PROSTATE NEOANTIGENS AND THEIR USES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of United States Provisional Application Serial Number 62/913,969, filed 11 October 2019, United States Provisional Application Serial Number 62/883,786, filed 07 August 2019, United States Provisional Application Serial Number 62/851,273, filed 22 May 2019, and United States Provisional Application Serial Number 62/790,673, filed 10 January 2019, the entire content of the aforementioned applications are incorporated herein by reference in its entirety.

SEQUENCE LISTING

This application contains a Sequence Listing submitted via EFS-Web, the entire content of which incorporated herein by reference in its entirety. The ASCII text file, created on 16 Debember 2019, is named JBI6160WOPCT2_ST25.txt and is 554 kilobytes in size.

BACKGROUND

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.

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.

Therefore, a need remains for therapies against a prostate cancer, including hormone sensitive, hormone naive high risk, and castration-resistant prostate cancers.

BRIEF SUMMARY

The disclosure provides an isolated polynucleotide encoding a polypeptide 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 or 447, or fragments thereof.

The disclosure also provides an isolated polynucleotide comprising a polynucleotide sequence 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 or 540, or fragments thereof.

The disclosure also provides an isolated heterologous polynucleotide comprising two 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 and 458, and fragments thereof.

The disclosure also provides an isolated heterologous polynucleotide encoding a heterologous polypeptide comprising two 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 and 447, and fragments thereof.

The disclosure also provides an isolated polypeptide comprising 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 or 447, or fragments thereof.

The disclosure also provides an isolated heterologous polypeptide comprising two 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 and 447, and fragments thereof.

The disclosure also provides a vector comprising

a polynucleotide encoding one or more polypeptides 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 or 447, or fragments thereof;

one or more polynucleotides 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 or 458, or fragments thereof;

a heterologous polynucleotide encoding a heterologous polypeptide comprising two 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 and 447, and fragments thereof; or

a heterologous polynucleotide comprising two 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 and 458, and fragments thereof.

The disclosure also provides a recombinant human adenovirus (rAd) comprising the vector of the disclosure.

The disclosure also provides a recombinant modified vaccinia Ankara (rMVA) comprising the vector of the disclosure.

The disclosure also provides a recombinant great ape adenovirus (rGAd) comprising the vector of the disclosure.

The disclosure also provides a cell comprising or transduced with the vector of the disclosure or the recombinant virus of the disclosure.

The disclosure also provides a vaccine comprising the polynucleotide of the disclosure.

The disclosure also provides a vaccine comprising the heterologous polynucleotide of the disclosure.

The disclosure also provides a vaccine comprising the polypeptide of the disclosure.

The disclosure also provides a vaccine comprising the heterologous polypeptide of the disclosure.

The disclosure also provides a vaccine comprising the vector of the disclosure.

The disclosure also provides a vaccine comprising the rAd of the disclosure.

The disclosure also provides a vaccine comprising the rMVA of the disclosure.

The disclosure also provides a vaccine comprising the rGAd of the disclosure.

The disclosure also provides a vaccine comprising the cell of the disclosure.

The disclosure also provides a pharmaceutical composition comprising the vaccine of the disclosure and a pharmaceutically acceptable carrier or adjuvant.

The disclosure also provides a kit comprising one or more vaccines of the disclosure or the pharmaceutical composition of the disclosure.

The disclosure also provides a method of preventing or treating a prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of one or more vaccines of the disclosure or one or more pharmaceutical composition of the disclosure.

The disclosure also provides a method of inducing an immune response against one or more polypeptides 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 or 447, or fragments thereof in a subject, comprising administering to the subject one or more vaccines of the disclosure.

The disclosure also provides an isolated heterologous polynucleotide encoding 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.

The disclosure also provides an isolated heterologous polypeptide comprising 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.

The disclosure also provides a vector comprising:

a heterologous polynucleotide encoding a heterologous polypeptide comprising 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;

a heterologous polynucleotide comprising two 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, 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 and 540, and fragments thereof;

a heterologous polynucleotide comprising 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 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 and 499, and fragments thereof;

a heterologous polynucleotide comprising 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 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 and 540, and fragments thereof;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NOs: 541, 550, 554, 555, 556, 623 or 624;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NOs: 543, 552, 557, 558, 559, 625 or 626;

a heterologous polynucleotide comprising a polynucleotide sequence of SEQ ID NO: 542 or SEQ ID NO: 551; or

a heterologous polynucleotide comprising the polynucleotide sequence of SEQ ID NO: 544 or SEQ ID NO: 553.

The disclosure also provides a recombinant human adenovirus (rAd), comprising:

a heterologous polynucleotide encoding a heterologous polypeptide comprising 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;

a heterologous polynucleotide comprising 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 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 and 178, and fragments thereof;

a heterologous polynucleotide comprising 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 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 and 499 and fragments thereof; or

a heterologous polynucleotide comprising 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 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 and 540, and fragments thereof.

The disclosure also provides a recombinant great ape adenovirus (rGAd), comprising:

a heterologous polynucleotide encoding a heterologous polypeptide comprising 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;

a heterologous polynucleotide comprising 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 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 and 178, and fragments thereof;

a heterologous polynucleotide comprising 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 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 and 499, and fragments thereof; or

a heterologous polynucleotide comprising 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 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 and 540, and fragments thereof.

The disclosure also provides a recombinant modified vaccinia Ankara (rMVA), comprising:

a heterologous polynucleotide encoding a heterologous polypeptide comprising 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;

a heterologous polynucleotide comprising 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 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 and 178, and fragments thereof;

a heterologous polynucleotide comprising 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 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 and 499, and fragments thereof; or

a heterologous polynucleotide comprising 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 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 and 540, and fragments thereof.

The disclosure also provides a vaccine comprising a rAd, comprising

a heterologous polynucleotide encoding a heterologous polypeptide comprising 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;

a heterologous polynucleotide comprising 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 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 and 178, and fragments thereof;

a heterologous polynucleotide comprising 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 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 and 499, and fragments thereof; or

a heterologous polynucleotide comprising 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 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 and 540, and fragments thereof.

The disclosure also provides a vaccine comprising rGAd20, comprising

a heterologous polynucleotide encoding a heterologous polypeptide comprising 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;

a heterologous polynucleotide comprising 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 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 and 178, and fragments thereof;

a heterologous polynucleotide comprising 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 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 and 499, and fragments thereof; or

a heterologous polynucleotide comprising 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 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 and 540, and fragments thereof.

The disclosure also provides a vaccine comprising rMVA comprising

a heterologous polynucleotide encoding a heterologous polypeptide comprising 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;

a heterologous polynucleotide comprising 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 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 and 178, and fragments thereof;

a heterologous polynucleotide comprising 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 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 and 499, and fragments thereof; or

a heterologous polynucleotide comprising 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 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 and 540, and fragments thereof.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide comprising 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.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising:

administering to the subject a therapeutically effective amount of a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide comprising 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

administering to the subject a therapeutically effective amount of a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide comprising 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.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NOs: 541, 550, 554, 555, 556, 623 or 624.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NOs: 543, 552, 557, 558, 559, 625 or 626.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject

a first vaccine comprising a first heterologous polynucleotide encoding a first heterologous polypeptide, wherein the first heterologous polypeptide comprises two 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 and 177; and

a second vaccine comprising a second heterologous polynucleotide encoding a second heterologous polypeptide, wherein the second heterologous polypeptide comprises two 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 and 177; wherein the first heterologous polypeptide and the second heterologous polypeptide have distinct amino acid sequences.

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject

a first vaccine comprising a first heterologous polynucleotide encoding a first heterologous polypeptide, wherein the first heterologous polypeptide comprises two 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 and 177; and

a second vaccine comprising a second heterologous polynucleotide encoding a second heterologous polypeptide, wherein the second heterologous polypeptide comprises two 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 and 177; wherein the first heterologous polypeptide and the second heterologous polypeptide have distinct amino acid sequences.

The disclosure also provides an isolated proteinaceous molecule that specifically binds the polypeptide of the disclosure.

The disclosure also provides a method of preventing or treating a prostate cancer in a subject, comprising administering to the subject the proteinaceous molecule of the disclosure.

It is to be understood, that the above embodiments of the invention encompass polypeptides comprising, in addition to the specifically recited polypeptides and fragments thereof, also additional polypeptide sequences, including one or more polypeptides different from those specifically recited. Similarly, the above embodiments of the invention also encompass polynucleotides comprising, in addition to the specifically recited polynucleotides and fragments thereof, also additional polynucleotide sequences, including one or more polynucleotides different from those specifically recited.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a cartoon of chimeric read-through fusions between Gene A and Gene B. Neoantigenic peptide sequences arise at the breakpoint junction.

FIG. 2 shows a cartoon of gene fusions resulting from chromosomal alteration, such as DNA translocations.

FIG. 3 shows a cartoon of splice variants with alternative 5' or 3' splice sites, retained introns, excluded exons or alternative terminations or insertions.

FIG. 4 shows the cartoon for approach of identification of splice variants.

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

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

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

FIG. 5D shows a flow cytometry dot plot depicting $TNF\alpha^{+}IFN\gamma^{+}CD8^{+}$ T cell frequencies in PBMC samples after stimulation with P98.

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

FIG. 6 shows 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.

FIG. 7 shows the number of prostate cancer patients whose PBMC samples demonstrated a positive $CD4^+$ immune response to the specified neoantigens.

FIG. 8 shows the number of prostate cancer patients whose PBMC samples demonstrated a positive $CD8^+$ immune response to the specified neoantigens.

DETAILED DESCRIPTION

Definitions

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.

It is to be understood that the terminology used herein is for describing particular embodiments only and is not intended to be limiting. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains.

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.

As used in this specification and the appended claims, 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.

The transitional terms "**comprising**," "**consisting essentially of**," and "**consisting of**" are intended to connote their generally accepted meanings in the patent vernacular; that is, (i) "comprising," which is synonymous with "including," "containing," or "characterized by," is inclusive or open-ended and does not exclude additional, unrecited elements or method steps; (ii) "consisting of" excludes any element, step, or ingredient not specified in the claim; and (iii) "consisting essentially of" limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed disclosure. Embodiments described in terms of the phrase "comprising" (or its equivalents) also provide as embodiments those independently described in terms of "consisting of" and "consisting essentially of."

As used in this specification and the appended claims, 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.

"**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.

"**Polynucleotide**" refers to a synthetic molecule comprising a chain of nucleotides covalently linked by a sugar-phosphate backbone or other equivalent covalent chemistry. cDNA is a typical example of a polynucleotide.

"**Polypeptide**" or "**protein**" refers to a molecule that comprises at least two amino acid residues linked by a peptide bond to form a polypeptide. Small polypeptides of less than 50 amino acids may be referred to as "**peptides**".

"**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.

"**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 heterologous polynucleotide. In-frame heterologous polynucleotide encodes a heterologous polypeptide encoded by both the first polynucleotide and the second polynucleotide.

"**Immunogenic**" refers to a polypeptide that comprises one or more immunogenic fragments.

"**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.

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

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

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

"**Vector**" refers to a polynucleotide capable of being duplicated within a biological system or that can be moved between such systems. Vector polynucleotides typically contain elements, such as origins of replication, polyadenylation signal or selection markers, that

function to facilitate the duplication or maintenance of these polynucleotides in a biological system. Examples of such biological systems may include a cell, virus, animal, plant, and reconstituted biological systems utilizing biological components capable of duplicating a vector. The polynucleotide comprising a vector may be DNA or RNA molecules or a hybrid of these.

"**Expression vector**" refers to a vector that can be utilized in a biological system or in a reconstituted biological system to direct the translation of a polypeptide encoded by a polynucleotide sequence present in the expression vector.

"**Viral vector**" refers to a vector construct that includes at least one polynucleotide element of viral origin and has the capacity to be packaged into a viral vector particle.

"**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.

"**Prevalence**" refers to a percentage of a population studied harboring a prostate neoantigen.

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

"**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).

"**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.

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

"**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.

"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.

"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.

"**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.

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

"**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**".

"**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.

"**Specifically binds**", "**specific binding**", "**specifically binding**" or "**binds**" refer to a proteinaceous molecule binding to an antigen or an epitope within the antigen (e.g. to prostate neoantigen) with greater affinity than for other antigens. Typically, the proteinaceous molecule binds to the antigen or the epitope within the antigen with an equilibrium dissociation constant (K_D) of about $1x10^{-7}$ M or less, for example about $5x10^{-8}$ M or less, about $1x10^{-8}$ M or less, about $1x10^{-9}$ M or less, about $1x10^{-10}$ M or less, about $1x10^{-11}$ M or less, or about $1x10^{-12}$ M or less, typically with the K_D that is at least one hundred fold less than its K_D for binding to a non-specific antigen (e.g., BSA, casein). In the context of the prostate neoantigens described here, "specific binding" refers to binding of the proteinaceous molecule to the prostate neoantigen without detectable binding to a wild-type protein the neoantigen is a variant of.

"**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.

"**Antibody**" refers to an immunoglobulin molecule including monoclonal antibodies including murine, human, humanized and chimeric monoclonal antibodies, antigen-binding fragments, bispecific or multispecific antibodies, dimeric, tetrameric or multimeric antibodies, single chain antibodies, domain antibodies and any other modified configuration of the immunoglobulin molecule that comprises an antigen binding site of the required specificity.

"**Full length antibody**" is comprised of two heavy chains (HC) and two light chains (LC) inter-connected by disulfide bonds as well as multimers thereof (e.g. IgM). Each heavy chain is comprised of a heavy chain variable domain (VH) and a heavy chain constant domain, the heavy chain constant domain comprised of subdomains CH1, hinge, CH2 and CH3. Each light chain is comprised of a light chain variable domain (VL) and a light chain constant domain (CL). The VH and the VL may be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with framework regions (FR). Each VH and VL is composed of three CDRs and four FR segments, arranged from amino-to-carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4.

"**Complementarity determining regions (CDR)**" are antibody regions that bind an antigen. There are three CDRs in the VH (HCDR1, HCDR2, HCDR3) and three CDRs in the VL (LCDR1, LCDR2, LCDR3). CDRs may be defined using various delineations such as Kabat (Wu et al. (1970) J Exp Med 132: 211-50; Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991), Chothia (Chothia et al. (1987) J Mol Biol 196: 901-17), IMGT (Lefranc et al. (2003) Dev Comp Immunol 27: 55-77) and AbM (Martin and Thornton J Bmol Biol 263: 800-15, 1996). The correspondence between the various delineations and variable region numbering are described (see e.g. Lefranc et al. (2003) Dev Comp Immunol 27: 55-77; Honegger and Pluckthun, J Mol Biol (2001) 309:657-70; International ImMunoGeneTics (IMGT) database; Web resources, http://www_imgt_org). Available programs such as abYsis by UCL Business PLC may be used to delineate CDRs. The term "CDR", "HCDR1", "HCDR2", "HCDR3", "LCDR1", "LCDR2" and "LCDR3" as used herein includes CDRs defined by any of the methods described supra, Kabat, Chothia, IMGT or AbM, unless otherwise explicitly stated in the specification.

Immunoglobulins may be assigned to five major classes, IgA, IgD, IgE, IgG and IgM, depending on the heavy chain constant region amino acid sequence. IgA and IgG are further sub-classified as isotypes IgA1, IgA2, IgG1, IgG2, IgG3 and IgG4. Antibody light chains of any vertebrate species may be assigned to one of two clearly distinct types, namely kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

"**Antigen-binding fragment**" refers to a portion of an immunoglobulin molecule that retains the antigen binding properties of the parental full length antibody. Exemplary antigen-binding fragments are heavy chain complementarity determining regions (HCDR) 1, 2 and/or 3,

light chain complementarity determining regions (LCDR) 1, 2 and/or 3, the VH, the VL, the VH and the VL, Fab, F(ab')2, Fd and Fv fragments as well as domain antibodies (dAb) consisting of either one VH domain or one VL domain. The VH and the VL domains may be linked together via a synthetic linker to form various types of single chain antibody designs in which the VH/VL domains pair intramolecularly, or intermolecularly in those cases when the VH and VL domains are expressed by separate chains, to form a monovalent antigen binding site, such as single chain Fv (scFv) or diabody; described for example in Int. Pat. Publ. No. WO1998/44001, Int. Pat. Publ. No. WO1988/01649, Int. Pat. Publ. No. WO1994/13804 or Int. Pat. Publ. No. WO1992/01047.

"**Monoclonal antibody**" refers to an antibody obtained from a substantially homogenous population of antibody molecules, i.e., the individual antibodies comprising the population are identical except for possible well-known alterations such as removal of C-terminal lysine from the antibody heavy chain or post-translational modifications such as amino acid isomerization or deamidation, methionine oxidation or asparagine or glutamine deamidation. Monoclonal antibodies typically bind one antigenic epitope. A bispecific monoclonal antibody binds two distinct antigenic epitopes. Monoclonal antibodies may have heterogeneous glycosylation within the antibody population. Monoclonal antibody may be monospecific or multispecific such as bispecific, monovalent, bivalent or multivalent.

"**Humanized antibody**" refers to an antibody in which at least one CDR is derived from non-human species and at least one framework is derived from human immunoglobulin sequences. Humanized antibody may include substitutions in the frameworks so that the frameworks may not be exact copies of expressed human immunoglobulin or human immunoglobulin germline gene sequences.

"**Human antibody**" refers to an antibody that is optimized to have minimal immune response when administered to a human subject. Variable regions of human antibody are derived from human immunoglobulin sequences. If human antibody contains a constant region or a portion of the constant region, the constant region is also derived from human immunoglobulin sequences. Human antibody comprises heavy and light chain variable regions that are "derived from" sequences of human origin if the variable regions of the human antibody are obtained from a system that uses human germline immunoglobulin or rearranged immunoglobulin genes. Such exemplary systems are human immunoglobulin gene libraries displayed on phage, and transgenic non-human animals such as mice or rats carrying human immunoglobulin loci. "Human antibody" typically contains amino acid differences when compared to the immunoglobulins expressed in humans due to differences between the systems used to obtain the human antibody and human immunoglobulin loci, introduction of somatic mutations or intentional introduction of substitutions into the frameworks or CDRs, or both. Typically, "human antibody" is at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%,

95%, 96%, 97%, 98% or 99% identical in amino acid sequence to an amino acid sequence encoded by human germline immunoglobulin or rearranged immunoglobulin genes. In some cases, "human antibody" may contain consensus framework sequences derived from human framework sequence analyses, for example as described in Knappik et al., (2000) J Mol Biol 296:57-86, or a synthetic HCDR3 incorporated into human immunoglobulin gene libraries displayed on phage, for example as described in Shi et al., (2010) J Mol Biol 397:385-96, and in Int. Patent Publ. No. WO2009/085462. Antibodies in which at least one CDR is derived from a non-human species are not included in the definition of "human antibody".

"**Alternative scaffold**" refers to a single chain protein framework that contains a structured core associated with variable domains of high conformational tolerance. The variable domains tolerate variation to be introduced without compromising scaffold integrity, and hence the variable domains can be engineered and selected for binding to a specific antigen.

"**Chimeric antigen receptor**" or "**CAR**" refers to engineered T cell receptors which graft a ligand or antigen specificity onto T cells (for example naïve T cells central memory T cells effector memory T cells or combinations thereof). CARs are also known as artificial T-cell receptors, chimeric T-cell receptors or chimeric immunoreceptors. CARs comprise an extracellular domain capable of binding to an antigen, a transmembrane domain and at least one intracellular domain. CAR intracellular domain comprises a polypeptide known to function as a domain that transmits a signal to cause activation or inhibition of a biological process in a cell. The transmembrane domain comprises any peptide or polypeptide known to span the cell membrane and that can function to link the extracellular and signaling domains. A chimeric antigen receptor may optionally comprise a hinge domain which serves as a linker between the extracellular and transmembrane domains.

"**T cell receptor**" or "**TCR**" refers to a molecule capable of recognizing a peptide when presented by an MHC molecule. Naturally-occurring TCR heterodimer consists of an alpha (α) and beta (β) chain in around 95% of T-cells, whereas around 5% of T-cells have TCRs consisting of gamma (γ) and delta (δ) chains. Each chain of a natural TCR is a member of the immunoglobulin superfamily and possesses one N-terminal immunoglobulin (Ig)-variable (V) domain, one Ig-constant (C) domain, a transmembrane/cell membrane-spanning region, and a short cytoplasmic tail at the C- terminal end. The variable domain of both the TCR a chain and β chain have three hypervariable or complementarity determining regions (CDRs), CDR1, CDR2 and CDR3, which are responsible for recognizing processed antigens presented on MHC.

TCR may be a full length α/β or γ/δ heterodimer or a soluble molecule comprising a portion of the extracellular domain of the TCR that retains binding the peptide/MHC complex. TCR may be engineered into a single chain TCR.

"**T cell receptor complex**" or "**TCR complex**" refers to a known TCR complex comprising of a TCRα and TCRβ chains, CD3ε, CD3γ, CD3δ and CD3ζ molecules. In some instances, TCRα and TCRβ chains are replaced by TCRγ and TCRδ chains. The amino acid sequences of the various proteins forming the TCR complex are well-known.

"**T cell**" and "**T lymphocyte**" are interchangeable and used synonymously herein. T cell includes thymocytes, naïve T lymphocytes, memory T cells, immature T lymphocytes, mature T lymphocytes, resting T lymphocytes, or activated T lymphocytes. A T cell can be a T helper (Th) cell, for example a T helper 1 (Th1) or a T helper 2 (Th2) cell. The T cell can be a helper T cell (HTL; $CD4^+$ T cell) $CD4^+$ T cell, a cytotoxic T cell (CTL; $CD8^+$ T cell), a tumor infiltrating cytotoxic T cell (TIL; $CD8^+$ T cell), $CD4^+CD8^+$ T cell, or any other subset of T cells. Also included are "NKT cells", which refer to a specialized population of T cells that express a semi-invariant αβ T-cell receptor, but also express a variety of molecular markers that are typically associated with NK cells, such as NK1.1. NKT cells include $NK1.1^+$ and $NK1.1^-$, as well as $CD4^+$, $CD4^-$, $CD8^+$ and $CD8^-$ cells. The TCR on NKT cells is unique in that it recognizes glycolipid antigens presented by the MHC I-like molecule CD Id. NKT cells can have either protective or deleterious effects due to their abilities to produce cytokines that promote either inflammation or immune tolerance. Also included are "gamma-delta T cells (γδ T cells)," which refer to a specialized population that to a small subset of T cells possessing a distinct TCR on their surface, and unlike the majority of T cells in which the TCR is composed of two glycoprotein chains designated α- and β-TCR chains, the TCR in γδ T cells is made up of a γ-chain and a δ-chain . γδ T cells can play a role in immunosurveillance and immunoregulation, and were found to be an important source of IL-17 and to induce robust $CD8^+$ cytotoxic T cell response. Also included are "regulatory T cells" or "Tregs" which refer to T cells that suppress an abnormal or excessive immune response and play a role in immune tolerance. Tregs are typically transcription factor Foxp3-positive $CD4^+$T cells and can also include transcription factor Foxp3-negative regulatory T cells that are IL-10-producing $CD4^+$T cells.

"**Natural killer cell**" or "**NK cell**" refers to a differentiated lymphocyte with a CD 16^+ $CD56^+$ and/or $CD57^+$ TCR^- phenotype. NKs are characterized by their ability to bind to and kill cells that fail to express "self" MHC/HLA antigens by the activation of specific cytolytic enzymes, the ability to kill tumor cells or other diseased cells that express a ligand for NK activating receptors, and the ability to release protein molecules called cytokines that stimulate or inhibit the immune response.

"**About**" means within an acceptable error range for the particular value as determined by one of ordinary 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.

"**Antigen presenting cell**" (APC) refers to any cell that presents on its surface an antigen in association with a major histocompatibility complex molecule, either MHC class I or MHC class II molecule, or both.

"**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.

"**Facilitator element**" refers to any polynucleotide or polypeptide element that is operably linked to a polynucleotide or a polypeptide, and include promoters, enhancers, polyadenylation signals, stop codons, protein tags, such as histidine tag, and the like. Facilitator elements herein include regulatory elements.

"**Distinct**" in the context of polypeptide or polynucleotide sequences refers to polypeptide or polynucleotide sequences that are not identical.

Compositions of matter

The disclosure relates to prostate neoantigens, polynucleotides encoding them, vectors, host cells, vaccines comprising the neoantigens or polynucleotides encoding the neoantigens, proteinaceous molecules binding the prostate neoantigens, and methods of making and using them. The disclosure also provides vaccines comprising the prostate neoantigens of the disclosure that are prevalent in a population of prostate cancer patients, thereby providing a pan-vaccine that may be useful to treating a broad population of patients having diagnosed with various stages of prostate cancer, such as localized or metastasized prostate cancer.

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 to develop a therapy amenable to

treatment of a spectrum of prostate cancer patients. One or more neoantigens or polynucleotides encoding the neoantigens of the disclosure may also be used for diagnostic or prognostic purposes.

Polypeptides and polynucleotides of the disclosure

The disclosure provides an isolated polynucleotide encoding a polypeptide 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 or 447, or fragments thereof.

The disclosure also provides an isolated polynucleotide comprising a polynucleotide sequence 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 or 458, or fragments thereof

The disclosure also provides an isolated polynucleotide comprising a polynucleotide sequence 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, 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 or 540, or fragments thereof.

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

The polynucleotides and the heterologous polynucleotides of the disclosure encode the prostate neoantigens and heterologous polypeptides comprising two or more prostate neoantigens described herein. The polynucleotides and the heterologous polynucleotides of the disclosure are useful in generating the polypeptides, the heterologous polypeptides, the vectors, the recombinant viruses, the cells and the vaccines of the disclosure. The polynucleotides and the heterologous polynucleotides of the disclosure may be utilized as therapeutics by delivering them to a subject having a prostate cancer using various technologies, including viral vectors as described herein or other delivery technologies as also described herein.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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..

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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..

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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..

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 387 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 388 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 389 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 390 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 391 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 392 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 393 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 394 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 395 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 396 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 397 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 398 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 399 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 400 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 401 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 402 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 403 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 404 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 405 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 406 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 407 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 408 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 426 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 427 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 428 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 429 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 430 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 431 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 432 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 433 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 434 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 435 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide of SEQ ID NO: 436 or a fragment thereof.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

The disclosure also provides an isolated polynucleotide encoding the polypeptide 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.

In some embodiments, the heterologous polynucleotide is an in-frame heterologous polynucleotide.

The disclosure also provides an isolated heterologous polynucleotide encoding a heterologous polypeptide comprising two 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 and 447, and fragments thereof.

The disclosure also provides an isolated heterologous polynucleotide encoding a heterologous polypeptide comprising two 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 and 177, and fragments thereof.

The disclosure also provides an isolated heterologous polynucleotide comprising two 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 and 458, and fragments thereof.

The disclosure also provides an isolated heterologous polynucleotide comprising two 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 and 178, and fragments thereof.

For expression in various hosts, the polynucleotides may be codon-optimized utilizing known methods. For example, for adenoviral expression, a heterologous polynucleotide comprising at least one of the polynucleotides 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 or 499 may be used. For expression in a modified vaccinia Ankara (MVA), a heterologous polynucleotide comprising at least one of the polynucleotides 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 or 540 may be used.

The disclosure also provides an isolated heterologous polynucleotide comprising two 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 and 499, and fragments thereof.

The disclosure also provides an isolated heterologous polynucleotide comprising two 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 and 540, and fragments thereof.

In some embodiments, the isolated heterologous polynucleotide is an in-frame heterologous polynucleotide.

In some embodiments, the fragments comprise about 6-24 amino acids in length.

In some embodiments, the fragments 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 amino acids. In some embodiments, the fragments comprise about 7 amino acids. In some embodiments, the fragments comprise about 8 amino acids. In some embodiments, the fragments comprise about 9 amino acids. In some embodiments, the fragments comprise about 10 amino acids. In some embodiments, the fragments comprise about 11 amino acids. In some embodiments, the fragments comprise about 12 amino acids. In some embodiments, the fragments comprise about 13 amino acids. In some embodiments, the fragments comprise about 14 amino acids. In some embodiments, the fragments comprise about 15 amino acids. In some embodiments, the fragments comprise about 16 amino acids. In some embodiments, the fragments comprise about 17 amino acids. In some embodiments, the fragments comprise about 18 amino acids. In some embodiments, the fragments comprise about 19 amino acids. In some embodiments, the fragments comprise about 20 amino acids. In some embodiments, the fragments comprise about 21 amino acids. In some embodiments, the fragments comprise about 22 amino acids. In some embodiments, the fragments comprise about 23 amino acids. In some embodiments, the fragments comprise about 24 amino acids. In some embodiments, the fragments comprise about 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.

In some embodiments, the fragments comprise 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.

In some embodiments, the fragments comprise 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.

In some embodiments, the fragments are immunogenic fragments.

Immunogenic fragments in general are peptides that activate T cells, for example those that induce cytotoxic T cells when presented on MHC. Methods for assessing activation of T cells and/or induction of cytotoxic T lymphocytes are well known. In an exemplary assay, PBMCs isolated from a prostate cancer patient are cultured *in vitro* in the presence of a test neoantigen or fragments thereof and IL-25. The cultures may be replenished periodically with IL-15 and IL-2 and cultured for an additional 12 days. On day 12, the cultures are re-stimulated with the test neoantigen or fragments thereof and the following day T cell activation may be assessed by measuring a percentage of $IFN\gamma^{+}TNA\alpha^{+}$ $CD8^{+}$ cells when compared to a control culture.

The disclosure also provides an isolated polypeptide comprising 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 or 447, or fragments thereof.

In some embodiments, the polypeptide is encoded by a polynucleotide sequence 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 or 458, or fragments thereof.

In some embodiments, the polypeptide is encoded by a polynucleotide sequence 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 or 499, or fragments thereof.

In some embodiments, the polypeptide is encoded by a polynucleotide sequence 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 or 540, or fragments thereof.

The polypeptides and the heterologous polypeptides of the disclosure comprise one or more prostate neoantigens described herein. The polypeptides and the heterologous polypeptides of the disclosure are useful in generating the recombinant viruses, the cells and the vaccines of the disclosure and proteinaceous molecules that specifically bind the one or more prostate neoantigens of the disclosure or may be used directly as therapeutic agents by delivering them to a subject having a prostate cancer using various technologies. The two or more neoantigens (e.g. polypeptides) may be incorporated into the vaccine in any order using standard cloning methods.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 1 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 3 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 5 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 7 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 9 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 11 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 13 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 15 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 17 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 19 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 21 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 23 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 25 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 27 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 29 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 31 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 33 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 35 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 37 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 39 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 41 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 43 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 45 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 47 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 49 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 51 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 53 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 55 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 57 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 59 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 61 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 63 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 65 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 67 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 69 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 71 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 73 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 75 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 77 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 79 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 81 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 83 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 85 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 87 or fragments thereof

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 89 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 91 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 93 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 95 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 97 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 99 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 101 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 103 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 105 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 107 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 109 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 111 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 113 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 115 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 117 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 119 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 121 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 123 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 125 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 127 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 129 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 131 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 133 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 135 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 137 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 139 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 141 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 143 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 145 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 147 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 149 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 151 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 153 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 155 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 157 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 159 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 161 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 163 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 165 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 167 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 169 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 171 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 173 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 175 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 177 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 179 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 181 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 183 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 185 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 187 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 189 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 191 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 193 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 195 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 197 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 199 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 201 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 203 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 205 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 207 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 209 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 211 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 213 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 215 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 217 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 219 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 221 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 223 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 225 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 227 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 229 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 231 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 233 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 235 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 237 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 239 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 241 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 243 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 245 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 247 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 249 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 251 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 253 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 255 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 257 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 259 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 261 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 263 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 265 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 267 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 269 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 271 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 273 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 275 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 277 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 279 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 281 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 283 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 285 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 287 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 289 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 291 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 293 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 295 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 297 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 299 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 301 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 303 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 305 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 307 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 309 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 311 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 313 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 315 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 317 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 319 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 321 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 323 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 325 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 327 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 329 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 331 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 333 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 335 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 337 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 339 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 341 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 343 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 345 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 347 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 349 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 351 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 353 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 355 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 357 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 359 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 361 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 363 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 365 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 367 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 369 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 371 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 373 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 375 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 379 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 381 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 383 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 385 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 387 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 388 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 389 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 390 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 391 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 392 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 393 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 394 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 395 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 396 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 397 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 398 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 399 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 400 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 401 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 402 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 403 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 404 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 405 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 406 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 407 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 408 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 426 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 427 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 428 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 429 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 430 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 431 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 432 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 433 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 434 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 435 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 436 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 437 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 438 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 439 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 440 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 441 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 442 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 443 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 444 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 445 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 446 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 447 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 560 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 561 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 562 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 563 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 564 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 565 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 566 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 567 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 568 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 569 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 570 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 571 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 572 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 573 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 574 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 575 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 576 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 577 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 578 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 579 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 580 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 581 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 582 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 583 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 584 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 585 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 586 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 587 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 588 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 589 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 590 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 591 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 592 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 593 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 594 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 595 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 596 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 597 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 598 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 599 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 600 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 601 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO:602 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 603 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 604 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 605 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 606 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 607 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 608 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 609 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 610 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 611 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 612 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 613 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 614 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 615 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 616 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 617 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 618 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 619 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 620 or fragments thereof.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence of SEQ ID NO: 621 or fragments thereof.

The disclosure also provides an isolated heterologous polypeptide comprising two 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 and 447, and fragments thereof.

In some embodiments, the heterologous polypeptide is encoded by two or more polynucleotides selected from the group consisting of 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 and 458, and fragments thereof.

In some embodiments, the fragments are about 6-25 amino acids in length, such as about 8-25 amino acids in length.

In some embodiments, the fragments comprise polypeptides 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.

In some embodiments, the fragments comprise polypeptides 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.

The disclosure also provides an isolated heterologous polypeptide comprising two 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 and 177, and fragments thereof.

In some embodiments, the heterologous polypeptide is encoded by a heterologous polynucleotide comprising two 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 and 178, and fragments thereof.

In some embodiments, the heterologous polypeptide is encoded by a heterologous polynucleotide comprising two 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 and 499, and fragments thereof.

In some embodiments, the heterologous polypeptide is encoded by a heterologous polynucleotide comprising two 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 and 540, and fragments thereof.

Variants of engineered polynucleotides, polypeptides, heterologous polynucleotides and heterologous polypeptides of the disclosure

Variants of the polynucleotides, polypeptides, heterologous polynucleotides and heterologous polypeptides or fragments thereof are within the scope of the disclosure. For example, variants may comprise one or more substitutions, deletions or insertions, as long as the variants retain or have improved characteristics (such as immunogenicity or stability) when compared to the parent. In some embodiments, the sequence identity may be about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% between the parent and the variant. In some embodiments, variants are generated by conservative substitutions.

In some embodiments, the identity is about 80%. In some embodiments, the identity is about 85%. In some embodiments, the identity is about 90%. In some embodiments, the identity is about 91%. In some embodiments, the identity is about 91%. In some embodiments, the identity is about 92%. In some embodiments, the identity is about 93%. In some embodiments, the identity is about 94%. In some embodiments, the identity is 94%. In some embodiments, the identity is about 95%. In some embodiments, the identity is about 96%. In some embodiments, the identity is about 97%. In some embodiments, the identity is about 98%. In some embodiments, the identity is about 99%.

In some embodiments, variants may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 substitutions, deletions or insertions, as long as the variants retain or have improved characteristics (such as immunogenicity or stability) when compared to the parent.

The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = number of identical positions/total number of positions ×100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The percent identity between two amino acid sequences may be determined using the algorithm of E. Meyers and W. Miller (*Comput Appl Biosci* 4:11-17 (1988)) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences may be determined using the Needleman and Wunsch (*J Mol Biol* 48:444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available at http_//_www_gcg_com), using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

The variants of the polypeptides or the heterologous polypeptides or fragments thereof containing one ammo acid alteration generally retain similar tertiary structure and antigenicity relative to the parent. In some instances, the variant may also contain at least one amino acid alteration that causes the variant to have increased antigenicity, increased binding affinity to TCR or to antibody, or both. The variants of the polypeptides or the heterologous polypeptides may also have improved ability to bind to a HLA molecule.

The variants of the disclosure may be engineered to contain conservative substitutions. Conservative substitutions are herein defined as exchanges within one of the following five groups: Group 1-small aliphatic, nonpolar or slightly polar residues (Ala, Ser, Thr, Pro, Gly); Group 2-polar, negatively charged residues and their amides (Asp, Asn, Glu, Gin); Group 3-polar, positively charged residues (His, Arg, Lys); Group 4-large, aliphatic, nonpolar residues (Met, Leu, lie, Val, Cys); and Group 5-large, aromatic residues (Phe, Tyr, Trp).

The variants of the disclosure may be engineered to contain less conservative substitutions, such as the replacement of one amino acid by another that has similar characteristics but is somewhat different in size, such as replacement of an alanine by an isoleucine residue. The variants of the disclosure may also be engineered to contain highly non-conservative substitutions which may involve substituting an acidic amino acid for one that is polar, or even for one that is basic in character.

Additional substitutions that may be made to generate variants of the disclosure include substitutions may involve structures other than the common L-amino acids. Thus, D-amino and non-standard amino acids (i.e., other than the common naturally occurring proteinogenic amino

acids) may also be used for substitution purposes to produce variants with enhanced immunogenicity when compared to the parent.

If substitutions at more than one position are found to result in polypeptides or heterologous polypeptides with substantially equivalent or greater immunogenicity, then combinations of those substitutions may be tested to determine if the combined substitutions result in additive or synergistic effects on the immunogenicity of the variant.

The amino acid residues that do not substantially contribute to interactions with the TCR may be modified by replacement with other amino acid whose incorporation does not substantially affect T-cell reactivity and does not eliminate binding to the relevant MHC. The amino acid residues that do not substantially contribute to interactions with the TCR may also be deleted as long as the deletion does not substantially affect T-cell reactivity and does not eliminate binding to the relevant MHC.

In addition, the polypeptides or the heterologous polypeptides or fragments thereof or variants may be further modified to improve stability and/or binding to MHC molecules in order to elicit a stronger immune response. Methods for such an optimization of a peptide sequence are well known in the art and include, for example, the introduction of reverse peptide bonds or non-peptide bonds. In a reverse peptide bond amino acid residues are not joined by peptide (—CO—NH—) linkages but the peptide bond is reversed. Such retro-inverso peptidomimetics may be made using methods known in the art. This approach involves making pseudopeptides containing changes involving the backbone, and not the orientation of side chains. Retro-inverse peptides, which contain NH—CO bonds instead of CO—NH peptide bonds are much more resistant to proteolysis. Additional non-peptide bond that may be used are, for example, —CH_2—NH, —CH_2S—, —CH_2CH_2—, —CH=CH—, —$COCH_2$—, —$CH(OH)CH_2$—, and —CH_2SO—.

The polypeptides or the heterologous polypeptides or fragments thereof, or variants of the disclosure may be synthesized with additional chemical groups present at their amino and/or carboxy termini, to enhance the stability, bioavailability, and/or affinity of the peptides. For example, hydrophobic groups such as carbobenzoxyl, dansyl, or t-butyloxycarbonyl groups may be added to the amino terminus. Likewise, an acetyl group or a 9-fluorenylmethoxy-carbonyl group may be placed at the amino termini. Additionally, the hydrophobic group, t-butyloxycarbonyl, or an amido group may be added to the carboxy termini.

Further, the polypeptides or the heterologous polypeptides or fragments thereof, or variants of the disclosure may be synthesized to alter their steric configuration. For example, the D-isomer of one or more of the amino acid residues of the peptide may be used, rather than the usual L-isomer.

Similarly, the polypeptides or the heterologous polypeptides or fragments thereof, or variants of the disclosure may be modified chemically by reacting specific amino acids either before or after synthesis of the polypeptides or the heterologous polypeptides or fragments thereof, or variants of the disclosure. Examples for such modifications are well known in the art and are summarized e.g. in R. Lundblad, Chemical Reagents for Protein Modification, 3rd ed. CRC Press, 2004 (Lundblad, 2004). Chemical modifications of amino acids include modification by acylation, amidation, pyridoxylation of lysine, reductive alkylation, trinitrobenzylation of amino groups with 2,4,6-trinitrobenzene sulphonic acid (TNBS), amide modification of carboxyl groups and sulphydryl modification by performic acid oxidation of cysteine to cysteic acid, formation of mercurial derivatives, formation of mixed disulphides with other thiol compounds, reaction with maleimide, carboxymethylation with iodoacetic acid or iodoacetamide and carbamoylation with cyanate at alkaline pH, although without limitation thereto. In this regard, the skilled person is referred to Chapter 15 of Current Protocols In Protein Science, Eds. Coligan et al. (John Wiley and Sons NY 1995-2000) (Coligan et a l., 1995) for more extensive methodology relating to chemical modification of proteins.

Briefly, modification of e.g. arginyl residues in proteins is often based on the reaction of vicinal dicarbonyl compounds such as phenylglyoxal, 2,3-butanedione, and 1,2-cyclohexanedione to form an adduct. Another example is the reaction of methylglyoxal with arginine residues. Cysteine can be modified without concomitant modification of other nucleophilic sites such as lysine and histidine. As a result, a large number of reagents are available for the modification of cysteine. The websites of companies such as Sigma-Aldrich (http_://www_sigma-aldrich.com) provide information on specific reagents. Selective reduction of disulfide bonds in proteins is also common. Disulfide bonds can be formed and oxidized during the heat treatment of biopharmaceuticals. Woodward's Reagent K may be used to modify specific glutamic acid residues. N-(3-(dimethylamino)propyl)-N′-ethylcarbodiimide can be used to form intra-molecular crosslinks between a lysine residue and a glutamic acid residue. For example, diethylpyrocarbonate is a reagent for the modification of histidyl residues in proteins. Histidine can also be modified using 4-hydroxy-2-nonenal. The reaction of lysine residues and other α-amino groups is, for example, useful in binding of peptides to surfaces or the cross-linking of proteins/peptides. Lysine is the site of attachment of poly(ethylene)glycol and the major site of modification in the glycosylation of proteins. Methionine residues in proteins can be modified with e.g. iodoacetamide, bromoethylamine, and chloramine T. Tetranitromethane and N-acetylimidazole can be used for the modification of tyrosyl residues. Cross-linking via the formation of dityrosine can be accomplished with hydrogen peroxide/copper ions. Recent studies on the modification of tryptophan have used N-bromosuccinimide, 2-hydroxy-5-nitrobenzyl bromide or 3-bromo-3-methyl-2-(2-nitrophenylmercapto)-3H-indole (BPNS-skatole).

Successful modification of therapeutic proteins and peptides with PEG is often associated with an extension of circulatory half-life while cross-linking of proteins with glutaraldehyde, polyethylene glycol diacrylate and formaldehyde is used for the preparation of hydrogels. Chemical modification of allergens for immunotherapy is often achieved by carbamylation with potassium cyanate.

The disclosure provides an isolated polypeptide that is about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the polypeptide 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 or 447.

The disclosure also provides an isolated polypeptide comprising a polypeptide sequence having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 substitutions, deletions or insertions when compared to the polypeptide 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 or 447.

The disclosure also provides an isolated polynucleotide that is about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide 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 or 458.

The disclosure also provides an isolated polypeptide that is about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the polypeptide 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.

The disclosure also provides an isolated polypeptide that is about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the polypeptide 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.

The disclosure also provides an isolated polypeptide that is about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the polypeptide of SEQ ID NOs: 541, 550, 554, 555, 556, 623 or 624.

The disclosure also provides an isolated polynucleotide that is about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the polypeptide of SEQ ID NOs: 542 or SEQ ID NO: 551.

The disclosure also provides an isolated polypeptide that is about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the polypeptide of SEQ ID NOs: 543, 552, 557, 558, 559, 625 or 626.

The disclosure also provides an isolated polynucleotide that is about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the polypeptide of SEQ ID NOs: 544 or SEQ ID NO: 553.

The disclosure also provides an isolated polypeptide comprising 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 and 447, and fragments thereof, wherein the polypeptide comprises one or more reverse peptide bonds.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence 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, wherein the polypeptide comprises one or more reverse peptide bonds.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence 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, wherein the polypeptide comprises one or more reverse peptide bonds.

The disclosure also provides an isolated heterologous polypeptide comprising an amino acid sequence of SEQ ID NOs: 541, 550, 554, 555, 556, 623 or 624, wherein the heterologous polypeptide comprises one or more reverse peptide bonds.

The disclosure also provides an isolated heterologous polypeptide comprising an amino acid sequence of SEQ ID NOs: EQ ID NOs: 543, 552, 557, 558, 559, 625 or 626, wherein the heterologous polypeptide comprises one or more reverse peptide bonds.

In some embodiments, the reverse peptide bond comprises NH—CO bond.

In some embodiments, the reverse peptide bond comprises CH_2—NH, —CH_2S—, —CH_2CH_2—, —CH=CH—, —$COCH_2$—, —CH(OH)CH_2—, or —CH_2SO— bond.

The disclosure also provides an isolated polypeptide comprising 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 and 447, and fragments thereof, wherein the polypeptide comprises one or more chemical modifications.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence 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, wherein the polypeptide comprises one or more chemical modifications.

The disclosure also provides an isolated polypeptide comprising an amino acid sequence 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, wherein the polypeptide comprises one or more chemical modifications.

The disclosure also provides an isolated heterologous polypeptide comprising an amino acid sequence of SEQ ID NOs: 541, 550, 554, 555, 556, 623 or 624, wherein the heterologous polypeptide comprises one or more chemical modifications.

The disclosure also provides an isolated heterologous polypeptide comprising an amino acid sequence of SEQ ID NOs: EQ ID NOs: 543, 552, 557, 558, 559, 625 or 626, wherein the heterologous polypeptide comprises one or more chemical modifications.

In some embodiments, the one or more chemical modification comprises modification with carbobenzoxyl, dansyl, t-butyloxycarbonyl, 9-fluorenylmethoxy-carbonyl or D-isomer of an amino acid.

Methods of making polynucleotides and polypeptides of the disclosure

The polynucleotides of the disclosure or variants may be in the form of RNA or in the form of DNA obtained by cloning or produced synthetically. The DNA may be double- stranded or single-stranded.

Methods of generating polynucleotides and heterologous polynucleotides of the disclosure or variants are known in the art and include chemical synthesis, enzymatic synthesis (e.g. *in vitro* transcription), enzymatic or chemical cleavage of a longer precursor, chemical synthesis of smaller fragments of the polynucleotides followed by ligation of the fragments or known PCR methods. The polynucleotide sequence to be synthesized may be designed with the appropriate codons for the desired amino acid sequence. In general, preferred codons may be selected for the intended host in which the sequence will be used for expression.

Methods of making polypeptides and heterologous polypeptides of the disclosure are known in the art and include standard molecular biology techniques for cloning and expression of the polypeptides and chemical synthesis of the polypeptides.

Peptides may be synthesized by the Fmoc-polyamide mode of solid-phase peptide synthesis as disclosed by Lukas et al. (Lukas et al., 1981) and by references as cited therein. Temporary N-amino group protection is afforded by the 9-fluorenylmethyloxycarbonyl (Fmoc) group. Repetitive cleavage of this highly base-labile protecting group is done using 20% piperidine in N, N-dimethylformamide. Side-chain functionalities may be protected as their butyl ethers (in the case of serine threonine and tyrosine), butyl esters (in the case of glutamic acid and aspartic acid), butyloxycarbonyl derivative (in the case of lysine and histidine), trityl derivative (in the case of cysteine) and 4-methoxy-2,3,6-trimethylbenzenesulphonyl derivative (in the case of arginine). Where glutamine or asparagine are C-terminal residues, use is made of the 4,4′-dimethoxybenzhydryl group for protection of the side chain amido functionalities. The solid-phase support is based on a polydimethyl-acrylamide polymer constituted from the three monomers dimethylacrylamide (backbone-monomer), bisacryloylethylene diamine (cross linker) and acryloylsarcosine methyl ester (functionalizing agent). The peptide-to-resin cleavable linked agent used is the acid-labile 4-hydroxymethyl-phenoxyacetic acid derivative. All amino acid derivatives are added as their preformed symmetrical anhydride derivatives with the exception of asparagine and glutamine, which are added using a reversed N, N-dicyclohexyl-carbodiimide/1 hydroxybenzotriazole mediated coupling procedure. All coupling and deprotection reactions are monitored using ninhydrin, trinitrobenzene sulphonic acid or isotin test procedures. Upon

completion of synthesis, peptides are cleaved from the resin support with concomitant removal of side-chain protecting groups by treatment with 95% trifluoroacetic acid containing a 50% scavenger mix. Scavengers commonly used include ethanedithiol, phenol, anisole and water, the exact choice depending on the constituent amino acids of the peptide being synthesized. Also a combination of solid phase and solution phase methodologies for the synthesis of peptides is possible (see, for example, (Bruckdorfer et al., 2004), and the references as cited therein).

U.S. Pat. No. 4,897,445 provides a method for the solid phase synthesis of non-peptide bonds (—CH_2—NH) in polypeptide chains which involves polypeptides synthesized by standard procedures and the non-peptide bond synthesized by reacting an amino aldehyde and an amino acid in the presence of $NaCNBH_3$.

Vectors and recombinant viruses of the disclosure

The disclosure also provides a vector comprising a polynucleotide or a heterologous polynucleotide of the disclosure, or fragments or variants thereof.

In some embodiments, the vector is an expression vector. In some embodiments, the vector is a viral vector. The vectors of the disclosure may be utilized to generate recombinant viruses comprising the vectors of the disclosure or to express the polypeptides of the disclosure.

The disclosure also provides a vector comprising a polynucleotide encoding one or more polypeptides 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 or 447, or fragments thereof.

The disclosure also provides a vector comprising a polynucleotide encoding a polypeptide that is about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the polypeptide 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 or 447, or fragments thereof.

The disclosure also provides a vector comprising one or more polynucleotides 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 or 458, or fragments thereof.

The disclosure also provides a vector comprising a polynucleotide that is about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide 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 or 458, or fragments thereof.

The disclosure also provides a vector comprising a heterologous polynucleotide encoding a heterologous polypeptide comprising two 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 and 447, and fragments thereof.

The disclosure also provides a vector comprising a heterologous polynucleotide comprising two 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 and 458, and fragments thereof.

The disclosure also provides a vector comprising a heterologous polynucleotide encoding a heterologous polypeptide comprising two 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 and 177, and fragments thereof.

The disclosure also provides a vector comprising a heterologous polynucleotide comprising two 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 and 499, and fragments thereof.

The disclosure also provides a vector comprising a heterologous polynucleotide comprising two 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 and 540, and fragments thereof.

The disclosure also provides a recombinant virus comprising the vector of the disclosure. The disclosure also provides a recombinant adenovirus comprising the vector of the disclosure.

The disclosure also provides a recombinant human adenovirus (rAd) comprising the vector of the disclosure.

The disclosure also provides a recombinant human adenovirus derived from serotype 26 (rAd26) comprising the vector of the disclosure.

The disclosure also provides a recombinant great ape adenovirus (rGAd) comprising the vector of the disclosure.

In some embodiments, the rGAd is derived from GAd20. In some embodiments, the rGAd is derived from GAd19. In some embodiments, the rGAd is derived from GAd21. In some embodiments, the rGAd is derived from GAd25. In some embodiments, the rGAd is derived from GAd26. In some embodiments, the rGAd is derived from GAd27, In some embodiments, the rGAd is derived from GAd28. In some embodiments, the rGAd is derived from GAd29. In some embodiments, the rGAd is derived from GAd30. In some embodiments, the rGAd is derived from GAd31. 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.

SEQ ID NO: 622

CATCATCAATAATATACCTTATTTTGGATTGAGGCCAATATGATAATGAGGTGGGCG
GGGCGAGGCGGGGCGGGTGACGTAGGACGCGCGAGTAGGGTTGGGAGGTGTGGCG
GAAGTGTGGCATTTGCAAGTGGGAGGAGCTGACATGCAATCTTCCGTCGCGGAAAA
TGTGACGTTTTTGATGAGCGCCGCCTACCTCCGGAAGTGCCAATTTTCGCGCGCTTTT
CACCGGATATCGTAGTAATTTTGGGCGGGACCATGTAAGATTTGGCCATTTTCGCGC
GAAAAGTGAAACGGGGAAGTGAAAACTGAATAATAGGGCGTTAGTCATAGCGCGTA
ATATTTACCGAGGGCCGAGGGACTTTGACCGATTACGTGGAGGACTCGCCCAGGTGT
TTTTTACGTGAATTTCCGCGTTCCGGGTCAAAGTCTCCGTTTTTATTGTCGCCGTCAT
CTGACGCGGAGGGTATTTAAACCCGCTGCGCTCCTAAAGAGGCCACTCTTGAGTGCC

AGCGAGAAGAGTTTTCTCCTCCGCTCCGTTTCGGCGATCGAAAAATGAGACATTTAG
CCTGCACTCCGGGTCTTTTGTCCGGCCGGGCGGCGTCCGAGCTTTTGGACGCTTTGCT
CAATGAGGTTCTGAGCGATGATTTTCCGTCTACTACCCACTTTAGCCCACCTACTCTT
CACGAACTGTACGATCTGGATGTACTGGTGGATGTGAACGATCCCAACGAGGAGGC
GGTTTCTACGTTTTTTCCCGAGTCTGCGCTTTTGGCTGCCCAGGAGGGATTTGACCTA
CACACTCCGCCGCTGCCTATTTTAGAGTCTCCGCTGCCGGAGCCCAGTGGTATACCTT
ATATGCCTGAACTGCTTCCCGAAGTGGTAGACCTGACCTGCCACGAGCCGGGCTTTC
CGCCCAGCGACGATGAGGGTGAGCCTTTTGCTTTAGACTATGCTGAGATACCTGGGC
TCGGTTGCAGGTCTTGTGCATATCATCAGAGGGTTACCGGAGACCCCGAGGTTAAGT
GTTCGCTGTGCTATATGAGGCTGACCTCTTCCTTTATCTACAGTAAGTTTTTTTGTGT
AGGTGGGCTTTTTGGGTAGGTGGGTTTTGTGGCAGGACAGGTGTAAATGTTGCTTGT
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CGAGCCGGATCCCGAGCCTCCTCGCAGGCCAAGGAAATTACCTTCCATTTTGTGCAA
GCCTAAGACACCTGTGAGGACCAGCGAGGCGGACAGCACTGACTCTGGCACTTCTA
CCTCTCCTCCTGAAATTCACCCAGTGGTTCCTCTGGGTATACATAGACCTGTTGCTGT
TAGAGTTTGCGGGCGACGCCCTGCAGTAGAGTGCATTGAGGACTTGCTTAACGATCC
CGAGGGACCTTTGGACTTGAGCATTAAACGCCCTAGGCAATAAACCCCACCTAAGTA
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ACTTGACCCCAATGGAGGCGTGGGGGTGCTTGGAGGAGTTTGCGGACGTGCGCCGTT
TGCTGGACGAGAGCTCTAGCAATACCTATAGTATTTGGAGGTATCTGTGGGGCTCTA
CTCAGGCCAAGTTGGTCTTCAGAATTAAGCAGGATTACAAGTGCGATTTTGAAGAGC
TTTTTAGTTCCTGTGGTGAGCTTTTGCAATCCTTGAATCTGGGCCACCAGGCTATCTT
CCAGGAAAAGGTTCTCTCGACTTTGGATTTTTCCACTCCCGGGCGCACCGCCGCTTGT
GTGGCTTTTGTGTCTTTTGTGCAAGATAAATGGAGCGGGGAGACCCACCTGAGTCAC
GGCTACGTGCTGGATTTCATGGCGATGGCTCTTTGGAGGGCTTACAACAAATGGAAG
ATTCAGAAGGAACTGTACGGTTCCGCCCTACGTCGTCCACTTCTGCAGCGGCAGGGG
CTGATGTTTCCCGACCATCGCCAGCATCAGAATCTGGAAGACGAGCGAGCGGAGAA
GATCAGCTTGAGAGCCGGCCTGGACCCTCCTCAGGAGGAATGAATCTCCCGCAGGT
GGTTGAGCTGTTTCCCGAACTGAGACGGGTCCTGACTATCAGGGAGGATGGTCAGTT
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CTTACCACTTACTGGCTTCAGCCCGGGGATGATTGGGAAGAGGCGATTAGGGTGTAT
GCAAAGGTGGCCCTGCGGCCCGATTGCAAGTATAAGATTACTAAGTTGGTTAATATT

AGAAACTGCTGCTATATTTCTGGAAACGGGGCCGAAGTGGAGATAGATACTGAGGA
CAGGGTGGCTATTAGGTGTTGCATGATAAACATGTGGCCCGGGATACTGGGGATGG
ATGGGGTGATATTTATGAATGTGAGGTTCACGGGCCCCAACTTTAATGGTACGGTGT
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CCTGTATAGAGGCCTGGACCGATGTAAAGGTTCGAGGTTGTTCCTTTTATAGCTGTT
GGAAGGCGGTGGTGTGTCGCCCTAAAAGCAGGGGTTCTGTGAAGAAATGCTTGTTTG
AAAGGTGCACCCTAGGTATCCTTTCTGAGGGCAACTCCAGGGTGCGCCATAATGTGG
CTTCGAACTGCGGTTGCTTCATGCAAGTGAAGGGGGTGAGCGTTATCAAGCATAACT
CGGTCTGTGGAAACTGCGAGGATCGCGCCTCTCAGATGCTGACCTGCTTTGATGGCA
ACTGTCACCTGTTGAAGACCATTCATATAAGCAGTCACCCCAGAAAGGCCTGGCCCG
TGTTTGAGCATAACATTCTGACCCGCTGTTCCTTGCATCTGGGGGTCAGGAGGGGTA
TGTTCCTGCCTTACCAGTGTAACTTTAGCCACACTAAAATCCTGCTGGAACCCGAGT
GCATGACTAAGGTCAGCCTGAATGGTGTGTTTGATGTGAGTCTGAAGATTTGGAAGG
TGCTGAGGTATGATGAGACCAGGACCAGGTGCCGACCCTGCGAGTGCGGCGGCAAG
CACATGAGAAATCAGCCTGTGATGTTGGATGTGACCGAGGAGCTTAGGCCTGACCAT
CTGGTGCTGGCCTGCACCAGGGCCGAGTTTGGGTCTAGCGATGAGGATACCGATTGA
GGTGGGTAAGGTGGGCGTGGCTAGCAGGGTGGGCGTGTATAAATTGGGGGTCTAAG
GGGTCTCTCTGTTTGTCTTGCAACAGCCGCCGCCATGAGCGACACCGGCAACAGCTT
TGATGGAAGCATCTTTAGTCCCTATCTGACAGTGCGCATGCCTCACTGGGCCGGAGT
GCGTCAGAATGTGATGGGTTCCAACGTGGATGGACGTCCCGTTCTGCCTTCAAATTC
GTCTACTATGGCCTACGCGACCGTGGGAGGAACTCCGCTGGACGCCGCGACCTCCGC
CGCCGCCTCCGCCGCCGCCGCGACCGCGCGCAGCATGGCTACGGACCTTTACAGCTC
TTTGGTGGCGAGCAGCGCGGCCTCTCGCGCGTCTGCTCGGGATGAGAAACTGACTGC
TCTGCTGCTTAAACTGGAAGACTTGACCCGGGAGCTGGGTCAACTGACCCAGCAGGT
TTCCAGCTTGCGTGAGAGCAGCCTTGCCTCCCCCTAATGGCCCATAATATAAATAAA
AGCCAGTCTGTTTGGATTAAGCAAGTGTATGTTCTTTATTTAACTCTCCGCGCGCGGT
AAGCCCGGGACCAGCGGTCTCGGTCGTTTAGGGTGCGGTGGATTTTTTCCAACACGT
GGTACAGGTGGCTCTGGATGTTTAGATACATGGGCATGAGTCCATCCCTGGGGTGGA
GGTAGCACCACTGCAGAGCTTCGTGCTCGGGGGTGGTGTTGTATATGATCCAGTCGT
AGCAGGAGCGCTGGGCGTGGTGCTGAAAAATGTCCTTAAGCAAGAGGCTTATAGCT
AGGGGGAGGCCCTTGGTGTAAGTGTTTACAAATCTGCTTAGCTGGGAGGGGTGCATC
CGGGGGGATATGATGTGCATCTTGGACTGGATTTTTAGGTTGGCTATGTTCCCGCCC
AGATCCCTTCTGGGATTCATGTTGTGCAGGACCACCAGCACGGTATATCCAGTGCAC
TTGGGAAATTTATCGTGGAGCTTAGACGGGAATGCATGGAAGAACTTGGAGACGCC
CTTGTGGCCTCCCAGATTTTCCATACATTCGTCCATGATGATGGCAATGGGCCCGTG
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GGTGAGGTCATCATAGGACATCTTTACGAATCGGGGGCGAAGGGTCCCGGACTGGG
GGATGATGGTACCCTCGGGCCCCGGGGCGTAGTTCCCCTCACAGATCTGCATCTCCC
AGGCTTTCATTTCAGAGGGAGGGATCATATCCACCTGCGGGGCGATGAAAAAGACA
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CTTTCCACAGCCGGTGGGCCCATATATCACGCCTATCACCGGCTGCAGCTGGTAGTT
AAGAGAGCTGCAGCTGCCGTCCTCCCGGAGCAGGGGGGCCACCTCGTTGAGCATAT
CCCTGACGTGGATGTTCTCCCTGACCAGTTCCGCCAGAAGGCGCTCGCCGCCCAGCG
AAAGCAGCTCTTGCAAGGAAGCAAAATTTTTCAGCGGTTTCAGGCCATCGGCCGTGG
GCATGTTTTTCAGCGTCTGGGTCAGCAGCTCCAGCCTGTCCCAGAGCTCGGTGATGT
GCTCTACGGCATCTCGATCCAGCAGATCTCCTCGTTTCGCGGGTTGGGGCGGCTTTC
GCTGTAGGGCACCAGCCGATGGGCGTCCAGCGGGGCCAGAGTCATGTCCTTCCATG
GGCGCAGGGTCCTCGTCAGGGTGGTCTGGGTCACGGTGAAGGGGTGCGCTCCGGGT
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TCTTCGCCCTGCGCGTCGGCCAGGTAGCATTTGACCATGGTCTCGTAGTCGAGACCC
TCGGCGGCGTGCCCCTTGGCGCGGAGCTTTCCCTTGGAGGTGGCGCCGCACGAGGGG
CACTGCAGGCTCTTCAGGGCGTAGAGCTTGGGAGCGAGAAACACGGACTCTGGGGA
GTAGGCGTCCGCGCCGCAGGCCGAGCAGACCGTCTCGCATTCCACCAGCCAAGTGA
GTTCCGGGCGGTCAGGGTCAAAAACCAGGTTGCCCCCATGCTTTTTGATGCGTTTCTT
ACCTTGGCTCTCCATGAGGCGGTGTCCCTTCTCGGTGACGAAGAGGCTGTCCGTGTC
CCCGTAGACCGACTTCAGGGGCCTGTCTTCCAGCGGAGTGCCTCTGTCCTCCTCGTA
GAGAAACTCTGACCACTCTGAGACGAAGGCCCGCGTCCAGGCCAGGACGAAGGAGG
CCACGTGGGAGGGGTAGCGGTCGTTGTCCACTAGCGGGTCCACCTTCTCCAGGGTGT
GCAGGCACATGTCCCCCTCCTCCGCGTCCAGAAAAGTGATTGGCTTGTAGGTGTAGG
ACACGTGACCGGGGGTTCCCAACGGGGGGGTATAAAAGGGGGTGGGTGCCCTTTCA
TCTTCACTCTCTTCCGCATCGCTGTCTGCGAGAGCCAGCTGCTGGGGTAAGTATTCCC
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AGAAAACACTATTTTTTTGTTATCAAGCTTGGTGGCGAATGACCCGTAGAGGGCGTT
GGAGAGCAGCTTGGCGATGGAGCGCAGGGTCTGGTTTTTGTCGCGGTCGGCTCGCTC
CTTGGCCGCGATGTTGAGTTGCACGTACTCGCGGGCCACGCACTTCCACTCGGGGAA
CACGGTGGTGCGCTCGTCTGGGATCAGGCGCACCCTCCAGCCGCGGTTGTGCAGGGT
GACCATGTCGACGCTGGTGGCGACCTCACCGCGCAGACGCTCGTTGGTCCAGCAGA
GGCGGCCGCCCTTGCGCGAGCAGAAGGGGGGTAGGGGGTCCAGCTGGTCCTCGTTT
GGGGGGTCCGCGTCGATGGTAAAGACCCCGGGGAGCAGGCGCGGGTCAAAGTAGTC
GATCTTGCAAGCTTGCATGTCCAGAGCCCGCTGCCATTCGCGGGCGGCGAGCGCGCG
CTCGTAGGGGTTGAGGGGCGGGCCCCAGGGCATGGGGTGGGTGAGCGCGGAGGCGT

ACATGCCGCAGATGTCATACACGTACAGGGGTTCCCTGAGGATACCGAGGTAGGTG
GGGTAGCAGCGCCCCCCGCGGATGCTGGCGCGCACGTAGTCATAGAGCTCGTGGGA
GGGGGCCAGCATGTTGGGCCCGAGGTTGGTGCGCTGGGGGCGCTCGGCGCGGAAGA
CGATCTGCCTGAAGATGGCGTGGGAGTTGGAGGAGATGGTGGGCCGCTGGAAGACG
TTGAAGCTTGCTTCTTGCAAGCCCACGGAGTCCCTGACGAAGGAGGCGTAGGACTCG
CGCAGCTTGTGCACCAGCTCGGCGGTGACCTGGACGTCGAGCGCACAGTAGTCGAG
GGTCTCGCGGATGATGTCATACCTATCCTCCCCCTTCTTTTTCCACAGCTCGCGGTTG
AGGACGAACTCTTCGCGGTCTTTCCAGTACTCTTGGAGGGGAAACCCGTCCGTGTCC
GAACGGTAAGAGCCTAGCATGTAGAACTGGTTGACGGCCTGGTAGGGGCAGCAGCC
CTTCTCCACGGGCAGCGCGTAGGCCTGCGCCGCCTTGCGGAGGGAGGTGTGGGTGA
GGGCGAAAGTGTCCCTGACCATGACTTTGAGGTATTGATGTCTGAAGTCTGTGTCAT
CGCAGCCGCCCTGTTCCCACAGGGTGTAGTCCGTGCGCTTTTTGGAGCGCGGGTTGG
GCAGGGAGAAGGTGAGGTCATTGAAGAGGATCTTCCCCGCTCGAGGCATGAAGTTT
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The disclosure also provides a recombinant chimpanzee adenovirus derived from serotype 20 (rChAd20) comprising the vector of the disclosure.

The disclosure also provides a recombinant modified vaccinia Ankara (rMVA) comprising the vector of the disclosure.

The vector may be a vector intended for expression of the polynucleotide or the heterologous polynucleotide of the disclosure in any host, such as bacteria, yeast or a mammal. Suitable expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors contain selection markers such as ampicillin-resistance, hygromycin-resistance, tetracycline resistance, kanamycin resistance or neomycin resistance to permit detection of those cells transformed or transduced with the desired DNA sequences. Exemplary vectors are plasmids, cosmids, phages, viral vectors, transposons or artificial chromosomes.

Vectors of the disclosure may be circular or linear. They may be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems can be derived, e.g., from ColE1, SV40, 2μ plasmid, λ, bovine papilloma virus, and the like.

The recombinant expression vectors can be designed for either transient expression, for stable expression, or for both. Also, the recombinant expression vectors can be made for constitutive expression or for inducible expression.

Further, the recombinant expression vectors can be made to include a suicide gene. "Suicide gene" refers to a gene that causes the cell expressing the suicide gene to die. The suicide gene can be a gene that confers sensitivity to an agent, e.g., a drug, upon the cell in which the gene is expressed, and causes the cell to die when the cell is contacted with or exposed to the agent. Suicide genes are known in the art and include, for example, the Herpes Simplex Virus (HSV) thymidine kinase (TK) gene, cytosine deaminase, purine nucleoside phosphorylase, and nitroreductase.

The vectors may also comprise selection markers, which are well known in the art. Selection markers include positive and negative selection marker. Marker genes include biocide resistance, e.g., resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host to provide prototrophy, and the like. Exemplary marker genes include antibiotic resistance genes (e.g., neomycin resistance gene, a hygromycin resistance gene, a kanamycin resistance gene, a tetracycline resistance gene, a penicillin resistance gene, histidinol resistance gene, histidinol x resistance gene), glutamine synthase genes, HSV-TK, HSV-TK derivatives for ganciclovir selection, or bacterial purine nucleoside phosphorylase gene for 6-methylpurine selection (Gadi et al., 7 *Gene Ther*. 1738-1743 (2000)). A nucleic acid sequence encoding a selection marker or the cloning site may be upstream or downstream of a nucleic acid sequence

encoding a polypeptide of interest or cloning site. .

Suitable vectors are known; many are commercially available for generating recombinant constructs. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene, La Jolla, Calif., USA); pTrc99A, pKK223-3, pKK233-3, pDR540, and pRIT5 (Pharmacia, Uppsala, Sweden). Eukaryotic: pWLneo, pSV2cat, pOG44, PXR1, pSG (Stratagene), pSVK3, pBPV, pMSG and pSVL (Pharmacia). Transposon vectors: Sleeping Beauty transposon and PiggyBac transposon.

In some embodiments, the vector is a viral vector. Viral vectors are derived from naturally occurring virus genomes, which typically are modified to be replication incompetent, e.g. non-replicating. Non-replicating viruses require the provision of proteins in trans for replication. Typically, those proteins are stably or transiently expressed in a viral producer cell line, thereby allowing replication of the virus. The viral vectors are, thus, typically infectious and non-replicating. Viral vectors may be adenovirus vectors, adeno-associated virus (AAV) vectors (e.g., AAV type 5 and type 2), alphavirus vectors (e.g., Venezuelan equine encephalitis virus (VEE), Sindbis virus (SIN), Semliki forest virus (SFV), and VEE-SIN chimeras), herpes virus vectors (e.g. vectors derived from cytomegaloviruses, like rhesus cytomegalovirus (RhCMV)), arena virus vectors (e.g. lymphocytic choriomeningitis virus (LCMV) vectors), measles virus vectors, pox virus vectors (e.g., vaccinia virus, modified vaccinia virus Ankara (MVA), NYVAC (derived from the Copenhagen strain of vaccinia), and avipox vectors: canarypox (ALVAC) and fowlpox (FPV) vectors), vesicular stomatitis virus vectors, retrovirus vectors, lentivirus vectors, viral like particles, baculoviral vectors and bacterial spores.

Adenovirus vectors

Adenovirus vectors 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.

Human adenovirus vectors 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).

Great ape adenovirus vectors 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, ChAdI7, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd55, ChAd63, ChAd73, ChAd82, ChAd83, ChAd146, ChAd147, PanAd1, PanAd2, or PanAd3.

Adenovirus vectors 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. Vectors based on Ad26 are described for example, in Int. Pat. Publ. No. WO2007/104792. Vectors based on Ad35 are described for example in U.S. Pat. No. 7,270,811 and Int. Pat. Publ. No. WO2000/70071. Vectors based on 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. Vectors based on PanAd1, PanAd2, PanAd3, ChAd55, ChAd73, ChAd83, ChAd146, and ChAd147 are described in Int. Pat. Publ. No. WO2010/086189.

Adenovirus vectors 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 El 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. Adenovirus vectors 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 vector of the disclosure may comprise a functional deletion or a complete removal of the E1 region and at least part of the E3 region. The adenovirus vector of the disclosure 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 El, e.g. 911 or PER.C6 cells (see, e.g., U.S. Pat. No. 5,994,128), El -transformed amniocytes (See, e.g., EP 1230354), E 1-transformed A549 cells (see e.g. Int. Pat. Publ. No. WO1998/39411, U.S. Pat. No. 5,891,690). Exemplary vectors that may be used are 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, provided that E4 open reading frame 6/7 is not deleted (see e.g. U.S. Pat. No. 9,750,801)

In some embodiments, the adenovirus vector is a human adenovirus (Ad) vector. In some embodiments, the Ad vector is derived from Ad5. In some embodiments, the Ad vector is

derived from Ad11. In some embodiments, the Ad vector is derived from Ad26. In some embodiments, the Ad vector is derived from Ad34. In some embodiments, the Ad vector is derived from Ad35. In some embodiments, the Ad vector is derived from Ad48. In some embodiments, the Ad vector is derived from Ad49. In some embodiments, the Ad vector is derived from Ad50.

In some embodiments, the adenovirus vector is a great ape adenovirus (GAd) vector. In some embodiments, the GAd vector is derived from GAd20. In some embodiments, the GAd vector is derived from GAd19. In some embodiments, the GAd vector is derived from GAd21. In some embodiments, the GAd vector is derived from GAd25. In some embodiments, the GAd vector is derived from GAd26. In some embodiments, the GAd vector is derived from GAd27. In some embodiments, the GAd vector is derived from GAd28. In some embodiments, the GAd vector is derived from GAd29. In some embodiments, the GAd vector is derived from GAd30. In some embodiments, the GAd vector is derived from GAd31. In some embodiments, the GAd vector is derived from ChAd4. In some embodiments, the GAd vector is derived from ChAd5. In some embodiments, the GAd vector is derived from ChAd6. In some embodiments, the GAd vector is derived from ChAd7. In some embodiments, the GAd vector is derived from ChAd8. In some embodiments, the GAd vector is derived from ChAd9. In some embodiments, the GAd vector is derived from ChAd20. In some embodiments, the GAd vector is derived from ChAd22. In some embodiments, the GAd vector is derived from ChAd24. In some embodiments, the GAd vector is derived from ChAd26. In some embodiments, the GAd vector is derived from ChAd30. In some embodiments, the GAd vector is derived from ChAd31. In some embodiments, the GAd vector is derived from ChAd32. In some embodiments, the GAd vector is derived from ChAd33. In some embodiments, the GAd vector is derived from ChAd37. In some embodiments, the GAd vector is derived from ChAd38. In some embodiments, the GAd vector is derived from ChAd44. In some embodiments, the GAd vector is derived from ChAd55. In some embodiments, the GAd vector is derived from ChAd63. In some embodiments, the GAd vector is derived from ChAd68. In some embodiments, the GAd vector is derived from ChAd73. In some embodiments, the GAd vector is derived from ChAd82. In some embodiments, the GAd vector is derived from ChAd83.

The polypeptide or the heterologous polypeptide of the disclosure may be inserted into a site or region (insertion region) in the vector that does not affect virus viability of the resultant recombinant virus. The polypeptide or the heterologous polypeptide of the disclosure 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 polypeptide or the heterologous polypeptide of the disclosure in the mammalian host cells that the vector is

being prepared for use may be operatively linked to the polypeptide or the heterologous polypeptide of the disclosure. "**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.

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 adenoviral vectors 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.

Poxvirus vectors

Poxvirus (*Poxviridae*) vectors 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).

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.

Vectors and viruses of the disclosure 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.

"**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 vector is derived from MVA 476 MG/14/78 . In some embodiments, the MVA vector is derived from MVA-571. In some embodiments, the MVA vector is derived from MVA-572. In some embodiments, the MVA vector is derived from MVA-574. In some embodiments, the MVA vector is derived from MVA-575. In some embodiments, the MVA vector is derived from MVA-BN.

The polynucleotide or the heterologous polynucleotide of the disclosure may be inserted into a site or region (insertion region) in the MVA vector that does not affect virus 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 virus 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.

Recombinant poxviral particles such as rMVA are 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. rMVA 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).

Other viral vectors and recombinant viruses

Other viral vectors include vectors derived from human adeno-associated viruses, such as AAV-2 (adeno-associated virus type 2). An attractive feature of AAV vectors is that they do not express any viral genes. The only viral DNA sequences included in the AAV vectors 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, AAV vectors 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. AAV vectors 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 Ad5. Vectors 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).

Retroviral vectors 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 vectors 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 retroviral vector 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 retroviral vector. 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 polynucleotides of the disclosure 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 retroviral vector 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).

Regulatory elements

The polynucleotide or the heterologous polynucleotide of the disclosure may be operably linked to one or more regulatory elements in the vector. The regulatory elements may comprise promoters, enhancers, polyadenylation signals, repressors and the like.

Some of the commonly used enhancer and promoter sequences in expression vectors and viral vectors are, for example, human cytomegalovirus (hCMV), vaccinia P7.5 early/late promoter, CAG, SV40, mouse CMV (mCMV), EF-l and hPGK promoters. Due to its high potency and moderate size of ca. 0.8 kB, the hCMV promoter is one of the most commonly used of these promoters. The hPGK promoter is characterized by a small size (ca. 0.4 kB), but it is less potent than the hCMV promoter. On the other hand, the CAG promoter consisting of a cytomegalovirus early enhancer element, promoter, first exon and intron of chicken beta-actin gene, and splice acceptor of the rabbit beta-globin gene, can direct very potent gene expression that is comparable to the hCMV promoter, but its large size makes it less suitable in viral vectors where space constraints can be a significant concern, e.g., in adenoviral vectors (AdV), adeno-associated viral vectors (AAV) or lentiviral vectors (LVs).

Additional promoters that may be used are Aotine Herpesvirus 1 major immediate early promoter (AoHV-1 promoter) described in Int. Pat. Publ. No. WO2018/146205. The promoter may be operably coupled to a repressor operator sequence, to which a repressor protein can bind in order to repress expression of the promoter in the presence of the repressor protein. In certain embodiments, the repressor operator sequence is a TetO sequence or a CuO sequence (see e.g. US9790256).

In certain cases, it may be desirable to express at least two separate polypeptides from the same vector. In this case each polynucleotide may be operably linked to the same or different promoter and/or enhancer sequences, or well-known bicistronic expression systems for example by utilizing internal ribosome entry site (IRES) from encephalomyocarditis virus may be used. Alternatively, bidirectional synthetic promoters may be used, such as a hCMV-rhCMV promoter and other promoters described in Int. Pat. Publ. No. WO2017/220499.

Polyadenylation signals may be derived from SV40 or bovine growth hormone (BGH).

The vectors of the disclosure may be generated using known techniques.

Exemplary regulatory elements that may be included into the vector comprise vaccinia P7.5 early/late promoter (SEQ ID NO: 630), TetOCMV promoter (SEQ ID NO: 628), BGH poly A site (SEQ ID NO: 629), T cell enhancer (SEQ ID NO: 546).

The disclosure also provides an isolated viral vector comprising a heterologous polynucleotide encoding a heterologous polypeptide comprising two 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 and 447, and fragments thereof.

In some embodiments, the viral vector comprises a heterologous polynucleotide comprising two 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 and 458, and fragments thereof.

The disclosure also provides an isolated viral vector comprising a heterologous polynucleotide encoding a heterologous polypeptide comprising two 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 and 177, and fragments thereof.

In some embodiments, the viral vector comprises a heterologous polynucleotide comprising two 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 and 178, and fragments thereof.

In some embodiments, the viral vector comprises a heterologous polynucleotide comprising two 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 and 499.

In some embodiments, the viral vector comprises a heterologous polynucleotide comprising two 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 and 540.

In some embodiments, the viral vector is an adenovirus vector.

In some embodiments, the viral vector is derived from a human adenovirus.

In some embodiments, the viral vector is derived from Ad26.

In some embodiments, the viral vector is derived from a great ape adenovirus.

In some embodiments, the vial vector is derived from GAd20.

In some embodiments, the viral vector is derived from ChAd20.

In some embodiments, the viral vector is a poxvirus vector.

In some embodiments, the viral vector is derived from modified vaccinia Ankara (MVA).

In some embodiments, the viral vector is derived from GAd19.

In some embodiments, the viral vector is derived from GAd21.

In some embodiments, the viral vector is derived from GAd25.

In some embodiments, the viral vector is derived from GAd26.

In some embodiments, the viral vector is derived from GAd27.

In some embodiments, the viral vector is derived from GAd28.

In some embodiments, the viral vector is derived from GAd29.

In some embodiments, the viral vector is derived from GAd30.

In some embodiments, the viral vector is derived from GAd31.

The disclosure also provides a recombinant human adenovirus serotype 26 (rAd26) comprising a viral vector comprising a heterologous polynucleotide encoding a heterologous polypeptide comprising two 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 and 447, and fragments thereof.

In some embodiments, the rAd26 comprises a viral vector comprising two 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 and 458, and fragments thereof.

The disclosure also provides a rAd26 comprising a heterologous polynucleotide encoding a heterologous polypeptide comprising two 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 and 177, and fragments thereof.

In some embodiments, the rAd26 comprises a heterologous polynucleotide comprising two 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 and 178, and fragments thereof.

In some embodiments, the rAd26 comprises a heterologous polynucleotide comprising two 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 and 499, and fragments thereof.

In some embodiments, the rAd26 comprises a heterologous polynucleotide comprising two 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 and 540, and fragments thereof.

The disclosure also provides a recombinant great ape adenovirus (rGAd) comprising a viral vector comprising a heterologous polynucleotide encoding a heterologous polypeptide

comprising two 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 and 447, and fragments thereof.

In some embodiments, the rGAd comprises a viral vector comprising a heterologous polynucleotide comprising two 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 and 458, and fragments thereof.

The disclosure also provides a rGAd comprising a heterologous polynucleotide encoding a heterologous polypeptide comprising two 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 and 177, and fragments thereof.

In some embodiments, the rGAd comprises a heterologous polynucleotide comprising two 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 and 178, and fragments thereof.

In some embodiments, the rGAd comprises a heterologous polynucleotide comprising two 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 and 499, and fragments thereof.

In some embodiments, the rGAd comprises a heterologous polynucleotide comprising two 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 and 540, and fragments thereof.

The disclosure also provides a recombinant modified vaccinia Ankara (rMVA) comprising a viral vector comprising a heterologous polynucleotide encoding a heterologous polypeptide comprising two 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 and 447, and fragments thereof.

In some embodiments, the rMVA comprises a viral vector comprisinga heterologous polypeptide comprising two 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 and 458, and fragments thereof.

The disclosure also provides a rMVA comprising a heterologous polynucleotide encoding a heterologous polypeptide comprising two 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 and 177, and fragments thereof.

In some embodiments, the rMVA comprises a heterologous polynucleotide comprising two 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 and 178, and fragments thereof.

In some embodiments, the rMVA comprises a heterologous polynucleotide comprising two 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 and 499, and fragments thereof.

In some embodiments, the rMVA comprises a heterologous polynucleotide comprising two 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 and 540, and fragments thereof.

Cells of the disclosure

The disclosure also provides a cell comprising or transduced with one or more vectors of the disclosure or one or more recombinant viruses of the disclosure. In some embodiments, the cell is a host cell.

Suitable cells include prokaryotic and eukaryotic cells, e.g., mammalian cells, yeast, fungi and bacteria (such as *E. coli*), such as Hek 293, CHO, PER.C6, PER.C6 TetO or chicken embryonic fibroblast (CEF) cells. The cell can be used *in vitro*, such as for research or for production of the polypeptides or viruses, or the cell can be used *in vivo*. In some embodiments, the cell is a muscle cell. In some embodiments, the cell is an antigen presenting cell (APC). Suitable antigen presenting cells include dendritic cells, B lymphocytes, monocytes and macrophages.

"**Transduced**", "**transformed**", or "**transfected**" refers to introduction of polynucleotides, heterologous polynucleotides or vectors of the disclosure inside a cell. The

introduced polynucleotide may or may not be integrated into chromosomal DNA making up the genome of the cell. "**Cell**" refers to the original cell and any progeny or clonal cell line established from the original cell.

The cells that are transfected with the polynucleotides or vectors of the disclosure may typically be obtained through cell culture repositories such as ATCC. APCs may be obtained from the peripheral blood using leukopheresis and "FICOLL/HYPAQUE" density gradient centrifugation (stepwise centrifugation through Ficoll and discontinuous Percoll density gradients). APCs may be isolated, cultured and engineered using known methods. For example, immature and mature dendritic cells may be generated from peripheral blood mononuclear cells (PBMCs) using known methods. In an exemplary method, isolated PBMCs are pre -treated to deplete T- and B-cells by means of an immunomagnetic technique. Lymphocyte -depleted PBMC are then cultured for in RPMI medium 9 e.g., about 7 days), supplemented with human plasma (preferably autologous plasma) and GM-CSF/IL-4, to generate dendritic cells. Dendritic cells are nonadherent when compared to their monocyte progenitors. Thus, on approximately day 7, non-adherent cells are harvested for further processing. The dendritic cells derived from PBMC in the presence of GM-CSF and IL-4 are immature, in that they can lose the nonadherence property and revert back to macrophage cell fate if the cytokine stimuli are removed from the culture. The dendritic cells in an immature state are effective in processing native protein antigens for the MHC class II restricted pathway (Romani, et al., J. Exp. Med. 169: 1169, 1989). Further maturation of cultured dendritic cells is accomplished by culturing for 3 days in a macrophage -conditioned medium (CM), which contains the necessary maturation factors. Mature dendritic cells are less able to capture new proteins for presentation but are much better at stimulating resting T cells (both CD4 and CD8) to grow and differentiate. Mature dendritic cells can be identified by their change in morphology, such as the formation of more motile cytoplasmic processes; by their nonadherence; by the presence of at least one of the following markers: CD83, CD68, HLA-DR or CD86; or by the loss of Fc receptors such as CD115 (reviewed in Steinman, Annu. Rev. Immunol. 9: 271, 1991). Mature dendritic cells can be collected and analyzed using typical cytofluorography and cell sorting techniques and devices, such as FACScan and FACStar. Primary antibodies used for flow cytometry are those specific to cell surface antigens of mature dendritic cells and are commercially available. Secondary antibodies can be biotinylated Igs followed by FITC- or PE -conjugated streptavidin. The vectors and recombinant viruses of the disclosure can be introduced into cells including APCs using the methods known in the art, including, but not limited to, transfection, electroporation, fusion, microinjection, viral-based delivery, or cell-based delivery.

Vaccines and pharmaceutical compositions of the disclosure

The polypeptides or the heterologous polypeptides or fragments thereof, or the polynucleotides encoding them may be delivered into the subject utilizing any known delivery vehicle suitable for administering to the subject. It is expected that the polypeptides, the heterologous polypeptides or fragments thereof will be immunogenic in the subject regardless of the delivery vehicle used. It is expected that the various viral strains will be equally effective in mediating delivery of the polypeptides or the heterologous polypeptides in human subjects and therefore can be interchangeable. For example, it is expected that the various MVA strains disclosed herein would be equally efficient in potentiating immune responses against antigens encoded by the same transgene cassette. Similarly, it is expected that the various adenovirus strains would be interchangeable with no or minimal changes in their ability to potentiate immune responses against antigens encoded by the same transgene cassette. The polynucleotide may be DNA or RNA, or derivatives thereof. RNA may be in the form of oligonucleotide RNA, tRNA (transfer RNA), snRNA (small nuclear RNA), rRNA (ribosomal RNA), mRNA (messenger RNA), antisense RNA, siRNA (small interfering RNA), self-replicating RNA, ribozymes, chimeric sequences, or derivatives of these groups.

The disclosure also provides a vaccine comprising the polynucleotide of the disclosure.

In some embodiments, the polynucleotide is DNA.

In some embodiments, the polynucleotides is RNA.

In some embodiments, RNA is mRNA, or self-replicating RNA.

In some embodiments, RNA is self-replicating RNA.

The disclosure also provides a vaccine comprising the heterologous polynucleotide of the disclosure.

In some embodiments, the heterologous polynucleotide is DNA.

In some embodiments, the heterologous polynucleotides is RNA.

In some embodiments, RNA is mRNA, or self-replicating RNA.

In some embodiments, RNA is self-replicating RNA.

The disclosure also provides a vaccine comprising the polypeptide of the disclosure.

The disclosure also provides a vaccine comprising the heterologous polypeptide of the disclosure.

The disclosure also provides a vaccine comprising the vector of the disclosure.

The disclosure also provides a vaccine comprising the rAd26 of the disclosure.

The disclosure also provides a vaccine comprising the rMVA of the disclosure.

The disclosure also provides a vaccine comprising the rGAd of the disclosure.

The disclosure also provides a vaccine comprising the rGAd20 of the disclosure.

The disclosure also provides a vaccine comprising the ChAd20 of the disclosure.

The disclosure also provides a vaccine comprising the cell of the disclosure.

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 formulation 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 MgCl2, 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.

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.

"**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 or viral vectors described herein.

A pharmaceutical composition according to the disclosure may in certain embodiments be the vaccine of the disclosure.

Similarly, the polynucleotides, the heterologous polynucleotides, the polypeptides and the heterologous polypeptides of the disclosure may be formulated into pharmaceutical compositions comprising the polynucleotides, the heterologous polynucleotides, the polypeptides and the heterologous polypeptides and the pharmaceutically acceptable excipients.

Kits

The disclosure also provides a kit comprising one or more vaccines of the disclosure. The disclosure also provides a kit comprising one or more recombinant viruses of the disclosure. The kits may be used to facilitate performing the methods described herein. In some embodiments, the kit further comprises reagents to facilitate entry of the vaccines of the disclosure into a cell, such as lipid-based formulations or viral packaging materials.

In some embodiments, the kit comprises the rAd26 of the disclosure. In some embodiments, the kit comprises the rMVA of the disclosure. In some embodiments, the kit comprises the rGAd of the disclosure. In some embodiments, the kit comprises the rAd26 of the disclosure and the rMVA of the disclosure. In some embodiments, the kit comprises the rGAd of the disclosure and the rMVA of the disclosure. In some embodiments, the kit comprises the rAd26 of the disclosure and the rGAd of the disclosure. In some embodiments, the kit comprises one or more polynucleotides of the disclosure. In some embodiments, the kit comprises one or more heterologous polynucleotides of the disclosure. In some embodiments, the kit comprises one or more polypeptides of the disclosure. In some embodiments, the kit comprises one or more heterologous polypeptides of the disclosure. In some embodiments, the kit comprises one or more vectors of the disclosure. In some embodiment, the kit comprises one or more cells of the disclosure.

Non-viral delivery vehicles

Nanoparticles

The polypeptides or the heterologous polypeptides or fragments thereof, the polynucleotides encoding them or vectors comprising the polynucleotides of the disclosure may be attached to nanoparticles for delivery to a subject. Delivery of the polypeptides or the heterologous polypeptides or fragments thereof , the polynucleotides encoding them or the vectors comprising the 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.

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).

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 polypeptides or the heterologous polypeptides or fragments thereof of the disclosure 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.

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.

The polynucleotides, the heterologous polynucleotides, the polypeptides and the heterologous 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).

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).

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 l50nm, such as about 20 to about 40nm, about 30 to about l40nm, about 40 to about l30nm, about 50 to about l20nm, about 60 to about 110nm, about 70 to about 100nm, or about 80 to about 90nm 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.

The nanoparticles may contain replicons that encode the polypeptides or the heterologous 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.

"**RNA replicon**" (or "self-replication RNA") refer 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 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, WO2019143949A2,

Other molecules suitable for complexing with the polynucleotides, the heterologous polynucleotides, the polypeptides and the heterologous 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).

Prostate neoantigen/HLA complexes

The disclosure also provides a protein complex comprising a prostate neoantigen and HLA. The disclosure also provides a protein complex comprising a fragment of the prostate neoantigen and HLA. The disclosure also provides a protein complex comprising a variant of the prostate neoantigen and HLA. The disclosure also provides a protein complex comprising a variant of a fragment of the prostate neoantigen and HLA.

In some embodiments, the prostate neoantigen comprises the polypeptide 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 or 447.

In some embodiments, the fragment of the prostate neoantigen comprises an amino acid sequences 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 and 621.

In some embodiments, the fragment of the prostate neoantigen comprises an amino acid sequence 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.

In some embodiments, HLA is class I HLA. In some embodiments, HLA is class II HLA. In some embodiments, HLA is HLA-A. In some embodiments, HLA is HLA-B. In some embodiments, HLA is HLA-C. In some embodiments, HLA is HLA-DP. In some embodiments,

HLA is HLA-DQ. In some embodiments, HLA is HLA-DR. In some embodiments, HLA is HLA-A*01:01, A*02:01, A*03:01, A*24:02, B*07:02 or B*08:01.

The complex of the prostate neoantigen and HLA may be used to for example isolate cognate T cells *in vitro* or *in vivo*. The complex of the prostate neoantigen and HLA may also be conjugated to a detectable label and used as a detection agent to detect, visualize or isolate cognate TCR or T cells expressing the cognate TCR. The complex of the prostate neoantigen and HLA may also be conjugated to a cytotoxic agent and used to deplete or reduce the number of cells expressing the cognate TCR. The complex may be in its native configuration or alternatively the prostate neoantigen and/or the HLA may be engineered.

Engineering concepts include covalent coupling of the peptide to the HLA, for example by using covalent linkers that may be cleavable. The prostate neoantigen and HLA complex may be a monomer or a multimer. The prostate neoantigen and HLA complex may be coupled to a toxin or a detection agent. Various engineering concepts include expressing the complex as a covalent prostate neoantigen- β2-α2-α1-β1 chain or prostate neoantigen-β chain, *e.g.* as a soluble complex. Linkers which are at least 15 amino acids long may be used between the prostate neoantigen and the HLA. Alternatively, the complex may be expressed as covalently coupled prostate neoantigen-single chain β1-α1. The prostate neoantigen/HLA complex may also be expressed as a full length HLAαβ chains to which the prostate neoantigen is covalently coupled to the N-terminus of the α chain or alternatively the prostate neoantigen is associated with the αβ chain via non-covalent interactions. Various expression formats are disclosed in US5976551, US5734023, US5820866, US7141656B2, US6270772B1 and US7074905B2. Additionally the HLA may be expressed as a single chain construct which is mutated at α1 chain or stabilized via disulfide bonds via α2 and β2 domains as described in US8377447B2 and US8828379B2. The prostate neoantigen or fragment thereof may be coupled to the HLA via light sensitive or periodate sensitive cleavable linkers as described in US9079941B2. The prostate neoantigen/HLA complexes may be engineered into multimeric format. Multimeric formats may be generated by incorporating a reactive side chain to the C-terminus of the HLA α or β chain to facilitate cross-linking of two or more prostate neoantigen/HLA complexes, as described in US7074904B2. Alternatively, a biotinylation recognition sequence BirA may be incorporated to the C-terminus of the HLA α or β chain which is subsequently biotinylated and the multimer is formed by binding to avidin/streptavidin as described in US563536. Multimeric prostate neoantigen/HLA complexes may further be generated utilizing Fc fusions, coupling the prostate neoantigen/HLA complexes in dextran carriers, oligomerizing the via coiled-coil domains, utilizing additional biotinylation peptides or conjugating the prostate neoantigen/HLA complexes onto nanoparticles or chelate carrier as is described in US6197302B1, US6268411B1,

US20150329617A1, EP1670823B1, EP1882700B1, EP2061807B1, US20120093934A1, US20130289253A1, US20170095544A1, US20170003288A1 and WO2017015064A1.

Proteinaceous molecules that bind the prostate neoantigens or prostate neoantigen/HLA complexes

The disclosure also provides a proteinaceous molecule comprising an antigen binding domain that specifically binds the polypeptide 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 or 447, or fragment thereof. The proteinaceous molecule has insubstantial binding to a wild-type protein the neoantigen is derived from.

The disclosure also provides a proteinaceous molecule comprising an antigen binding domain that specifically binds a prostate neoantigen/HLA complex, wherein the prostate neoantigen comprises a polypeptide 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 or 447, or fragments thereof.

Exemplary fragments comprise amino acid sequences 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.

Other exemplary fragments comprise amino acid sequences 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.

HLA may comprise class I or class II.

HLA may comprise HLA-A, HLA-B or HLA-C.

HLA may comprise HLA-DP, HLA-DQ or HLA-DR.

HLA may comprise class I alleles HLA-A*01:01, A*02:01, A*03:01, A*24:02, B*07:02 or B*08:01.

In some embodiments, the proteinaceous molecule is an antigen binding fragment of an antibody.

In some embodiments, the proteinaceous molecule is a multispecific molecule. In some embodiments, the proteinaceous molecule is a bispecific molecule. In some embodiments, the proteinaceous molecule is a trispecific molecule. In some embodiments, the multispecific molecule binds two or more distinct prostate neoantigens. In some embodiments, the multispecific molecule binds a prostate neoantigen and a T cell receptor (TCR) complex. In some embodiments, the multispecific molecule binds two or more distinct prostate neoantigens and a T cell receptor (TCR) complex.

In some embodiments, the proteinaceous molecule is an antibody.

In some embodiments, the proteinaceous molecule is a multispecific antibody. In some embodiments, the proteinaceous molecule is a bispecific antibody. In some embodiments, the proteinaceous molecule is a trispecific antibody. In some embodiments, the proteinaceous molecule is a T cell redirecting molecule.

In instances where the prostate neoantigen of the disclosure is part of an extracellular domain of a protein, the prostate neoantigen may be used as a tumor associated antigen for recruiting T cells to tumors or targeting CAR-T and other cellular therapies to tumor utilizing antigen binding domains that selectively bind the prostate neoantigen on tumor cells.

In instances in which the prostate neoantigen is part of an intracellular domain, antigen binding domains having the ability to be delivered into intracellular compartments conjugated to cytotoxic agent or a therapeutic agent may be used as therapeutics. Alternatively, cells engineered to express cognate TCR which bind the prostate neoantigen/HLA complex may be used as therapeutics.

In some embodiments, the proteinaceous molecule is an alternative scaffold.

In some embodiments, the proteinaceous molecule is a chimeric antigen receptor (CAR).

In some embodiments, the proteinaceous molecule is a T cell receptor (TCR).

Binding of the proteinaceous molecule to the prostate neoantigen or the prostate neoantigen/HLA complex of the disclosure may be determined experimentally using any suitable method. Such methods may utilize ProteOn XPR36, Biacore 3000 or KinExA instrumentation, ELISA or competitive binding assays known to those skilled in the art. The measured binding may vary if measured under different conditions (e.g., osmolarity, pH). Thus, measurements of affinity and other binding parameters (e.g., K_D, K_{on}, K_{off}) are typically made with standardized conditions and a standardized buffer, such as the buffer described herein**.** Skilled in the art will appreciate that the internal error for affinity measurements for example using Biacore 3000 or ProteOn (measured as standard deviation, SD) may typically be within 5-33% for measurements within the typical limits of detection. "**Insubstantial**" refers to binding that is 100-fold less when compared to the measured binding of the proteinaceous molecule to the prostate neoantigen of the disclosure. The proteinaceous molecule of the disclosure may further be characterized for their activity and function using know methods and those described herein, such as ability of the proteinaceous molecules to kill cells expressing the prostate neoantigens or prostate neoantigen/HLA complexes.

Antibodies and antigen binding domains

Antibodies and antigen binding domains that specifically bind the prostate neoantigens or the prostate neoantigen/HLA complexes may be generated using known methods. Such

antibodies may include immunoglobulin molecules of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2) or subclass.

For example, the hybridoma method of Kohler and Milstein, *Nature* 256:495, 1975 may be used to generate monoclonal antibodies. In the hybridoma method, a mouse or other host animal, such as a hamster, rat or monkey, is immunized with one or more prostate neoantigens, or prostate neoantigen/HLA complexes followed by fusion of spleen cells from immunized animals with myeloma cells using standard methods to form hybridoma cells (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986)). Colonies arising from single immortalized hybridoma cells are screened for production of antibodies with desired properties, such as specificity of binding and affinity for the prostate neoantigen of the disclosure.

Various host animals may be used to produce the antibodies. For example, Balb/c mice, rats or chickens may be used to generate antibodies containing the VH/VL pair, and llama and alpaca may be used to generated heavy chain only (VHH) antibodies using standard immunization protocols. The antibodies made in non-human animals may be humanized using various technologies to generate more human-like sequences.

Exemplary humanization techniques including selection of human acceptor frameworks are known and include CDR grafting (U.S. Patent No. 5,225,539), SDR grafting (U.S. Patent No. 6,818,749), resurfacing (Padlan, (1991) *Mol Immunol* 28:489-499), Specificity Determining Residues Resurfacing (U.S. Patent Publ. No. 2010/0261620), human framework adaptation (U.S. Patent No. 8,748,356) and superhumanization (U.S. Patent No. 7,709, 226). In these methods, CDRs of parental antibodies are transferred onto human frameworks that may be selected based on their overall homology to the parental frameworks, based on similarity in CDR length, or canonical structure identity, or any combination thereof.

Humanized antibodies may be further optimized to improve their selectivity or affinity to a desired antigen by incorporating altered framework support residues to preserve binding affinity (backmutations) by techniques such as those described in Int. Patent Publ. Nos. WO1090/007861 and WO1992/22653, or by introducing variation at any of the CDRs for example to improve affinity of the antibody.

Transgenic animals, such as mice or rats carrying human immunoglobulin (Ig) loci in their genome may be used to generate human antibodies against the prostate neoantigens or the prostate neoantigen/HLA complexes, and are described in for example U.S. Patent No. 6,150,584, Int. Patent Publ. No. WO99/45962, Int. Patent Publ. Nos. WO2002/066630, WO2002/43478, WO2002/043478 and WO1990/04036, Lonberg *et al* (1994) *Nature* 368:856-9; Green *et al* (1994) *Nature Genet.* 7:13-21; Green & Jakobovits (1998) *Exp. Med.* 188:483-95; Lonberg and Huszar (1995) *Int Rev Immunol* 13:65-93; Bruggemann *et al.,* (1991) *Eur J*

Immunol 21:1323- 1326; Fishwild *et al.,* (1996) *Nat Biotechnol* 14:845-851; Mendez *et al.,* (1997) *Nat Genet* 15:146-156; Green (1999) *J Immunol Methods* 231:11-23; Yang *et al.,* (1999) *Cancer Res* 59:1236-1243; Brüggemann and Taussig (1997) *Curr Opin Biotechnol* 8:455-458. The endogenous immunoglobulin loci in such animal may be disrupted or deleted, and at least one complete or partial human immunoglobulin locus may be inserted into the genome of the animal using homologous or non-homologous recombination, using transchromosomes, or using minigenes. Companies such as Regeneron (http://_www_regeneron_com), Harbour Antibodies (http://_www_harbourantibodies_com), Open Monoclonal Technology, Inc. (OMT) (http://_www_omtinc_net), KyMab (http://_www_kymab_com), Trianni (http://_www.trianni_com) and Ablexis (http://_www_ablexis_com) may be engaged to provide human antibodies directed against a selected antigen using technologies as described above.

Human antibodies may be selected from a phage display library, where the phage is engineered to express human immunoglobulins or portions thereof such as Fabs, single chain antibodies (scFv), domain antibodies or unpaired or paired antibody variable regions (Knappik *et al.,* (2000) *J Mol Biol* 296:57-86; Krebs *et al.*, (2001) *J Immunol Meth* 254:67-84; Vaughan *et al.,* (1996) *Nature Biotechnology* 14:309-314; Sheets *et al.*, (1998) *PITAS (USA)* 95:6157-6162; Hoogenboom and Winter (1991) *J Mol Biol* 227:381; Marks *et al.*, (1991) *J Mol Biol* 222:581). The antibodies of the disclosure may be isolated for example from phage display library expressing antibody heavy and light chain variable regions as heterologous polypeptides with bacteriophage pIX coat protein as described in Shi *et al.,* (2010) *J Mol Biol* 397:385-96, and Int. Patent Publ. No. WO09/085462). The libraries may be screened for phage binding to the prostate neoantigen or the prostate neoantigen/HLA complex and the obtained positive clones may be further characterized, the Fabs isolated from the clone lysates, and expressed as full length IgGs. Such phage display methods for isolating human antibodies are described in for example: U.S. Patent Nos. 5,223,409, 5,403,484, 5,571,698, 5,427,908, 5, 580,717, 5,969,108, 6,172,197, 5,885,793; 6,521,404; 6,544,731; 6,555,313; 6,582,915 and 6,593,081. The antibodies may further be tested for their binding to the HLA/neoantigen complex or to the neoantigen alone.

Preparation of immunogenic antigens and monoclonal antibody production may be performed using any suitable technique, such as recombinant protein production or by chemical synthesis of peptides. The immunogenic antigens may be administered to an animal in the form of purified protein, or protein mixtures including whole cells or cell or tissue extracts, or the antigen may be formed *de novo* in the animal's body from nucleic acids encoding said antigen or a portion thereof.

Antigen binding domains that specifically bind the prostate neoantigen or prostate neoantigen/HLA complexes may also be derived from the antibodies described herein. Antigen

binding domains include single chain antibodies, Fab fragments, Fv fragments, single-chain Fv fragments (scFv), VHH domains, VH, VL, alternative scaffolds (e.g. non-antibody antigen binding domains), a divalent antibody fragment such as an (Fab)2′-fragment, F(ab′) fragments, disulfide-linked Fvs (sdFv), intrabodies, minibodies, diabodies, triabodies and decabodies.

Bispecific and multispecific antibodies that specifically bind the prostate neoantigen or prostate neoantigen/HLA complexes and a second antigen may be generated using known methods. The second antigen may be a T cell receptor complex (TCR complex). The second antigen may be CD3 within the TCR complex. The bispecific and multispecific antibodies that specifically bind the prostate neoantigen or prostate neoantigen/HLA complexes of the disclosure and the second antigen may be engineered into any multivalent format using any known antigen binding domains format that specifically bind the prostate neoantigens or prostate neoantigen/HLA complexes and the second antigen. The antigen binding domain that specifically bind the prostate neoantigen or prostate neoantigen/HLA complex may be conjugated to one or more Fc domains or fragment thereof, or optionally to other scaffolds such as half-life extending moieties including albumin, PEG or transferrin.

Multispecific antibodies that specifically bind two or more prostate neoantigens may provide a benefit in terms of improved specificity in targeting tumor cells expressing the prostate neoantigens.

The antigen binding domains that specifically bind the prostate neoantigen or prostate neoantigen/HLA complexes may be engineered into full length multispecific antibodies which are generated using Fab arm exchange, in which substitutions are introduced into two monospecific bivalent antibodies within the Ig constant region CH3 domain which promote Fab arm exchange *in vitro*. In the methods, two monospecific bivalent antibodies are engineered to have certain substitutions at the CH3 domain that promote heterodimer stability; the antibodies are incubated together under reducing conditions sufficient to allow the cysteines in the hinge region to undergo disulfide bond isomerization; thereby generating the bispecific antibody by Fab arm exchange. The incubation conditions may optimally be restored to non-reducing. Exemplary reducing agents that may be used are 2- mercaptoethylamine (2-MEA), dithiothreitol (DTT), dithioerythritol (DTE), glutathione, tris(2-carboxyethyl)phosphine (TCEP), L-cysteine and beta-mercaptoethanol, preferably a reducing agent selected from the group consisting of: 2-mercaptoethylamine, dithiothreitol and tris(2-carboxyethyl)phosphine. For example, incubation for at least 90 min at a temperature of at least 20°C in the presence of at least 25 mM 2-MEA or in the presence of at least 0.5 mM dithiothreitol at a pH of from 5-8, for example at pH of 7.0 or at pH of 7.4 may be used.

CH3 mutations that may be used include technologies such as Knob-in-Hole mutations (Genentech), electrostatically-matched mutations (Chugai, Amgen, NovoNordisk, Oncomed), the

Strand Exchange Engineered Domain body (SEEDbody) (EMD Serono), Duobody® mutations (Genmab), and other asymmetric mutations (e.g. Zymeworks).

Knob-in-hole mutations are disclosed for example in WO1996/027011 and include mutations on the interface of CH3 region in which an amino acid with a small side chain (hole) is introduced into the first CH3 region and an amino acid with a large side chain (knob) is introduced into the second CH3 region, resulting in preferential interaction between the first CH3 region and the second CH3 region. Exemplary CH3 region mutations forming a knob and a hole are T366Y/F405A, T366W/F405W, F405W/Y407A, T394W/Y407T, T394S/Y407A, T366W/T394S, F405W/T394S and T366W/T366S_L368A_Y407V.

Heavy chain heterodimer formation may be promoted by using electrostatic interactions by substituting positively charged residues on the first CH3 region and negatively charged residues on the second CH3 region as described in US2010/0015133, US2009/0182127, US2010/028637 or US2011/0123532.

Other asymmetric mutations that can be used to promote heavy chain heterodimerization are L351Y_F405A_Y407V/T394W, T366I_K392M_T394W/F405A_Y407V, T366L_K392M_T394W/F405A_Y407V, L351Y_Y407A/T366A_K409F, L351Y_Y407A/T366V_K409F, Y407A/T366A_K409F, or T350V_L351Y_F405A_Y407V/T350V_T366L_K392L_T394W as described in US2012/0149876 or US2013/0195849 (Zymeworks).

SEEDbody mutations involve substituting select IgG residues with IgA residues to promote heavy chai heterodimerization as described in US20070287170.

Other exemplary mutations that may be used are R409D_K370E/D399K_E357K, S354C_T366W/Y349C_ T366S_L368A_Y407V, Y349C_T366W/S354C_T366S_L368A_Y407V, T366K/L351D, L351K/Y349E, L351K/Y349D, L351K/L368E, L351Y_Y407A/T366A_K409F, L351Y_Y407A/T366V_K409F, K392D/D399K, K392D/ E356K, K253E_D282K_K322D/D239K_E240K_K292D, K392D_K409D/D356K_D399K as described in WO2007/147901, WO 2011/143545, WO2013157954, WO2013096291 and US2018/0118849.

Duobody® mutations (Genmab) are disclosed for example in US9150663 and US2014/0303356 and include mutations F405L/K409R, wild-type/F405L_R409K, T350I_K370T_F405L/K409R, K370W/K409R, D399AFGHILMNRSTVWY/K409R, T366ADEFGHILMQVY/K409R, L368ADEGHNRSTVQ/K409AGRH, D399FHKRQ/K409AGRH, F405IKLSTVW/K409AGRH and Y407LWQ/K409AGRH.

Additional bispecific or multispecific structures into which the antigen binding domains that specifically bind the prostate neoantigen or prostate neoantigen/HLA complexes can be incorporated include Dual Variable Domain Immunoglobulins (DVD) (Int. Pat. Publ. No.

WO2009/134776; DVDs are full length antibodies comprising the heavy chain having a structure VH1-linker-VH2-CH and the light chain having the structure VL1-linker-VL2-CL; linker being optional), structures that include various dimerization domains to connect the two antibody arms with different specificity, such as leucine zipper or collagen dimerization domains (Int. Pat. Publ. No. WO2012/022811, U.S. Pat. No. 5,932,448; U.S. Pat. No. 6,833,441), two or more domain antibodies (dAbs) conjugated together, diabodies, heavy chain only antibodies such as camelid antibodies and engineered camelid antibodies, Dual Targeting (DT)-Ig (GSK/Domantis), Two-in-one Antibody (Genentech), Cross-linked Mabs (Karmanos Cancer Center), mAb2 (F-Star) and CovX-body (CovX/Pfizer), IgG-like Bispecific (InnClone/Eli Lilly), Ts2Ab (MedImmune/AZ) and BsAb (Zymogenetics), HERCULES (Biogen Idec) and TvAb (Roche), ScFv/Fc Fusions (Academic Institution), SCORPION (Emergent BioSolutions/Trubion, Zymogenetics/BMS), Dual Affinity Retargeting Technology (Fc-DART) (MacroGenics) and Dual$(ScFv)_2$-Fab (National Research Center for Antibody Medicine--China), Dual-Action or Bis-Fab (Genentech), Dock-and-Lock (DNL) (ImmunoMedics), Bivalent Bispecific (Biotecnol) and Fab-Fv (UCB-Celltech). ScFv-, diabody-based, and domain antibodies, include but are not limited to, Bispecific T Cell Engager (BiTE) (Micromet), Tandem Diabody (Tandab) (Affimed), Dual Affinity Retargeting Technology (DART) (MacroGenics), Single-chain Diabody (Academic), TCR-like Antibodies (AIT, ReceptorLogics), Human Serum Albumin ScFv Fusion (Merrimack) and COMBODY (Epigen Biotech), dual targeting nanobodies (Ablynx), dual targeting heavy chain only domain antibodies.

Alternative scaffolds

Alternative scaffolds (also referred to as antibody mimetics) that specifically bind the prostate neoantigen or prostate neoantigen/HLA complexes may be generated using various scaffolds known in the art and described herein. Alternative scaffolds may be monobodies, designed to incorporate the fibronectin type III domain (Fn3) of fibronectin or tenascin as a protein scaffold (U.S. Pat. No. 6,673,901; U.S. Pat. No. 6,348,584) or synthetic FN3 domains such as tencon as described in U.S. Pat. Publ. No. 2010/0216708 and U.S. Pat. Pub. No. 2010/0255056. Additional alternative scaffolds comprise Adnectin™, an iMab, an Anticalin®, an EETI-II/AGRP, a Kunitz domain, a thioredoxin peptide aptamer, an Affibody®, a DARPin, an Affilin, a Tetranectin, a Fynomer, and an Avimer. Alternative scaffolds are single chain polypeptidic frameworks that contains a highly structured core associated with variable domains of high conformational tolerance allowing insertions, deletions, or other substitutions within the variable domains. Libraries introducing diversity to one or more variable domains, and in some instances to the structured core, may be generated using known protocols and the resulting libraries may be screened for binding to the neoantigen of the disclosure, and the identified

binders may be further characterized for their specificity using known methods. Alternative scaffold may be derived from Protein A, in particular, the Z-domain thereof (affibodies), ImmE7 (immunity proteins), BPTI/APPI (Kunitz domains), Ras-binding protein AF-6 (PDZ-domains), charybdotoxin (Scorpion toxin), CTLA-4, Min-23 (knottins), lipocalins (anticalins), neokarzinostatin, a fibronectin domain, an ankyrin consensus repeat domain, or thioredoxin (Skerra, A., "Alternative Non-Antibody Scaffolds for Molecular Recognition," *Curr. Opin. Biotechnol.* 18:295-304 (2005); Hosse et al., "A New Generation of Protein Display Scaffolds for Molecular Recognition," *Protein Sci.* 15:14-27 (2006); Nicaise et al., "Affinity Transfer by CDR Grafting on a Nonimmunoglobulin Scaffold," *Protein Sci.* 13:1882-1891 (2004); Nygren and Uhlen, "Scaffolds for Engineering Novel Binding Sites in Proteins," *Curr. Opin. Struc. Biol.* 7:463-469 (1997).

Chimeric antigen receptors (CAR)

CARs may be generated that bind the prostate neoantigens or the prostate neoantigen/HLA complex by incorporating an antigen binding domain that specifically binds the prostate neoantigens or the prostate neoantigen/HLA complex to the extracellular domain of the CAR. CARs are genetically engineered receptors. These engineered receptors can be readily inserted into and expressed by immune cells, including T cells in accordance with techniques known in the art. With a CAR, a single receptor can be programmed to both recognize a specific antigen and, when bound to that antigen, activate the immune cell to attack and destroy the cell bearing that antigen. When these antigens exist on tumor cells, an immune cell that expresses the CAR can target and kill the tumor cell.

The CAR typically comprises an extracellular domain that binds the antigen (e.g. the prostate neoantigen or the prostate neoantigen/HLA complex), an optional linker, a transmembrane domain, and a cytosolic domain comprising a costimulatory domain and/or a signaling domain.

The extracellular domain of the CAR may contain any polypeptide that specifically binds the desired antigen (e.g. prostate neoantigen). The extracellular domain may comprise a scFv, a portion of an antibody or an alternative scaffold. The CARs may also be engineered to bind two or more desired antigens that may be arranged in tandem and separated by linker sequences. For example, one or more domain antibodies, scFvs, llama VHH antibodies or other VH only antibody fragments may be organized in tandem via a linker to provide bispecificity or multispecificity to the CAR.

The transmembrane domain of the CAR may be derived from the transmembrane domain of CD8, an alpha, beta or zeta chain of a T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137,

CD154, KIRDS2, OX40, CD2, CD27, LFA-1 (CDI la, CD18), ICOS (CD278), 4-1 BB (CD137), 4-1 BBL, GITR, CD40, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRFI), CD160, CD1 9, IL2R beta, IL2R gamma, IL7R a, ITGA1, VLA1 , CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CDI Id, ITGAE, CD103, ITGAL, CDI la, LFA-1 , ITGAM, CDI lb, ITGAX, CDI lc, ITGB1, CD29, ITGB2, CD1 8, LFA-1, ITGB7, TNFR2, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRT AM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D, and/or NKG2C.

The intracellular costimulatory domain of CAR may be derived from the intracellular domains of one or more co-stimulatory molecules. Co-stimulatory molecules are well-known cell surface molecules other than antigen receptors or Fc receptors that provide a second signal required for efficient activation and function of T lymphocytes upon binding to antigen. Exemplary co-stimulatory domains that can be used in CARs are intracellular domains of 4-1BB, CD2, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD83, CD134 (OX40), CD150 (SLAMF1), CD152 (CTLA4), CD223 (LAG3), CD270 (HVEM), CD278 (ICOS), DAP10, LAT, NKD2C SLP76, TRIM, BTLA, GITR, CD226, HVEM, and ZAP70.

The intracellular signaling domain of the CAR may be derived from the signaling domains of for example CD3ζ, CD3ε, CD22, CD79a, CD66d, CD39 DAP10, DAP12, Fc epsilon receptor I gamma chain (FCER1G), FcR β, CD3δ, CD3γ, CD5, CD226, or CD79B. "**Intracellular signaling domain**" refers to the part of the CAR polypeptide that participates in transducing the message of effective CAR binding to a target antigen into the interior of the immune effector cell to elicit effector cell function, e.g., activation, cytokine production, proliferation and cytotoxic activity, including the release of cytotoxic factors to the CAR-bound target cell, or other cellular responses elicited following antigen binding to the extracellular CAR domain.

The optional linker of the CAR positioned between the extracellular domain and the transmembrane domain may be a polypeptide of about 2 to 100 amino acids in length. The linker may include or be composed of flexible residues such as glycine and serine so that the adjacent protein domains are free to move relative to one another. Longer linkers may be used when it is desirable to ensure that two adjacent domains do not sterically interfere with one another. Linkers may be cleavable or non-cleavable. Examples of cleavable linkers include 2A linkers (for example T2A), 2A-like linkers or functional equivalents thereof and combinations thereof. The linker may also be derived from a hinge region or portion of the hinge region of any immunoglobulin. Non-limiting examples of linkers include a part of human CD8α chain, partial

extracellular domain of CD28, FcyRIlla receptor, IgG, IgM, IgA, IgD, IgE, an Ig hinge, or functional fragment thereof.

Exemplary CARs that may be used are for example CAR that contains an extracellular domain that binds the prostate neoantigen of the disclosure, CD8 transmembrane domain and CD3ζ signaling domain. Other exemplary CARs contain an extracellular domain that binds the prostate neoantigen of the disclosure, CD8 or CD28 transmembrane domain, CD28, 41BB or OX40 costimulatory domain and CD3ζ signaling domain.

The CARs are generated by standard molecular biology techniques. The extracellular domain that binds the desired antigen may be derived from antibodies or their antigen binding fragments generated using the technologies described herein.

T cell receptor (TCR)

TCRs may be generated that bind the prostate neoantigen/HLA complexes. The TCRs may be identified based on T cell binding to the prostate neoantigen/HLA complex, isolating the T cell and sequencing the TCR expressed in the T cells. The identified TCRs may be identified from αβ T cells or γδ T cells. The identified TCRs may be further engineered to improve their affinity, stability, solubility or the like. TCRs may be affinity matured utilizing the same technologies utilized to affinity mature immunoglobulins. TCRs may be expressed as soluble TCRs which have been cysteine stabilized, they can be stabilized by engineering mutations onto α/β interaction surface, for example G192R on α chain and R208G on β chain. TCRs may also be stabilized by engineering cysteine residues which form disulfide bonds into TCR constant domain, by introducing mutations into the hydrophobic core, such as at positions 11, 13,19, 21, 53, 76, 89, 91 or 94 of α chain, utilizing domain swaps including swaps between α and β chain V domains, transmembrane domains or constant domains as described in US7329731, US7871817B2, US7569664, US9133264, US9624292, US20120252742A1, US2016/0130319, EP3215164A1, EP3286210A1, WO2017091905A1 or US9884075.

Cells expressing the CARs or the TCRs of the disclosure

Cells expressing the CARs or the TCRs that specifically bind the prostate neoantigens of the disclosure of the prostate neoantigen/HLA complexes of the disclosure are within the scope of the disclosure. The disclosure also provides isolated cells comprising the CAR of the disclosure or the TCR of the disclosure. In some embodiments, the isolated cells are transduced with the CAR or the TCR of the disclosure, resulting in constitutive expression of the CAR or the TCR of the disclosure on the surface of the cell. The cells expressing the CAR or the TCR of the disclosure may further be engineered to express one or more co-stimulatory molecules. Exemplary co-stimulatory molecules are CD28, ICOS, LIGHT, GITR, 4-1BB and OX40. The

cells expressing the CAR or the TCR of the disclosure may further be engineered to produce one or more cytokines or chemokines or proinflammatory mediators, such as TNFα, IFNγ, IL-2, IL-3, IL-6, IL-7, IL-11, IL-12, IL-15, IL-17 or IL-21. The cells may have their endogenous TCR locus and/or HLA locus inactivated using known gene editing technologies. In some embodiment, the cell comprising the CAR or the TCR of the disclosure is a T cell, a natural killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell (Treg), a human embryonic stem cell, a lymphoid progenitor cell, a T cell-precursor cell, a pluripotent stem cell or induced pluripotent stem cell (iPSC) from which lymphoid cells may be differentiated.

In some embodiments, the isolated cell comprising the CAR or the TCR of the disclosure is a T cell. The T cell may be any T cell, such as a cultured T cell, e.g., a primary T cell, or a T cell from a cultured T cell line, e.g., Jurkat, SupT1, etc., or a T cell obtained from a mammal. If obtained from a mammal, the T cell can be obtained from any source, including to bone marrow, blood, lymph node, thymus, or other tissues or fluids. T cells may also be enriched for or purified. The T cell may be a human T cell. The T cell may be a T cell isolated from a human. The T cell can be any type of T cell and may be of any developmental stage, including, $CD4^+CD8^+$ double positive T cells, $CD8^+$ T cells (*e.g.,* cytotoxic T cells), $CD4^+$ helper T cells, *e.g.,* Th1 and Th2 cells, peripheral blood mononuclear cells (PBMCs), peripheral blood leukocytes (PBLs), tumor infiltrating cells, memory T cells, naïve T cells, and the like. The T cell may be a $CD8^+$ T cell or a $CD4^+$ T cell. The T cell may be an αβ T cell or a γδ T cell.

In some embodiments, the isolated cell comprising the CAR of the disclosure or the TCR of the disclosure is a NK cell.

In some embodiments, the isolated cell comprising the CAR of the disclosure or the TCR of the disclosure is a αβ T cell.

In some embodiments, the isolated cell comprising the CAR of the disclosure or the TCR of the disclosure is a γδ T cell.

In some embodiments, the isolated cell comprising the CAR of the disclosure or the TCR of the disclosure is a $CD8^+$ cytotoxic T lymphocyte

In some embodiments, the isolated cell comprising the CAR of the disclosure or the TCR of the disclosure is a human embryonic stem cell.

In some embodiments, the isolated cell comprising the CAR of the disclosure or the TCR of the disclosure is a lymphoid progenitor cell.

In some embodiments, the isolated cell comprising the CAR of the disclosure or the TCR of the disclosure is a pluripotent stem cell.

In some embodiments, the isolated cell comprising the CAR of the disclosure or the TCR of the disclosure is an induced pluripotent stem cell (iPSC).

The cells of the disclosure may be generated by introducing a lentiviral vector comprising a desired CAR or TCR into the cells using known methods. The cells of the disclosure are able to replicate *in vivo* resulting in long-term persistence that can lead to sustained tumor control.

Conjugates with cytotoxic agents, drugs, detectable labels, and the like

The polypeptides, the heterologous polypeptide and the proteinaceous molecules binding them may be conjugated to a cytotoxic agent, therapeutics, detectable labels and the like. These molecules are referred herein to immunoconjugates. The immunoconjugates comprising the prostate neoantigens may be used to detect, deliver payload or kill cells expressing a HLA molecule that binds the prostate neoantigen. The immunoconjugates comprising the antibodies, antigen binding fragments or alternative scaffolds which specifically bind the prostate neoantigen or the prostate neoantigen/HLA complex may be used to detect, deliver payload or kill cells that express the prostate neoantigen on their surface in the context or a larger protein or in complex with HLA, or detect intracellular prostate neoantigens after lysis of the cells.

In some embodiments, the immunoconjugate comprises a detectable label.

In some embodiments, the immunoconjugate comprises a cytotoxic agent.

In some embodiments, the immunoconjugate comprises a therapeutic.

Detectable labels include compositions that can be visualized via spectroscopic, photochemical, biochemical, immunochemical, or chemical means. Detectable labels may also include cytotoxic agents, an cytotoxic agents may include detectable labels.

Exemplary detectable labels include radioactive isotopes, magnetic beads, metallic beads, colloidal particles, fluorescent dyes, electron-dense reagents, enzymes (for example, as commonly used in an ELISA), biotin, digoxigenin, haptens, luminescent molecules, chemiluminescent molecules, fluorochromes, fluorophores, fluorescent quenching agents, colored molecules, radioactive isotopes, scintillates, avidin, streptavidin, protein A, protein G, antibodies or fragments thereof, polyhistidine, Ni^{2+}, Flag tags, myc tags, heavy metals, enzymes, alkaline phosphatase, peroxidase, luciferase, electron donors/acceptors, acridinium esters, and colorimetric substrates.

A detectable label may emit a signal spontaneously, such as when the detectable label is a radioactive isotope. In other cases, the detectable label emits a signal as a result of being stimulated by an external field.

Exemplary radioactive isotopes may be γ-emitting, Auger-emitting, β-emitting, an alpha-emitting or positron-emitting radioactive isotope. Exemplary radioactive isotopes include ^{3}H, ^{11}C, ^{13}C, ^{15}N, ^{18}F, ^{19}F, ^{55}Co, ^{57}Co, ^{60}Co, ^{61}Cu, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{68}Ga, ^{72}As, ^{75}Br, ^{86}Y, ^{89}Zr, ^{90}Sr, ^{94m}Tc, ^{99m}Tc, ^{115}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{211}At, ^{212}Bi, ^{213}Bi, ^{223}Ra, ^{226}Ra, ^{225}Ac and ^{227}Ac.

Exemplary metal atoms are metals with an atomic number greater than 20, such as calcium atoms, scandium atoms, titanium atoms, vanadium atoms, chromium atoms, manganese atoms, iron atoms, cobalt atoms, nickel atoms, copper atoms, zinc atoms, gallium atoms, germanium atoms, arsenic atoms, selenium atoms, bromine atoms, krypton atoms, rubidium atoms, strontium atoms, yttrium atoms, zirconium atoms, niobium atoms, molybdenum atoms, technetium atoms, ruthenium atoms, rhodium atoms, palladium atoms, silver atoms, cadmium atoms, indium atoms, tin atoms, antimony atoms, tellurium atoms, iodine atoms, xenon atoms, cesium atoms, barium atoms, lanthanum atoms, hafnium atoms, tantalum atoms, tungsten atoms, rhenium atoms, osmium atoms, iridium atoms, platinum atoms, gold atoms, mercury atoms, thallium atoms, lead atoms, bismuth atoms, francium atoms, radium atoms, actinium atoms, cerium atoms, praseodymium atoms, neodymium atoms, promethium atoms, samarium atoms, europium atoms, gadolinium atoms, terbium atoms, dysprosium atoms, holmium atoms, erbium atoms, thulium atoms, ytterbium atoms, lutetium atoms, thorium atoms, protactinium atoms, uranium atoms, neptunium atoms, plutonium atoms, americium atoms, curium atoms, berkelium atoms, californium atoms, einsteinium atoms, fermium atoms, mendelevium atoms, nobelium atoms, or lawrencium atoms.

In some embodiments, the metal atoms may be alkaline earth metals with an atomic number greater than twenty.

In some embodiments, the metal atoms may be lanthanides.

In some embodiments, the metal atoms may be actinides.

In some embodiments, the metal atoms may be transition metals.

In some embodiments, the metal atoms may be poor metals.

In some embodiments, the metal atoms may be gold atoms, bismuth atoms, tantalum atoms, and gadolinium atoms.

In some embodiments, the metal atoms may be metals with an atomic number of 53 (i.e. iodine) to 83 (i.e. bismuth).

In some embodiments, the metal atoms may be atoms suitable for magnetic resonance imaging.

The metal atoms may be metal ions in the form of +1, +2, or +3 oxidation states, such as Ba^{2+}, Bi^{3+}, Cs^{+}, Ca^{2+}, Cr^{2+}, Cr^{3+}, Cr^{6+}, Co^{2+}, Co^{3+}, Cu^{+}, Cu^{2+}, Cu^{3+}, Ga^{3+}, Gd^{3+}, Au^{+}, Au^{3+}, Fe^{2+}, Fe^{3+}, F^{3+}, Pb^{2+}, Mn^{2+}, Mn^{3+}, Mn^{4+}, Mn^{7+}, Hg^{2+}, Ni^{2+}, Ni^{3+}, Ag^{+}, Sr^{2+}, Sn^{2+}, Sn^{4+}, and Zn^{2+}. The metal atoms may comprise a metal oxide, such as iron oxide, manganese oxide, or gadolinium oxide.

Suitable dyes include any commercially available dyes such as, for example, 5(6)-carboxyfluorescein, IRDye 680RD maleimide or IRDye 800CW, ruthenium polypyridyl dyes, and the like.

Suitable fluorophores are fluorescein isothiocyanate (FITC), fluorescein thiosemicarbazide, rhodamine, Texas Red, CyDyes (e.g., Cy3, Cy5, Cy5.5), Alexa Fluors (e.g., Alexa488, Alexa555, Alexa594; Alexa647), near infrared (NIR) (700-900 nm) fluorescent dyes, and carbocyanine and aminostyryl dyes.

The immunoconjugates comprising a detectable label may be used as an imaging agent.

In some embodiments, the cytotoxic agent is a chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

In some embodiments, the cytotoxic agent is daunomycin, doxorubicin, methotrexate, vindesine, bacterial toxins such as diphtheria toxin, ricin, geldanamycin, maytansinoids or calicheamicin. The cytotoxic agent may elicit their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition.

In some embodiments, the cytotoxic agent is an enzymatically active toxin such as diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), *momordica charantia* inhibitor, curcin, crotin, *sapaonaria officinalis* inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

In some embodiments, the cytotoxic agent is a radionuclide, such as ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re.

In some embodiments, the cytotoxic agent is dolastatins or dolostatin peptidic analogs and derivatives, auristatin or monomethyl auristatin phenylalanine. Exemplary molecules are disclosed in U.S. Pat No. 5,635,483 and 5,780,588. Dolastatins and auristatins have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke et al (2001) Antimicrob Agents and Chemother. 45(12):3580-3584) and have anticancer and antifungal activity. The dolastatin or auristatin drug moiety may be attached to the antibody of the invention through the N (amino) terminus or the C (carboxyl) terminus of the peptidic drug moiety (WO02/088172), or via any cysteine engineered into the antibody.

The immunoconjugates may be made using known methods.

In some embodiments, the detectable label is complexed with a chelating agent.

The detectable label, cytotoxic agent or therapeutic may be linked directly, or indirectly via a linker, to the polypeptides, the heterologous polypeptides or the proteinaceous molecules that bind the polypeptides or the heterologous polypeptides. Suitable linkers are known in the art and include, for example, prosthetic groups, non-phenolic linkers (derivatives of N-succimidyl-benzoates; dodecaborate), chelating moieties of both macrocyclics and acyclic chelators, such as derivatives of 1,4,7,10-tetraazacyclododecane-1,4,7,10,tetraacetic acid (DOTA), derivatives of

diethylenetriaminepentaacetic avid (DTPA), derivatives of S-2-(4-Isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) and derivatives of 1,4,8,11-tetraazacyclodocedan-1,4,8,11-tetraacetic acid (TETA), N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis(p-azidobenzoyl)hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene) and other chelating moieties. Suitable peptide linkers are well known.

Methods of treatment, uses and administration

The various molecules of the disclosure that can be used to introduce the prostate neoantigens of the disclosure into a subject, e.g. the polynucleotides, the heterologous polynucleotides, the polypeptides, the heterologous polypeptides, the vectors, the recombinant viruses and vaccines of the disclosure may be used to treat prostate cancer in a subject. The various molecules of the disclosure that may be used to introduce the prostate neoantigens of the disclosure into a subject, e.g. the polynucleotides, the heterologous polynucleotides, the polypeptides, the heterologous polypeptides, the vectors, the recombinant viruses and vaccines of the disclosure may also be used to prevent prostate cancer in a subject. The proteinaceous molecules that specifically bind the prostate neoantigens of the disclosure or the prostate neoantigen/HLA complexes of the disclosure can be used to treat or prevent prostate cancer in the subject.

The prostate cancer neoantigens identified herein are present at a frequency of at least about 1% or more, about 2% or more, about 3% or more, about 4% or more, about 5% or more, about 6% or more, about 7% or more, about 8% or more, about 9% or more, about 10% or more, about 11% or more, about 12% or more, about 13% or more, about 14% or more, about 15% or more, about 16% or more about 17% or more, about 18% or more, about 19% or more, about 20% or more, about 21% or more, about 22% or more, about 23% or more, about24% or more, about 25% or more, about 26% or more, about 27% or more, about 28% or more, about 29% or more, about 30% or more, about 35% or more, about 40% or more, about 45% or more, about 50% or more, about 55% or more, about 60% or more, about 65% or more or about 70% or more in a population of subjects having the prostate cancer.

The disclosure provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of one or more vaccines of the disclosure.

The disclosure also provides a use of the vaccine of the disclosure for the preparation of a medicament for treating or preventing a prostate cancer.

The disclosure also provides a use of the vaccine of the disclosure for the preparation of a pharmaceutical composition for treating or preventing a prostate cancer.

The disclosure also provides the vaccine of the disclosure for use in treating or preventing a prostate cancer.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of one or more pharmaceutical compositions of the disclosure.

In some embodiments, the vaccine comprises the rAd26 of the disclosure.

In some embodiments, the vaccine comprises the rMVA of the disclosure.

In some embodiments, the vaccine comprises the rGAd of the disclosure.

In some embodiments, the vaccine comprises the rGAd20 of the disclosure.

In some embodiments, the vaccine comprises the rCh20 of the disclosure.

In some embodiments, the vaccine comprises the rAd26 of the disclosure and rMVA of the disclosure.

In some embodiments, the vaccine comprises the rGAd20 of the disclosure and rMVA of the disclosure.

In some embodiments, the vaccine comprises the heterologous polypeptide of the disclosure.

In some embodiments, the vaccine comprises the heterologous polynucleotide of the disclosure.

In some embodiments, the vaccine comprises the polynucleotide of the disclosure.

In some embodiments, the vaccine comprises the polypeptide of the disclosure.

"**Prostate cancer**" 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.

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.

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 ≥4.0 ng/mL. In some instances, elevated PSA may refer to level off ≥ 3.0 ng/mL. PSA levels may also be compared to post-androgen deprivation therapy levels.

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

The disclosure also provides a method of treating prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a first vaccine comprising the rAd26 of the disclosure for priming the immune response; and administering to the subject a therapeutically effective amount of a second vaccine comprising the rMVA of the disclosure for boosting the immune response; thereby treating the prostate cancer in the subject.

The disclosure also provides a method of treating prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a first vaccine comprising the rGAd of the disclosure for priming the immune response; and administering to the subject a therapeutically effective amount of a second vaccine comprising the rMVA of the disclosure for boosting the immune response; thereby treating the prostate cancer in the subject.

The disclosure also provides a method of treating prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a first vaccine comprising the rGAd20 of the disclosure for priming the immune response; and administering to the subject a therapeutically effective amount of a second vaccine comprising the rMVA of the disclosure for boosting the immune response; thereby treating the prostate cancer in the subject.

The disclosure also provides a method of preventing prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a first vaccine comprising the rAd26 of the disclosure for priming the immune response; and administering to the subject a therapeutically effective amount of a second vaccine comprising the rMVA of the disclosure for boosting the immune response; thereby preventing the prostate cancer in the subject.

The disclosure also provides a method of preventing prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a first vaccine comprising the rGAd of the disclosure for priming the immune response; and administering to the subject a therapeutically effective amount of a second vaccine comprising the rMVA of the

disclosure for boosting the immune response; thereby preventing the prostate cancer in the subject.

The disclosure also provides a method of preventing prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a first vaccine comprising the rGAd20 of the disclosure for priming the immune response; and administering to the subject a therapeutically effective amount of a second vaccine comprising the rMVA of the disclosure for boosting the immune response; thereby preventing the prostate cancer in the subject.

The disclosure also provides a method of enhancing an immune response in a subject, comprising administering to the subject an immunologically effective amount of a first vaccine comprising the rAd26 of the disclosure for priming the immune response; and administering to the subject an immunologically effective amount of a second vaccine comprising the rMVA of the disclosure for boosting the immune response; thereby enhancing the immune response in the subject.

The disclosure also provides a method of enhancing an immune response in a subject, comprising administering to the subject an immunologically effective amount of a first vaccine comprising the rGAd of the disclosure for priming the immune response; and administering to the subject an immunologically effective amount of a second vaccine comprising the rMVA of the disclosure for boosting the immune response; thereby enhancing the immune response in the subject.

The disclosure also provides a method of enhancing an immune response in a subject, comprising administering to the subject an immunologically effective amount of a first vaccine comprising the rGAd20 of the disclosure for priming the immune response; and administering to the subject an immunologically effective amount of a second vaccine comprising the rMVA of the disclosure for boosting the immune response; thereby enhancing the immune response in the subject.

The disclosure also provides a method of treating prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a first vaccine comprising the rAd26 of the disclosure for priming the immune response; and administering to the subject a therapeutically effective amount of a second vaccine comprising the rMVA of the disclosure for boosting the immune response; thereby treating the prostate cancer in the subject, wherein the rAd26 and the rMVA comprise a heterologous polynucleotide encoding one or more polypeptides 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 or 447, or fragments thereof.

The disclosure also provides a method of treating prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a first vaccine comprising the rGAd of the disclosure for priming the immune response; and administering to the subject a therapeutically effective amount of a second vaccine comprising the rMVA of the disclosure for boosting the immune response; thereby treating the prostate cancer in the subject, wherein the rGAd and the rMVA comprise a heterologous polynucleotide encoding one or more polypeptides 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 or 447, or fragments thereof.

The disclosure also provides a method of treating prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a first vaccine comprising the rGAd20 of the disclosure for priming the immune response; and administering to the subject a therapeutically effective amount of a second vaccine comprising the rMVA of the disclosure for boosting the immune response; thereby treating the prostate cancer in the subject, wherein the rGAd20 and the rMVA comprise a heterologous polynucleotide encoding one or more polypeptides 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 or 447, or fragments thereof.

The disclosure also provides a method of preventing prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a first vaccine comprising the rAd26 of the disclosure for priming the immune response; and administering to the subject a therapeutically effective amount of a second vaccine comprising the rMVA of the disclosure for boosting the immune response; thereby preventing the prostate cancer in the subject, wherein the rAd26 and the rMVA comprise a heterologous polynucleotide encoding one or more polypeptides 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 or 447, or fragments thereof.

The disclosure also provides a method of preventing prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a first vaccine comprising the rGAd of the disclosure for priming the immune response; and administering to the subject a therapeutically effective amount of a second vaccine comprising the rMVA of the disclosure for boosting the immune response; thereby preventing the prostate cancer in the subject, wherein the rGAd and the rMVA comprise a heterologous polynucleotide encoding one

or more polypeptides 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 or 447, or fragments thereof.

The disclosure also provides a method of preventing prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a first vaccine comprising the rGAd20 of the disclosure for priming the immune response; and administering to the subject a therapeutically effective amount of a second vaccine comprising the rMVA of the disclosure for boosting the immune response; thereby preventing the prostate cancer in the subject, wherein the rGAd20 and the rMVA comprise a heterologous polynucleotide encoding one or more polypeptides 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 or 447, or fragments thereof.

The disclosure also provides a method of enhancing an immune response in a subject, comprising administering to the subject an immunologically effective amount of a first vaccine comprising the rAd26 of the disclosure for priming the immune response; and administering to the subject an immunologically effective amount of a second vaccine comprising the rMVA of

the disclosure for boosting the immune response; thereby enhancing the immune response in the subject, wherein the rAd26 and the rMVA comprise a heterologous polynucleotide encoding one or more polypeptides 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 or 447, or fragments thereof.

The disclosure also provides a method of enhancing an immune response in a subject, comprising administering to the subject an immunologically effective amount of a first vaccine comprising the rGAd of the disclosure for priming the immune response; and administering to the subject an immunologically effective amount of a second vaccine comprising the rMVA of the disclosure for boosting the immune response; thereby enhancing the immune response in the subject, wherein the rGAd and the rMVA comprise a heterologous polynucleotide encoding one or more polypeptides 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 or 447, or fragments thereof.

The disclosure also provides a method of enhancing an immune response in a subject, comprising administering to the subject an immunologically effective amount of a first vaccine

comprising the rGAd20 of the disclosure for priming the immune response; and administering to the subject an immunologically effective amount of a second vaccine comprising the rMVA of the disclosure for boosting the immune response; thereby enhancing the immune response in the subject, wherein the rGAd20 and the rMVA comprise a heterologous polynucleotide encoding one or more polypeptides 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 or 447, or fragments thereof.

In some embodiments, the subject is suspected to have or is suspected to develop prostate cancer.

The vaccines of the disclosure may be typically administered by intramuscular or subcutaneous injection. However other modes of administration such as intravenous, cutaneous, intradermal or nasal can be envisaged as well. Intramuscular administration of the vaccines can be achieved by using a needle. An alternative is the use of a needleless injection device to administer the composition (using, e.g., Biojector(TM)) or a freeze-dried powder containing the vaccine.

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, 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.

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

The vaccines of the disclosure are administered to a subject, giving rise to an immune response in the subject. An amount of the vaccine to in induce a detectable immune response is

defined to be 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.

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.

In one exemplary regimen, the adenovirus vector 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 vector 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.

In one exemplary regimen, the rMVA of the disclosure 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 $lxl0^7$ $TCID_{50}$ to $lx10^9$ $TCID_{50}$ (50% Tissue Culture Infective Dose) or Inf.U. (Infectious Unit). The rMVA may be administered in a volume ranging between 0.25 and 1.0 ml.

Boosting compositions may be administered two or more times, weeks or months after administration of the priming composition, for example, about 1 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.

Prostate neoantigen combinations for vaccine therapy

The identified neoantigens described herein can be further validated and prioritized for their inclusion into a universal prostate cancer vaccine. The considerations for inclusion are, for example, higher expression in prostate cancer tissues vs. normal prostate tissue and other normal tissues (such as liver, kidney, pancreas, ovary, prostate, mammary gland, colon, stomach, skeletal muscle and lung), or undetectable expression in normal tissues, ability of the neoantigens or their fragments to mediate activation of $CD8^+$ T cells in known assays, binding to HLA, demonstrated

in vivo processing and presentation to HLA of peptide fragments derived from the neoantigens and sufficient prevalence in prostate cancer subjects.

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. 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 utilizing 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 heterologous polynucleotides encoding heterologous 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 orders providing heterologous polypeptides of 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.

The disclosure provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide, wherein the heterologous polypeptide comprises two 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 and 177, and fragments thereof.

The disclosure also provides a vaccine comprising a heterologous polynucleotide comprising two 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 and 178, and fragments thereof.

In some embodiments, the fragments comprise polypeptides of SEQ ID NOs: 387, 388, 390, 392, 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, 621, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710 or 711.

In some embodiments, the vaccine is capable of eliciting a cellular immune response in a subject administered the vaccine.

In some embodiments, the cellular immune response is specific against a fragment of one or more polypeptides 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 or 177.

In some embodiments, the cellular immune response is activation of vaccine-specific $CD8^+$ T cells, $CD4^+$ T cells, or $CD8^+$ T cells and $CD4^+$ T cells, wherein activation is assessed by increased production of TNFα, IFNγ, or TNFα and IFNγ by $CD8^+$ T cells, $CD4^+$ T cells, or $CD8^+$ T cells and $CD4^+$ T cells.

In some embodiments, the heterologous 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.

In some embodiments, the heterologous polypeptide comprises an amino acid sequence of SEQ ID NOs: 541, 550, 554, 555, 556, 623 or 624.

In some embodiments, the heterologous polynucleotide comprises a polynucleotide sequence of SEQ ID NO: 542 or SEQ ID NO: 551.

In some embodiments, the heterologous polypeptide comprises the amino acid sequence of SEQ ID NOs: 543, 552, 557, 558, 559, 625 or 626.

In some embodiments, the heterologous polynucleotide comprises the polynucleotide sequence of SEQ ID NO: 544 or SEQ ID NO: 553.

In some embodiments, the heterologous polynucleotide is DNA or RNA.

In some embodiments, RNA is mRNA or self-replicating RNA.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 541.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 550.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 554.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 555.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 556.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 623.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 624.

The disclosure also provides a vaccine comprising a heterologous polynucleotide of SEQ ID NO: 542.

The disclosure also provides a vaccine comprising a heterologous polynucleotide of SEQ ID NO: 551.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 543.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 552.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 557.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 558.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 559.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 625.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 626.

The disclosure also provides a vaccine comprising a heterologous polynucleotide of SEQ ID NOs: 544.

The disclosure also provides a vaccine comprising a heterologous polynucleotide of SEQ ID NOs: 553.

In some embodiments, the vaccine is a recombinant virus.

In some embodiments, the recombinant virus is derived from adenovirus (Ad), poxvirus, adeno-associated virus (AAV) or retrovirus.

In some embodiments, 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).

In some embodiments, the recombinant virus is derived from GAd20.

In some embodiments, the recombinant virus derived from GAd20 comprises a polynucleotide sequence of SEQ ID NO: 713.

In some embodiments, the recombinant virus is derived from MVA.

In some embodiments, the recombinant virus is derived from hAd26.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 541, wherein the vaccine is a recombinant virus derived from GAd20.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 550, wherein the vaccine is a recombinant virus derived from GAd20.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 554, wherein the vaccine is a recombinant virus derived from GAd20.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 555, wherein the vaccine is a recombinant virus derived from GAd20.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 556, wherein the vaccine is a recombinant virus derived from GAd20.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 623, wherein the vaccine is a recombinant virus derived from GAd20.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 624, wherein the vaccine is a recombinant virus derived from GAd20.

The disclosure also provides a vaccine comprising a polynucleotide sequence of SEQ ID NO: 713, wherein the vaccine is a recombinant virus derived from GAd20.

The disclosure also provides a vaccine comprising a heterologous polynucleotide of SEQ ID NOs: 542, wherein the vaccine is a recombinant virus derived from GAd20.

The disclosure also provides a vaccine comprising a heterologous polynucleotide

of SEQ ID NOs: 551, wherein the vaccine is a recombinant virus derived from GAd20.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 543, wherein the vaccine is a recombinant virus derived from MVA.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 552, wherein the vaccine is a recombinant virus derived from MVA.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 557, wherein the vaccine is a recombinant virus derived from MVA.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 558, wherein the vaccine is a recombinant virus derived from MVA.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 559, wherein the vaccine is a recombinant virus derived from MVA.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 625, wherein the vaccine is a recombinant virus derived from MVA.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 626, wherein the vaccine is a recombinant virus derived from MVA.

The disclosure also provides a vaccine comprising a heterologous polynucleotide of SEQ ID NOs: 544, wherein the vaccine is a recombinant virus derived from MVA.

The disclosure also provides a vaccine comprising a heterologous polynucleotide of SEQ ID NOs: 553, wherein the vaccine is a recombinant virus derived from MVA.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NOs: 541, 550, 554, 555, 556, 623 or 624.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 541.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a vaccine

comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 550.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 554.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 555.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 556.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 623.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 624.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NOs: 543, 552, 557, 558, 559, 625 or 626.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 543.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 552.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a vaccine

comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 557.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 558.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 559.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 625.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject a therapeutically effective amount of a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 626.

The disclosure also provides a use of a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NOs: 541, 550, 554, 555, 556, 623 or 624 for the preparation of a medicament for treating or preventing a prostate cancer.

The disclosure also provides a use of a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 541 for the preparation of a medicament for treating or preventing a prostate cancer.

The disclosure also provides a use of a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 550 for the preparation of a medicament for treating or preventing a prostate cancer.

The disclosure also provides a use of a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 554 for the preparation of a medicament for treating or preventing a prostate cancer.

The disclosure also provides a use of a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 555 for the preparation of a medicament for treating or preventing a prostate cancer.

The disclosure also provides a use of a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 556 for the preparation of a medicament for treating or preventing a prostate cancer.

The disclosure also provides a use of a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 623 for the preparation of a medicament for treating or preventing a prostate cancer.

The disclosure also provides a use of a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 624 for the preparation of a medicament for treating or preventing a prostate cancer.

The disclosure also provides a use of a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NOs: 543, 552, 557, 558, 559, 625 or 626 for the preparation of a medicament for treating or preventing a prostate cancer.

The disclosure also provides a use of a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 543 for the preparation of a medicament for treating or preventing a prostate cancer.

The disclosure also provides a use of a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 552 for the preparation of a medicament for treating or preventing a prostate cancer.

The disclosure also provides a use of a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 557 for the preparation of a medicament for treating or preventing a prostate cancer.

The disclosure also provides a use of a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 558 for the preparation of a medicament for treating or preventing a prostate cancer.

The disclosure also provides a use of a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 559 for the preparation of a medicament for treating or preventing a prostate cancer.

The disclosure also provides a use of a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 625 for the preparation of a medicament for treating or preventing a prostate cancer.

The disclosure also provides a use of a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 626 for the preparation of a medicament for treating or preventing a prostate cancer.

The disclosure also provides a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NOs: 541, 550, 554, 555, 556, 623 or 624 for use in treating or preventing a prostate cancer.

The disclosure also provides a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 541 for use in treating or preventing a prostate cancer.

The disclosure also provides a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 550 for use in treating or preventing a prostate cancer.

The disclosure also provides a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 554 for use in treating or preventing a prostate cancer.

The disclosure also provides a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 555 for use in treating or preventing a prostate cancer.

The disclosure also provides a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 556 for use in treating or preventing a prostate cancer.

The disclosure also provides a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 623 for use in treating or preventing a prostate cancer.

The disclosure also provides a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 624 for use in treating or preventing a prostate cancer.

The disclosure also provides a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NOs: 543, 552, 557, 558, 559, 625 or 626 for use in treating or preventing a prostate cancer.

The disclosure also provides a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 543 for use in treating or preventing a prostate cancer.

The disclosure also provides a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 552 for use in treating or preventing a prostate cancer..

The disclosure also provides a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 557 for use in treating or preventing a prostate cancer.

The disclosure also provides a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 558 for use in treating or preventing a prostate cancer.

The disclosure also provides a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 559 for use in treating or preventing a prostate cancer.

The disclosure also provides a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 625 for use in treating or preventing a prostate cancer.

The disclosure also provides a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 626 for use in treating or preventing a prostate cancer.

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.

In some embodiments, the vaccine comprising the heterologous polynucleotide sequence encoding a heterologous polypeptide of SEQ ID NOs: 541 or 550 is administered as a prime.

In some embodiments, the vaccine comprising the heterologous polynucleotide sequence encoding a heterologous polypeptide of SEQ ID NOs 543 or 552 is administered as a boost.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising

administering to the subject a therapeutically effective amount of a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide comprising 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

administering to the subject a therapeutically effective amount of a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide comprising 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.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject

a first vaccine comprising a first heterologous polynucleotide encoding a first heterologous polypeptide, wherein the first heterologous polypeptide comprises two 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 and 177; and

a second vaccine comprising a second heterologous polynucleotide encoding a second heterologous polypeptide, wherein the second heterologous polypeptide comprises two 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 and 177.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject

a first vaccine comprising a first heterologous polynucleotide encoding a first heterologous polypeptide, wherein the first heterologous polypeptide comprises two 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 and 177; and

a second vaccine comprising a second heterologous polynucleotide encoding a second heterologous polypeptide, wherein the second heterologous polypeptide comprises two 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 and 177; wherein the first heterologous polypeptide and the second heterologous polypeptide have distinct amino acid sequences.

In some embodiments, the polypeptides are organized in a first order and in a second order.

In some embodiments, the polypeptides organized in the first order comprises a heterologous polypeptide of SEQ ID NO: 541 or SEQ ID NO: 550.

In some embodiments, the polypeptides organized in the second order comprises a heterologous polypeptide of SEQ ID NO: 543 or SEQ ID NO: 552.

In some embodiments, the heterologous polynucleotide is DNA or RNA.

In some embodiments, RNA is mRNA or self-replicating RNA.

In some embodiments, the vaccine is a recombinant virus.

In some embodiments, the recombinant virus is derived from adenovirus, poxvirus, adeno-associated virus or retrovirus.

In some embodiments, the polypeptides organized in the first order are encoded by a polynucleotide of SEQ ID NO: 542 or SEQ ID NO: 551.

In some embodiments, the polypeptides organized in the second order are encoded by a polynucleotide of SEQ ID NO: 544 or SEQ ID NO: 553.

In some embodiments, the prime comprises the recombinant virus derived from GAd20 comprising a heterologous polypeptide of SEQ ID NO: 541 or SEQ ID NO: 550.

In some embodiments, the boost comprises the recombinant virus derived from MVA comprising the heterologous polypeptide of SEQ ID NO: 543 or SEQ ID NO: 552.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject

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

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

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject

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

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

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject

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

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

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject
a first vaccine comprising a first heterologous polynucleotide encoding a first heterologous polypeptide, wherein the first heterologous polypeptide comprises an amino acid sequence of SEQ ID NO: 541, wherein the first vaccine is a recombinant Gad20; and
a second vaccine comprising a second heterologous polynucleotide encoding a second heterologous polypeptide, wherein the second heterologous polypeptide comprises the amino acid sequence of SEQ ID NO: 543, wherein the second vaccine is a recombinant MVA.

The disclosure also provides a method of treating or preventing a prostate cancer in a subject, comprising administering to the subject
a first vaccine comprising a first heterologous polynucleotide encoding a first heterologous polypeptide, wherein the first heterologous polypeptide comprises an amino acid sequence of SEQ ID NO: 550, wherein the first vaccine is a recombinant GAd20; and
a second vaccine comprising a second heterologous polynucleotide encoding a second heterologous polypeptide, wherein the second heterologous polypeptide comprises the amino acid sequence of SEQ ID NO: 552, wherein the second vaccine is a recombinant MVA.

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NOs: 541, 550, 554, 555, 556, 623 or 624.

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 541.

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 550.

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 554.

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 555.

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 556.

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 623.

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 624.

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NOs: 543, 552, 557, 558, 559, 625 or 626.

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 543.

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 552.

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 557.

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 558.

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 559.

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 625.

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 626.

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject

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

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

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject

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

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

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject

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

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

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject

a first vaccine comprising a first heterologous polynucleotide encoding a first heterologous polypeptide, wherein the first heterologous polypeptide comprises an amino acid sequence of SEQ ID NO: 541, wherein the first vaccine is a recombinant Gad20; and
a second vaccine comprising a second heterologous polynucleotide encoding a second heterologous polypeptide, wherein the second heterologous polypeptide comprises the amino acid sequence of SEQ ID NO: 543, wherein the second vaccine is a recombinant MVA.

The disclosure also provides a method of inducing an immune response in a subject, comprising administering to the subject
a first vaccine comprising a first heterologous polynucleotide encoding a first heterologous polypeptide, wherein the first heterologous polypeptide comprises an amino acid sequence of SEQ ID NO: 550, wherein the first vaccine is a recombinant GAd20; and
a second vaccine comprising a second heterologous polynucleotide encoding a second heterologous polypeptide, wherein the second heterologous polypeptide comprises the amino acid sequence of SEQ ID NO: 552, wherein the second vaccine is a recombinant MVA.

The disclosure also provides a first vaccine comprising a first heterologous polynucleotide encoding a first heterologous polypeptide, wherein the first heterologous polypeptide comprises two 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 and 177, and a second vaccine comprising a second heterologous polynucleotide encoding a second heterologous polypeptide, wherein the second heterologous polypeptide comprises two 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 and 177 for use in treating or preventing a prostate cancer.

The disclosure also provides a first vaccine comprising a first heterologous polynucleotide encoding a first heterologous polypeptide, wherein the first heterologous polypeptide comprises an amino acid sequence of SEQ ID NOs: 541, 550, 554, 555, 556, 623 or 624, and a second vaccine comprising a second heterologous polynucleotide encoding a second heterologous polypeptide, wherein the second heterologous polypeptide comprises the amino acid sequence of SEQ ID NOs: 543, 552, 557, 558, 559, 625 or 626 for use in treating or preventing a prostate cancer.

The disclosure also provides a first vaccine comprising a first heterologous polynucleotide encoding a first heterologous polypeptide, wherein the first heterologous polypeptide comprises an amino acid sequence of SEQ ID NO: 541, and a second vaccine comprising a second heterologous polynucleotide encoding a second heterologous polypeptide,

wherein the second heterologous polypeptide comprises the amino acid sequence of SEQ ID NO: 543 for use in treating or preventing a prostate cancer.

The disclosure also provides a first vaccine comprising a first heterologous polynucleotide encoding a first heterologous polypeptide, wherein the first heterologous polypeptide comprises an amino acid sequence of SEQ ID NO: 550m and a second vaccine comprising a second heterologous polynucleotide encoding a second heterologous polypeptide, wherein the second heterologous polypeptide comprises the amino acid sequence of SEQ ID NO: 552 for use in treating or preventing a prostate cancer.

The disclosure also provides a first vaccine comprising a first heterologous polynucleotide encoding a first heterologous polypeptide, wherein the first heterologous polypeptide comprises an amino acid sequence of SEQ ID NO: 541, wherein the first vaccine is a recombinant Gad20; and

a second vaccine comprising a second heterologous polynucleotide encoding a second heterologous polypeptide, wherein the second heterologous polypeptide comprises the amino acid sequence of SEQ ID NO: 543, wherein the second vaccine is a recombinant MVA for use in treating or preventing a prostate cancer.

The disclosure also provides a first vaccine comprising a first heterologous polynucleotide encoding a first heterologous polypeptide, wherein the first heterologous polypeptide comprises an amino acid sequence of SEQ ID NO: 550, wherein the first vaccine is a recombinant GAd20; and a second vaccine comprising a second heterologous polynucleotide encoding a second heterologous polypeptide, wherein the second heterologous polypeptide comprises the amino acid sequence of SEQ ID NO: 552, wherein the second vaccine is a recombinant MVA for use in treating or preventing a prostate cancer.

The disclosure also provides a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NOs: 541, 550, 554, 555, 556, 623 or 624 for use in treating or preventing a prostate cancer.

The disclosure also provides a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 541 for use in inducing an immune response.

The disclosure also provides a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 550 for use in inducing an immune response.

The disclosure also provides a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 554 for use in inducing an immune response.

The disclosure also provides a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 555 for use in inducing an immune response.

The disclosure also provides a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 556 for use in inducing an immune response.

The disclosure also provides a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 623 for use in inducing an immune response.

The disclosure also provides a vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 624 for use in inducing an immune response.

The disclosure also provides a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NOs: 543, 552, 557, 558, 559, 625 or 626 for use in inducing an immune response.

The disclosure also provides a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 543 for use in inducing an immune response.

The disclosure also provides a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 552 for use in inducing an immune response.

The disclosure also provides a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 557 for use in inducing an immune response.

The disclosure also provides a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 558 for use in inducing an immune response.

The disclosure also provides a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 559 for use in inducing an immune response.

The disclosure also provides a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 625 for use in inducing an immune response.

The disclosure also provides a vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 626 for use in inducing an immune response.

The disclosure also provides a first vaccine comprising a first heterologous polynucleotide encoding a first heterologous polypeptide, wherein the first heterologous polypeptide comprises an amino acid sequence of SEQ ID NOs: 541, 550, 554, 555, 556, 623 or 624, and a second vaccine comprising a second heterologous polynucleotide encoding a second heterologous polypeptide, wherein the second heterologous polypeptide comprises the amino acid sequence of SEQ ID NOs: 543, 552, 557, 558, 559, 625 or 626 for use in inducing an immune response.

The disclosure also provides a first vaccine comprising a first heterologous polynucleotide encoding a first heterologous polypeptide, wherein the first heterologous polypeptide comprises an amino acid sequence of SEQ ID NO: 541, and a second vaccine comprising a second heterologous polynucleotide encoding a second heterologous polypeptide, wherein the second heterologous polypeptide comprises the amino acid sequence of SEQ ID NO: 543 for use in inducing an immune response.

The disclosure also provides a first vaccine comprising a first heterologous polynucleotide encoding a first heterologous polypeptide, wherein the first heterologous polypeptide comprises an amino acid sequence of SEQ ID NO: 550, and a second vaccine comprising a second heterologous polynucleotide encoding a second heterologous polypeptide, wherein the second heterologous polypeptide comprises the amino acid sequence of SEQ ID NO: 552 for use in inducing an immune response.

The disclosure also provides a first vaccine comprising a first heterologous polynucleotide encoding a first heterologous polypeptide, wherein the first heterologous polypeptide comprises an amino acid sequence of SEQ ID NO: 541, wherein the first vaccine is a recombinant Gad20, and a second vaccine comprising a second heterologous polynucleotide encoding a second heterologous polypeptide, wherein the second heterologous polypeptide comprises the amino acid sequence of SEQ ID NO: 543, wherein the second vaccine is a recombinant MVA for use in inducing an immune response.

The disclosure also provides a first vaccine comprising a first heterologous polynucleotide encoding a first heterologous polypeptide, wherein the first heterologous polypeptide comprises an amino acid sequence of SEQ ID NO: 550, wherein the first vaccine is a recombinant GAd20, and

a second vaccine comprising a second heterologous polynucleotide encoding a second heterologous polypeptide, wherein the second heterologous polypeptide comprises the amino acid sequence of SEQ ID NO: 552, wherein the second vaccine is a recombinant MVA for use in inducing an immune response.

In some embodiments, the subject has, is suspected to have or is suspected to develop prostate cancer. Prostate cancer diagnosis performed by a licensed physician.

In some embodiments, prostate cancer is a relapsed prostate cancer, a refractory prostate cancer, a metastatic prostate cancer or a castration resistant prostate cancer, or any combination thereof.

In some embodiments, the subject is treatment naïve.

In some embodiments, the subject has received androgen deprivation therapy.

In some embodiments, the first vaccine is administered one or more times to the subject.

In some embodiments, the second vaccine is administered one or more times to the subject.

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 16 weeks prior to administering the second vaccine.

In some embodiments, the prostate cancer is a relapsed prostate cancer, a refractory prostate cancer, a metastatic prostate cancer, or a castration resistant prostate cancer, or any combination thereof.

In some embodiments, the method comprises further administering an additional cancer therapeutic agent to the subject.

Combination therapies

The vaccines of the disclosure may be used in combination with at least one additional cancer therapeutic agent for treating prostate cancer.

The additional cancer therapeutic agent may be a surgery, a chemotherapy, an androgen deprivation therapy, radiation therapy, targeted therapy or a checkpoint inhibitor, or any combination thereof.

Exemplary chemotherapeutic agents are 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, and mitoxantrone, vinblastine, vincristine, vindesine, vinorelbine, paclitaxel, docetaxel.

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

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.

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.

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.

In some embodiments, one or more vaccines or the disclosure is 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.

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.

In some embodiments, one or more vaccines of the disclosure 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.

Kits

The disclosure also provides a kit comprising:

a first vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NOs: 541, 550, 554, 555, 556, 623 or 624; and

a second vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NOs: 543, 552, 557, 558, 559, 625 or 626.

The disclosure also provides a kit comprising:

a first vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 541; and

a second vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 543.

The disclosure also provides a kit comprising:

a first vaccine comprising a recombinant virus derived from GAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 550; and

a second vaccine comprising a recombinant virus derived from MVA comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NO: 552.

Heterologous polypeptides encoding or heterologous polypeptides comprising a combination of select prostate neoantigens

The disclosure also provides an isolated heterologous polypeptide comprising 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 VPFRELKNVSVLEGLRQGRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPV KSSI (SEQ ID NO: 309);
AS51 comprising the amino acid sequence GMECTLGQVGAPSPRREEDGWRGGHSRFKADVPAPQGPCWGGQPGSAPSSAPPEQS LLD (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 VPFRELKNQRTAQGAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAGRGPEA GGGVLQPQRPAPEKPGCPCRRGQPRLHTVKMWRA (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 CHLFLQPQVGTPPPHTASARAPSGPPHPHESCPAGRRPARAAQTCARRQHGLPGCEE AGTARVPSLHLHLHQAALGAGRGRGWGEACAQVPPSRG (SEQ ID NO: 297);
AS23 comprising the amino acid sequence KIQNKNCPD (SEQ ID NO: 285);
MS1 comprising the amino acid sequence HYKLIQQPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCSTNDICVTANFCISVTF LKPCFLLHEASASQ (SEQ ID NO: 437);
MS3 comprising the amino acid sequence RTALTHNQDFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITPQLGGQSDSSQ

PLLTTGRPQGWQDQALRHTQQASPASCATITIPIHSAALGDHSGDPGPAWDTCPPLP LTTLIPRAPPPYGDSTARSWPSRCGPLG (SEQ ID NO: 439);

MS6 comprising the amino acid sequence YAYKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFLSRALRALHVL WNGFQLHCQ (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.

In some embodiments, the isolated heterologous polypeptide further comprises 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 WGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLPLRTLAGCLARTAHL RPGAESLPQPQLHCT (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 LWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSARTCCGTKWPSSQEAALGLGSG LLRFSCGTAAIR (SEQ ID NO: 223), and fragments thereof.

The disclosure also provides an isolated heterologous polypeptide comprising 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 WGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLPLRTLAGCLARTAHLRPGAESLPQPQLHCT (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 LWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSARTCCGTKWPSSQEAALGLGSGLLRFSCGTAAIR (SEQ ID NO: 223), and fragments thereof.

In some embodiments, the isolated heterologous polypeptide further comprises 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 VPFRELKNQRTAQGAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAGRGPEA GGGVLQPQRPAPEKPGCPCRRGQPRLHTVKMWRA (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 CHLFLQPQVGTPPPHTASARAPSGPPHPHESCPAGRRPARAAQTCARRQHGLPGCEE AGTARVPSLHLHLHQAALGAGRGRGWGEACAQVPPSRG (SEQ ID NO: 297);
AS23 comprising the amino acid sequence KIQNKNCPD (SEQ ID NO: 285);
MS1 comprising the amino acid sequence HYKLIQQPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCSTNDICVTANFCISVTF LKPCFLLHEASASQ (SEQ ID NO: 437);
MS3 comprising the amino acid sequence RTALTHNQDFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITPQLGGQSDSSQ PLLTTGRPQGWQDQALRHTQQASPASCATITIPIHSAALGDHSGDPGPAWDTCPPLP LTTLIPRAPPPYGDSTARSWPSRCGPLG (SEQ ID NO: 439);
MS6 comprising the amino acid sequence YAYKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFLSRALRALHVL WNGFQLHCQ (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.

In some embodiments, the isolated heterologous polypeptide further comprises 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 QNLQNGGGSRSSATLPGRRRRRWLRRRRQPISVAPAGPPRRPNQKPNPPGGARCVIMRPTWPGTSAFT (SEQ ID NO: 177), and fragments thereof.

The disclosure also provides a heterologous polypeptide comprising two 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 and 177, and fragments thereof.

The disclosure also provides an isolated heterologous polynucleotide comprising two or more polynucleotides selected from the group consisting of
the polynucleotide sequence of TGGAAATTCGAGATGAGCTACACGGTGGGTGGCCCGCCTCCCCATGTTCATGCTAGACCCAGGCATTGGAAAACTGATAGA (SEQ ID NO: 276) (encoding AS18);
the polynucleotide sequence of TATGAAGCAGGGATGACTCTGGGAGGTAAGATACTTTTCTTTCTCTTCCTCCTCCTTCCTCTCTCCCCCTTCTCCCTCATTTTC (SEQ ID NO: 382) (encoding P87);
the polynucleotide sequence of GATGGCCACTCCTACACATCCAAGGTGAATTGTTTACTCCTTCAAGATGGGTTCCATGGCTGTGTGAGCATCACCGGGGCAGCTGGAAGAAGAAACCTGAGCATCTTCCTGTTCTTGATGCTGTGCAAATTGGAGTTCCATGCTTGT (SEQ ID NO: 334) (encoding AS55);
the polynucleotide sequence of ACAGGGGGCAAAAGCACCTGCTCGGCTCCTGGCCCTCAGTCTCTCCCCTCCACTCCATTCTCCACCTACCCACAGTGGGTCATTCTGATCACCGAACTG (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
GTGCCCTTCCGGGAGCTCAAGAACGTGAGTGTCCTGGAGGGGCTCCGTCAAGGC
CGGCTTGGGGGTCCCTGTTCATGTCACTGCCCAAGACCTTCCCAGGCCAGGCTCA
CGCCAGTGGATGTGGCAGGTCCCTTCTTGTGTCTGGGGGATCCTGGGCTGTTCCC
CCCAGTCAAGAGCAGTATC (SEQ ID NO: 310) (encoding AS43);
the polynucleotide sequence of
GGCATGGAGTGCACCCTGGGGCAGGTGGGTGCCCCGTCCCCTCGGAGGGAGGAG
GACGGTTGGCGTGGGGGCCACAGCCGATTCAAGGCTGATGTACCAGCACCGCAG
GGACCCTGCTGGGGTGGCCAACCTGGCTCTGCCCCCTCCTCAGCTCCTCCTGAAC
AGTCATTATTAGAT (SEQ ID NO: 326) (encoding AS51);
the polynucleotide sequence of
GGCAACACCACCCTCCAGCAGCTGGGTGAGGCCTCCCAGGCGCCCTCAGGCTCC
CTCATCCCTCTGAGGCTGCCTCTGCTCTGGGAAGTGAGGGGC ((SEQ ID NO: 272)
(encoding AS16);
the polynucleotide sequence of
GAGGCCTTCCAGAGGGCCGCTGGTGAGGGCGGCCCGGGCCGCGGTGGGGCACG
GCGCGGTGCCAGGGTGTTGCAGAGCCCCTTTTGCAGGGCAGGAGCTGGGGAGTG
GTTAGGACATCAGTCCCTCAGG (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
GTGCCCTTCCGGGAGCTCAAGAACCAGAGAACAGCACAAGGGGCTCCTGGGATC
CACCACGCGGCTTCCCCCGTTGCTGCCAACCTCTGCGACCCGGCGAGACACGCA
CAGCACACACGCATCCCCTGCGGCGCTGGCCAAGTGCGTGCTGGCCGAGGTCCC
GAAGCAGGTGGTGGAGTACTACAGCCACAGAGGCCTGCCCCCGAGAAGCCTGG
GTGTCCCTGCCGGAGAGGCCAGCCCAGGCTGCACACCGTGAAGATGTGGAGGGC
G (SEQ ID NO: 262) (encoding AS11);
the polynucleotide sequence of AAGAGAAGTTTTGCTGTCACGGAGAGGATCATC
(SEQ ID NO: 266) (encoding AS13);
the polynucleotide sequence of
TTCAAGAAGTTCGACGGCCCTTGTGGTGAGCGCGGCGGCGGGCGCACGGCTCGA
GCTCTGTGGGCGCGCGGCGACAGCGTCCTGACTCCTGCCCTCGACCCCCAGACC

CCTGTCAGGGCGCCCTCCCTGACCCGAGCCGCAGCTGCCGTG (SEQ ID NO: 318) (encoding AS47);
the polynucleotide sequence of CTTGTACTTGGTGTATTGAGCGGGCACAGTGGCTCACGCCTA (SEQ ID NO: 256) (encoding AS8);
the polynucleotide sequence of CAGTGGCAGCACTACCACCGGTCAGGTGAGGCCGCAGGGACTCCCCTCTGGAGA CCCACAAGAAAC (SEQ ID NO: 278) (encoding AS19);
the polynucleotide sequence of TGCCACCTCTTCCTGCAGCCCCAGGTTGGCACCCCCCCCCCCCACACTGCCAGTG CTCGAGCCCCCAGTGGTCCACCCCACCCTCATGAAAGTTGCCCTGCAGGGCGAA GACCTGCGAGAGCTGCGCAGACATGTGCACGCCGACAGCACGGACTTCCTGGCT GTGAAGAGGCTGGTACAGCGCGTGTTCCCAGCCTGCACCTGCACCTGCACCAGG CCGCCCTCGGAGCAGGAAGGGGCCGTGGGTGGGGAGAGGCCTGTGCCCAAGTA CCCCCCTCAAGAGGC (SEQ ID NO: 298) (encoding AS37);
the polynucleotide sequence of AAAATTCAGAATAAAAATTGTCCAGAC (SEQ ID NO: 286) (encoding AS23);
the polynucleotide sequence of CACTACAAATTAATTCAACAACCCATATCCCTCTTCTCCATCACTGATAGGCTCC ATAAGACGTTCAGTCAGCTGCCCTCGGTCCATCTCTGCTCAATCACCTTCCAGTG GGGACACCCGCCCATTTTCTGCTCAACAAATGATATCTGTGTCACGGCCAACTTC TGCATCTCGGTCACATTCCTTAAACCGTGCTTCCTCCTACATGAGGCATCTGCCT CACAG (SEQ ID NO: 448) (encoding MS1);
the polynucleotide sequence of AGGACCGCCCTGACACACAATCAGGACTTCTCTATCTACAGGCTCTGTTGCAAG AGGGGGTCCCTCTGCCACGCTTCCCAGGCCAGATCCCCGGCTTTCCCGAAGCCG GTCAGACCTCTTCCTGCCCCCATCACCAGAATCACCCCCCAACTGGGGGGACAA TCTGACTCGAGTCAACCCCTTCTCACTACGGGAAGACCTCAGGGGTGGCAAGAT CAAGCTCTTAGACACACCCAGCAAGCCAGTCCTGCCTCTTGTGCCACCATCACCA TTCCCATCCACTCAGCTGCCCTTGGTGACCACTCCGGAGACCCTGGTCCAGCCTG GGACACCTGCCCGCCGCTGCCGCTCACTACCCTCATCCCCCGAGCTCCCCCGCCG TATGGAGACAGCACTGCCAGGTCCTGGCCCTCCCGCTGTGGGCCCCTCGGC (SEQ ID NO: 450) (encoding MS3);
the polynucleotide sequence of TATGCTTACAAGGACTTTCTCTGGTGTTTTCCTTTTTCTTTAGTTTTTCTCCAAGAG ATTCAAATCTGCTGCCATGTTAGCTGTCTTTGCTGTATCTGCTGTAGTACACGAA

TATGCCTTGGCTGTTTGCTTGAGCTTTTTCTATCCCGTGCTCTTCGTGCTCTTCAT GTTCTTTGGAATGGCTTTCAACTTCATTGTCAA (SEQ ID NO: 453) (encoding MS6); the polynucleotide sequence of ACCATGCCTGCTATTTTAAAGTTACAGAAGAATTGTCTTCTCTCCTTA (SEQ ID NO: 455) (encoding MS8); and the polynucleotide sequence of TATGAAGCAGGGATGACTCTGGGAGAAAAATTCCGGGTTGGCAATTGCAAGCAT CTCAAAATGACCAGACCC (SEQ ID NO: 380) (encoding P82); and fragments thereof.

In some embodiments, the isolated heterologous polynucleotide further comprises 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 ACAGAAATTTCATGTTGCACCCTGAGCAGTGAGGAGAATGAATACCTTCCAAGA CCAGAGTGGCAGCTCCAG (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 TGGGGGATGGAGTTGGCAGCGTCTCGGAGGTTCTCCTGGGACCACCACTCCGCC GGGGGGCCGCCCAGAGTGCCAAGCGTCCGATCCGGCGCCGCCCAAGTGCAGCCC AAGGACCCGCTCCCGCTCCGCACCCTGGCAGGCTGCCTAGCCAGGACTGCGCAC

CTGCGCCCTGGGGCGGAGTCCTTACCCCAACCCCAGCTTCACTGCACA (SEQ ID NO: 226) (encoding FUS8);
the polynucleotide sequence of CACGTGGTGGGCTATGGCCACCTTGATACTTCCGGGTCATCCTCCTCCTCCTCCT GGCCC (SEQ ID NO: 346) (encoding FUS15);
the polynucleotide sequence of AACAGCAAGATGGCTTTGAACTCATTAAACTCCATTGATGATGCACAGTTGACA AGAATTGCCCCTCCAAGATCTCATTGCTGTTTCTGGGAAGTAAACGCTCCT (SEQ ID NO: 354) (encoding P35);
the polynucleotide sequence of AAAATGCACTTCTCCCTCAAGGAGCACCCACCGCCCCCTTGCCCGCCT (SEQ ID NO: 236) (encoding FUS19); and
the polynucleotide sequence of CTGTGGTTCCAGAGCAGTGAGCTGTCCCCGACGGGAGCGCCATGGCCCAGCCGC CGCCCGACGTGGAGGGGGACGACTGTCTCCCCGCGTACCGCCACCTCTTCTGCCC GGACCTGCTGCGGGACAAAGTGGCCTTCATCACAGGAGGCGGCTCTGGGATTGG GTTCCGGATTGCTGAGATTTTCATGCGGCACGGCTGCCATACGG (SEQ ID NO: 224) (encoding FUS7), and fragments thereof.

The disclosure also provides an isolated heterologous polynucleotide comprising two 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 ACAGAAATTTCATGTTGCACCCTGAGCAGTGAGGAGAATGAATACCTTCCAAGA CCAGAGTGGCAGCTCCAG (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 TGGGGGATGGAGTTGGCAGCGTCTCGGAGGTTCTCCTGGGACCACCACTCCGCC GGGGGGCCGCCCAGAGTGCCAAGCGTCCGATCCGGCGCCGCCCAAGTGCAGCCC AAGGACCCGCTCCCGCTCCGCACCCTGGCAGGCTGCCTAGCCAGGACTGCGCAC CTGCGCCCTGGGGCGGAGTCCTTACCCCAACCCCAGCTTCACTGCACA (SEQ ID NO: 226) (encoding FUS8);

the polynucleotide sequence of CACGTGGTGGGCTATGGCCACCTTGATACTTCCGGGTCATCCTCCTCCTCCTCCT GGCCC (SEQ ID NO: 346) (encoding FUS15);

the polynucleotide sequence of AACAGCAAGATGGCTTTGAACTCATTAAACTCCATTGATGATGCACAGTTGACA AGAATTGCCCCTCCAAGATCTCATTGCTGTTTCTGGGAAGTAAACGCTCCT (SEQ ID NO: 354) (encoding P35);

the polynucleotide sequence of AAAATGCACTTCTCCCTCAAGGAGCACCCACCGCCCCCTTGCCCGCCT (SEQ ID NO: 236) (encoding FUS19); and

the polynucleotide sequence of CTGTGGTTCCAGAGCAGTGAGCTGTCCCCGACGGGAGCGCCATGGCCCAGCCGC CGCCCGACGTGGAGGGGGACGACTGTCTCCCCGCGTACCGCCACCTCTTCTGCCC GGACCTGCTGCGGGACAAAGTGGCCTTCATCACAGGAGGCGGCTCTGGGATTGG GTTCCGGATTGCTGAGATTTTCATGCGGCACGGCTGCCATACGG (SEQ ID NO: 224) (encoding FUS7).

In some embodiments, the isolated heterologous polynucleotide further comprises one or more polynucleotides or one or more at least 24 nucleotide long contiguous fragments of the one or more polynucleotides, wherein the polynucleotides comprise

the polynucleotide sequence of TGGAAATTCGAGATGAGCTACACGGTGGGTGGCCCGCCTCCCCATGTTCATGCT AGACCCAGGCATTGGAAAACTGATAGA (SEQ ID NO: 276) (encoding AS18);

the polynucleotide sequence of
TATGAAGCAGGGATGACTCTGGGAGGTAAGATACTTTTCTTTCTCTTCCTCCTCC
TTCCTCTCTCCCCCTTCTCCCTCATTTTC (SEQ ID NO: 382) (encoding P87);
the polynucleotide sequence of
GATGGCCACTCCTACACATCCAAGGTGAATTGTTTACTCCTTCAAGATGGGTTCC
ATGGCTGTGTGAGCATCACCGGGGCAGCTGGAAGAAGAAACCTGAGCATCTTCC
TGTTCTTGATGCTGTGCAAATTGGAGTTCCATGCTTGT (SEQ ID NO: 334) (encoding AS55);
the polynucleotide sequence of
ACAGGGGGCAAAAGCACCTGCTCGGCTCCTGGCCCTCAGTCTCTCCCCTCCACTC
CATTCTCCACCTACCCACAGTGGGTCATTCTGATCACCGAACTG (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
GTGCCCTTCCGGGAGCTCAAGAACGTGAGTGTCCTGGAGGGGCTCCGTCAAGGC
CGGCTTGGGGGTCCCTGTTCATGTCACTGCCCAAGACCTTCCCAGGCCAGGCTCA
CGCCAGTGGATGTGGCAGGTCCCTTCTTGTGTCTGGGGGATCCTGGGCTGTTCCC
CCCAGTCAAGAGCAGTATC (SEQ ID NO: 310) (encoding AS43);
the polynucleotide sequence of
GGCATGGAGTGCACCCTGGGGCAGGTGGGTGCCCCGTCCCCTCGGAGGGAGGAG
GACGGTTGGCGTGGGGGCCACAGCCGATTCAAGGCTGATGTACCAGCACCGCAG
GGACCCTGCTGGGGTGGCCAACCTGGCTCTGCCCCCTCCTCAGCTCCTCCTGAAC
AGTCATTATTAGAT (SEQ ID NO: 326) (encoding AS51);
the polynucleotide sequence of
GGCAACACCACCCTCCAGCAGCTGGGTGAGGCCTCCCAGGCGCCCTCAGGCTCC
CTCATCCCTCTGAGGCTGCCTCTGCTCTGGGAAGTGAGGGGC ((SEQ ID NO: 272) (encoding AS16);
the polynucleotide sequence of
GAGGCCTTCCAGAGGGCCGCTGGTGAGGGCGGCCCGGGCCGCGGTGGGGCACG
GCGCGGTGCCAGGGTGTTGCAGAGCCCCTTTTGCAGGGCAGGAGCTGGGGAGTG
GTTAGGACATCAGTCCCTCAGG (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 GTGCCCTTCCGGGAGCTCAAGAACCAGAGAACAGCACAAGGGGCTCCTGGGATC CACCACGCGGCTTCCCCCGTTGCTGCCAACCTCTGCGACCCGGCGAGACACGCA CAGCACACACGCATCCCCTGCGGCGCTGGCCAAGTGCGTGCTGGCCGAGGTCCC GAAGCAGGTGGTGGAGTACTACAGCCACAGAGGCCTGCCCCCGAGAAGCCTGG GTGTCCCTGCCGGAGAGGCCAGCCCAGGCTGCACACCGTGAAGATGTGGAGGGC G (SEQ ID NO: 262) (encoding AS11);
the polynucleotide sequence of AAGAGAAGTTTTGCTGTCACGGAGAGGATCATC (SEQ ID NO: 266) (encoding AS13);
the polynucleotide sequence of TTCAAGAAGTTCGACGGCCCTTGTGGTGAGCGCGGCGGCGGGCGCACGGCTCGA GCTCTGTGGGCGCGCGGCGACAGCGTCCTGACTCCTGCCCTCGACCCCCAGACC CCTGTCAGGGCGCCCTCCCTGACCCGAGCCGCAGCTGCCGTG (SEQ ID NO: 318) (encoding AS47);
the polynucleotide sequence of CTTGTACTTGGTGTATTGAGCGGGCACAGTGGCTCACGCCTA (SEQ ID NO: 256) (encoding AS8);
the polynucleotide sequence of CAGTGGCAGCACTACCACCGGTCAGGTGAGGCCGCAGGGACTCCCCTCTGGAGA CCCACAAGAAAC (SEQ ID NO: 278) (encoding AS19);
the polynucleotide sequence of TGCCACCTCTTCCTGCAGCCCCAGGTTGGCACCCCCCCCCCCCACACTGCCAGTG CTCGAGCCCCCAGTGGTCCACCCCACCCTCATGAAAGTTGCCCTGCAGGGCGAA GACCTGCGAGAGCTGCGCAGACATGTGCACGCCGACAGCACGGACTTCCTGGCT GTGAAGAGGCTGGTACAGCGCGTGTTCCCAGCCTGCACCTGCACCTGCACCAGG CCGCCCTCGGAGCAGGAAGGGGCCGTGGGTGGGGAGAGGCCTGTGCCCAAGTA CCCCCCTCAAGAGGC (SEQ ID NO: 298) (encoding AS37);
the polynucleotide sequence of AAAATTCAGAATAAAAATTGTCCAGAC (SEQ ID NO: 286) (encoding AS23);

the polynucleotide sequence of CACTACAAATTAATTCAACAACCCATATCCCTCTTCTCCATCACTGATAGGCTCC ATAAGACGTTCAGTCAGCTGCCCTCGGTCCATCTCTGCTCAATCACCTTCCAGTG GGGACACCCGCCCATTTTCTGCTCAACAAATGATATCTGTGTCACGGCCAACTTC TGCATCTCGGTCACATTCCTTAAACCGTGCTTCCTCCTACATGAGGCATCTGCCT CACAG (SEQ ID NO: 448) (encoding MS1);

the polynucleotide sequence of AGGACCGCCCTGACACACAATCAGGACTTCTCTATCTACAGGCTCTGTTGCAAG AGGGGGTCCCTCTGCCACGCTTCCCAGGCCAGATCCCCGGCTTTCCCGAAGCCG GTCAGACCTCTTCCTGCCCCCATCACCAGAATCACCCCCCAACTGGGGGGACAA TCTGACTCGAGTCAACCCCTTCTCACTACGGGAAGACCTCAGGGGTGGCAAGAT CAAGCTCTTAGACACACCCAGCAAGCCAGTCCTGCCTCTTGTGCCACCATCACCA TTCCCATCCACTCAGCTGCCCTTGGTGACCACTCCGGAGACCCTGGTCCAGCCTG GGACACCTGCCCGCCGCTGCCGCTCACTACCCTCATCCCCCGAGCTCCCCCGCCG TATGGAGACAGCACTGCCAGGTCCTGGCCCTCCCGCTGTGGGCCCCTCGGC (SEQ ID NO: 450) (encoding MS3);

the polynucleotide sequence of TATGCTTACAAGGACTTTCTCTGGTGTTTTCCTTTTTCTTTAGTTTTTCTCCAAGAG ATTCAAATCTGCTGCCATGTTAGCTGTCTTTGCTGTATCTGCTGTAGTACACGAA TATGCCTTGGCTGTTTGCTTGAGCTTTTTCTATCCCGTGCTCTTCGTGCTCTTCAT GTTCTTTGGAATGGCTTTCAACTTCATTGTCAA (SEQ ID NO: 453) (encoding MS6);

the polynucleotide sequence of ACCATGCCTGCTATTTTAAAGTTACAGAAGAATTGTCTTCTCTCCTTA (SEQ ID NO: 455) (encoding MS8); and

the polynucleotide sequence of TATGAAGCAGGGATGACTCTGGGAGAAAAATTCCGGGTTGGCAATTGCAAGCAT CTCAAAATGACCAGACCC (SEQ ID NO: 380) (encoding P82), and fragments thereof.

In some embodiments, the isolated heterologous polynucleotide further comprises one or more polynucleotides selected from the group consisting of

the polynucleotide sequence of ATTGCGAGAGAGCTGCATCAGTTCGCTTTTGACCTGCTAATCAAGTCACAC (SEQ ID NO: 168) (encoding M84);

the polynucleotide sequence of CAGCCCGACTCCTTTGCAGCCTTGCACTCTAGCCTCAATGAACTGGGAGAG (SEQ ID NO: 172) (encoding M86);

the polynucleotide sequence of TTTGTGCAAGGCAAAGACTGGGGATTAAAGAAATTCATCCGTAGAGATTTT (SEQ ID NO: 20) (encoding M10);
the polynucleotide sequence of TTTGTGCAAGGCAAAGACTGGGGAGTCAAGAAATTCATCCGTAGAGATTTT (SEQ ID NO: 24) (encoding M12); and
the polynucleotide sequence of CAGAACCTGCAGAATGGAGGGGGGAGCAGGTCTTCAGCCACACTGCCGGGGCG GCGGCGGCGGCGGTGGCTGCGGCGGCGGCGGCAGCCAATATCAGTAGCTCCTGC GGGGCCCCCTCGCCGACCAAACCAAAAACCAAACCCACCTGGCGGTGCGAGGTG TGTGATTATGAGACCAACGTGGCCAGGAACCTCCGCATTCACA (SEQ ID NO: 178) (encoding FR1); and fragments thereof.

The disclosure also provides a heterologous polynucleotide encoding two 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 and 177, and fragments thereof.

The disclosure also provides a heterologous polynucleotide comprising two 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 and 178, and fragments thereof.

The disclosure also provides a heterologous polynucleotide comprising two 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 and 499, and fragments thereof. The polynucleotides are useful in expression in adenovirus.

The disclosure also provides a heterologous polynucleotide comprising two 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 and 540, and fragments thereof. The polynucleotides are useful in expression in MVA.

For example, a heterologous polynucleotide of SEQ ID NO: 542 encoding a heterologous polypeptide of SEQ ID NO: 541 by may be used to generate adenovirus-based prostate cancer vaccines, such as those based on rGAd20. Alternatively, heterologous polynucleotides encoding heterologous polypeptides of SEQ ID NOs: 550, 554, 555, 556, 623 or

624 may also be used in adenovirus-based vaccines. In addition to the heterologous polynucleotide encoding the combination of the 41 neoantigens, additional facilitator elements may be included to the 5' or 3' of the heterologous polynucleotide, such as regulatory sequences, tags, and the like. Exemplary facilitator elements include CMV promoter, vaccinia P7.5 promoter, TetO repressor, Kozak sequence, a polynucleotide encoding a T cell enhancer (TCE), a polynucleotide encoding a histidine tag, one or more stop codons, a polyadenylation signal or a promoter sequence. Exemplary polynucleotide sequences of the facilitator elements comprise CMVTetO promoter comprising SEQ ID NO: 628, Kozak sequence comprising SEQ ID NO: 545, the polynucleotide encoding the TCE comprising SEQ ID NO: 546, the polynucleotide encoding the histidine tag comprising SEQ ID NO: 547, a BGH polyadenylation site comprising SEQ ID NO: 629, the one or more stop codons comprising the polynucleotide sequence of TAGTAA, the CMV promoter comprising a polynucleotide of SEQ ID NO: 628 or the vaccinia P7.5 promoter comprising a polynucleotide of SEQ ID NO: 630. Thus the heterologous polynucleotide comprising one or more additional facilitator sequences comprises the polynucleotide sequence of SEQ ID NO: 551, encoding the polypeptide of SEQ ID NO: 550.

In some embodiments, the isolated heterologous polypeptide comprises the amino acid sequence of SEQ ID NO: 541.

In some embodiments, the heterologous polypeptide is encoded by the heterologous polynucleotide of SEQ ID NO: 542.

In some embodiments, the heterologous polynucleotide further comprises one or more facilitator elements.

In some embodiments, the facilitator elements 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 Kozak sequence comprises SEQ ID NO: 545, the polynucleotide encoding the TCE comprises SEQ ID NO: 546, the polynucleotide encoding the histidine tag comprises SEQ ID NO: 547 and the one or more stop codons comprises the polynucleotide sequence of TAGTAA.

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.

The disclosure also provides an isolated heterologous polypeptide comprising the amino acid sequence of SEQ ID NO: 550.

In some embodiments, the heterologous polypeptide is encoded by the heterologous polynucleotide of SEQ ID NO: 551.

The disclosure also provides an isolated heterologous polypeptide comprising the amino acid sequence of SEQ ID NO: 554.

In some embodiments, the isolated heterologous polypeptide comprises the amino acid sequence of SEQ ID NO: 555.

The disclosure also provides an isolated heterologous polypeptide comprising the amino acid sequence of SEQ ID NO: 556.

In some embodiments, the isolated heterologous polypeptide comprises the amino acid sequence of SEQ ID NO: 623.

The disclosure also provides an isolated heterologous polypeptide comprising the amino acid sequence of SEQ ID NO: 624.

In some embodiments, the heterologous polynucleotide is DNA. In some embodiments, the heterologous polynucleotide is RNA. In some embodiments, the heterologous polynucleotide is mRNA. In some embodiments, the heterologous polynucleotide is self-replicating RNA.

The heterologous polynucleotide of SEQ ID NO: 544 encoding the heterologous polypeptide of SEQ ID NO: 543 may be used to engineer MVA-based prostate cancer vaccines. Alternatively, the heterologous polypeptides of SEQ ID NOs: 557, 558, 559, 625 or 626 may also be used. In addition to the heterologous polynucleotide encoding the combination of the 41 neoantigens, additional facilitator elements may be included to the 5' or 3' of the heterologous polynucleotide, such as regulatory sequences, tags, and the like. Exemplary facilitator sequences include Kozak sequence, a polynucleotide encoding a T cell enhancer (TCE), a polynucleotide encoding a histidine tag, one or more stop codons, a polyadenylation signal or a promoter sequence. Exemplary polynucleotide sequences of the facilitator elements comprise Kozak sequence comprising SEQ ID NO: 545, the polynucleotide encoding the TCE comprising SEQ ID NO: 546, the polynucleotide encoding the histidine tag comprising SEQ ID NO: 547 and the one or more stop codons comprising the polynucleotide sequence of TAGTAA. Thus a heterologous polynucleotide comprising one or more additional facilitator elements comprises the polynucleotide sequence of SEQ ID NO: 553, encoding the polypeptide of SEQ ID NO: 552.

The disclosure also provides an isolated heterologous polypeptide comprising the amino acid sequence of SEQ ID NO: 543.

In some embodiments, the heterologous polypeptide is encoded by the heterologous polynucleotide of SEQ ID NO: 544.

In some embodiments, the heterologous polynucleotide further comprises one or more facilitator elements.

In some embodiments, the facilitator elements 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 Kozak sequence comprises SEQ ID NO: 545, the polynucleotide encoding the TCE comprises SEQ ID NO: 546, the polynucleotide encoding the histidine tag comprises SEQ ID NO: 547 and the one or more stop codons comprises the polynucleotide sequence of TAGTAA.

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. In some embodiments, the isolated heterologous polypeptide comprises the amino acid sequence of SEQ ID NO: 552.

In some embodiments, the heterologous polypeptide is encoded by the heterologous polynucleotide of SEQ ID NO: 553.

The disclosure also provides an isolated heterologous polypeptide comprising the amino acid sequence of SEQ ID NO: 557.

The disclosure also provides an isolated heterologous polypeptide comprising the amino acid sequence of SEQ ID NO: 558.

The disclosure also provides an isolated heterologous polypeptide comprising the amino acid sequence of SEQ ID NO: 559.

The disclosure also provides an isolated heterologous polypeptide comprising the amino acid sequence of SEQ ID NO: 625.

The disclosure also provides an isolated heterologous polypeptide comprising the amino acid sequence of SEQ ID NO: 626.

In some embodiments, the heterologous polynucleotide is DNA. In some embodiments, the heterologous polynucleotide is RNA. In some embodiments, the heterologous polynucleotide is mRNA. In some embodiments, the heterologous polynucleotide is self-replicating RNA.

One or more fragments of the 41 prostate 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) may also be used to generate a prostate cancer vaccine, as long as the one or more fragments are immunogenic. Exemplary such fragments that may be used are polypeptides of SEQ ID NOs: 387, 388, 390, 392, 405, 406, 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, 621, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695,

696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709. Immunogenicity of the fragments can be tested using assays described herein.

In some embodiments, the first N-terminal amino acid residue of the fragment is absent.

The disclosure also provides an isolated heterologous polynucleotide encoding the heterologous polypeptide of the disclosure.

In some embodiments, the first 5' codon of the fragment is absent.

The disclosure also provides a vector comprising a heterologous polynucleotide encoding two 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 and 177, and fragments thereof.

The disclosure also provides a vector comprising a heterologous polynucleotide comprising two 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 and 178, and fragments thereof.

The disclosure also provides a vector comprising a heterologous polynucleotide comprising two 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 and 499, and fragments thereof. The polynucleotides are useful in expression in adenovirus.

The disclosure also provides a vector comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 541. In some embodiments, the heterologous polynucleotide comprises the polynucleotide of SEQ ID NO: 542.

The disclosure also provides a vector comprising the heterologous polynucleotide of SEQ ID NO: 542.

The disclosure also provides a vector comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 550. In some embodiments, the heterologous polynucleotide comprises the polynucleotide of SEQ ID NO: 551.

The disclosure also provides a vector comprising the heterologous polynucleotide of SEQ ID NO: 551.

The disclosure also provides a vector comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 554.

The disclosure also provides a vector comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 555.

The disclosure also provides a vector comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 556.

The disclosure also provides a vector comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 623.

The disclosure also provides a vector comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 624.

In some embodiments, the vector is a viral vector.

In some embodiments, the viral vector is an adenovirus vector.

In some embodiments, the adenovirus vector is derived from a human adenovirus, optionally from Ad5, Ad7, Ad11, Ad26, Ad34, Ad35, Ad48, Ad49 or Ad50.

In some embodiments, the adenovirus vector is derived from Ad26.

In some embodiments, the adenovirus vector is derived from a great ape adenovirus, optionally from GAd20, GAd19, GAd21, GAd25, GAd26, GAd27, GAd28, GAd29, GAd30, GAd31, ChAd3, ChAd4, ChAd5, ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd1, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd55, ChAd63, ChAd73, ChAd82, ChAd83, ChAd146, ChAd147, PanAd1, PanAd2 or PanAd3.

In some embodiments, the adenovirus vector is derived from GAd20.

The disclosure also provides a rGAd20 comprising a heterologous polynucleotide encoding a heterologous polypeptide comprising two 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 and 177, and fragments thereof.

The disclosure also provides a rGAd20 comprising a heterologous polynucleotide comprising two 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 and 178, and fragments thereof.

The disclosure also provides a rGAd20 comprising a heterologous polynucleotide comprising two 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 and 499, and fragments thereof. The polynucleotides are useful in expression in adenovirus.

The disclosure also provides a rGAd20 comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 541. In some embodiments, the heterologous polynucleotide comprises SEQ ID NO: 542.

The disclosure also provides a rGAd20 comprising the heterologous polynucleotide of SEQ ID NO: 542.

The disclosure also provides a rGAd20 comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 550. In some embodiments, the heterologous polynucleotide comprises SEQ ID NO: 551.

The disclosure also provides a rGAd20 comprising the heterologous polynucleotide of SEQ ID NO: 551.

The disclosure also provides a rGAd20 comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 554.

The disclosure also provides a rGAd20 comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 555.

The disclosure also provides a rGAd20 comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 556.

The disclosure also provides a rGAd20 comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 623.

The disclosure also provides a rGAd20 comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 624.

The disclosure also provides a cell comprising or transduced with the rGAd20, wherein the rGAd20 comprises:

a heterologous polynucleotide encoding a heterologous polypeptide comprising two 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 and 177, and fragments thereof;

a heterologous polynucleotide comprising two 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 and 499, and fragments thereof;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 541;

heterologous polynucleotide of SEQ ID NO: 542;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 550;

a heterologous polynucleotide of SEQ ID NO: 551;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 554;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 555;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 556;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 623; or

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 624.

The disclosure also provides a vaccine comprising the rGAd20, wherein the rGAd20 comprises:

a heterologous polynucleotide encoding a heterologous polypeptide comprising two 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 and 177, and fragments thereof;

a heterologous polynucleotide comprising two 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 and 499, and fragments thereof;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 541;

a heterologous polynucleotide of SEQ ID NO: 542;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 550;

a heterologous polynucleotide of SEQ ID NO: 551;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 554;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 555;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 556;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 623; or

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 624.

The disclosure also provides a vector comprising a heterologous polynucleotide comprising two 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 and 178, and fragments thereof.

The disclosure also provides a vector comprising a heterologous polynucleotide comprising two 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 and 540, and fragments thereof. The polynucleotides are useful in expression in MVA.

The disclosure also provides a vector comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 543. In some embodiments, the heterologous polynucleotide comprises SEQ ID NO: 544.

The disclosure also provides a vector comprising a heterologous polynucleotide of SEQ ID NO: 544.

The disclosure also provides a vector comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 552. In some embodiments, the heterologous polynucleotide comprises SEQ ID NO: 553.

The disclosure also provides a vector comprising a heterologous polynucleotide of SEQ ID NO: 553.

The disclosure also provides a vector comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 557.

The disclosure also provides a vector comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 558.

The disclosure also provides a vector comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 559.

The disclosure also provides a vector comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 625.

The disclosure also provides a vector comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 626.

In some embodiments, the vector is a viral vector.

In some embodiments, the viral vector is MVA.

In some embodiments, the MVA vector is derived from MVA 476 MG/14/78, MVA-571, MVA-572, MVA-574, MVA-575 or MVA-BN.

The disclosure also provides a rMVA comprising a heterologous polynucleotide encoding a heterologous polypeptide comprising two 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 and 177, and fragments thereof;

The disclosure also provides a rMVA comprising a heterologous polynucleotide comprising two 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 and 178, and fragments thereof.

The disclosure also provides a rMVA comprising a heterologous polynucleotide comprising two 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 and 540, and fragments thereof. The polynucleotides are useful in expression in MVA.

The disclosure also provides a rMVA comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 543. In some embodiments, the heterologous polynucleotide comprises SEQ ID NO: 544.

The disclosure also provides a rMVA comprising the heterologous polynucleotide of SEQ ID NO: 544.

The disclosure also provides a rMVA comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 552. In some embodiments, the heterologous polynucleotide comprises SEQ ID NO: 553.

The disclosure also provides a rMVA comprising the heterologous polynucleotide of SEQ ID NO: 553.

The disclosure also provides a rMVA comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 557.

The disclosure also provides a rMVA comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 558.

The disclosure also provides a rMVA comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 559.

The disclosure also provides a rMVA comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 625.

The disclosure also provides a rMVA comprising a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 626.

The disclosure also provides a cell comprising or transduced with the rMVA, wherein the rMVA comprises:

a heterologous polynucleotide encoding a heterologous polypeptide comprising two 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 and 177, and fragments thereof;

a heterologous polynucleotide comprising two 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 and 540;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 543;

a heterologous polynucleotide of SEQ ID NO: 544;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 552;

a heterologous polynucleotide of SEQ ID NO: 553;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 557;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 558;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 559;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 625; or

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 626.

The disclosure also provides a vaccine comprising the rMVA, wherein the MVA comprises:

a heterologous polynucleotide encoding a heterologous polypeptide comprising two 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 and 177, and fragments thereof;

a heterologous polynucleotide comprising two 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 and 540;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 543;

a heterologous polynucleotide of SEQ ID NO: 544;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 552;

a heterologous polynucleotide of SEQ ID NO: 553;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 557;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 558;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 559;

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 625; or

a heterologous polynucleotide encoding the heterologous polypeptide of SEQ ID NO: 626.

The disclosure also provides a vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide comprising 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.

In some embodiments, the heterologous polynucleotide comprises 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 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 and 178, and fragments thereof.

In some embodiments, the fragments comprise peptides of SEQ ID NOs: 387, 388, 390, 392, 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.

In some embodiments, the heterologous polypeptide comprises SEQ ID NOs: 541, 550, 554, 555, 556, 623 or 624.

In some embodiments, the heterologous polynucleotide comprises SEQ ID NO: 542 or SEQ ID NO: 551.

In some embodiments, the heterologous polypeptide comprises SEQ ID NOs: 543, 552, 557, 558, 559, 625 or 626.

In some embodiments, the heterologous polynucleotide comprises SEQ ID NO: 544 or SEQ ID NO: 553.

In some embodiments, the heterologous polynucleotide is DNA or RNA.

In some embodiments, RNA is mRNA or self-replicating RNA.

In some embodiments, the vaccine is a recombinant virus.

In some embodiments, the recombinant virus is derived from adenovirus, poxvirus, adeno-associated virus or retrovirus.

In some embodiments, 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).

In some embodiments, the recombinant virus is derived from GAd20.

In some embodiments, the recombinant virus is derived from MVA.

In some embodiments, the recombinant virus is derived from Ad26.

The disclosure also provides a vaccine comprising a heterologous polynucleotide sequence encoding a heterologous polypeptide of SEQ ID NO: 541 or 550.

In some embodiments, the heterologous polynucleotide comprises SEQ ID NO: 542 or 551.

In some embodiments, the vaccine is a recombinant virus derived from GAd20.

The disclosure also provides a vaccine comprising a heterologous polynucleotide sequence encoding a heterologous polypeptide of SEQ ID NO: 543 or 552.

In some embodiments, the heterologous polynucleotide comprises SEQ ID NO: 544 or 553.

In some embodiments, the vaccine is a recombinant virus derived from MVA.

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

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")**

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.

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.

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.

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")**

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.

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).

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.

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")**

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.

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).

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.

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**

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.

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). SEP

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.

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.

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).

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

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.

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.

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.

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

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

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.

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

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.

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

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

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

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

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.

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

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**SRSSATLPGRRRRRRWLRRRRQPISVAPAGPPRRPNQKPNPPGGARCVIMRPTWPGTSAFT	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	Cagaacctgcagaatggaggggggagcaggtcttcagccacactgccggggcggcggcggcggcggtggctgcggcggcggcggcagccaatatcagtagctcctgcggggccccctcgccgaccaaaccaaaaaccaaacccacctggcggtgcgaggtgtgtgattatgagaccaacgtggccaggaactccgcattcaca	178
FR2	cagaacctgcagaatggaggggggGgagcaggtcttcagccacactgccggggcggcggcggcggcggtggctgcggcggcggcggcagccaatatcagtag	180
FR3	aaccaagagaaagaggcagAaaaaaactattga	182
FR4	gatgctgctgtcagtcccaggggctgcagcacaggcaggggagagggaatctggggtggtggcagtctcccctgagaaaagtgagagtccccaaaaggaggatggtttatcatcccagttga	184
FR5	tttgtcgtttctgttgtgaAaaaaaacaggacttgccccctttcgtctatttgtcagacgaatgttacaatttactggcaataaagttttggatagaccttaa	186
FR6	agaagcagaagatcggctataaaaagataatg	188
FR7	agtataattgtacaAGagagagagagagcagagagaagggtcagaagaggccaagtgatggaaatagtggattaa	190
FR8	aataacttggtcacagAaaaaaaacacagaaatgtgcaatgtcatgatgcagttgaggaaaatggccaatcatcctttattacatcgccaatattacacagctgaaa	192
FR9	cacccacagaggaaaaggcgggggtccctccgagcccacccctggctctcggccccaggatgcaactgtgcacccagcttgccagatttttccccattacacccccagtgtggcatatccttggtccccagaggcacacccctttgatc	194

FR10	gccagccggcaccatctgtgggggcaacagcgggcacccccgcaccacccccgtgcccacc tgttcacccaggaccctgcgatgcctcccctgacc	196
FR11	gggcctggggaggctgggggcccctcaccccaaggcgggtgagc	198
FR12	ggcgggggcccagcggctcaggggaggctcccacttctccttcagccctgaggacatgaaa	200
FR13	ggaaatccagatgtcccacccccgtcctcttcaaggcagatcagagcgagagttctttgtcaagtg ggtag	202
FR14	gctttttgttactgtggggaaaaagttccttagga	204
FR15	acgctgcacccggactccggcaacggctgctacttgcgccgccagaagcg	206
FR16	acgctgcacccggactccggcaacatgtacggctgctacttgcgccgccagaa	208
FR17	ctgcacccggactccggcaacatgtgctgctacttgcgccgccagaagcgc	210

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	LQERMELLACGAERGAGGWGGGGGGGGGDRRGGGGSAPALADFAGGRG	239

Table 6.

Neoantigen ID	Polynucleotide sequence	SEQ ID NO:
FUS1	TGCGGGGCCTCTGCCTGTGATGTCTCCCTCATTGCTATGGACAGTGCT	212
FUS2	ACCGAATACAACCAGAAATTACAAGTGAATCAATTTAGTGAATCCAAA	214
FUS3	ACAGAAATTTCATGTTGCACCCTGAGCAGTGAGGAGAATGAATACCTTCCAAGACCAGAGTGGCAGCTCCAG	216
FUS4	GGGCTGGTGTCCTTCGGGGAGCACTTTTGTCTGCCCTGCGCCCTCTGCCA	218
FUS5	AACAGCAAGATGGCTTTGAACTCAGAAGCCTTATCAGTTGTGAGTGAG	220
FUS6	TGTGAGGAGCGCGGCGCGGCAGGAAGCCTTATCAGTTGTGAG	222
FUS7	CTGTGGTTCCAGAGCAGTGAGCTGTCCCCGACGGGAGCGCCATGGCCCAGCCGCCGCCCGACGTGGAGGGGGACGACTGTCTCCCCGCGTACCGCCACCTCTTCTGCCCGGACCTGCTGCGGGACAAAGTGGCCTTCATCACAGGAGGCGGCTCTGGGATTGGGTTCCGGATTGCTGAGATTTTCATGCGGCACGGCTGCCATACGG	224

FUS8	TGGGGGATGGAGTTGGCAGCGTCTCGGAGGTTCTCCTGGG ACCACCACTCCGCCGGGGGGCCGCCCAGAGTGCCAAGCGT CCGATCCGGCGCCGCCCAAGTGCAGCCCAAGGACCCGCTC CCGCTCCGCACCCTGGCAGGCTGCCTAGCCAGGACTGCGCA CCTGCGCCCTGGGGCGGAGTCCTTACCCCAACCCCAGCTTC ACTGCACA	226
FUS9	AAGGAACAGATTTTAGCTGTGGCCAGTCTCGTTTCCTCTCA GTCCATCCACCCTTCATGGGGCCAGAGCCCTCTCTCCAGAA TC	228
FUS10	CTGGAGCTGGAGCTGTCGGAAGGAGTCTGCTTCAGATTAA GA	230
FUS11	CAGCAGCTAAGGATATTTTGTGCAGCCATGGCCTCCAACGA AGATTTCTCCA	232
FUS15	CACGTGGTGGGCTATGGCCACCTTGATACTTCCGGGTCATC CTCCTCCTCCTCCTGGCCC	346
FUS18	GACGGGTTTAGCGGCAGCCTCTTCGCAGTTGTCACCAGACG CTGTTACTTCCTAAAATGGCGGACAATCTTCCCACAGAGTT TGATGTGGTTA	234
FUS19	AAAATGCACTTCTCCCTCAAGGAGCACCCACCGCCCCCTTG CCCGCCT	236
FUS23	GATCTGCGCCGGGTCGCCACATACTGCGCTCCTTTACCCTC ATCGTGGAGGCCTGGGACTGGGACAACGATACCACCCCGA ATGAGGAGCTGC	238
FUS24	TTGCAGGAGCGGATGGAGTTGCTTGCCTGCGGAGCCGAGC GCGGGGCCGGCGGCTGGGGGGGAGGCGGTGGCGGCGGCG GCGGCGACCGAAGAGGAGGAGGAGGAAGCGCGCCAGCTC TTGCAGACTTTGCAGGCGGCCGAGGG	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		

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	CTGACGTTTTTAGATTTCATCCAGGTAACGTTGAGAGTAA TGTCAGGATCTCAAATGGAAAACGGAAGTTCCTATTTTTT CAAGCCCTTTTCATGGGGTCTGGGGGTGGGACTCTCGGCC TGGCTGTGTGTAATGTTAACT	242
AS2	TTCATGATTGGAGAACTTGTAGGTGAGTTGTGTTGCCAAC TCACTTTCCGTTTACCTTTCCTCGAGAGTCTTTGTCAAGCT GTAGTTACACAGGCTTTGAGGTTTAACCCATCTTTTCAGG AAGTTTGTATTTATCAAGACACTGATCTCATG	244
AS3	GTTGCTATGATGGTTCCTGATAGACAGGTTCATTATGACT TTGGATTG	246
AS4	TGGTGTCCGCTGGATCTTAGACTCGGTTCCACTGGATGTC TCACATGCAGACATCATCAAACGTCACATGAG	248
AS5	GTCGTGGGAAGGCGTCATGAAACAGCTCCTCAACCCCTG CTGGTGCCCGACCGAGCTGGTGGTGAAGGGGGAGCA	250
AS6	GACTACTGGGCTCAAAAGGAGAAGATCAGCATCCCCAGA ACACACCTGTGT	252
AS7	GACTACTGGGCTCAAAAGGAGAAGGGATCATCTTCATTC CTGCGACCATCCTGT	254
AS8	CTTGTACTTGGTGTATTGAGCGGGCACAGTGGCTCACGCC TA	256
AS9	CCTGTCCCAACTGCTACACCTGGGGTAAGATCAGTGACTA GTCCCCAGGGGCTGGGCCTTTTCCTTAAGTTTATT	258
AS10	AAGGAAAATGATGTCCGTGAGGTCTGTGATGTGTATTTAC AAATGCAGATCTTCTTCCATTTTAAGTTCAGAAGTTACTT TCAT	260
AS11, AS33	GTGCCCTTCCGGGAGCTCAAGAACCAGAGAACAGCACAA GGGGCTCCTGGGATCCACCACGCGGCTTCCCCCGTTGCTG CCAACCTCTGCGACCCGGCGAGACACGCACAGCACACAC GCATCCCCTGCGGCGCTGGCCAAGTGCGTGCTGGCCGAG GTCCCGAAGCAGGTGGTGGAGTACTACAGCCACAGAGGC CTGCCCCCGAGAAGCCTGGGTGTCCCTGCCGGAGAGGCC AGCCCAGGCTGCACACCGTGAAGATGTGGAGGGCG	262

AS12	TTTGCTAGAAAAATGCTGGAGAAGGTACACAGACAACAC CTACAGCTTTCCCACAATAGCCAGGAA	264
AS13	AAGAGAAGTTTTGCTGTCACGGAGAGGATCATC	266
AS14	ATGTTCCTTAGAAAGGAGCAGCAGGTGGGTCCCCACAGC TTTTCTATGCTT	268
AS15	GTGCTGCGCTTTCTGGACTTAAAGGTGAGATACCTGCACT CT	270
AS16	GGCAACACCACCCTCCAGCAGCTGGGTGAGGCCTCCCAG GCGCCCTCAGGCTCCCTCATCCCTCTGAGGCTGCCTCTGC TCTGGGAAGTGAGGGGC	272
AS17	GGACTGAACTTAAATACTGATAGACCAGGTGGTTACAGC TATTCAATTTGGTGGAAAAACAATGCCAAGAACAGA	274
AS18	TGGAAATTCGAGATGAGCTACACGGTGGGTGGCCCGCCT CCCCATGTTCATGCTAGACCCAGGCATTGGAAAACTGAT AGA	276
AS19	CAGTGGCAGCACTACCACCGGTCAGGTGAGGCCGCAGGG ACTCCCCTCTGGAGACCCACAAGAAAC	278
AS20	AAGGTCCTCAACGAGATCGATGCGGTAGTTACCGTCCCTC CCTCCCTGTCTACCTCCCAGATACCGCAGGGCTGCTGCAT CATATTG	280
AS21	GCCAATCTGAAAGGCACCCTGCAGGTGAGGAGTGGGCAG GCAGTGAGTCCACGC	282
AS22	CTCCAGGCGGCTGCCTCGGGCCAAGGCAAGCAGGGCGTC CCTTGTCCCTGGGGTTGCTGTGCCTACGCTGAGAGTCCCC GGGCCCTGATTTCGGGAGATGCTCCATCACAGGTGGAGC GGGAGGTGCCGGGCCCCTGCCTCAACACGCATTCTCTCTC CCACAGATCCCCACAGCTCCCAGGCCTTCCACACCCCAA GCAGCCTTCTGTT	284
AS23	AAAATTCAGAATAAAAATTGTCCAGAC	286
AS32	GGCGAGGTGGAGCTCTCAGAGGGCGGTGAGGGCCAGCG GCACCTTGCATTTCCCTGGGCCTGCTCTGGGCCGGGCTGG AGAGGGGTGTGCTGTGCTGCTGTGGAGCCTGCT	288
AS63	ATTGAAATGAAAAAACTGTTAAAAAGT	290
AS34	AAGATGCGGGCCATCCAGGCCGAGGGTGGGCACGGGCA GGCCTGCTGTGGAGGGGCCTGGGGATGGGCACCGGGGGA CGGGGGCCCCCAGGGGATGCTCACGCATACTCTGCCCAC CCTGGGCTTCCAGAGCGCCTGGACATGGAGAAGAGAAGA TGCAGACAGAGCCTGGAGGACTCCGAAAGCCTGCGCATC AAGGAGGTGGAGCATA	292
AS35	CTGCTGGAGCCCTTCCGCCGCGGTGAGCCCGGGCCCCGC GGGCTGCTCTCGGGAAGTTCCCGCGGAGGGGAGGGGCCT GGCCGTTCGATCGAGGCTGCACCCGCCACACCTTTGCCCT GTTGCCGCAAGAACCCTTGTCGGCCCCAGCCTTCCAGATT TTTGCCTCCTAGGGTATTGTTAGTGATCATTCTTCCCAAA CTGGATTGTCCAAAACTTGGGTTC	294
AS36	CCCTCGGGGCGGAGAACCAAACGGTTAGTTACCCTGCGT TCTGGCTGCGCCATACAATGCTGGCATCCTCGTGCCGGCC CAGTTCCCTCAGCGCTTCCTCACACAGAGAGGCCCCCAA	296

	GGCTTGTCAGGGGAGCAGCAGATCCCAGGACAGTGACCC TGGGACGGAGCCCTGCAGTCATGCCTCGGGCCCCTGCG	
AS37	TGCCACCTCTTCCTGCAGCCCCAGGTTGGCACCCCCCCCC CCCACACTGCCAGTGCTCGAGCCCCCAGTGGTCCACCCCA CCCTCATGAAAGTTGCCCTGCAGGGCGAAGACCTGCGAG AGCTGCGCAGACATGTGCACGCCGACAGCACGGACTTCC TGGCTGTGAAGAGGCTGGTACAGCGCGTGTTCCCAGCCT GCACCTGCACCTGCACCAGGCCGCCCTCGGAGCAGGAAG GGGCCGTGGGTGGGGAGAGGCCTGTGCCCAAGTACCCCC CTCAAGAGGC	298
AS38	AAGGAGCTCAAGCTGGAGCAGCAGGTGGGTGGGCAGGG CTTGAGAGGGGTGGGCCAAGGGGTGCGTGGCGGCTTCGT GACCCTCACTACCCATACCCCGTTCCCCTCCCAGGAAGCT GCAGAGCGGGAGTCTAAA	300
AS39	GGAGAAATCAGCCAGGAAGAGGTGCCTCCCTCCCGCCAC CTGGGCGTCTCATGGGGTGCTGGGGTGTGGGCGGGCCTC ACCCTCGGGGCCTCTGCACCCCCTAACTCTAGCTTCCCCT CAGGTGCTGAGCTACAGCCAGTTGTGTGCTGCATTAGGA GTGACACAAGACAGCCCCGACCCCCCGACTTTCCTCAGC ACAGGGGAGATCCACGCCTTCCTCAGCTCTCCCTCGGGGC GGAGAACCAAACGGTTAGTTACCCTGCGTTCTGGCTGCG CCATACAATGCTGGCATCCTCGTGCCGGCCCAGTTCCCTC AGCGCTTCCTCACACAGAGAGGCCCCCAAGGCTTGTCAG GGGAGCAGCAGATCCCAGGACAGTGACCCTGGGACGGA GCCCTGCAGTCATGCCTCGGGCCCCTGCGTAACCTCCACT GTCTCCAGCCCAGGTCTCCTTCCTCAGAGGCTATTGCCTC TCGCTCTGACTGGGCTCCCTGTGGAGGAAGATGGTTTCGA GCACGCGGGAGCC	302
AS40	GACTGCATGCTCAGCGAGGAAGGTGGGCAGGCGCGGCGG GGTGGATCCCTCTGCTCCTTAGCTGCCCACACCATTGCCT CGGCAGCCCGAGGTCGCTTCCTCTCCAGGCTCTCCAATTT CTGTGCCGTAGTTAAAGCGAGCAGGGGCGCCCCTTCCTG CACCTGGGAG	304
AS41	GAGGCCTTCCAGAGGGCCGCTGGTGAGGGCGGCCCGGGC CGCGGTGGGGCACGGCGCGGTGCCAGGGTGTTGCAGAGC CCCTTTTGCAGGGCAGGAGCTGGGGAGTGGTTAGGACAT CAGTCCCTCAGG	306
AS42	CCTGAGCCCAGGAGACTGAGCCCAGGTGAGCCCAGGGGG CGACCCCGGAAGGGCTGGGGGATCTGGGGTTTGTGTGGA GCGCGGGTGGGGCCCAAGGCTTGGCGG	308
AS43	GTGCCCTTCCGGGAGCTCAAGAACGTGAGTGTCCTGGAG GGGCTCCGTCAAGGCCGGCTTGGGGGTCCCTGTTCATGTC ACTGCCCAAGACCTTCCCAGGCCAGGCTCACGCCAGTGG ATGTGGCAGGTCCCTTCTTGTGTCTGGGGGATCCTGGGCT GTTCCCCCCAGTCAAGAGCAGTATC	310
AS44	TTTGTGAGCCTGACTGCCATCCAGATGGCATCGTCGGCCA CTCCCTGGGGGAGGTGGCCTGTGGCTACGCCGACGGCTG CCTGTCCCAGGAGGAGGCCGTCCTCGCTGCCTACTGGAG GGGACAGTGCATCAAAGAAGCCCATCTCCCGCCGGGCGC CATGGCAGCCGTGGGCTTGTCCTGGGAGGAGTGTAAACA GCGCTGCCCCCCGGGCGTGGTGCCCGCCTGCCACAACTCC AAGGACACAGTCACCATCTCGGGACCTCAGGCCCCGGTG TTTGAGTTCGTGGAGCAGC	312

AS45	CCAGTTGCCATTAAACCTGGTACAGGGCCGCCCAATAAC TCCAGTATACACGGTGGCTCCAAACGTTCAGAGAATTCCT ACTGCCGGGATCTACGGGGCCAGTTACGTGCCATTTGCTG CTCCAGCTACAGCCACGATCGCCACACTACAGAAGAACG CGGCAGCCGCGGCCGCCATGTATGGAGGATACGCAGGCT ACATACCTCAGGCCTTCCCTGCTGCTGCCATTCAGGTCCC CATCCCCGACGTCTACCAGACATACTGAGGCTGGTGACC AGCACGAAGACAGACCACACAAACACCACTGAAGGAAC GCTTGACTATTTA	314
AS46	AAGTGGAACAAGAACTGGACAGCCACACTCGGGGCTCTT ACAATCAGGGGTCATAAGCTGCTATGTCACCTACCTCACC TTCTCAGCTCTGTCCAGCAAACCTGCAGAAGTAGTTCTAG A	316
AS47	TTCAAGAAGTTCGACGGCCCTTGTGGTGAGCGCGGCGGC GGGCGCACGGCTCGAGCTCTGTGGGCGCGCGGCGACAGC GTCCTGACTCCTGCCCTCGACCCCCAGACCCCTGTCAGGG CGCCCTCCCTGACCCGAGCCGCAGCTGCCGTG	318
AS48	GAAAATGCCAGCCTAGTGTTTACAGGATCCAACAGCCCC ATACCAGCCTGCGAACTGAGTAGTCACCCAGCTCATGGT ATCAGTCCTTGGATACCCTCACCTGGAAATGAACATTTCC ATGGCATAAAGAAGCAAGTAAAGGCAATAAAAGTAGAA	320
AS49	CGGCTGACGCAACGGCTGGTCCAGGGCTGGACCCCAATG GAGAACAGGTGGTGTGGCAGGCGAGCGGGTGGGCAGCC CGCATCATCCAGCACGAGATGGACCACCTGCAGGGCTGC CTGTTTATTGACAAAATGGACAGCAGGACGTTCACAAAC GTCTATTGGA	322
AS50	GAAAATTCAGGCAACGCCTCGCGTTGGCTGCATGTACCA AGTAGTTCAGACGATTGGCTCGGATGGAAAAAATCTTCT GCAATTACTTCCAATTCC	324
AS51	GGCATGGAGTGCACCCTGGGGCAGGTGGGTGCCCCGTCC CCTCGGAGGGAGGAGGACGGTTGGCGTGGGGGCCACAGC CGATTCAAGGCTGATGTACCAGCACCGCAGGGACCCTGC TGGGGTGGCCAACCTGGCTCTGCCCCCTCCTCAGCTCCTC CTGAACAGTCATTATTAGAT	326
AS52	AAAGGGAGTGTGGAGAGGCGCTCGGTGAGCCTGGGGCAT CCTGCTGAGGGTTGGGCATGGGCAGAGAGGAGCCTCCAG CCAGGCATGACCACAGCCAACACAGGCTGCCTCTCATTC CACCACAGAGGGTGCCTCCTCCCTGTTTTGCCCAAATTAC ACTGTGGGCTAGGTGGACTACCTCTTGTCAGAGCTAAAG AAATCAAGCGAGTGCAGAGGGCAGGGGAGAGTTCGCTGC CTGTGAAGGGCCTTCTCACCGTCGCTTCGGCTGTCATCGC AGTCCTGTGGGGTAGGCCAAGCGAGGTCACAGGAGAAAA TGAGGCTCAGCATGAT	328
AS53	TTTGGCCTCACCACACTTGCAGGTAGAAGCTCCCACGGG ACCTCAGGACTGAGGGCAGCCACACACACCAAGTCTGGG GACGGTGGCCAGGGGGCTGCCAGGCAGTGTGAGAAGCTC CTGGAGCTGGCCCGGGCTACCAGACCCTGGGGGAGGAGT ACGTCAGCATCATCCAGGTGGACCCATCGCGGATACATG TGCCCTCCTCGCTGCGCCGTGGCGTGCTGG	330
AS54	ATTATTGACAGCGACAAGATAATGGCAGTGTGCATGGGG TGCCTGCTCACACGTCATGTGCAATGCCAGGCCATGGAG ATGCAACAG	332
AS55	GATGGCCACTCCTACACATCCAAGGTGAATTGTTTACTCC TTCAAGATGGGTTCCATGGCTGTGTGAGCATCACCGGGG	334

	CAGCTGGAAGAAGAAACCTGAGCATCTTCCTGTTCTTGAT GCTGTGCAAATTGGAGTTCCATGCTTGT	
AS56	CTACTAAATGCAGAAGATTACCGGTGTGCCATTCATTCAA AAGAGATTTATCTTCTTTCCCCCTCCCCCCACCAGGCAAT GGACAAGTTTTCTCTCTGCTGCATCAACTGCAATCTATGT TTACATGTATTCCTTTTACTACTATTTTTTCAAAACAAAGA TGTATGGCTTATTTCAAACATCATTTTACTTTGGATATATG GCGGTATT	336
AS57	ACAGGGGGCAAAAGCACCTGCTCGGCTCCTGGCCCTCAG TCTCTCCCCTCCACTCCATTCTCCACCTACCCACAGTGGG TCATTCTGATCACCGAACTG	338
AS58	GTGGAGACCCTGGAGAATGCCAACAGCTTCTCCAGCGGG ATCCAGCCACTCCTCTGTTCCCTGATTGGCCTGGAGAATC CCACC	340
AS59	GCTGGGGCTGGAACAATTTCCGAAGGTAGTGTTCTCCATG GTCAGAGGCTGGAGTGTGATGCCAGACGTTTTTTTGGGTG TGGGACTACAATACTGGCAGAGTGGGAGCACCAT	342
AS55.1	GATGGCCACTCCTACACATCCAAGGTGAATTGTTTACTCC TTCAAGATGGGTTCCATGGCTGTGTGAGCATCACCGGGG CAGCTGGAAGAAGAAACCTGAGCATCTTCCTGTTCTTGAT GCTGTGCAAATTGGAGTTCCATGCT	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

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**FPVGA EGTF**PAAATQRGVVRPA	351
P35	TMPRSS2-ERG	**NSKMALNS**LNSIDDAQLTRIAPPRSHCCFWEVNAP	353
P37	TSTD1-F11R	MAG**GVLRRLLCR**EPDRDGDKGASRE ETVVPLHIGDPVVLPGIGQCYSALF	355
P46	TP53 (R213D)	DDRNTFDIVWW**CPMSRLRLAL**TVPPS TTTTCVTVPAWAA	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	352

		CTCAGCGGGGTGTCGTTCGGCCGGC GTG	
P35	TMPRSS2-ERG	AACAGCAAGATGGCTTTGAACTCATT AAACTCCATTGATGATGCACAGTTGA CAAGAATTGCCCCTCCAAGATCTCAT TGCTGTTTCTGGGAAGTAAACGCTCC T	354
P37	TSTD1-F11R	ATGGCTGGAGGAGTCCTTCGGCGGC TGTTGTGTCGGGAGCCTGATCGCGAT GGGGACAAAGGCGCAAGTCGAGAGG AAACTGTTGTGCCTCTTCATATTGGC GATCCTGTTGTGCTCCCTGGCATTGG GCAGTGTTACAGTGCACTCTTCT	356
P46	TP53 (R213D)	GATGACAGAAACACTTTCGACATAG TGTGGTGGTGCCCTATGAGCCGCCTG AGGTTGGCTCTGACTGTACCACCATC CACTACAACTACATGTGTAACAGTTC CTGCATGGGCGGCATGA	358
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 CACTACAACTACATGTGTAACAGTTC CTGCATGGGCGGCATGA	358

Example 4: HLA binding predictions

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.

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-	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	CPMSRLRLA	394
P48	AR.p.H875Y	Missense	YQFTFDLLI	395
P50	AR (W742C)	Missense	IQYSCMGLM	396
P56	SPOP (F102C)	Missense	RAKCKFSIL	397
P58	AR (Q903H)	Missense	HVPKILSGK	398
P59	FOXA1 (F254V)	Missense	NMVENGCYL	399
P60	FOXA1.p.F266L	Missense	YLRRQKRLK	400
P61	FOXA1.p.R261G	Missense	CYLGRQKRF	401
P73	TP53 (G266E)	Missense	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	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

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

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Example 6: Binding of neoantigens to HLA

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

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.

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.

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.

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.

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.

Table 20 shows the polynucleotide sequences encoding the corresponding longer neoantigens.

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**ISLFSITDRLHKTFSQLPSVHLCSIT FQWGHPPIFCSTNDICVTANFCISVTFLKPCFLL HEASASQ	437
MS2	CHD7	**WTDIVKQS**VSTNCISIKKGSYTKLFSLVFLIFC WPLIIQL	438
MS3	TESK1	**RTALTHNQ**DFSIYRLCCKRGSLCHASQARSP AFPKPVRPLPAPITRITPQLGGQSDSSQPLLTTG RPQGWQDQALRHTQQASPASCATITIPIHSAA LGDHSGDPGPAWDTCPPLPLTTLIPRAPPPYG DSTARSWPSRCGPLG	439
MS4	PPIP5K2	**LRYGALCN**VSRISYFSLTNIFNFVIKSLTAIFTV KF	440
MS5	SRPK2	**RKERNIRK**SESTLRLSPFPTPAPSGAPAAAQG KVVRVPGPAGGLVPRDAGARLLPPAGGPGGG AAAGEGRAGRGRFPSITEPRPRDLPPRVATGR RAGGRRKGAGQGVRTRPLPASWPGGRGPFRK GPRRLPLGSGPPAAGVQRLRCSHLSRGPRRRR GRVCGRACVSPPLPPRPPPVGLSAENLSWLSS GLPRACSWREFSPETCAFRLSGLDSKLSARVE RDLGALRAPGSRAAQGGGRVRGSRSEWKTRP WRPPPAWPLTRAGGPLPKNPFLESCSETAQRR RVFSFSTPLS	441
MS6	SOAT1	**YAYKDFLW**CFPFSLVFLQEIQICCHVSCLCCIC CSTRICLGCLLELFLSRALRALHVLWNGFQLH CQ	442
MS7	PDE4D	**SINKATIT**GKKDLELILHVSRKKPFLPRVNITP TPISCCNLKMLKKFFLLYIIISIIDLTNCLSCYLE HFYRFTFFTDVHYF	443
MS8	ZYG11A	**TMPAILKL**QKNCLLSL	444
MS9	SYNE2	**PYYSALSG**NSWVPSTLESDPFGYVFSPLATRP ALNDQESILWPTLTSVVSCALSCPSLNLPENW LTLITGGMKGGKKMKFTFRH	445
MS10	USP21	**GLRNLGNT**VRAILLSFLSKRNVKWCWGWGK PTSLGKACGRRALKLF	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

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

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 (VPFRELKNQRTAQGAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAGRGPEAGGGVLQPQRPAPEKPGCPCRRGQPRLHTVKMWRA; 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
(YAYKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFLSRALRALHVLWN
GFQLHCQ; 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
(WGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLPLRTLAGCLARTAHLRP
GAESLPQPQLHCT; 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).

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	CACNA1 D	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	CTGTGGTTCCAGTCCAGCGAGCTGTCTCCTACTGGTGCCCCTTGGCCATCTAGACGCCCTACTTGGAGAGGCACCACCGTGTCACCAAGAACCGCCACAAGCAGCGCCAGAACCTGTTGTGGCACAAAGTGGCCCTCCAGCCAAGAAGCCGCTCTCGGACTTGGAAGCGGACTGCTGAGGTTCTCTTGTGGAACCGCCGCCATTCGG
M84	AR-T878A	167	495	ATCGCTAGAGAGCTGCACCAGTTCGCCTTCGACCTGCTGATCAAGAGCCAC
M86	AR-L702H	171	496	CAGCCTGATTCTTTTGCCGCACTGCACAGCTCCCTGAACGAGCTGGGAGAG
M10	SPOP-F133L	19	497	TTCGTGCAAGGCAAGGATTGGGGCCTCAAAAAGTTTATCCGCAGAGACTTC
M12	SPOP-W133V	23	498	TTTGTGCAGGGCAAAGACTGGGGCGTGAAGAAGTTCATCCGGCGGGACTTC
FR1	ZFHX3	177	499	CAGAACCTGCAGAACGGCGGAGGCTCTAGAAGCTCTGCTACACTTCCTGGCAGGCGGCGGAGAAGATGGCTGAGAAGAAGGCGGCAGCCTATCTCTGTGGCTCCTGCTGGACCTCCTAGACGGCCCAACCAGAAGCCTAATCCTCCTGGCGGAGCCAGATGCGTGATCATGAGGCCTACATGGCCTGGCACCAGCGCCTTCACC

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	TGGAAGTTCGAGATGAGCTACACCGTTGGCGGCCCTCCACCACATGTTCACGCCAGACCTAGACACTGGAAAACCGACAGA
P87	AR-Intron	381	501	TACGAGGCCGGCATGACACTCGGAGGCAAGATCCTGTTCTTCCTGTTCCTGCTGCTCCCTCTGAGCCCCTTCAGCCTGATCTTT
AS55	SPOC	333	461	GATGGCCACAGCTACACCAGCAAAGTGAACTGCCTCCTGCTGCAGGATGGCTTCCACGGCTGTGTGTCTATTACTGGCGCCGCTGGCAG

				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	CACNA1 D	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

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.

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.

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.

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.

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

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

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

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

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

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.

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.

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

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.

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.

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

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

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**.

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**.

Kozak sequence: CGCGACTTCGCCGCC; **SEQ ID NO: 545**

Polynucleotide encoding the TCE:

ATGGGCCAGAAAGAGCAGATCCACACACTGCAGAAAAACAGCGAGCGGATGAGCA AGCAGCTGACCAGATCTTCTCAGGCCGTG; **SEQ ID NO: 546**

Polynucleotide encoding the serine-histidine tag: AGCCATCACCATCACCACCAT; **SEQ ID NO: 547**

Two stop codons (TAGTAA)

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

Viral vector production

GAd20 viral vector production

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.

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.

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.

CMV promoter with TetO sites: **SEQ ID NO: 628**

Ccattgcatacgttgtatccatatcataatatgtacatttatattggctcatgtccaacattaccgccatgttgacattgattattgactagttattaat
agtaatcaattacggggtcattagttcatagcccatatatggagttccgcgttacataacttacggtaaatggcccgcctggctgaccgcccaa
cgacccccgcccattgacgtcaataatgacgtatgttcccatagtaacgccaatagggactttccattgacgtcaatgggtggagtatttacgg
taaactgcccacttggcagtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaatgacggtaaatggcccgcctggcattatg
cccagtacatgaccttatgggactttcctacttggcagtacatctacgtattagtcatcgctattaccatggtgatgcggttttggcagtacatcaa
tgggcgtggatagcggtttgactcacggggatttccaagtctccaccccattgacgtcaatgggagtttgttttggcaccaaaatcaacggga
ctttccaaaatgtcgtaacaactccgccccattgacgcaaatgggcggtaggcgtgtacggtgggaggtctatataagcagagctctccctat
cagtgatagagatctccctatcagtgatagagatcgtcgacgagctcgtttagtgaaccgtcagatcgcctggagacgccatccacgctgttt
tgacctccatagaagacaccgggaccgatccagcctccgcggccgggaacggtgcattggaacgcggattccccgtgccaagagtga

BGH polyA **SEQ ID NO: 629**

ctgtgccttctagttgccagccatctgttgtttgcccctcccccgtgccttccttgaccctggaaggtgccactcccactgtcctttcctaataaaa
tgaggaaattgcatcgcattgtctgagtaggtgtcattctattctgggggggtggggtggggcaggacagcaagggggaggattgggaaga
caatagcaggcatgctggggatgcggtgggctctatggcc

MVA viral vector production

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.

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.

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 occured 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.

P7.5 early/late promoter **SEQ ID NO: 630**

GATCACTAATTCCAAACCCACCCGCTTTTTATAGTAAGTTTTTCACCCATAAATAATA
AATACAATAATTAATTTCTCGTAAAAGTAGAAAATATATTCTAATTTATTGCACGGT
AAGGAAGTAGAATCATAAAGAACAGTGACGGATC

neoGAd20 protein **SEQ ID NO: 541 (no TCE, no HIS tag)**

QNLQNGGGSRSSATLPGRRRRRWLRRRRQPISVAPAGPPRRPNQKPNPPGGARCVIMRP
TWPGTSAFTKRSFAVTERIIDYWAQKEKGSSSFLRPSCDYWAQKEKISIPRTHLCLVLGV
LSGHSGSRLYEAGMTLGGKILFFLFLLLPLSPFSLIFTEISCCTLSSEENEYLPRPEWQLQVP
FRELKNVSVLEGLRQGRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSIT
GGKSTCSAPGPQSLPSTPFSTYPQWVILITELGMECTLGQVGAPSPRREEDGWRGGHSRF
KADVPAPQGPCWGGQPGSAPSSAPPEQSLLDWKFEMSYTVGGPPPHVHARPRHWKTDR
DGHSYTSKVNCLLLQDGFHGCVSITGAAGRRNLSIFLFLMLCKLEFHACKIQNKNCPDFK

KFDGPCGERGGGRTARALWARGDSVLTPALDPQTPVRAPSLTRAAAAVHYKLIQQPISL
FSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCSTNDICVTANFCISVTFLKPCFLLHEASAS
QCHLFLQPQVGTPPPHTASARAPSGPPHPHESCPAGRRPARAAQTCARRQHGLPGCEEA
GTARVPSLHLHLHQAALGAGRGRGWGEACAQVPPSRGVLRFLDLKVRYLHSQWQHYH
RSGEAAGTPLWRPTRNVPFRELKNQRTAQGAPGIHHAASPVAANLCDPARHAQHTRIPC
GAGQVRAGRGPEAGGGVLQPQRPAPEKPGCPCRRGQPRLHTVKMWRAVAMMVPDRQ
VHYDFGLGVPGDSTRRAVRRMNTFYEAGMTLGEKFRVGNCKHLKMTRPNSKMALNSE
ALSVVSECGASACDVSLIAMDSAFVQGKDWGVKKFIRRDFYAYKDFLWCFPFSLVFLQE
IQICCHVSCLCCICCSTRICLGCLLELFLSRALRALHVLWNGFQLHCQTEYNQKLQVNQF
SESKSLYHREKQLIAMDSAICEERGAAGSLISCETMPAILKLQKNCLLSLRTALTHNQDFS
IYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITPQLGGQSDSSQPLLTTGRPQGWQD
QALRHTQQASPASCATITIPIHSAALGDHSGDPGPAWDTCPPLPLTTLIPRAPPPYGDSTA
RSWPSRCGPLGGNTTLQQLGEASQAPSGSLIPLRLPLLWEVRGQPDSFAALHSSLNELGEI
ARELHQFAFDLLIKSHFVQGKDWGLKKFIRRDFWGMELAASRRFSWDHHSAGGPPRVP
SVRSGAAQVQPKDPLPLRTLAGCLARTAHLRPGAESLPQPQLHCTLWFQSSELSPTGAP
WPSRRPTWRGTTVSPRTATSSARTCCGTKWPSSQEAALGLGSGLLRFSCGTAAIRKMHF
SLKEHPPPPCPPEAFQRAAGEGGPGRGGARRGARVLQSPFCRAGAGEWLGHQSLRHVV
GYGHLDTSGSSSSSSWPNSKMALNSLNSIDDAQLTRIAPPRSHCCFWEVNAP

neoGAd20 polynucleotide SEQ ID NO: 542 (no TCE, no HIS tag)

CAGAACCTGCAGAACGGCGGAGGCTCTAGAAGCTCTGCTACACTTCCTGGCAGGCG
GCGGAGAAGATGGCTGAGAAGAAGGCGGCAGCCTATCTCTGTGGCTCCTGCTGGAC
CTCCTAGACGGCCCAACCAGAAGCCTAATCCTCCTGGCGGAGCCAGATGCGTGATCA
TGAGGCCTACATGGCCTGGCACCAGCGCCTTCACCAAGAGAAGCTTTGCCGTGACCG
AGCGGATCATCGACTATTGGGCTCAAAAAGAGAAGGGCAGCAGCAGCTTCCTGCGG
CCTAGCTGTGATTATTGGGCCCAGAAAGAAAAGATCAGCATCCCCAGAACACACCT
GTGCCTGGTGCTGGGAGTGCTGTCTGGACACTCTGGCAGCAGACTGTATGAGGCCGG
CATGACACTCGGCGGCAAGATCCTGTTCTTCCTGTTCCTGCTGCTCCCTCTGAGCCCC
TTCAGCCTGATCTTCACCGAGATCAGCTGCTGCACCCTGAGCAGCGAGGAAAACGA
GTACCTGCCTAGACCTGAGTGGCAGCTGCAGGTCCCCTTCAGAGAGCTGAAGAACGT
TTCCGTGCTGGAAGGCCTGAGACAGGGCAGACTTGGCGGCCCTTGTAGCTGTCACTG
CCCCAGACCTAGTCAGGCCAGACTGACACCTGTGGATGTGGCCGGACCTTTCCTGTG
TCTGGGAGATCCTGGCCTGTTTCCACCTGTGAAGTCCAGCATCACAGGCGGCAAGTC
CACATGTTCTGCCCCTGGACCTCAGAGCCTGCCTAGCACACCCTTCAGCACATACCC
TCAGTGGGTCATCCTGATCACCGAACTCGGCATGGAATGCACCCTGGGACAAGTGG
GAGCCCCATCTCCTAGAAGAGAAGAGGATGGCTGGCGCGGAGGCCACTCTAGATTC
AAAGCTGATGTGCCCGCTCCTCAGGGCCCTTGTTGGGGAGGACAACCTGGATCTGCC
CCATCTTCTGCCCCACCTGAACAGTCCCTGCTGGATTGGAAGTTCGAGATGAGCTAC
ACCGTCGGCGGACCTCCACCTCATGTTCATGCCAGACCTCGGCACTGGAAAACCGAC
AGAGATGGCCACAGCTACACCAGCAAAGTGAACTGCCTCCTGCTGCAGGATGGCTT
CCACGGCTGTGTGTCTATTACTGGCGCCGCTGGCAGACGGAACCTGAGCATCTTTCT
GTTTCTGATGCTGTGCAAGCTCGAGTTCCACGCCTGCAAGATCCAGAACAAGAACTG

CCCCGACTTCAAGAAGTTCGACGGCCCTTGCGGAGAAAGAGGCGGAGGCAGAACAG
CTAGAGCCCTTTGGGCTAGAGGCGACAGCGTTCTGACACCAGCTCTGGACCCTCAGA
CACCTGTTAGGGCCCCTAGCCTGACAAGAGCTGCCGCCGCTGTGCACTACAAGCTGA
TCCAGCAGCCAATCAGCCTGTTCAGCATCACCGACCGGCTGCACAAGACATTCAGCC
AGCTGCCAAGCGTGCACCTGTGCTCCATCACCTTCCAGTGGGGACACCCTCCTATCT
TTTGCTCCACCAACGACATCTGCGTGACCGCCAACTTCTGTATCAGCGTGACCTTCCT
GAAGCCTTGCTTTCTGCTGCACGAGGCCAGCGCCTCTCAGTGCCACTTGTTTCTGCAG
CCCCAAGTGGGCACACCTCCTCCACATACAGCCTCTGCTAGAGCACCTAGCGGCCCT
CCACATCCTCACGAATCTTGTCCTGCCGGAAGAAGGCCTGCCAGAGCCGCTCAAACA
TGTGCCAGACGACAGCACGGACTGCCTGGATGTGAAGAGGCTGGAACAGCCAGAGT
GCCTAGCCTGCACCTCCATCTGCATCAGGCTGCTCTTGGAGCCGGAAGAGGTAGAGG
ATGGGGCGAAGCTTGTGCTCAGGTGCCACCTTCTAGAGGCGTGCTGAGATTCCTGGA
CCTGAAAGTGCGCTACCTGCACAGCCAGTGGCAGCACTATCACAGATCTGGCGAAG
CCGCCGGAACACCCCTTTGGAGGCCAACAAGAAACGTGCCCTTCCGGGAACTGAAG
AACCAGAGAACAGCTCAGGGCGCTCCTGGAATCCACCATGCTGCTTCTCCAGTGGCC
GCCAACCTGTGTGATCCTGCCAGACATGCCCAGCACACCAGGATTCCTTGTGGCGCT
GGACAAGTGCGCGCTGGAAGAGGACCTGAAGCAGGCGGAGGTGTTCTGCAACCTCA
AAGACCCGCTCCTGAGAAGCCTGGCTGCCCTTGCAGAAGAGGACAGCCTAGACTGC
ACACCGTGAAAATGTGGCGAGCCGTGGCCATGATGGTGCCCGATAGACAGGTCCAC
TACGACTTTGGACTGGGCGTGCCAGGCGATAGCACTCGGAGAGCCGTCAGACGGAT
GAACACCTTTTACGAAGCCGGGATGACCCTGGGCGAGAAGTTCAGAGTGGGCAACT
GCAAGCACCTGAAGATGACCCGGCCTAACAGCAAGATGGCCCTGAATAGCGAGGCC
CTGTCTGTGGTGTCTGAATGTGGCGCCTCTGCCTGTGACGTGTCCCTGATCGCTATGG
ACTCCGCCTTTGTGCAGGGCAAAGACTGGGGCGTGAAGAAGTTCATCCGGCGGGAC
TTCTACGCCTACAAGGACTTCCTGTGGTGCTTCCCCTTCTCTCTGGTGTTCCTGCAAG
AGATCCAGATCTGCTGTCATGTGTCCTGCCTGTGCTGCATCTGCTGTAGCACCAGAA
TCTGCCTGGGCTGTCTGCTGGAACTGTTCCTGAGCAGAGCCCTGAGAGCACTGCACG
TGCTGTGGAACGGATTCCAGCTGCACTGCCAGACCGAGTACAACCAGAAACTGCAA
GTGAACCAGTTCAGCGAGAGCAAGAGCCTGTACCACCGGGAAAAGCAGCTCATTGC
CATGGACAGCGCCATCTGCGAAGAGAGAGGCGCCGCAGGATCTCTGATCTCCTGCG
AAACAATGCCCGCCATCCTGAAGCTGCAGAAGAATTGCCTCCTAAGCCTGCGAACC
GCTCTGACACACAACCAGGACTTCAGCATCTACAGACTGTGTTGCAAGCGGGGCTCC
CTGTGCCATGCAAGCCAAGCTAGAAGCCCCGCCTTTCCTAAACCTGTGCGACCTCTG
CCAGCTCCAATCACCAGAATTACCCCTCAGCTCGGCGGCCAGAGCGATTCATCTCAA
CCTCTGCTGACCACCGGCAGACCTCAAGGCTGGCAAGACCAAGCTCTGAGACACAC
CCAGCAGGCTAGCCCTGCCTCTTGTGCCACCATCACAATCCCCATCCACTCTGCCGCT
CTGGGCGATCATTCTGGCGATCCTGGACCAGCCTGGGACACATGTCCTCCACTGCCA
CTCACAACACTGATCCCTAGGGCTCCTCCACCTTACGGCGATTCTACCGCTAGAAGC
TGGCCCAGCAGATGTGGACCACTCGGAGGCAACACAACCCTCCAGCAACTGGGAGA
AGCCTCTCAGGCTCCTAGCGGCTCTCTGATCCCTCTCAGACTGCCTCTCCTGTGGGAA
GTTCGGGGCCAGCCTGATTCTTTTGCCGCACTGCACAGCTCCCTGAACGAGCTGGGA
GAGATCGCTAGAGAGCTGCACCAGTTCGCCTTCGACCTGCTGATCAAGAGCCACTTC
GTGCAAGGCAAGGATTGGGGCCTCAAAAAGTTTATCCGCAGAGACTTCTGGGGCAT
GGAACTGGCCGCCAGCAGAAGATTCAGCTGGGATCATCATAGCGCAGGCGGCCCAC
CTAGAGTGCCATCTGTTAGAAGCGGAGCTGCCCAGGTGCAGCCTAAAGATCCTCTGC
CACTGAGAACACTGGCCGGCTGCCTTGCTAGAACAGCCCATCTTAGACCTGGCGCCG
AGTCTCTGCCTCAGCCACAACTGCACTGTACCCTGTGGTTCCAGTCCAGCGAGCTGT
CTCCTACTGGTGCCCCTTGGCCATCTAGACGCCCTACTTGGAGAGGCACCACCGTGT
CACCAAGAACCGCCACAAGCAGCGCCAGAACCTGTTGTGGCACAAAGTGGCCCTCC
AGCCAAGAAGCCGCTCTCGGACTTGGAAGCGGACTGCTGAGGTTCTCTTGTGGAACC
GCCGCCATTCGGAAGATGCACTTTAGCCTGAAAGAACACCCTCCACCACCTTGTCCT
CCAGAGGCTTTCCAAAGAGCTGCTGGCGAAGGCGGACCTGGTAGAGGTGGTGCTAG

AAGAGGTGCTAGGGTGCTGCAGAGCCCATTCTGTAGAGCAGGCGCAGGCGAATGGC
TGGGCCATCAGAGTCTGAGACATGTCGTCGGCTACGGCCACCTGGATACAAGCGGA
AGCAGCTCTAGCTCCAGCTGGCCTAACTCAAAAATGGCTCTGAACAGCCTGAACTCC
ATCGACGACGCCCAGCTGACAAGAATCGCCCCTCCTAGATCTCACTGCTGCTTTTGG
GAAGTGAACGCCCCA

neoMVA protein **SEQ ID NO: 543 (no TCE, no HIS Tag)**

QNLQNGGGSRSSATLPGRRRRRWLRRRRQPISVAPAGPPRRPNQKPNPPGGARCVIMRP
TWPGTSAFTGMECTLGQVGAPSPRREEDGWRGGHSRFKADVPAPQGPCWGGQPGSAPS
SAPPEQSLLDDYWAQKEKISIPRTHLCWKFEMSYTVGGPPPHVHARPRHWKTDRDYWA
QKEKGSSSFLRPSCVPFRELKNVSVLEGLRQGRLGGPCSCHCPRPSQARLTPVDVAGPFL
CLGDPGLFPPVKSSITEISCCTLSSEENEYLPRPEWQLQYEAGMTLGGKILFFLFLLLPLSP
FSLIFLVLGVLSGHSGSRLKRSFAVTERIITGGKSTCSAPGPQSLPSTPFSTYPQWVILITEL
DGHSYTSKVNCLLLQDGFHGCVSITGAAGRRNLSIFLFLMLCKLEFHACQWQHYHRSGE
AAGTPLWRPTRNVAMMVPDRQVHYDFGLKIQNKNCPDVLRFLDLKVRYLHSVPFRELK
NQRTAQGAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAGRGPEAGGGVLQPQR
PAPEKPGCPCRRGQPRLHTVKMWRACHLFLQPQVGTPPPHTASARAPSGPPHPHESCPA
GRRPARAAQTCARRQHGLPGCEEAGTARVPSLHLHLHQAALGAGRGRGWGEACAQVP
PSRGHYKLIQQPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCSTNDICVTANFCISV
TFLKPCFLLHEASASQFKKFDGPCGERGGGRTARALWARGDSVLTPALDPQTPVRAPSL
TRAAAAVGVPGDSTRRAVRRMNTFCGASACDVSLIAMDSACEERGAAGSLISCESLYHR
EKQLIAMDSAIFVQGKDWGVKKFIRRDFTMPAILKLQKNCLLSLNSKMALNSEALSVVS
EYEAGMTLGEKFRVGNCKHLKMTRPTEYNQKLQVNQFSESKRTALTHNQDFSIYRLCC
KRGSLCHASQARSPAFPKPVRPLPAPITRITPQLGGQSDSSQPLLTTGRPQGWQDQALRH
TQQASPASCATITIPIHSAALGDHSGDPGPAWDTCPPLPLTTLIPRAPPPYGDSTARSWPSR
CGPLGYAYKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFLSRALRALH
VLWNGFQLHCQGNTTLQQLGEASQAPSGSLIPLRLPLLWEVRGNSKMALNSLNSIDDAQ
LTRIAPPRSHCCFWEVNAPFVQGKDWGLKKFIRRDFEAFQRAAGEGGPGRGGARRGAR
VLQSPFCRAGAGEWLGHQSLRWGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQP
KDPLPLRTLAGCLARTAHLRPGAESLPQPQLHCTIARELHQFAFDLLIKSHKMHFSLKEH
PPPPCPPHVVGYGHLDTSGSSSSSSWPQPDSFAALHSSLNELGELWFQSSELSPTGAPWPS
RRPTWRGTTVSPRTATSSARTCCGTKWPSSQEAALGLGSGLLRFSCGTAAIRS

neoMVA polynucleotide SEQ ID NO: 544

CAGAACCTGCAGAACGGCGGAGGCTCTAGAAGCTCTGCTACACTTCCTGGCAGGCG
GCGGAGAAGATGGCTGAGAAGAAGGCGGCAGCCTATCTCTGTGGCTCCTGCTGGAC
CTCCTAGACGGCCCAACCAGAAGCCTAATCCTCCTGGCGGAGCCAGATGCGTGATCA

TGAGGCCTACATGGCCTGGCACCAGCGCCTTTACCGGCATGGAATGTACACTGGGCC
AAGTGGGAGCCCCATCTCCTAGAAGAGAAGAGGATGGCTGGCGCGGAGGCCACTCT
AGATTCAAAGCTGATGTGCCCGCTCCTCAGGGCCCTTGTTGGGGAGGACAACCTGGA
TCTGCCCCATCTTCTGCCCCACCTGAACAGAGCCTGCTGGATGACTATTGGGCTCAA
AAAGAGAAGATCAGCATCCCCAGAACACACCTGTGCTGGAAGTTCGAGATGAGCTA
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neoGAd20 expression cassette protein SEQ ID NO: 550

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neoGAd20 expression cassette polynucleotide SEQ ID NO: 551

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neoMVA expression cassette protein SEQ ID NO: 552
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neoMVA expression cassette polynucleotide **SEQ ID NO: 553**

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ACAACCAGAAACTGCAAGTGAACCAGTTCAGCGAGAGCAAGCGGACCGCTCTGACC
CACAACCAGGACTTCAGCATCTACCGGCTGTGCTGCAAGAGGGGCTCTCTGTGTCAT
GCTAGCCAGGCTAGAAGCCCCGCCTTTCCTAAGCCTGTCAGACCTCTGCCTGCTCCT
ATCACCAGAATCACCCCTCAGCTCGGCGGCCAGTCTGATTCATCTCAGCCACTGCTG

ACCACCGGCAGACCTCAAGGATGGCAAGACCAGGCTCTGAGACACACACAGCAGGC
TAGCCCAGCCTCTTGCGCCACCATCACAATACCAATACATTCTGCCGCTCTGGGCGA
TCACAGCGGAGATCCTGGACCTGCCTGGGATACTTGTCCTCCTCTGCCCCTAACTAC
ACTGATCCCTAGGGCTCCTCCACCTTACGGCGATAGCACAGCCAGATCCTGGCCTAG
CAGATGTGGCCCTCTGGGCTACGCCTACAAGGACTTCCTGTGGTGCTTCCCCTTCTCT
CTGGTGTTCCTGCAAGAAATCCAGATCTGCTGTCACGTGTCCTGCCTGTGCTGTATCT
GCTGTAGCACCCGGATCTGTCTGGGCTGTCTGCTGGAACTGTTCCTGAGCAGAGCCC
TGAGAGCACTGCACGTGCTGTGGAACGGATTCCAGCTGCACTGCCAGGGCAACACC
ACACTGCAACAGCTGGGAGAAGCCTCTCAGGCCCCAAGCGGTTCTCTGATCCCTCTC
AGACTGCCCCTCCTGTGGGAAGTGCGGGGCAATTCTAAGATGGCTCTCAACAGCCTG
AACTCCATCGACGACGCCCAGCTGACAAGAATCGCCCCTCCAAGAAGCCACTGTTGC
TTTTGGGAAGTGAACGCCCCTTTTGTGCAGGGTAAAGATTGGGGCCTCAAAAAGTTT
ATCAGACGGGACTTCGAGGCTTTCCAGAGAGCAGCTGGCGAAGGCGGACCTGGCAG
AGGTGGTGCTAGAAGAGGTGCTAGAGTGCTGCAGAGCCCATTCTGTAGAGCTGGCG
CTGGCGAATGGCTGGGCCACCAATCTCTTAGATGGGGAATGGAACTGGCCGCTAGC
AGGCGGTTTAGCTGGGATCATCATTCTGCCGGCGGACCTCCAAGAGTGCCAAGCGTT
AGAAGCGGAGCAGCCCAGGTCCAGCCTAAAGATCCACTGCCACTGAGAACACTGGC
CGGCTGCCTTGCCAGAACAGCTCATCTTAGACCTGGCGCCGAAAGCCTGCCTCAACC
TCAGCTGCATTGCACAATCGCCAGAGAACTGCACCAGTTCGCCTTCGACCTGCTGAT
CAAGAGCCACAAGATGCACTTCTCACTGAAAGAGCACCCGCCACCGCCGTGCCCAC
CGCACGTTGTCGGCTATGGCCACCTGGATACAAGCGGCTCCTCTAGCAGTAGCTCCT
GGCCTCAGCCTGACAGCTTTGCTGCCCTGCATAGCTCCCTGAATGAGCTGGGCGAAC
TGTGGTTCCAGTCCAGCGAACTGTCTCCTACTGGCGCTCCATGGCCAAGCAGAAGGC
CTACTTGGAGAGGCACCACCGTGTCTCCAAGAACCGCTACAAGCAGCGCCAGAACC
TGTTGCGGCACAAAATGGCCCTCCAGCCAAGAAGCTGCCCTCGGACTTGGAAGCGG
ACTGCTGAGATTCAGCTGTGGCACAGCCGCCATCAGAAGCCATCACCATCACCACCA
TTAGTAAAGGCGCGCC

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

SEQ ID NO: 554
MGQKEQIHTLQKNSERMSKQLTRSSQAVQNLQNGGGSRSSATLPGRRRRRWLRRRRQP
ISVAPAGPPRRPNQKPNPPGGARCVIMRPTWPGTSAFTKRSFAVTERIILVLGVLSGHSGS
RLYEAGMTLGGKILFFLFLLLPLSPFSLIFTEISCCTLSSEENEYLPRPEWQLQVPFRELKN
VSVLEGLRQGRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSITGGKSTC
SAPGPQSLPSTPFSTYPQWVILITELGMECTLGQVGAPSPRREEDGWRGGHSRFKADVPA
PQGPCWGGQPGSAPSSAPPEQSLLDDYWAQKEKGSSSFLRPSCDYWAQKEKISIPRTHLC
WKFEMSYTVGGPPPHVHARPRHWKTDRGVPGDSTRRAVRRMNTFYEAGMTLGEKFRV
GNCKHLKMTRPNSKMALNSEALSVVSECGASACDVSLIAMDSAFVQGKDWGVKKFIRR
DFYAYKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFLSRALRALHVLW
NGFQLHCQTEYNQKLQVNQFSESKSLYHREKQLIAMDSAICEERGAAGSLISCETMPAIL
KLQKNCLLSLRTALTHNQDFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITPQL
GGQSDSSQPLLTTGRPQGWQDQALRHTQQASPASCATITIPIHSAALGDHSGDPGPAWD
TCPPLPLTTLIPRAPPPYGDSTARSWPSRCGPLGDGHSYTSKVNCLLLQDGFHGCVSITGA
AGRRNLSIFLFLMLCKLEFHACKIQNKNCPDFKKFDGPCGERGGGRTARALWARGDSVL
TPALDPQTPVRAPSLTRAAAAVHYKLIQQPISLFSITDRLHKTFSQLPSVHLCSITFQWGH
PPIFCSTNDICVTANFCISVTFLKPCFLLHEASASQCHLFLQPQVGTPPPHTASARAPSGPP
HPHESCPAGRRPARAAQTCARRQHGLPGCEEAGTARVPSLHLHLHQAALGAGRGRGW
GEACAQVPPSRGVLRFLDLKVRYLHSQWQHYHRSGEAAGTPLWRPTRNVPFRELKNQR
TAQGAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAGRGPEAGGGVLQPQRPAP
EKPGCPCRRGQPRLHTVKMWRAVAMMVPDRQVHYDFGLGNTTLQQLGEASQAPSGSL

IPLRLPLLWEVRGQPDSFAALHSSLNELGEIARELHQFAFDLLIKSHFVQGKDWGLKKFIR
RDFWGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLPLRTLAGCLARTAHL
RPGAESLPQPQLHCTLWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSARTCCGTKWP
SSQEAALGLGSGLLRFSCGTAAIRKMHFSLKEHPPPPCPPEAFQRAAGEGGPGRGGARRG
ARVLQSPFCRAGAGEWLGHQSLRHVVGYGHLDTSGSSSSSSWPNSKMALNSLNSIDDA
QLTRIAPPRSHCCFWEVNAP

SEQ ID NO: 555
MGQKEQIHTLQKNSERMSKQLTRSSQAVQNLQNGGGSRSSATLPGRRRRRWLRRRRQP
ISVAPAGPPRRPNQKPNPPGGARCVIMRPTWPGTSAFTKRSFAVTERIITEISCCTLSSEEN
EYLPRPEWQLQYEAGMTLGGKILFFLFLLLPLSPFSLIFDYWAQKEKGSSSFLRPSCVPFR
ELKNVSVLEGLRQGRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSITGG
KSTCSAPGPQSLPSTPFSTYPQWVILITELGMECTLGQVGAPSPRREEDGWRGGHSRFKA
DVPAPQGPCWGGQPGSAPSSAPPEQSLLDDYWAQKEKISIPRTHLCLVLGVLSGHSGSRL
WKFEMSYTVGGPPPHVHARPRHWKTDRDGHSYTSKVNCLLLQDGFHGCVSITGAAGR
RNLSIFLFLMLCKLEFHACKIQNKNCPDFKKFDGPCGERGGGRTARALWARGDSVLTPA
LDPQTPVRAPSLTRAAAAVHYKLIQQPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIF
CSTNDICVTANFCISVTFLKPCFLLHEASASQCHLFLQPQVGTPPPHTASARAPSGPPHPH
ESCPAGRRPARAAQTCARRQHGLPGCEEAGTARVPSLHLHLHQAALGAGRGRGWGEA
CAQVPPSRGVLRFLDLKVRYLHSQWQHYHRSGEAAGTPLWRPTRNVPFRELKNQRTAQ
GAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAGRGPEAGGGVLQPQRPAPEKP
GCPCRRGQPRLHTVKMWRAVAMMVPDRQVHYDFGLGVPGDSTRRAVRRMNTFYEAG
MTLGEKFRVGNCKHLKMTRPNSKMALNSEALSVVSECGASACDVSLIAMDSAFVQGK
DWGVKKFIRRDFYAYKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFLS
RALRALHVLWNGFQLHCQTEYNQKLQVNQFSESKSLYHREKQLIAMDSAICEERGAAG
SLISCETMPAILKLQKNCLLSLRTALTHNQDFSIYRLCCKRGSLCHASQARSPAFPKPVRP
LPAPITRITPQLGGQSDSSQPLLTTGRPQGWQDQALRHTQQASPASCATITIPIHSAALGD
HSGDPGPAWDTCPPLPLTTLIPRAPPPYGDSTARSWPSRCGPLGGNTTLQQLGEASQAPS
GSLIPLRLPLLWEVRGQPDSFAALHSSLNELGEIARELHQFAFDLLIKSHFVQGKDWGLK
KFIRRDFWGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLPLRTLAGCLAR
TAHLRPGAESLPQPQLHCTLWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSARTCCG
TKWPSSQEAALGLGSGLLRFSCGTAAIRKMHFSLKEHPPPPCPPEAFQRAAGEGGPGRG
GARRGARVLQSPFCRAGAGEWLGHQSLRHVVGYGHLDTSGSSSSSSWPNSKMALNSLN
SIDDAQLTRIAPPRSHCCFWEVNAP

SEQ ID NO: 556
MGQKEQIHTLQKNSERMSKQLTRSSQAVQNLQNGGGSRSSATLPGRRRRRWLRRRRQP
ISVAPAGPPRRPNQKPNPPGGARCVIMRPTWPGTSAFTKRSFAVTERIIDYWAQKEKGSS
SFLRPSCVPFRELKNVSVLEGLRQGRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGL
FPPVKSSILVLGVLSGHSGSRLYEAGMTLGGKILFFLFLLLPLSPFSLIFTEISCCTLSSEENE
YLPRPEWQLQTGGKSTCSAPGPQSLPSTPFSTYPQWVILITELGMECTLGQVGAPSPRREE
DGWRGGHSRFKADVPAPQGPCWGGQPGSAPSSAPPEQSLLDDYWAQKEKISIPRTHLC
WKFEMSYTVGGPPPHVHARPRHWKTDRDGHSYTSKVNCLLLQDGFHGCVSITGAAGR
RNLSIFLFLMLCKLEFHACKIQNKNCPDFKKFDGPCGERGGGRTARALWARGDSVLTPA
LDPQTPVRAPSLTRAAAAVHYKLIQQPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIF
CSTNDICVTANFCISVTFLKPCFLLHEASASQCHLFLQPQVGTPPPHTASARAPSGPPHPH
ESCPAGRRPARAAQTCARRQHGLPGCEEAGTARVPSLHLHLHQAALGAGRGRGWGEA
CAQVPPSRGVLRFLDLKVRYLHSQWQHYHRSGEAAGTPLWRPTRNVPFRELKNQRTAQ
GAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAGRGPEAGGGVLQPQRPAPEKP
GCPCRRGQPRLHTVKMWRAVAMMVPDRQVHYDFGLGVPGDSTRRAVRRMNTFYEAG
MTLGEKFRVGNCKHLKMTRPNSKMALNSEALSVVSECGASACDVSLIAMDSAFVQGK
DWGVKKFIRRDFYAYKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFLS

RALRALHVLWNGFQLHCQTEYNQKLQVNQFSESKSLYHREKQLIAMDSAICEERGAAG
SLISCETMPAILKLQKNCLLSLRTALTHNQDFSIYRLCCKRGSLCHASQARSPAFPKPVRP
LPAPITRITPQLGGQSDSSQPLLTTGRPQGWQDQALRHTQQASPASCATITIPIHSAALGD
HSGDPGPAWDTCPPLPLTTLIPRAPPPYGDSTARSWPSRCGPLGGNTTLQQLGEASQAPS
GSLIPLRLPLLWEVRGQPDSFAALHSSLNELGEIARELHQFAFDLLIKSHFVQGKDWGLK
KFIRRDFWGMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLPLRTLAGCLAR
TAHLRPGAESLPQPQLHCTLWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSARTCCG
TKWPSSQEAALGLGSGLLRFSCGTAAIRKMHFSLKEHPPPPCPPEAFQRAAGEGGPGRG
GARRGARVLQSPFCRAGAGEWLGHQSLRHVVGYGHLDTSGSSSSSSWPNSKMALNSLN
SIDDAQLTRIAPPRSHCCFWEVNAP

SEQ ID NO: 623
MGQKEQIHTLQKNSERMSKQLTRSSQAVGVPGDSTRRAVRRMNTFYEAGMTLGEKFR
VGNCKHLKMTRPNSKMALNSEALSVVSECGASACDVSLIAMDSAFVQGKDWGVKKFI
RRDFYAYKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLELFLSRALRALHV
LWNGFQLHCQTEYNQKLQVNQFSESKSLYHREKQLIAMDSAICEERGAAGSLISCETMP
AILKLQKNCLLSLRTALTHNQDFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITP
QLGGQSDSSQPLLTTGRPQGWQDQALRHTQQASPASCATITIPIHSAALGDHSGDPGPA
WDTCPPLPLTTLIPRAPPPYGDSTARSWPSRCGPLGGNTTLQQLGEASQAPSGSLIPLRLP
LLWEVRGQPDSFAALHSSLNELGEIARELHQFAFDLLIKSHFVQGKDWGLKKFIRRDFW
GMELAASRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLPLRTLAGCLARTAHLRPGA
ESLPQPQLHCTLWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSARTCCGTKWPSSQE
AALGLGSGLLRFSCGTAAIRKMHFSLKEHPPPPCPPEAFQRAAGEGGPGRGGARRGARV
LQSPFCRAGAGEWLGHQSLRHVVGYGHLDTSGSSSSSSWPNSKMALNSLNSIDDAQLTR
IAPPRSHCCFWEVNAPDGHSYTSKVNCLLLQDGFHGCVSITGAAGRRNLSIFLFLMLCKL
EFHACKIQNKNCPDFKKFDGPCGERGGGRTARALWARGDSVLTPALDPQTPVRAPSLTR
AAAAVHYKLIQQPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCSTNDICVTANFCIS
VTFLKPCFLLHEASASQCHLFLQPQVGTPPPHTASARAPSGPPHPHESCPAGRRPARAAQ
TCARRQHGLPGCEEAGTARVPSLHLHLHQAALGAGRGRGWGEACAQVPPSRGVLRFLD
LKVRYLHSQWQHYHRSGEAAGTPLWRPTRNVPFRELKNQRTAQGAPGIHHAASPVAA
NLCDPARHAQHTRIPCGAGQVRAGRGPEAGGGVLQPQRPAPEKPGCPCRRGQPRLHTV
KMWRAVAMMVPDRQVHYDFGLQNLQNGGGSRSSATLPGRRRRRWLRRRRQPISVAPA
GPPRRPNQKPNPPGGARCVIMRPTWPGTSAFTKRSFAVTERIILVLGVLSGHSGSRLYEA
GMTLGGKILFFLFLLLPLSPFSLIFTEISCCTLSSEENEYLPRPEWQLQVPFRELKNVSVLE
GLRQGRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSITGGKSTCSAPGP
QSLPSTPFSTYPQWVILITELGMECTLGQVGAPSPRREEDGWRGGHSRFKADVPAPQGPC
WGGQPGSAPSSAPPEQSLLDDYWAQKEKGSSSFLRPSCDYWAQKEKISIPRTHLCWKFE
MSYTVGGPPPHVHARPRHWKTDR

SEQ ID NO: 624
MGQKEQIHTLQKNSERMSKQLTRSSQAVGNTTLQQLGEASQAPSGSLIPLRLPLLWEVR
GQPDSFAALHSSLNELGEIARELHQFAFDLLIKSHFVQGKDWGLKKFIRRDFWGMELAA
SRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLPLRTLAGCLARTAHLRPGAESLPQPQ
LHCTLWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSARTCCGTKWPSSQEAALGLG
SGLLRFSCGTAAIRKMHFSLKEHPPPPCPPEAFQRAAGEGGPGRGGARRGARVLQSPFCR
AGAGEWLGHQSLRHVVGYGHLDTSGSSSSSSWPNSKMALNSLNSIDDAQLTRIAPPRSH
CCFWEVNAPGVPGDSTRRAVRRMNTFYEAGMTLGEKFRVGNCKHLKMTRPNSKMAL
NSEALSVVSECGASACDVSLIAMDSAFVQGKDWGVKKFIRRDFYAYKDFLWCFPFSLVF
LQEIQICCHVSCLCCICCSTRICLGCLLELFLSRALRALHVLWNGFQLHCQTEYNQKLQV
NQFSESKSLYHREKQLIAMDSAICEERGAAGSLISCETMPAILKLQKNCLLSLRTALTHN
QDFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITPQLGGQSDSSQPLLTTGRPQG
WQDQALRHTQQASPASCATITIPIHSAALGDHSGDPGPAWDTCPPLPLTTLIPRAPPPYGD

STARSWPSRCGPLGDGHSYTSKVNCLLLQDGFHGCVSITGAAGRRNLSIFLFLMLCKLEF
HACKIQNKNCPDFKKFDGPCGERGGGRTARALWARGDSVLTPALDPQTPVRAPSLTRA
AAAVHYKLIQQPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCSTNDICVTANFCISV
TFLKPCFLLHEASASQCHLFLQPQVGTPPPHTASARAPSGPPHPHESCPAGRRPARAAQT
CARRQHGLPGCEEAGTARVPSLHLHLHQAALGAGRGRGWGEACAQVPPSRGVLRFLDL
KVRYLHSQWQHYHRSGEAAGTPLWRPTRNVPFRELKNQRTAQGAPGIHHAASPVAAN
LCDPARHAQHTRIPCGAGQVRAGRGPEAGGGVLQPQRPAPEKPGCPCRRGQPRLHTVK
MWRAVAMMVPDRQVHYDFGLQNLQNGGGSRSSATLPGRRRRRWLRRRRQPISVAPAG
PPRRPNQKPNPPGGARCVIMRPTWPGTSAFTKRSFAVTERIITEISCCTLSSEENEYLPRPE
WQLQYEAGMTLGGKILFFLFLLLPLSPFSLIFDYWAQKEKGSSSFLRPSCVPFRELKNVSV
LEGLRQGRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSITGGKSTCSAP
GPQSLPSTPFSTYPQWVILITELGMECTLGQVGAPSPRREEDGWRGGHSRFKADVPAPQG
PCWGGQPGSAPSSAPPEQSLLDDYWAQKEKISIPRTHLCLVLGVLSGHSGSRLWKFEMS
YTVGGPPPHVHARPRHWKTDR

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

SEQ ID NO: 557
MGQKEQIHTLQKNSERMSKQLTRSSQAVQNLQNGGGSRSSATLPGRRRRRWLRRRRQP
ISVAPAGPPRRPNQKPNPPGGARCVIMRPTWPGTSAFTGMECTLGQVGAPSPRREEDGW
RGGHSRFKADVPAPQGPCWGGQPGSAPSSAPPEQSLLDDYWAQKEKISIPRTHLCWKFE
MSYTVGGPPPHVHARPRHWKTDRDYWAQKEKGSSSFLRPSCVPFRELKNVSVLEGLRQ
GRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSITEISCCTLSSEENEYLPR
PEWQLQYEAGMTLGGKILFFLFLLLPLSPFSLIFLVLGVLSGHSGSRLKRSFAVTERIITGG
KSTCSAPGPQSLPSTPFSTYPQWVILITELDGHSYTSKVNCLLLQDGFHGCVSITGAAGRR
NLSIFLFLMLCKLEFHACCHLFLQPQVGTPPPHTASARAPSGPPHPHESCPAGRRPARAA
QTCARRQHGLPGCEEAGTARVPSLHLHLHQAALGAGRGRGWGEACAQVPPSRGHYKLI
QQPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCSTNDICVTANFCISVTFLKPCFLL
HEASASQVAMMVPDRQVHYDFGLKIQNKNCPDVLRFLDLKVRYLHSVPFRELKNQRTA
QGAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAGRGPEAGGGVLQPQRPAPEK
PGCPCRRGQPRLHTVKMWRAQWQHYHRSGEAAGTPLWRPTRNFKKFDGPCGERGGGR
TARALWARGDSVLTPALDPQTPVRAPSLTRAAAAVGVPGDSTRRAVRRMNTFCGASAC
DVSLIAMDSACEERGAAGSLISCESLYHREKQLIAMDSAIFVQGKDWGVKKFIRRDFTM
PAILKLQKNCLLSLNSKMALNSEALSVVSEYEAGMTLGEKFRVGNCKHLKMTRPTEYN
QKLQVNQFSESKRTALTHNQDFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITP
QLGGQSDSSQPLLTTGRPQGWQDQALRHTQQASPASCATITIPIHSAALGDHSGDPGPA
WDTCPPLPLTTLIPRAPPPYGDSTARSWPSRCGPLGYAYKDFLWCFPFSLVFLQEIQICCH
VSCLCCICCSTRICLGCLLELFLSRALRALHVLWNGFQLHCQGNTTLQQLGEASQAPSGS
LIPLRLPLLWEVRGNSKMALNSLNSIDDAQLTRIAPPRSHCCFWEVNAPFVQGKDWGLK
KFIRRDFEAFQRAAGEGGPGRGGARRGARVLQSPFCRAGAGEWLGHQSLRWGMELAA
SRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLPLRTLAGCLARTAHLRPGAESLPQPQ
LHCTIARELHQFAFDLLIKSHKMHFSLKEHPPPPCPPHVVGYGHLDTSGSSSSSSWPQPDS
FAALHSSLNELGELWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSARTCCGTKWPSS
QEAALGLGSGLLRFSCGTAAIR

SEQ ID NO: 558
MGQKEQIHTLQKNSERMSKQLTRSSQAVDGHSYTSKVNCLLLQDGFHGCVSITGAAGR
RNLSIFLFLMLCKLEFHACQWQHYHRSGEAAGTPLWRPTRNVAMMVPDRQVHYDFGL
VLRFLDLKVRYLHSKIQNKNCPDVPFRELKNQRTAQGAPGIHHAASPVAANLCDPARHA
QHTRIPCGAGQVRAGRGPEAGGGVLQPQRPAPEKPGCPCRRGQPRLHTVKMWRACHLF
LQPQVGTPPPHTASARAPSGPPHPHESCPAGRRPARAAQTCARRQHGLPGCEEAGTARV

PSLHLHLHQAALGAGRGRGWGEACAQVPPSRGHYKLIQQPISLFSITDRLHKTFSQLPSV
HLCSITFQWGHPPIFCSTNDICVTANFCISVTFLKPCFLLHEASASQFKKFDGPCGERGGG
RTARALWARGDSVLTPALDPQTPVRAPSLTRAAAAVQNLQNGGGSRSSATLPGRRRRR
WLRRRRQPISVAPAGPPRRPNQKPNPPGGARCVIMRPTWPGTSAFTGMECTLGQVGAPS
PRREEDGWRGGHSRFKADVPAPQGPCWGGQPGSAPSSAPPEQSLLDDYWAQKEKISIPR
THLCWKFEMSYTVGGPPPHVHARPRHWKTDRDYWAQKEKGSSSFLRPSCVPFRELKNV
SVLEGLRQGRLGGPCSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSITEISCCTLSS
EENEYLPRPEWQLQYEAGMTLGGKILFFLFLLLPLSPFSLIFLVLGVLSGHSGSRLKRSFA
VTERIITGGKSTCSAPGPQSLPSTPFSTYPQWVILITELGVPGDSTRRAVRRMNTFCGASA
CDVSLIAMDSACEERGAAGSLISCESLYHREKQLIAMDSAIFVQGKDWGVKKFIRRDFT
MPAILKLQKNCLLSLNSKMALNSEALSVVSEYEAGMTLGEKFRVGNCKHLKMTRPTEY
NQKLQVNQFSESKRTALTHNQDFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRIT
PQLGGQSDSSQPLLTTGRPQGWQDQALRHTQQASPASCATITIPIHSAALGDHSGDPGPA
WDTCPPLPLTTLIPRAPPPYGDSTARSWPSRCGPLGYAYKDFLWCFPFSLVFLQEIQICCH
VSCLCCICCSTRICLGCLLELFLSRALRALHVLWNGFQLHCQGNTTLQQLGEASQAPSGS
LIPLRLPLLWEVRGNSKMALNSLNSIDDAQLTRIAPPRSHCCFWEVNAPFVQGKDWGLK
KFIRRDFEAFQRAAGEGGPGRGGARRGARVLQSPFCRAGAGEWLGHQSLRWGMELAA
SRRFSWDHHSAGGPPRVPSVRSGAAQVQPKDPLPLRTLAGCLARTAHLRPGAESLPQPQ
LHCTIARELHQFAFDLLIKSHKMHFSLKEHPPPPCPPHVVGYGHLDTSGSSSSSSWPQPDS
FAALHSSLNELGELWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSARTCCGTKWPSS
QEAALGLGSGLLRFSCGTAAIR

SEQ ID NO: 559
MGQKEQIHTLQKNSERMSKQLTRSSQAVGNTTLQQLGEASQAPSGSLIPLRLPLLWEVR
GNSKMALNSLNSIDDAQLTRIAPPRSHCCFWEVNAPFVQGKDWGLKKFIRRDFEAFQRA
AGEGGPGRGGARRGARVLQSPFCRAGAGEWLGHQSLRWGMELAASRRFSWDHHSAG
GPPRVPSVRSGAAQVQPKDPLPLRTLAGCLARTAHLRPGAESLPQPQLHCTIARELHQFA
FDLLIKSHKMHFSLKEHPPPPCPPHVVGYGHLDTSGSSSSSSWPQPDSFAALHSSLNELGE
LWFQSSELSPTGAPWPSRRPTWRGTTVSPRTATSSARTCCGTKWPSSQEAALGLGSGLL
RFSCGTAAIRDGHSYTSKVNCLLLQDGFHGCVSITGAAGRRNLSIFLFLMLCKLEFHACQ
WQHYHRSGEAAGTPLWRPTRNVAMMVPDRQVHYDFGLKIQNKNCPDVLRFLDLKVR
YLHSVPFRELKNQRTAQGAPGIHHAASPVAANLCDPARHAQHTRIPCGAGQVRAGRGP
EAGGGVLQPQRPAPEKPGCPCRRGQPRLHTVKMWRACHLFLQPQVGTPPPHTASARAP
SGPPHPHESCPAGRRPARAAQTCARRQHGLPGCEEAGTARVPSLHLHLHQAALGAGRG
RGWGEACAQVPPSRGHYKLIQQPISLFSITDRLHKTFSQLPSVHLCSITFQWGHPPIFCSTN
DICVTANFCISVTFLKPCFLLHEASASQFKKFDGPCGERGGGRTARALWARGDSVLTPAL
DPQTPVRAPSLTRAAAAVGVPGDSTRRAVRRMNTFCGASACDVSLIAMDSATEYNQKL
QVNQFSESKYEAGMTLGEKFRVGNCKHLKMTRPTMPAILKLQKNCLLSLNSKMALNSE
ALSVVSECEERGAAGSLISCESLYHREKQLIAMDSAIFVQGKDWGVKKFIRRDFRTALTH
NQDFSIYRLCCKRGSLCHASQARSPAFPKPVRPLPAPITRITPQLGGQSDSSQPLLTTGRPQ
GWQDQALRHTQQASPASCATITIPIHSAALGDHSGDPGPAWDTCPPLPLTTLIPRAPPPYG
DSTARSWPSRCGPLGYAYKDFLWCFPFSLVFLQEIQICCHVSCLCCICCSTRICLGCLLEL
FLSRALRALHVLWNGFQLHCQQNLQNGGGSRSSATLPGRRRRRWLRRRRQPISVAPAG
PPRRPNQKPNPPGGARCVIMRPTWPGTSAFTGMECTLGQVGAPSPRREEDGWRGGHSRF
KADVPAPQGPCWGGQPGSAPSSAPPEQSLLDDYWAQKEKISIPRTHLCWKFEMSYTVG
GPPPHVHARPRHWKTDRDYWAQKEKGSSSFLRPSCVPFRELKNVSVLEGLRQGRLGGP
CSCHCPRPSQARLTPVDVAGPFLCLGDPGLFPPVKSSITEISCCTLSSEENEYLPRPEWQLQ
YEAGMTLGGKILFFLFLLLPLSPFSLIFLVLGVLSGHSGSRLKRSFAVTERIITGGKSTCSAP
GPQSLPSTPFSTYPQWVILITEL

SEQ ID NO: 625

MGQKEQIHTLQKNSERMSKQLTRSSQAVGNTTLQQLGEASQAPSGSLIPLRLPLLWEVR
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SEQ ID NO: 713 The polynucleotide sequence of the full GAd20 incorporating the GAd20 expression cassette

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cccgccccgcctcgccccgcccacctcattatcatattggcctcaatccaaaataaggtatattattgatgatg

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

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*

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

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)

Claims

1. A vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide, wherein the heterologous polypeptide is selected from the group consisting of SEQ ID NOs: 541, 543, 550, 554, 555, 556, 623, 624, 552, 557, 558, 559, 625, and 626.

2. The vaccine of claim 1, wherein the vaccine is capable of eliciting a cellular immune response in a subject to be administered the vaccine.

3. The vaccine of claim 2, wherein the cellular immune response is activation of vaccine-specific $CD8^+$ T cells, $CD4^+$ T cells, or $CD8^+$ T cells and $CD4^+$ T cells, wherein activation is assessed by increased production of TNFα, IFNγ, or TNFα and IFNγ by $CD8^+$ T cells, $CD4^+$ T cells, or $CD8^+$ T cells and $CD4^+$ T cells relative to production of TNFα, IFNγ, or TNFα and IFNγ by $CD8^+$ T cells, $CD4^+$ T cells, or $CD8^+$ T cells and $CD4^+$ T cells from an unvaccinated subject.

4. The vaccine of any one of claims 1-3, wherein the heterologous polynucleotide comprises a promoter, an enhancer, a polyadenylation site, a Kozak sequence, a stop codon, a T cell enhancer (TCE), or any combination thereof.

5. The vaccine of claim 4, wherein the promoter comprises a CMV promoter or a vaccinia P7.5 promoter.

6. The vaccine of claim 4 or 5, wherein 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.

7. The vaccine of any one of claims 1-6, wherein the heterologous polynucleotide is DNA or RNA.

8. The vaccine of claim 7, wherein RNA is mRNA or self-replicating RNA.

9. The vaccine of any one of claims 1-7, wherein the vaccine is a recombinant virus derived from adenovirus (Ad), poxvirus, adeno-associated virus (AAV) or retrovirus.

10. The vaccine of claim 9, 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).

11. The vaccine of claim 10, wherein the recombinant virus is derived from GAd20.

12. The vaccine of claim 10, wherein the recombinant virus is derived from MVA.

13. The vaccine of claim 10, wherein the recombinant virus is derived from hAd26.

14. A vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NOs: 541, 550, 554, 555, 556, 623 or 624, wherein the vaccine is a recombinant virus derived from GAd20 or Ad26.

15. A vaccine comprising a heterologous polynucleotide encoding a heterologous polypeptide of SEQ ID NOs: 543, 552, 557, 558, 559, 625 or 626, wherein the vaccine is a recombinant virus derived from MVA.

16. Use of a therapeutically effective amount of one or more vaccines of any one of claims 1-15 in the manufacture of a medicament for the treatment or prevention of a prostate cancer in a subject.

17. A vaccine as claimed in any one of claims 1-15 for use in inducing an immune response in a subject.

18. The use of claim 16, wherein the prostate cancer is a relapsed prostate cancer, a refractory prostate cancer, a metastatic prostate cancer or a castration resistant prostate cancer, or any combination thereof.

19. The use of claim 16 or claim 18, wherein an additional cancer therapeutic agent is to be administered to the subject.

20. The use of claim 19, wherein the additional cancer therapeutic agent is 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, a 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, GPRC5D antibody, or any combination thereof.