, or modified vaccinia ankara (MVA).

Embodiment 61. The method of embodiment 60, wherein the recombinant virus is derived from GAd20.

Embodiment 62. The method of embodiment 60, wherein the recombinant virus is derived from MVA.

Embodiment 63. The method of embodiment 60, wherein the recombinant virus is derived from hAd26.

Embodiment 64. The method of embodiment 61 or 63, wherein the polynucleotide encodes a polypeptide of SEQ ID NOs: 541, 550, 554, 555, 556, 623, or 624.

Embodiment 65. The method of embodiment 62 or 63, wherein the polynucleotide encodes a polypeptide of SEQ ID NOs: 543, 552, 557, 558, 559, 625, or 626.

Embodiment 66. The method of any one of embodiments 38-65, wherein the prostate cancer is a localized prostate adenocarcinoma, a relapsed prostate cancer, a refractory prostate cancer, a metastatic prostate cancer, a castration resistant prostate cancer, or any combination thereof.

Embodiment 67. The method of any one of embodiments 38-66, wherein the subject is treatment naïve.

Embodiment 68. The method of any one of embodiments 38-66, wherein the subject has received androgen deprivation therapy.

Embodiment 69.The method of any one of embodiments 38-68, wherein the subject has an elevated level of prostate specific antigen (PSA).

Embodiment 70. A method for monitoring responsiveness of a subject having prostate cancer to a therapeutic agent, the method comprising:

(a) evaluating expression of one or more prostate cancer biomarkers, wherein the one or more prostate cancer biomarkers comprise: RCN1, STEAP1, PITX2, TIMP1, KLK4, KRT18, KLK3, ACPP, KLK2, TSPAN1, ATF3, SPDEF, NPY, SPINK1, HOXB13, FOXA1, KRT17, FOLH1, TNFRSF19, GREB1, KRT8, FLNC, GRHL2, RAB3B, JCHAIN, TMEFF2, AGR2, ACADL, AZGP1, MUC1, STEAP2, UGT2B17, METTL7A, HPN, NKX3-1, GNMT, ADAMTS15, HSD3B2, EPHA3, KCNN2, LGR5, IDO1, GPR39, C9orf152, MYBPC1, THBS2, ETV7, COL1A1, FGFR4, NR0B1, AR, ARv3, TMPRSS2:ERG, ROR1, FGF8, NKX2-2, EDIL3, RELN, FGF9, AKR1C4, CLUL1, KISS1R, CYP3A5, CYP17A1, SFRP4, HNF1A, CALCR, SYP, MSLN, or combinations thereof;

(b) administering a therapeutic agent to the subject; and

(c) evaluating the expression of the one or more prostate cancer biomarkers evaluated in step (a), wherein a decrease in the expression of the one or more prostate cancer biomarkers compared to the expression in step (a) is indicative of responsiveness to the therapeutic agent.

Embodiment 71. The method of embodiment 70, wherein the one or more prostate cancer biomarkers comprise: RCN1, STEAP1, PITX2, TIMP1, KLK4, KRT18, KLK3, ACPP, KLK2, TSPAN1, ATF3, SPDEF, NPY, SPINK1, HOXB13, FOXA1, KRT17, FOLH1, TNFRSF19, GREB1, KRT8, FLNC, GRHL2, RAB3B, JCHAIN, TMEFF2, AGR2, ACADL, AZGP1, MUC1, STEAP2, UGT2B17, METTL7A, HPN, NKX3-1, GNMT, ADAMTS15, HSD3B2, EPHA3, KCNN2, LGR5, IDO1, GPR39, C9orf152, MYBPC1, THBS2, ETV7, COL1A1, FGFR4, NR0B1, AR, ARv3, TMPRSS2:ERG, or combinations thereof.

Embodiment 72. The method of embodiment 70, wherein the one or more prostate cancer biomarkers comprise: HPN, ROR1, FLNC, GPR39, FGF8, NKX2-2, MUC1, NKX3-1, EDIL3, LGR5, FGFR4, STEAP1, ATF3, RELN, UGT2B17, KLK3, C9orf152, GNMT, METTL7A, FGF9, SPDEF, FOXA1, AKR1C4, GREB1, CLUL1, TMEFF2, HOXB13, KLK2, NPY, GRHL2, STEAP2, THBS2, KISS1R, KRT8, TNFRSF19, CYP3A5, KLK4, IDO1, FOLH1, NR0B1, EPHA3, CYP17A1, SFRP4, KRT18, TSPAN1, HNF1A, ADAMTS15, ACPP, CALCR, SYP, AZGP1, AR, ARv3, MSLN, TMPRSS2:ERG, and combinations thereof.

Embodiment 73. The method of embodiment 71, wherein the one or more prostate cancer biomarkers are from a plasma sample.

Embodiment 74. The method of embodiment 72, wherein the one or more prostate cancer biomarkers are from a blood sample.

Embodiment 75. The method of any one of embodiments 70-74, wherein the expression of the one or more prostate cancer biomarkers is evaluated by qPCR.

Embodiment 76. The method of embodiment 75, further comprising, prior to performing the qPCR, extracting RNA from the sample from the subject and synthesizing cDNA from the extracted RNA.

Embodiment 77. A method for preparing a cDNA from a subject with prostate cancer useful for analyzing an expression of prostate cancer neoantigens, the method comprising:

(a) extracting RNA from a sample from the subject;

(b) producing amplified cDNA from the RNA extracted in step (a) by:

(i) reverse transcribing the extracted RNA to produce the cDNA, and

(ii) amplifying the cDNA; and

(c) analyzing the amplified cDNA produced in step (b) for one or more prostate cancer neoantigens, wherein the cDNA encodes an amino acid sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, fragments of the preceding sequences, or any combination thereof.

Embodiment 78. The method of embodiment 77, wherein the cDNA encodes an amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, fragments of the preceding sequences, or any combination thereof.

Embodiment 79. The method of embodiment 77 or 78, wherein the cDNA encodes an amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, fragments of the preceding sequences, or any combination thereof.

Embodiment 80. The method of embodiment 79, wherein the cDNA encodes an amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, and 223.

Embodiment 81. A method of treating prostate cancer in a subject, the method comprising:

administering a therapeutically effective amount of a prostate cancer vaccine to the subject to thereby treat the prostate cancer, wherein the prostate cancer vaccine comprises a polynucleotide encoding one or more polypeptides selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, fragments of the preceding sequences, or any combination thereof.

Embodiment 82. The method of embodiment 81, wherein the prostate cancer vaccine comprises a polynucleotide encoding one or more polypeptides selected from the group consisting of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, and fragments thereof.

Embodiment 83. The method of embodiment 81 or 82, wherein the polynucleotide comprises:

a) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 276, 382, 334, 338, 270, 254, 310, 326, 272, 306, 252, 246, 262, 266, 318, 256, 278, 298, 286, 448, 450, 453, 455, 380, 344, 212, 350, 214, 216, 222, 220, 226, 346, 354, 236, 224, 168, 172, 20, 24, 178, and fragments thereof;

b) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, and fragments thereof; or

c) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 500, 501, 461, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 477, 519, 520, 521, 522, 523, 524, 525, 485, 486, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, and fragments thereof.

Embodiment 84. The method of any one of embodiments 81-83, wherein the polynucleotide encodes a polypeptide comprising the amino acid sequence of SEQ ID NOs: 541, 550, 554, 555, 556, 623, 624, 543, 552, 557, 558, 559, 625, or 626.

Embodiment 85. The method of embodiment 84, wherein the polynucleotide comprises the sequence of SEQ ID NOs: 542, 551, 544, or 553.

Embodiment 86. The method of any one of embodiments 81-85, wherein the polynucleotide is DNA or RNA.

Embodiment 87. The method of embodiment 86, wherein RNA is mRNA or self-replicating RNA.

Embodiment 88. The method of any one of embodiments 81-87, wherein the vaccine is a recombinant virus.

Embodiment 89. The method of embodiment 88, wherein the recombinant virus is derived from an adenovirus (Ad), a poxvirus, an adeno-associated virus (AAV), or a retrovirus.

Embodiment 90. The method of embodiment 89, wherein the recombinant virus is derived from hAd5, hAd7, hAd11, hAd26, hAd34, hAd35, hAd48, hAd49, hAd50, GAd20, GAd19, GAd21, GAd25, GAd26, GAd27, GAd28, GAd29, GAd30, GAd31, ChAd3, ChAd4, ChAd5, ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd55, ChAd63, ChAd73, ChAd82, ChAd83, ChAd146, ChAd147, PanAd1, PanAd2, PanAd3, Copenhagen vaccinia virus (W), New York Attenuated Vaccinia Virus (NYVAC), ALVAC, TROVAC, or modified vaccinia ankara (MVA).

Embodiment 91. The method of embodiment 89, wherein the recombinant virus is derived from GAd20.

Embodiment 92. The method of embodiment 89, wherein the recombinant virus is derived from MVA.

Embodiment 93. The method of embodiment 89, wherein the recombinant virus is derived from hAd26.

Embodiment 94. The method of embodiment 91 or 93, wherein the polynucleotide encodes a polypeptide of SEQ ID NOs: 541, 550, 554, 555, 556, 623, or 624.

Embodiment 95. The method of embodiment 92 or 93, wherein the polynucleotide encodes a polypeptide of SEQ ID NOs: 543, 552, 557, 558, 559, 625, or 626.

Embodiment 96. The method of any one of embodiments 81-95, wherein the prostate cancer is a localized prostate adenocarcinoma, a relapsed prostate cancer, a refractory prostate cancer, a metastatic prostate cancer or a castration resistant prostate cancer, or any combination thereof.

Embodiment 97. The method of any one of embodiments 81-96, wherein the subject is treatment naïve.

Embodiment 98. The method of any one of embodiments 81-96, wherein the subject has received androgen deprivation therapy.

Embodiment 99. The method of any one of embodiments 81-98, wherein the subject has an elevated level of prostate specific antigen (PSA).

We claim:

1. A method of diagnosing a subject with prostate cancer, the method comprising:

evaluating the presence of one or more prostate cancer neoantigens in a sample from the subject, the one or more prostate cancer neoantigens comprising the amino acid sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, fragments of the preceding sequences, or any combination thereof,

wherein the presence of the one or more prostate cancer neoantigens is indicative of prostate cancer in the subject.

2. The method of claim 1, wherein the one or more prostate cancer neoantigens comprise the amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, fragments of the preceding sequences, or any combination thereof.

3. The method of claim 1 or 2, wherein the one or more prostate cancer neoantigens comprise the amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, fragments of the preceding sequences, or any combination thereof.

4. The method of claim 3, wherein the method comprises evaluating the presence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, and 223.

5. The method of any one of the previous claims, wherein the presence of the one or more prostate cancer neoantigens is evaluated by qPCR.

6. The method of claim 5, further comprising, prior to performing the qPCR, extracting RNA from the sample from the subject and synthesizing cDNA from the extracted RNA.

7. The method of claim 6, wherein the RNA is produced from a DNA sequence comprising SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 380, 382, 384, 386, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 519, 520, 521, 522, 523, 524, 525, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, fragments of the preceding sequences, or any combination thereof.

8. The method of any one of the previous claims, wherein the sample comprises a prostate cancer tissue sample.

9. The method of any one of the previous claims, wherein the prostate cancer is a localized prostate adenocarcinoma, a relapsed prostate cancer, a refractory prostate cancer, a metastatic prostate cancer, a castration resistant prostate cancer, or any combination thereof.

10. The method of any one of the previous claims, wherein the subject is treatment naïve.

11. The method of any one of claims 1-9, wherein the subject has received androgen deprivation therapy.

12. The method of any one of the previous claims, wherein the subject has an elevated level of prostate specific antigen (PSA).

13. A method of treating prostate cancer in a subject, the method comprising:

a) evaluating the presence of one or more prostate cancer neoantigens in a sample from the subject, the one or more prostate cancer neoantigens comprising the amino acid sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, fragments of the preceding sequences, or any combination thereof; and

b) administering a therapeutically effective amount of a prostate cancer vaccine to the subject to thereby treat the prostate cancer.

14. The method of claim 13, wherein the one or more prostate cancer neoantigens comprise the amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, fragments of the preceding sequences, or any combination thereof.

15. The method of claim 13 or 14, wherein the one or more prostate cancer neoantigens comprise the amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, fragments of the preceding sequences, or any combination thereof.

16. The method of claim 15, wherein the method comprises evaluating the presence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, and 223.

17. The method of any one of claims 13-16, wherein the presence of the one or more prostate cancer neoantigens is evaluated by qPCR.

18. The method of claim 17, further comprising, prior to performing the qPCR, extracting RNA from the sample from the subject and synthesizing cDNA from the extracted RNA.

19. The method of any one of claims 13-18, wherein the sample comprises a prostate cancer tissue sample.

20. The method of any one of claims 13-19, wherein the prostate cancer vaccine comprises a polynucleotide encoding one or more polypeptides selected from the group consisting of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, and fragments thereof.

21. The method of claim 20, wherein the polynucleotide comprises:

a) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 276, 382, 334, 338, 270, 254, 310, 326, 272, 306, 252, 246, 262, 266, 318, 256, 278, 298, 286, 448, 450, 453, 455, 380, 344, 212, 350, 214, 216, 222, 220, 226, 346, 354, 236, 224, 168, 172, 20, 24, 178, and fragments thereof;
b) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, and fragments thereof; or
c) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 500, 501, 461, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 477, 519, 520, 521, 522, 523, 524, 525, 485, 486, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, and fragments thereof.

22. The method of any one of claims 13-21, wherein the polynucleotide encodes a polypeptide comprising the amino acid sequence of SEQ ID NOs: 541, 550, 554, 555, 556, 623, 624, 543, 552, 557, 558, 559, 625, or 626.

23. The method of claim 22, wherein the polynucleotide comprises a polynucleotide sequence of SEQ ID NOs: 542, 551, 544, or 553.

24. The method of any one of claims 13-23, wherein the polynucleotide is DNA or RNA.

25. The method of claim 24, wherein RNA is mRNA or self-replicating RNA.

26. The method of claim 13-25, wherein the vaccine is a recombinant virus.

27. The method of claim 26, wherein the recombinant virus is derived from an adenovirus (Ad), a poxvirus, an adeno-associated virus (AAV), or a retrovirus.

28. The method of claim 27, wherein the recombinant virus is derived from hAd5, hAd7, hAd11, hAd26, hAd34, hAd35, hAd48, hAd49, hAd50, GAd20, GAd19, GAd21, GAd25, GAd26, GAd27, GAd28, GAd29, GAd30, GAd31, ChAd3, ChAd4, ChAd5, ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd55, ChAd63, ChAd73, ChAd82, ChAd83, ChAd146, ChAd147, PanAd1, PanAd2, PanAd3, Copenhagen vaccinia virus (W), New York Attenuated Vaccinia Virus (NYVAC), ALVAC, TROVAC, or modified vaccinia ankara (MVA).

29. The method of claim 28, wherein the recombinant virus is derived from GAd20.

30. The method of claim 28, wherein the recombinant virus is derived from MVA.

31. The method of claim 28, wherein the recombinant virus is derived from hAd26.

32. The method of claim 29 or 31, wherein the polynucleotide encodes a polypeptide of SEQ ID NOs: 541, 550, 554, 555, 556, 623, or 624.

33. The method of claim 30 or 31, wherein the polynucleotide encodes a polypeptide of SEQ ID NOs: 543, 552, 557, 558, 559, 625, or 626.

34. The method of any one of claims 13-33, wherein the prostate cancer is a localized prostate adenocarcinoma, a relapsed prostate cancer, a refractory prostate cancer, a metastatic prostate cancer or a castration resistant prostate cancer, or any combination thereof.

35. The method of any one of claims 13-34, wherein the subject is treatment naïve.

36. The method of any one of claims 13-34, wherein the subject has received androgen deprivation therapy.

37.The method of any one of claims 13-36, wherein the subject has an elevated level of prostate specific antigen (PSA).

38. A method of treating prostate cancer in a subject, the method comprising:

a) evaluating the presence of one or more prostate cancer neoantigens in a sample from the subject, the one or more prostate cancer neoantigens comprising the amino acid sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, fragments of the preceding sequences, or any combination thereof; and;

b) evaluating expression of one or more prostate cancer biomarkers in a sample from the subject, wherein the one or more prostate cancer biomarkers comprise: RCN1, STEAP1, PITX2, TIMP1, KLK4, KRT18, KLK3, ACPP, KLK2, TSPAN1, ATF3, SPDEF, NPY, SPINK1, HOXB13, FOXA1, KRT17, FOLH1, TNFRSF19, GREB1, KRT8, FLNC, GRHL2, RAB3B, JCHAIN, TMEFF2, AGR2, ACADL, AZGP1, MUC1, STEAP2, UGT2B17, METTL7A, HPN, NKX3-1, GNMT, ADAMTS15, HSD3B2, EPHA3, KCNN2, LGR5, IDO1, GPR39, C9orf152, MYBPC1, THBS2, ETV7, COL1A1, FGFR4, NR0B1, AR, ARv3, TMPRSS2:ERG, ROR1, FGF8,

NKX2-2, EDIL3, RELN, FGF9, AKR1C4, CLUL1, KISS1R, CYP3A5, CYP17A1, SFRP4, HNF1A, CALCR, SYP, MSLN, or any combination thereof; and

c) administering a therapeutically effective amount of a prostate cancer vaccine to the subject.

39. The method of claim 38, further comprising, after administering the therapeutically effective amount of the prostate cancer vaccine, evaluating expression of the one or more prostate cancer biomarkers evaluated in step b), wherein a decrease in expression compared to the expression in step b) is indicative of responsiveness to the prostate cancer vaccine.

40. The method of claim 38 or 39, wherein the one or more prostate cancer neoantigens comprise the amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, fragments of the preceding sequences, or any combination thereof.

41. The method of any one of claims 38-40, wherein the one or more prostate cancer neoantigens comprise the amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, fragments of the preceding sequences, or any combination thereof.

42. The method of claim 41, wherein the method comprises evaluating the presence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, and 223.

43. The method of any one of claims 38-42, wherein the presence of the one or more prostate cancer neoantigens is evaluated by qPCR.

44. The method of claim 43, further comprising, prior to performing the qPCR, extracting RNA from the sample from the subject and synthesizing cDNA from the extracted RNA.

45. The method of any one of claims 38-44, wherein the one or more neoantigens are from a prostate cancer tissue sample.

46. The method of any one of claims 38-45, wherein the one or more prostate cancer biomarkers comprise: RCN1, STEAP1, PITX2, TIMP1, KLK4, KRT18, KLK3, ACPP, KLK2, TSPAN1, ATF3, SPDEF, NPY, SPINK1, HOXB13, FOXA1, KRT17, FOLH1, TNFRSF19, GREB1, KRT8, FLNC, GRHL2, RAB3B, JCHAIN, TMEFF2, AGR2, ACADL, AZGP1, MUC1, STEAP2, UGT2B17, METTL7A, HPN, NKX3-1, GNMT, ADAMTS15, HSD3B2, EPHA3, KCNN2, LGR5, IDO1, GPR39, C9orf152, MYBPC1, THBS2, ETV7, COL1A1, FGFR4, NR0B1, AR, ARv3, TMPRSS2:ERG, or combinations thereof.

47. The method of any one of claims 38-45, wherein the one or more prostate cancer biomarkers comprise: HPN, ROR1, FLNC, GPR39, FGF8, NKX2-2, MUC1, NKX3-1, EDIL3, LGR5, FGFR4, STEAP1, ATF3, RELN, UGT2B17, KLK3, C9orf152, GNMT, METTL7A, FGF9, SPDEF, FOXA1, AKR1C4, GREB1, CLUL1, TMEFF2, HOXB13, KLK2, NPY, GRHL2, STEAP2, THBS2, KISS1R, KRT8, TNFRSF19, CYP3A5, KLK4, IDO1, FOLH1, NR0B1, EPHA3, CYP17A1, SFRP4, KRT18, TSPAN1, HNF1A, ADAMTS15, ACPP, CALCR, SYP, AZGP1, AR, ARv3, MSLN, TMPRSS2:ERG, and combinations thereof.

48. The method of claim 46, wherein the one or more prostate cancer biomarkers are from a plasma sample.

49. The method of claim 47, wherein the one or more prostate cancer biomarkers are from a blood sample.

50. The method of any one of claims 38-49, wherein the expression of the one or more prostate cancer biomarkers is evaluated by qPCR.

51. The method of claim 50, further comprising, prior to performing the qPCR, extracting RNA from the sample from the subject and synthesizing cDNA from the extracted RNA.

52. The method of any one of claims 38-51, wherein the prostate cancer vaccine comprises a polynucleotide encoding one or more polypeptides selected from the group consisting of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, and fragments thereof.

53. The method of claim 52, wherein the polynucleotide comprises:

a) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 276, 382, 334, 338, 270, 254, 310, 326, 272, 306, 252, 246, 262, 266, 318, 256, 278, 298, 286, 448, 450, 453, 455, 380, 344, 212, 350, 214, 216, 222, 220, 226, 346, 354, 236, 224, 168, 172, 20, 24, 178, and fragments thereof;

b) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, and fragments thereof; or

c) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 500, 501, 461, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 477, 519, 520, 521, 522, 523, 524, 525, 485, 486, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, and fragments thereof.

54. The method of claim 52 or 53, wherein the polypeptide comprises the amino acid sequence of SEQ ID NOs: 541, 550, 554, 555, 556, 623, 624, 543, 552, 557, 558, 559, 625, or 626.

55. The method of claim 54, wherein the polynucleotide comprises a polynucleotide sequence of SEQ ID NOs: 542, 551, 544 or 553.

56. The method of any one of claims 52-55, wherein the polynucleotide is DNA or RNA.

57. The method of claim 56, wherein RNA is mRNA or self-replicating RNA.

58. The method of any one of claims 38-57, wherein the vaccine is a recombinant virus.

59. The method of claim 58, wherein the recombinant virus is derived from an adenovirus (Ad), a poxvirus, an adeno-associated virus (AAV), or a retrovirus.

60. The method of claim 59, wherein the recombinant virus is derived from hAd5, hAd7, hAd11, hAd26, hAd34, hAd35, hAd48, hAd49, hAd50, GAd20, GAd19, GAd21, GAd25, GAd26, GAd27, GAd28, GAd29, GAd30, GAd31, ChAd3, ChAd4, ChAd5, ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd55, ChAd63, ChAd73, ChAd82, ChAd83, ChAd146, ChAd147, PanAd1, PanAd2, PanAd3, Copenhagen vaccinia virus (W), New York Attenuated Vaccinia Virus (NYVAC), ALVAC, TROVAC, or modified vaccinia ankara (MVA).

61. The method of claim 60, wherein the recombinant virus is derived from GAd20.

62. The method of claim 60, wherein the recombinant virus is derived from MVA.

63. The method of claim 60, wherein the recombinant virus is derived from hAd26.

64. The method of claim 61 or 63, wherein the polynucleotide encodes a polypeptide of SEQ ID NOs: 541, 550, 554, 555, 556, 623, or 624.

65. The method of claim 62 or 63, wherein the polynucleotide encodes a polypeptide of SEQ ID NOs: 543, 552, 557, 558, 559, 625, or 626.

66. The method of any one of claims 38-65, wherein the prostate cancer is a localized prostate adenocarcinoma, a relapsed prostate cancer, a refractory prostate cancer, a metastatic prostate cancer, a castration resistant prostate cancer, or any combination thereof.

67. The method of any one of claims 38-66, wherein the subject is treatment naïve.

68. The method of any one of claims 38-66, wherein the subject has received androgen deprivation therapy.

69. The method of any one of claims 38-68, wherein the subject has an elevated level of prostate specific antigen (PSA).

70. A method for monitoring responsiveness of a subject having prostate cancer to a therapeutic agent, the method comprising:

(a) evaluating expression of one or more prostate cancer biomarkers, wherein the one or more prostate cancer biomarkers comprise: RCN1, STEAP1, PITX2, TIMP1, KLK4, KRT18, KLK3, ACPP, KLK2, TSPAN1, ATF3, SPDEF, NPY, SPINK1, HOXB13, FOXA1, KRT17, FOLH1, TNFRSF19, GREB1, KRT8, FLNC, GRHL2, RAB3B, JCHAIN, TMEFF2, AGR2, ACADL, AZGP1, MUC1, STEAP2, UGT2B17, METTL7A, HPN, NKX3-1, GNMT, ADAMTS15, HSD3B2, EPHA3, KCNN2, LGR5, IDO1, GPR39, C9orf152, MYBPC1, THBS2, ETV7, COL1A1, FGFR4, NR0B1, AR, ARv3, TMPRSS2:ERG, ROR1, FGF8, NKX2-2, EDIL3, RELN, FGF9, AKR1C4, CLUL1, KISS1R, CYP3A5, CYP17A1, SFRP4, HNF1A, CALCR, SYP, MSLN, or combinations thereof;

(b) administering a therapeutic agent to the subject; and

(c) evaluating the expression of the one or more prostate cancer biomarkers evaluated in step (a), wherein a decrease in the expression of the one or more prostate cancer biomarkers compared to the expression in step (a) is indicative of responsiveness to the therapeutic agent.

71. The method of claim 70, wherein the one or more prostate cancer biomarkers comprise: RCN1, STEAP1, PITX2, TIMP1, KLK4, KRT18, KLK3, ACPP, KLK2, TSPAN1, ATF3, SPDEF, NPY, SPINK1, HOXB13, FOXA1, KRT17, FOLH1, TNFRSF19, GREB1, KRT8, FLNC, GRHL2, RAB3B, JCHAIN, TMEFF2, AGR2, ACADL, AZGP1, MUC1, STEAP2, UGT2B17, METTL7A, HPN, NKX3-1, GNMT, ADAMTS15, HSD3B2, EPHA3, KCNN2, LGR5, IDO1, GPR39, C9orf152, MYBPC1, THBS2, ETV7, COL1A1, FGFR4, NR0B1, AR, ARv3, TMPRSS2:ERG, or combinations thereof.

72. The method of claim 70, wherein the one or more prostate cancer biomarkers comprise: HPN, ROR1, FLNC, GPR39, FGF8, NKX2-2, MUC1, NKX3-1, EDIL3, LGR5, FGFR4, STEAP1, ATF3, RELN, UGT2B17, KLK3, C9orf152, GNMT, METTL7A, FGF9, SPDEF, FOXA1, AKR1C4, GREB1, CLUL1, TMEFF2, HOXB13, KLK2, NPY, GRHL2, STEAP2, THBS2, KISS1R, KRT8, TNFRSF19, CYP3A5, KLK4, IDO1, FOLH1, NR0B1, EPHA3, CYP17A1, SFRP4, KRT18, TSPAN1, HNF1A, ADAMTS15, ACPP, CALCR, SYP, AZGP1, AR, ARv3, MSLN, TMPRSS2:ERG, and combinations thereof.

73. The method of claim 71, wherein the one or more prostate cancer biomarkers are from a plasma sample.

74. The method of claim 72, wherein the one or more prostate cancer biomarkers are from a blood sample.

75. The method of any one of claims 70-74, wherein the expression of the one or more prostate cancer biomarkers is evaluated by qPCR.

76. The method of claim 75, further comprising, prior to performing the qPCR, extracting RNA from the sample from the subject and synthesizing cDNA from the extracted RNA.

77. A method for preparing a cDNA from a subject with prostate cancer useful for analyzing an expression of prostate cancer neoantigens, the method comprising:

(a) extracting RNA from a sample from the subject;

(b) producing amplified cDNA from the RNA extracted in step (a) by:

(i) reverse transcribing the extracted RNA to produce the cDNA, and

(ii) amplifying the cDNA; and

(c) analyzing the amplified cDNA produced in step (b) for one or more prostate cancer neoantigens, wherein the cDNA encodes an amino acid sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, fragments of the preceding sequences, or any combination thereof.

78. The method of claim 77, wherein the cDNA encodes an amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, fragments of the preceding sequences, or any combination thereof.

79. The method of claim 77 or 78, wherein the cDNA encodes an amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, fragments of the preceding sequences, or any combination thereof.

80. The method of claim 79, wherein the cDNA encodes an amino acid sequence of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, and 223.

81. A method of treating prostate cancer in a subject, the method comprising:

administering a therapeutically effective amount of a prostate cancer vaccine to the subject to thereby treat the prostate cancer, wherein the prostate cancer vaccine comprises a

polynucleotide encoding one or more polypeptides selected from the group consisting of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 379, 381, 383, 385, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435,436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, fragments of the preceding sequences, or any combination thereof.

82. The method of claim 81, wherein the prostate cancer vaccine comprises a polynucleotide encoding one or more polypeptides selected from the group consisting of SEQ ID NOs: 275, 381, 333, 337, 269, 253, 309, 325, 271, 305, 251, 245, 261, 265, 317, 255, 277, 297, 285, 437, 439, 442, 444, 379, 343, 211, 349, 213, 215, 221, 219, 225, 345, 353, 235, 223, 167, 171, 19, 23, 177, and fragments thereof.

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

a) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 276, 382, 334, 338, 270, 254, 310, 326, 272, 306, 252, 246, 262, 266, 318, 256, 278, 298, 286, 448, 450, 453, 455, 380, 344, 212, 350, 214, 216, 222, 220, 226, 346, 354, 236, 224, 168, 172, 20, 24, 178, and fragments thereof;

b) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, and fragments thereof; or

c) one or more polynucleotides selected from the group consisting of SEQ ID NOs: 500, 501, 461, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 477, 519, 520, 521, 522, 523, 524, 525, 485, 486, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, and fragments thereof.

84. The method of any one of claims 81-83, wherein the polynucleotide encodes a polypeptide comprising the amino acid sequence of SEQ ID NOs: 541, 550, 554, 555, 556, 623, 624, 543, 552, 557, 558, 559, 625, or 626.

85. The method of claim 84, wherein the polynucleotide comprises the sequence of SEQ ID NOs: 542, 551, 544, or 553.

86. The method of any one of claims 81-85, wherein the polynucleotide is DNA or RNA.

87. The method of claim 86, wherein RNA is mRNA or self-replicating RNA.

88. The method of any one of claims 81-87, wherein the vaccine is a recombinant virus.

89. The method of claim 88, wherein the recombinant virus is derived from an adenovirus (Ad), a poxvirus, an adeno-associated virus (AAV), or a retrovirus.

90. The method of claim 89, wherein the recombinant virus is derived from hAd5, hAd7, hAd11, hAd26, hAd34, hAd35, hAd48, hAd49, hAd50, GAd20, GAd19, GAd21, GAd25, GAd26, GAd27, GAd28, GAd29, GAd30, GAd31, ChAd3, ChAd4, ChAd5, ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd55, ChAd63, ChAd73, ChAd82, ChAd83, ChAd146, ChAd147, PanAd1, PanAd2, PanAd3, Copenhagen vaccinia virus (W), New York Attenuated Vaccinia Virus (NYVAC), ALVAC, TROVAC, or modified vaccinia ankara (MVA).

91. The method of claim 89, wherein the recombinant virus is derived from GAd20.

92. The method of claim 89, wherein the recombinant virus is derived from MVA.

93. The method of claim 89, wherein the recombinant virus is derived from hAd26.

94. The method of claim 91 or 93, wherein the polynucleotide encodes a polypeptide of SEQ ID NOs: 541, 550, 554, 555, 556, 623, or 624.

95. The method of claim 92 or 93, wherein the polynucleotide encodes a polypeptide of SEQ ID NOs: 543, 552, 557, 558, 559, 625, or 626.

96. The method of any one of claims 81-95, wherein the prostate cancer is a localized prostate adenocarcinoma, a relapsed prostate cancer, a refractory prostate cancer, a metastatic prostate cancer or a castration resistant prostate cancer, or any combination thereof.

97. The method of any one of claims 81-96, wherein the subject is treatment naïve.

98. The method of any one of claims 81-96, wherein the subject has received androgen deprivation therapy.

99. The method of any one of claims 81-98, wherein the subject has an elevated level of prostate specific antigen (PSA).

FIG. 1

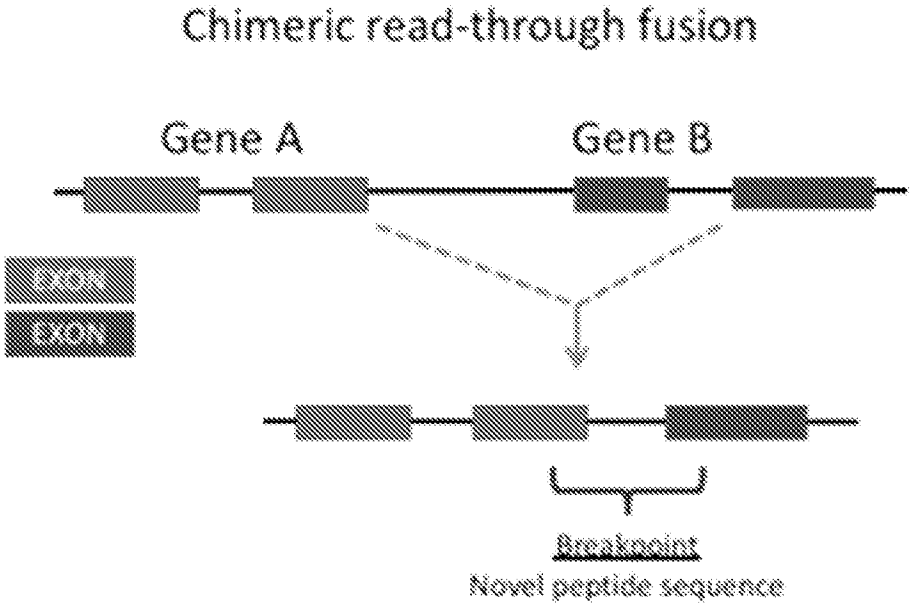

Chimeric read-through fusion
Gene A
Gene B
EXON
EXON
Breakpoint
Novel peptide sequence

FIG. 2

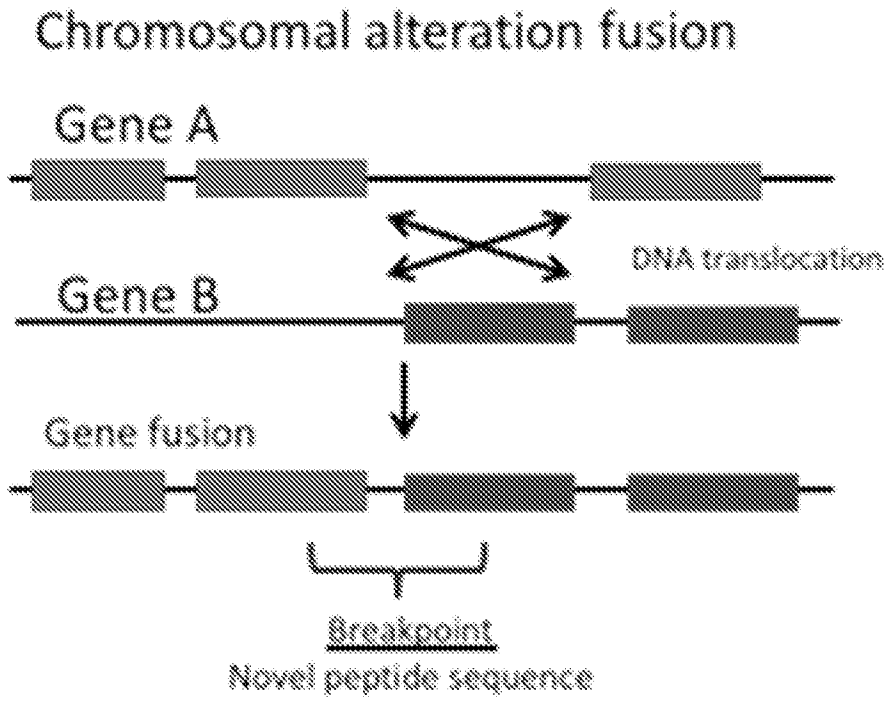

Chromosomal alteration fusion
Gene A
DNA translocation
Gene B
Gene fusion
Breakpoint
Novel peptide sequence

FIG. 3

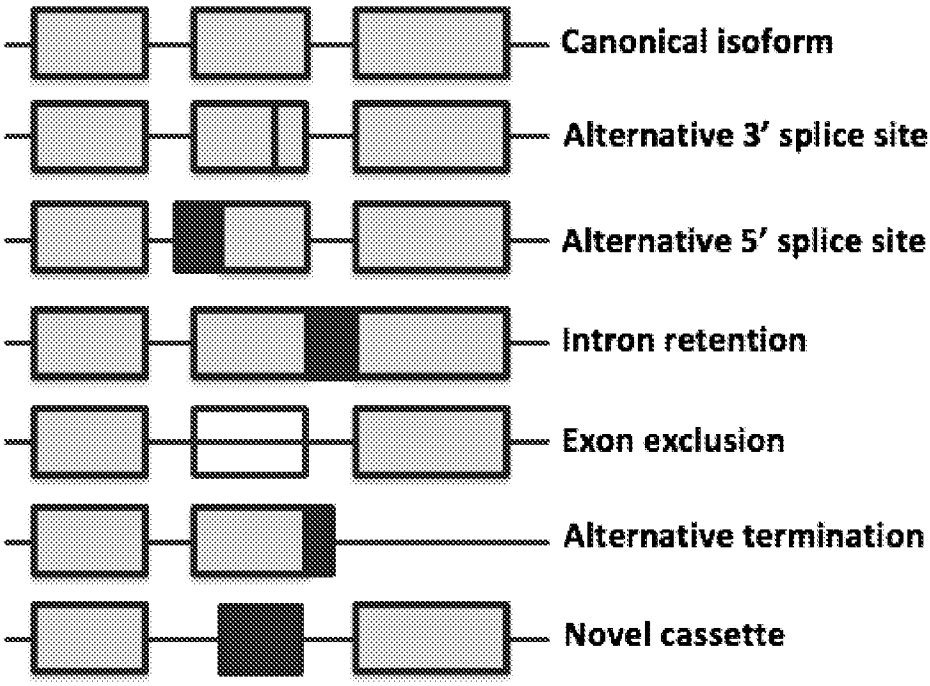

Canonical isoform
Alternative 3' splice site
Alternative 5' splice site
Intron retention
Exon exclusion
Alternative termination
Novel cassette

FIG. 4

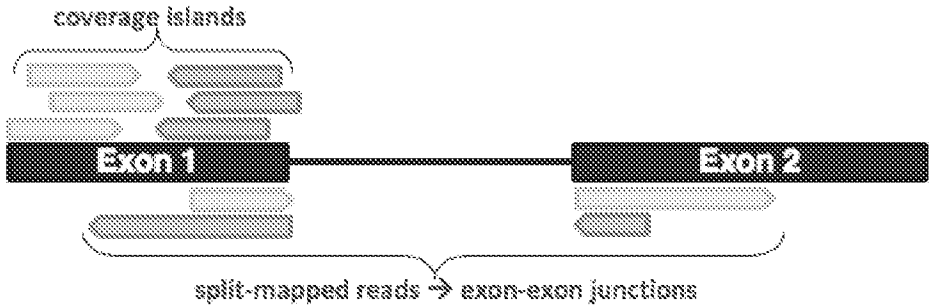

coverage islands
Exon 1
Exon 2
split-mapped reads → exon-exon junctions

FIG. 5A

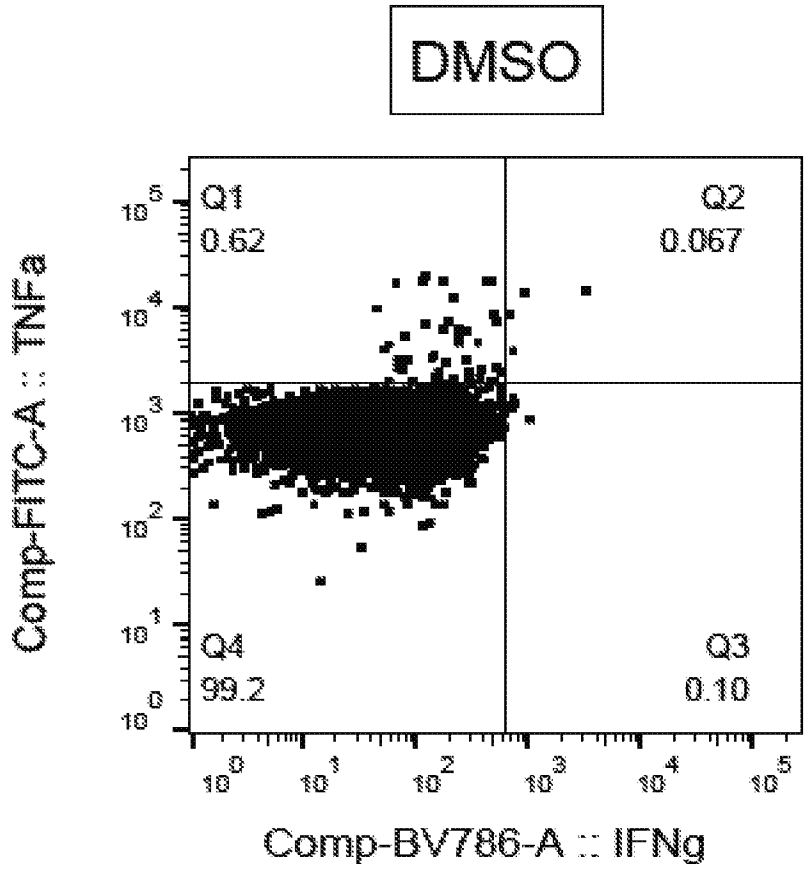

DMSO
Q1
0.62
Q2
0.067
Q4
99.2
Q3
0.10
Comp-FITC-A :: TNFa
Comp-BV786-A :: IFNg

FIG. 5B

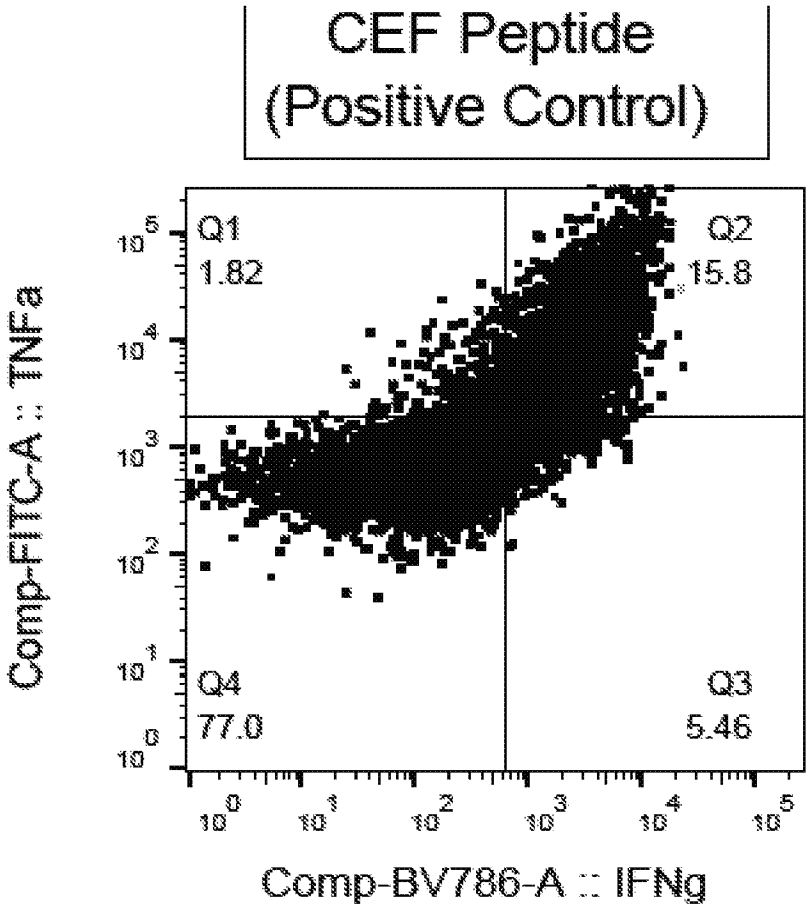

CEF Peptide
(Positive Control)
Q1
1.82
Q2
15.8
Q4
77.0
Q3
5.46
Comp-FITC-A :: TNFa
Comp-BV786-A :: IFNg

FIG. 5C

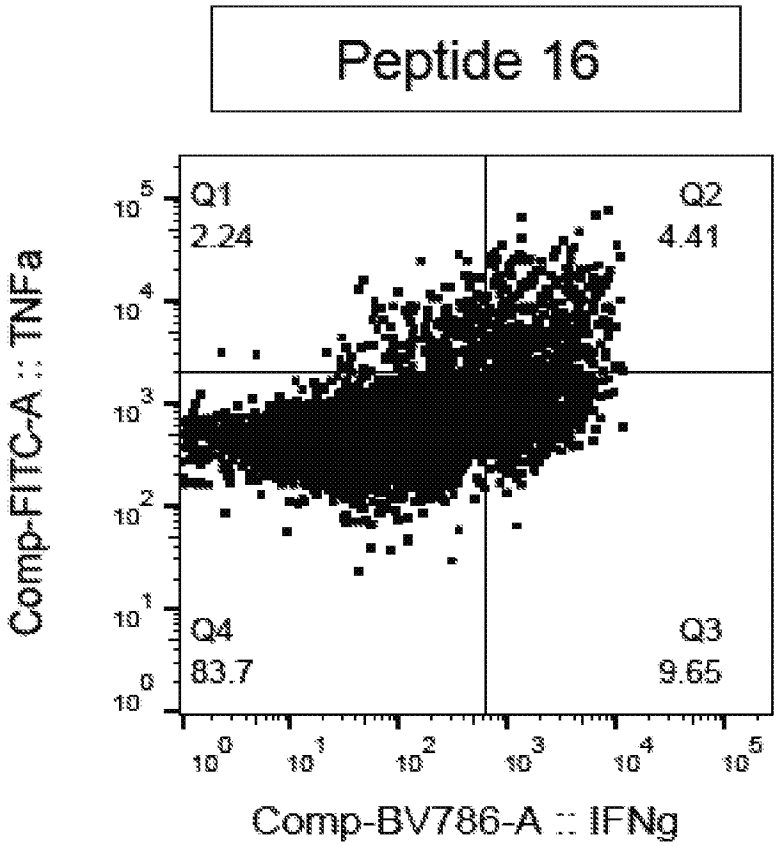

Peptide 16
Q1
2.24
Q2
4.41
Q4
83.7
Q3
9.65
Comp-FITC-A :: TNFa
Comp-BV786-A :: IFNg

FIG. 5D

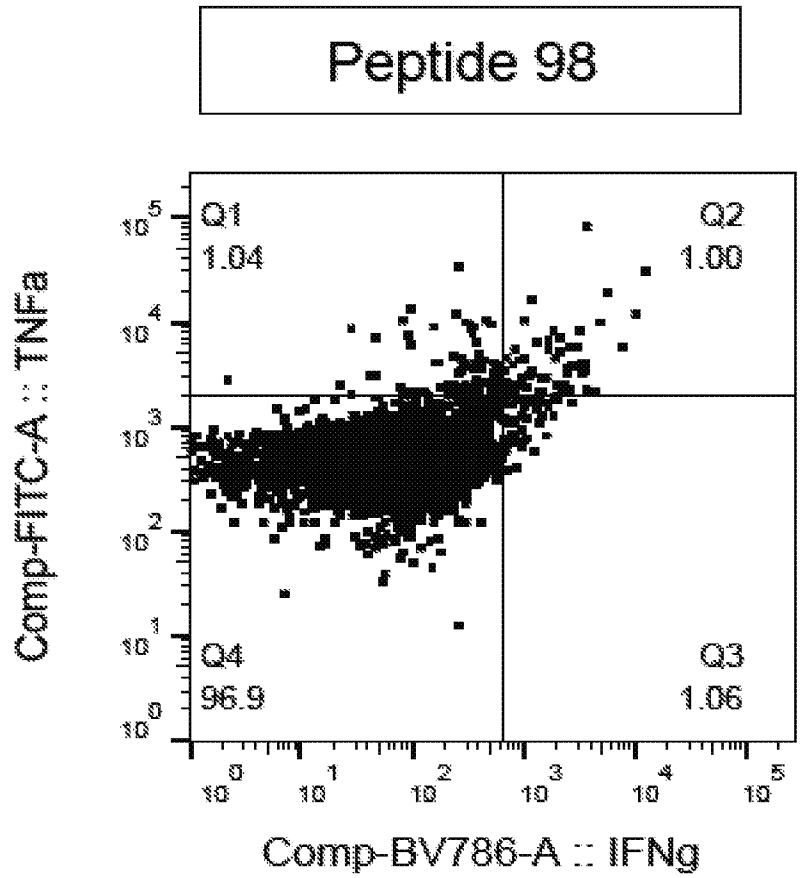

Peptide 98
Q1
1.04
Q2
1.00
Q4
96.9
Q3
1.06
Comp-FITC-A :: TNFa
Comp-BV786-A :: IFNg

FIG. 5E

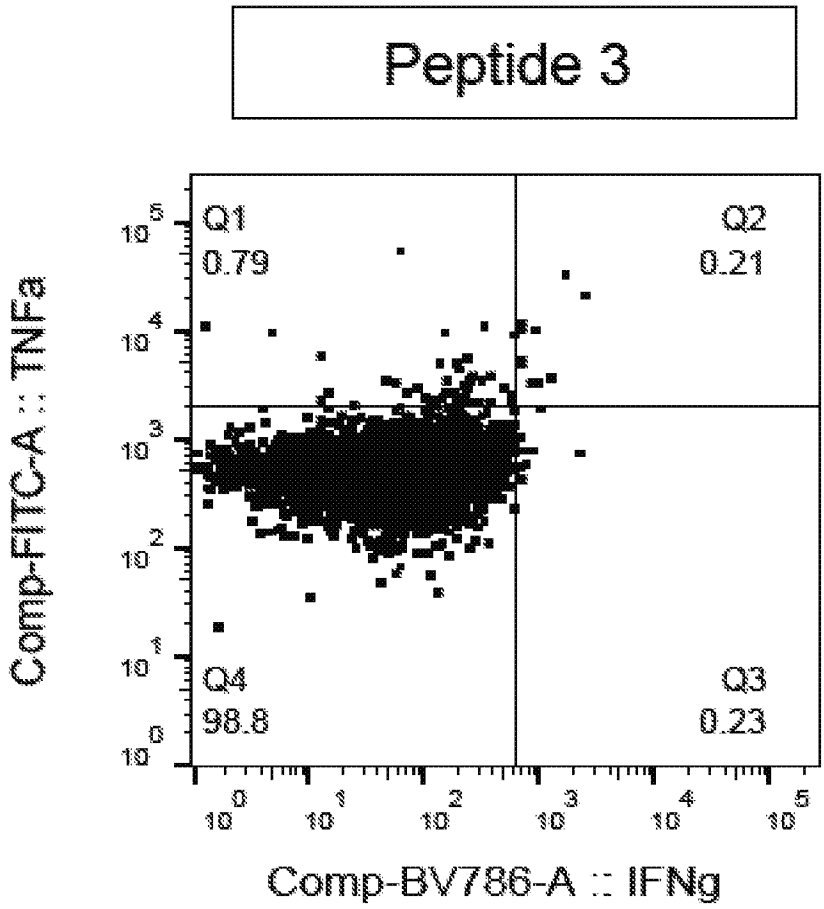

Peptide 3
Q1
0.79
Q2
0.21
Q4
98.8
Q3
0.23
Comp-FITC-A :: TNFa
Comp-BV786-A :: IFNg

FIG. 6

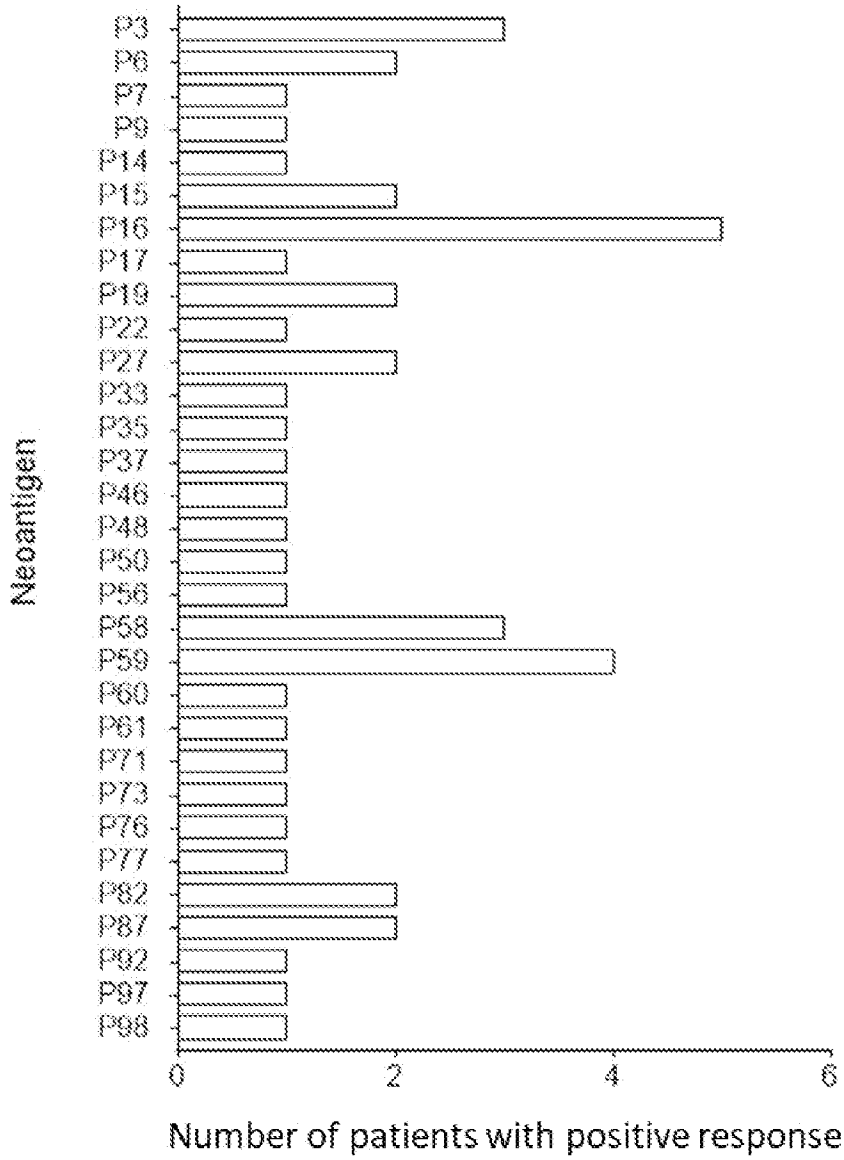

P3
P6
P7
P9
P14
P15
P16
P17
P19
P22
P27
P33
P35
P37
P46
P48
P50
P56
P58
P59
P60
P61
P71
P73
P76
P77
P82
P87
P92
P97
P98
Neoantigen
0
2
4
6
Number of patients with positive response

FIG. 7

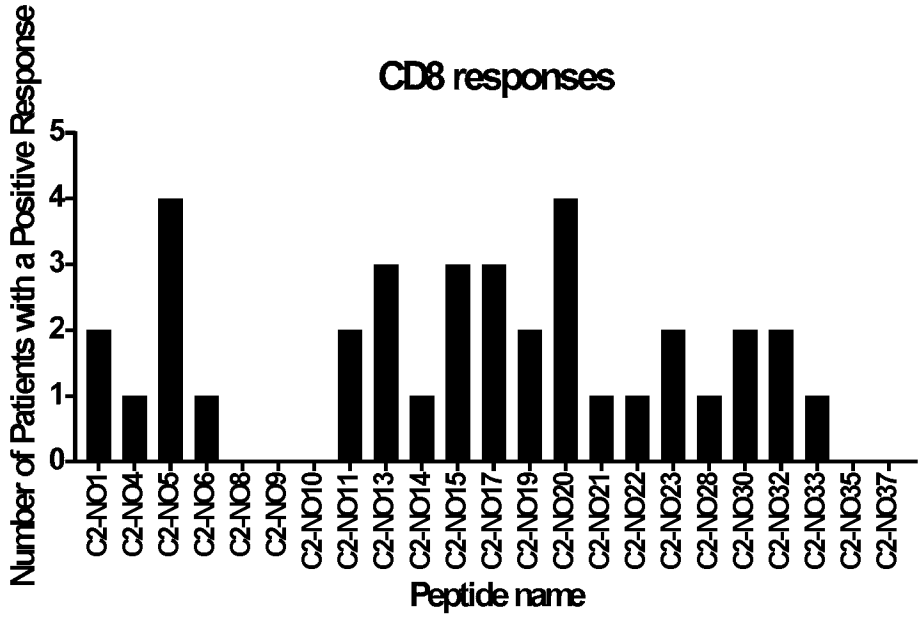
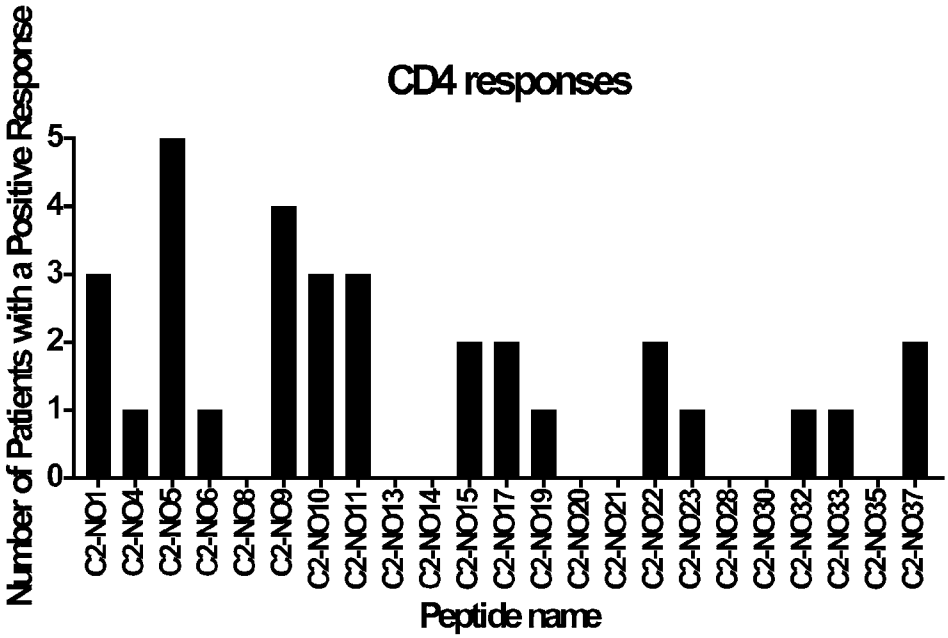

CD8 responses
Number of Patients with a Positive Response
0
1
2
3
4
5
C2-NO1
C2-NO4
C2-NO5
C2-NO6
C2-NO8
C2-NO9
C2-NO10
C2-NO11
C2-NO13
C2-NO14
C2-NO15
C2-NO17
C2-NO19
C2-NO20
C2-NO21
C2-NO22
C2-NO23
C2-NO28
C2-NO30
C2-NO32
C2-NO33
C2-NO35
C2-NO37
Peptide name

FIG. 8


CD4 responses
Number of Patients with a Positive Response
0
1
2
3
4
5
C2-NO1
C2-NO4
C2-NO5
C2-NO6
C2-NO8
C2-NO9
C2-NO10
C2-NO11
C2-NO13
C2-NO14
C2-NO15
C2-NO17
C2-NO19
C2-NO20
C2-NO21
C2-NO22
C2-NO23
C2-NO28
C2-NO30
C2-NO32
C2-NO33
C2-NO35
C2-NO37
Peptide name

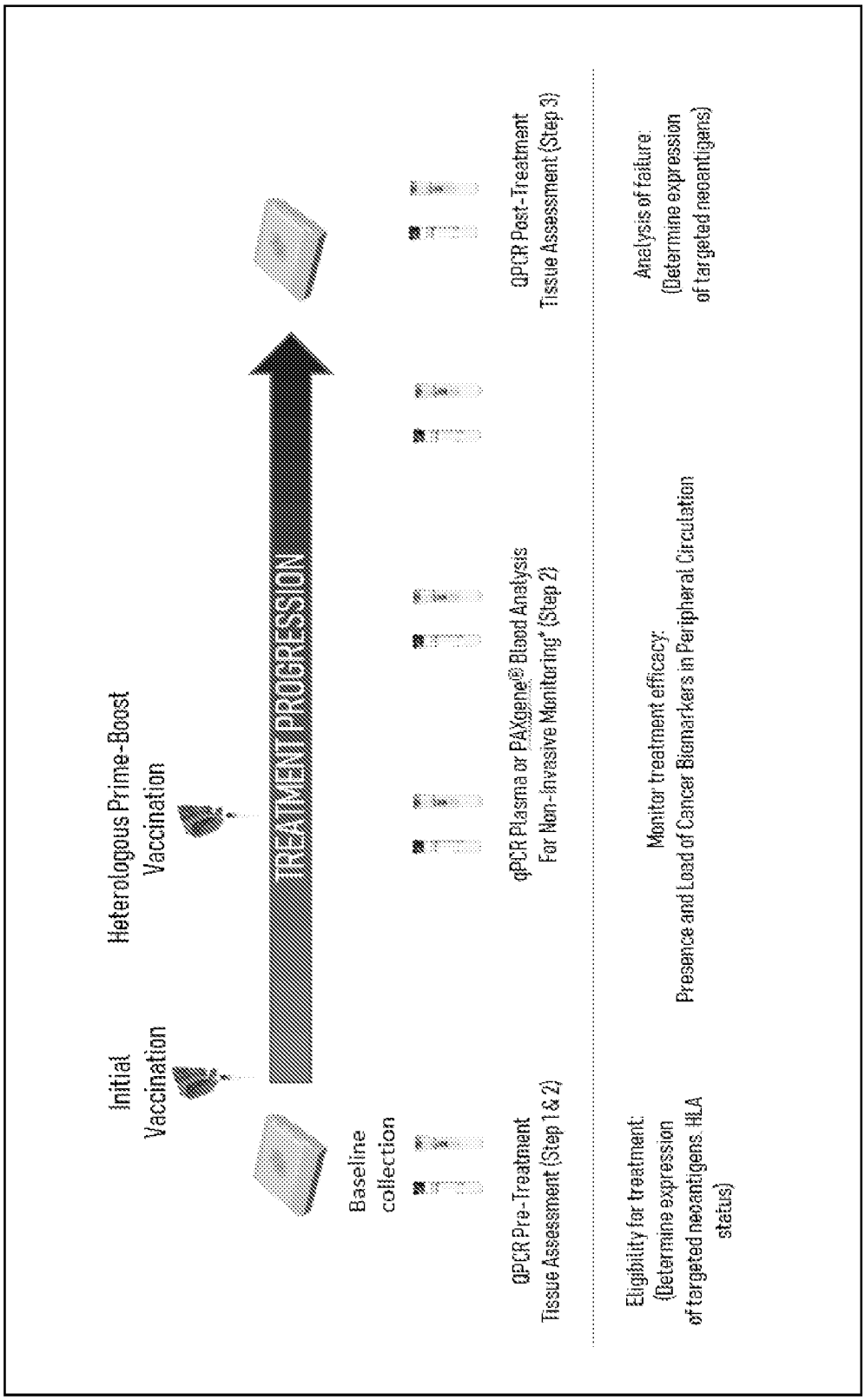

Initial Vaccination
Heterologous Prime-Boost Vaccination
TREATMENT PROGRESSION
Baseline collection
qPCR Pre-Treatment Tissue Assessment (Step 1 & 2)
qPCR Plasma or PAXgene® Blood Analysis For Non-invasive Monitoring* (Step 2)
qPCR Post-Treatment Tissue Assessment (Step 3)
Eligibility for treatment: (Determine expression of targeted neoantigens. HLA status)
Monitor treatment efficacy: Presence and Load of Cancer Biomarkers in Peripheral Circulation
Analysis of failure: (Determine expression of targeted neoantigens)

FIG. 9

FIG. 10

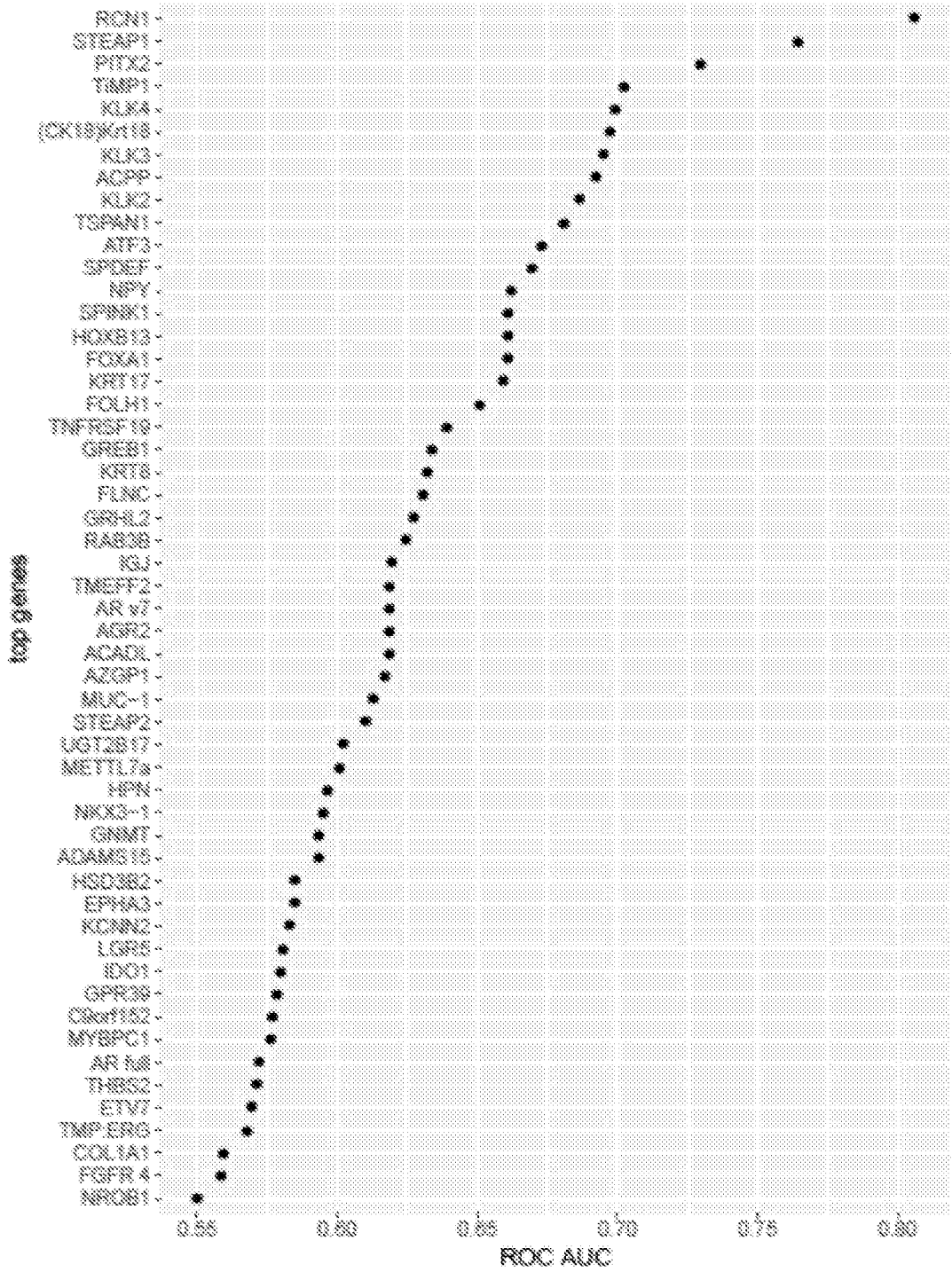

RCN1
STEAP1
PITX2
TIMP1
KLK4
(CK18)Ker18
KLK3
ACPP
KLK2
TSPAN1
ATF3
SPDEF
NPY
SPINK1
HOXB13
FOXA1
KRT17
FOLH1
TNFRSF19
GREB1
KRT8
FLNC
GRHL2
RAB3B
IGJ
TMEFF2
AR v7
AGR2
ACADL
AZGP1
MUC-1
STEAP2
UGT2B17
METTL7a
HPN
NKX3-1
GNMT
ADAMTS1
HSD3B2
EPHA3
KCNN2
LGR5
IDO1
GPR39
C8orf4
MYBPC1
AR fl
THBS2
ETV1
TMPRSS2:ERG
COL1A1
FGFR4
NPD009
top genes
0.55
0.60
0.65
0.70
0.75
0.80
ROC AUC

FIG. 11

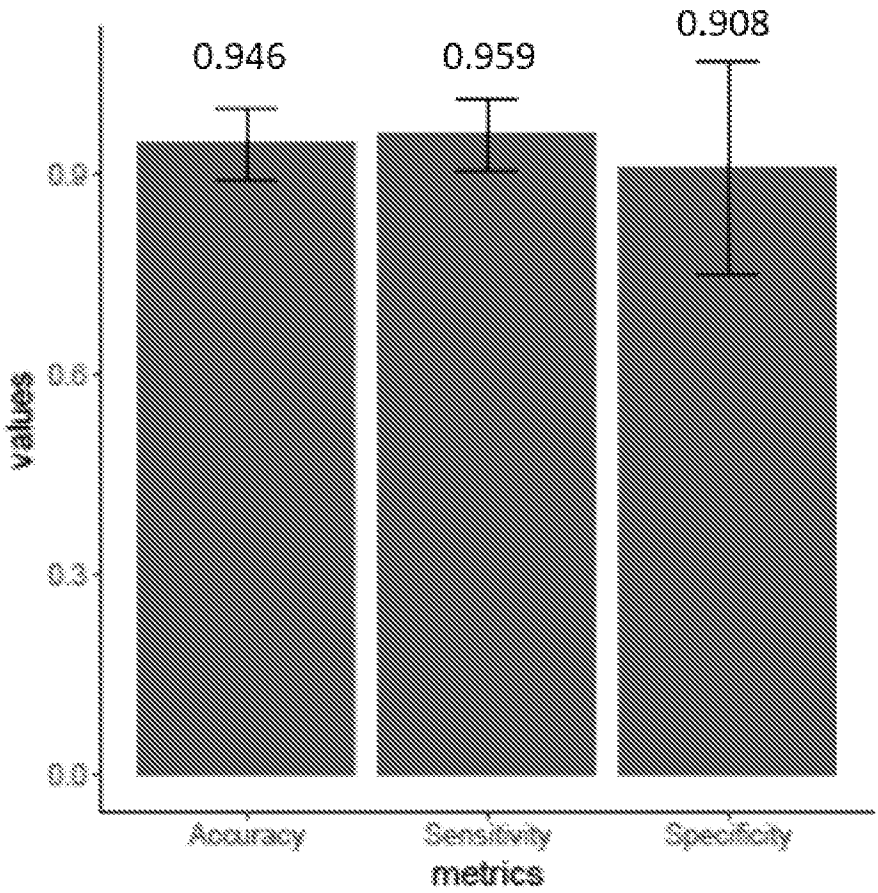

0.946
0.959
0.908
0.9
0.6
0.3
0.0
values
Accuracy
Sensitivity
Specificity
metrics

FIG. 12

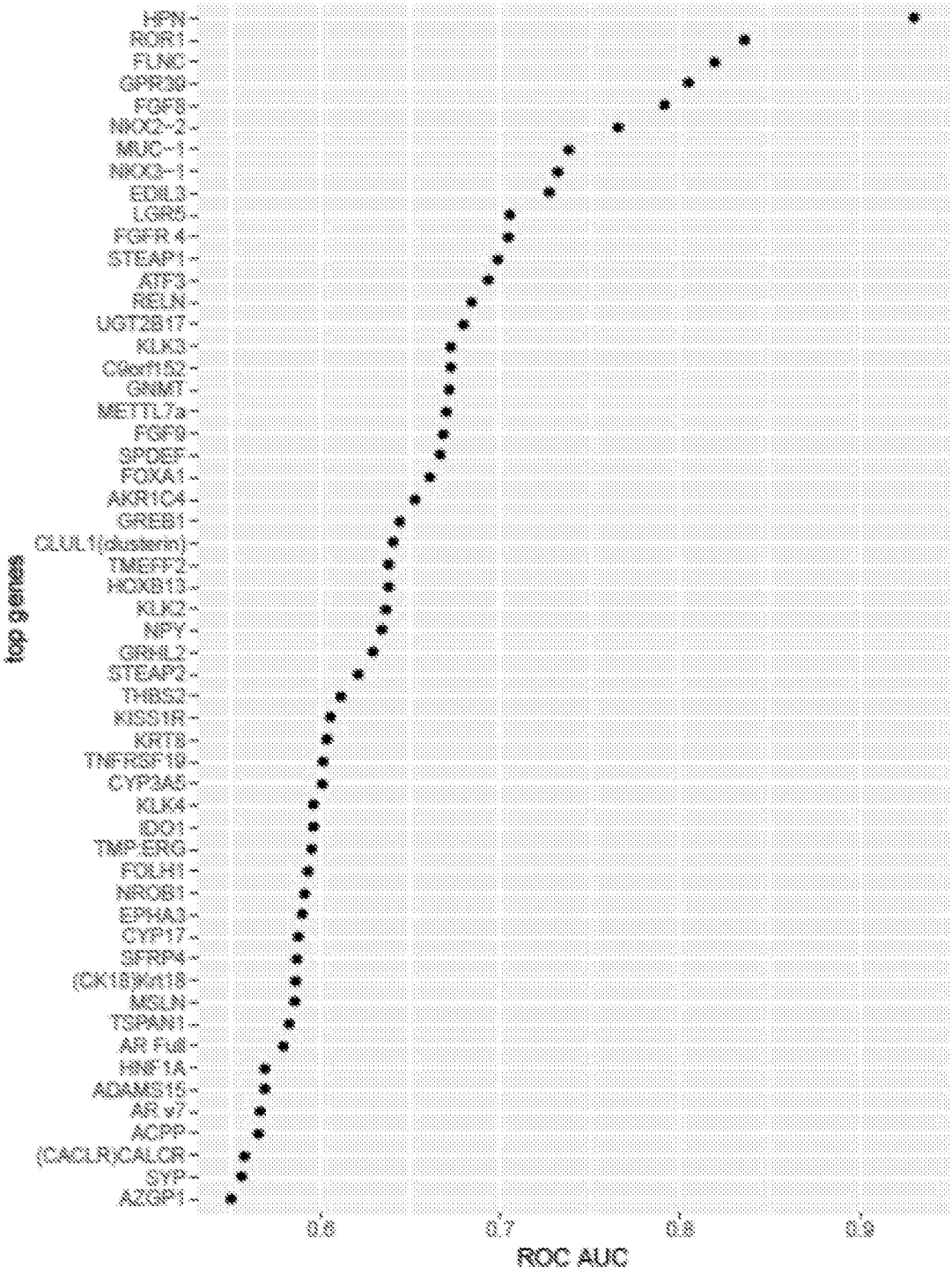

top genes
HPN
ROR1
FUNC
GPR39
FGF8
NKCC-2
MUC-1
NKCC-1
EDIL3
LGR5
FGFR 4
STEAP1
ATP3
RELN
UGT2B17
KLK3
C9orf152
GNMT
METTL7a
FGF9
SPOEF
FOXA1
AKR1C4
GREB1
CLUL1(clusterm)
TMEFF2
HOXB13
KLK2
NPY
GRHL2
STEAP2
TMBS2
KISS1R
KRT8
TNFRSF19
CYP3A5
KLK4
IDO1
TMP-ERG
FOLH1
NRG61
EPHA3
CYP17
SFRP4
(CK18)Krt18
MSLN
TSPAN1
AR Full
HNF1A
ADAMS15
AR v7
ACPP
(CACLR)CALCR
SYP
AZGP1
0.6
0.7
0.8
0.9
ROC AUC

FIG. 13

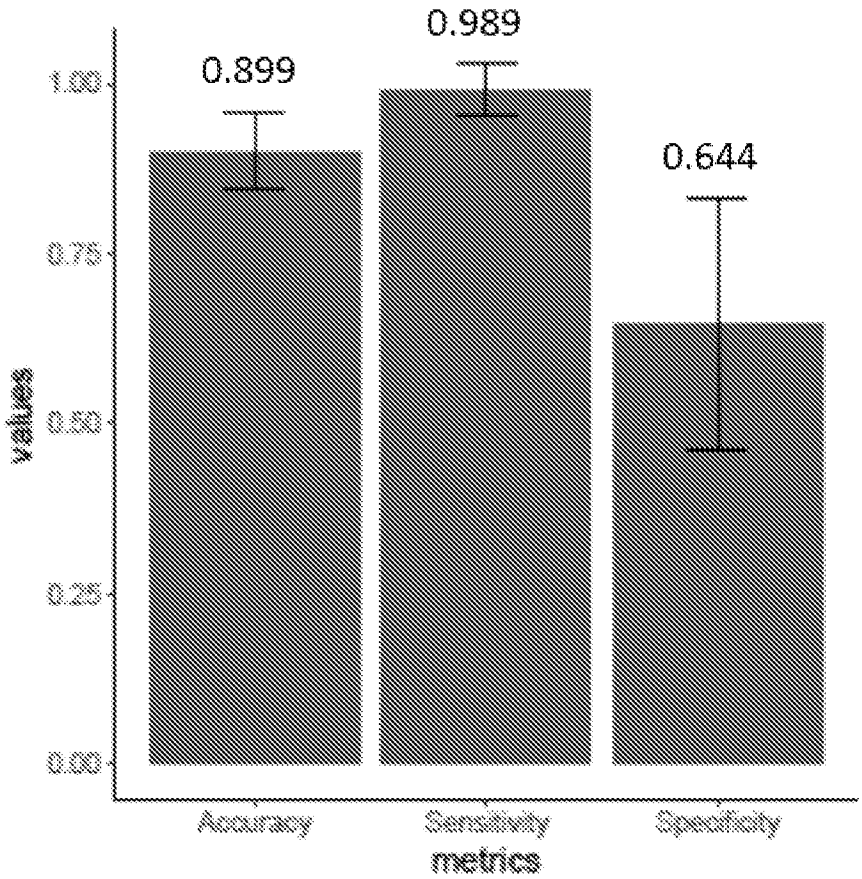
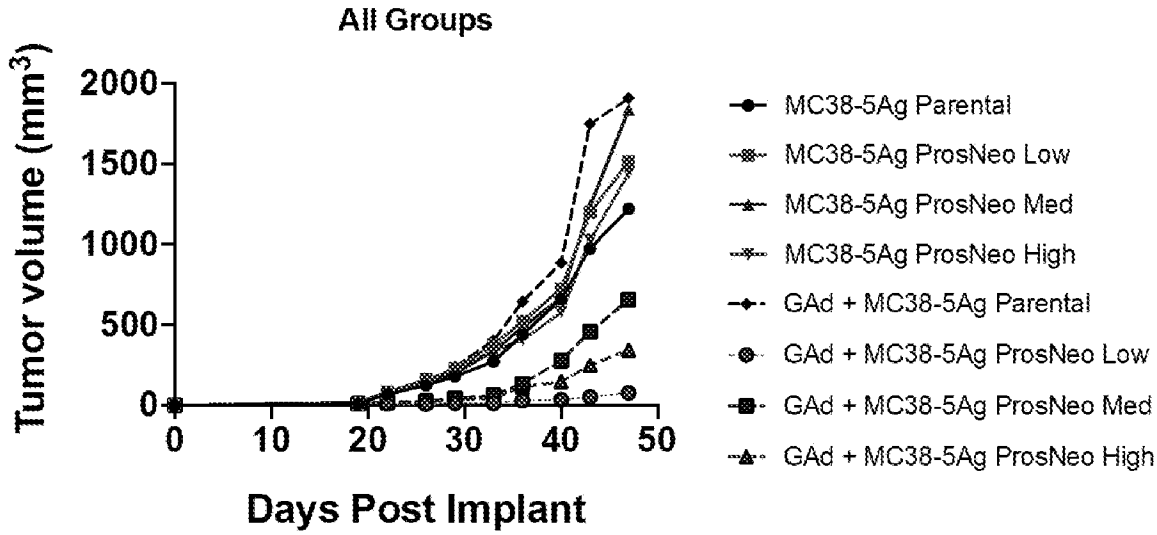

0.899
0.989
0.644
1.00
0.75
0.50
0.25
0.00
values
Accuracy
Sensitivity
Specificity
metrics

FIG. 14


All Groups
Tumor volume (mm3)
2000
1500
1000
500
0
0
10
20
30
40
50
Days Post Implant
MC38-5Ag Parental
MC38-5Ag ProsNeo Low
MC38-5Ag ProsNeo Med
MC38-5Ag ProsNeo High
GAd + MC38-5Ag Parental
GAd + MC38-5Ag ProsNeo Low
GAd + MC38-5Ag ProsNeo Med
GAd + MC38-5Ag ProsNeo High

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2021/055968

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/6886
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017/177207 A1 (BOSTONGENE LLC [US]) 12 October 2017 (2017-10-12) claim 1; figure 3; example 4; table 5 ----- -/--	13, 17-19, 24-31, 34-39, 43-51, 58-63, 66-69, 81, 86-93, 96-99

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 September 2021	15/11/2021

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Santagati, Fabio

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2021/055968

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JIN ZHANG ET AL: "INTEGRATE-neo: a pipeline for personalized gene fusion neoantigen discovery", BIOINFORMATICS, 24 November 2016 (2016-11-24), page btw674, XP055686986, GB ISSN: 1367-4803, DOI: 10.1093/bioinformatics/btw674 table S3	1,5-13, 17-19, 24-31, 34-39, 43-51, 58-63, 66-69, 77,81, 86-93, 96-99
Y	WO 2004/113571 A2 (EXONHIT THERAPEUTICS SA [FR]; EINSTEIN RICHARD [US] ET AL.) 29 December 2004 (2004-12-29) claims 1, 7, 8; figures 3,4; table 2; sequences 183-185	1,5-12, 77
A	WO 2006/056766 A2 (ST GEORGES ENTPR LTD [GB]; FENSKE CHRISTIANE DOROTHEA [GB] ET AL.) 1 June 2006 (2006-06-01) claims 1-6	1
Y	WO 2018/102585 A1 (ADVAXIS INC [US]; PETIT ROBERT [US] ET AL.) 7 June 2018 (2018-06-07) page 273 - page 274; table 21; sequence 721	1,5-12, 77
X,P	WO 2020/144615 A1 (JANSSEN BIOTECH INC [US]) 16 July 2020 (2020-07-16) the whole document	1,5-13, 17-19, 24-31, 34-39, 43-51, 58-63, 66-69, 77,81, 86-93, 96-99
A	MALEKZADEH PARISA ET AL: "Neoantigen screening identifies broad TP53 mutant immunogenicity in patients with epithelial cancers", THE JOURNAL OF CLINICAL INVESTIGATION, vol. 129, no. 3, 1 March 2019 (2019-03-01) , pages 1109-1114, XP055841521, GB ISSN: 0021-9738, DOI: 10.1172/JCI123791 Retrieved from the Internet: URL:https://www.jci.org/articles/view/123791/version/3/pdf/render.pdf> table 1	13,38,81

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2021/055968

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [X] furnished subsequent to the international filing date for the purposes of international search only:

      [X] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2021/055968

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.:
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:

see additional 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 an 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, 5-13, 17-19, 24-31, 34-39, 43-51, 58-63, 66-69, 77, 81, 86-93
96-99(all partially)

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 5-13, 17-19, 24-31, 34-39, 43-51, 58-63, 66-69, 77, 81, 86-93, 96-99(all partially)

A method of diagnosing or treating a subject with prostate cancer, the method comprising:evaluating the presence of one or more prostate cancer neoantigens in a sample from the subject, the one or more prostate cancer neoantigens comprising the amino acid sequence of SEQ ID NO: 1. A method for preparing a cDNA from a subject with prostate cancer useful for analyzing an expression of prostate cancer neoantigens, the method comprising:(a) extracting RNA from a sample from the subject;(b) producing amplified cDNA from the RNA extracted in step (a) by:(i) reverse transcribing the extracted RNA to produce the cDNA, and(ii) amplifying the cDNA; and(c) analyzing the amplified cDNA produced in step (b) for one or more prostate cancer neoantigens, wherein the cDNA encodes an amino acid sequence of SEQ ID NOs: 1. A method of treating prostate cancer in a subject, the method comprising:administering a therapeutically effective amount of a prostate cancer vaccine to the subject to thereby treat the prostate cancer, wherein the prostate cancer vaccine comprises a polynucleotide encoding one or more polypeptides selected from the group consisting of SEQ ID NOs: 1.

2-237. claims: 1-69, 77-99(all partially)

The same subject-matter as invention 1 except wherein the amino acid sequence is
inv 2: SEQ ID NO:3
inv 3: SEQ ID NO:5
.....
inv 237: SEQ ID NO:447

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238. claims: 70, 71, 73-76(all partially)

A method for monitoring responsiveness of a subject having prostate cancer to a therapeutic agent, the method comprising evaluating expression of one or more prostate cancer biomarkers, wherein the one or more prostate cancer biomarkers comprise RCN1.

239-306. claims: 70-76(partially)

The same subject-matter as invention 238 except wherein the one or more biomarkers comprise
inv 239: STEAP1
inv 240: PITX2
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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

inv 306: MSLN

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2021/055968

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