TREATMENT METHODS

CROSS REFERENCE TO RELATED APPLICATIONS

[001] This application claims the benefit of U.S. Provisional Application No. 62/583,233, filed November 8, 2017, U.S. Provisional Application No. 62/484,258, filed April 11, 2017 and U.S. Provisional Application No. 62/473,899, filed March 20, 2017, the contents of each of which are hereby incorporated by reference herein in their entirety.

BACKGROUND

[002] Cancer is characterized by proliferation of abnormal cells. Many treatments include costly and painful surgeries and chemotherapies. Although there is a growing interest in cancer therapies that target cancerous cells using a patient's own immune system, such therapies have had limited success.

SUMMARY

[002a] In an aspect of the present disclosure, there is provided a method of selecting tumor antigens, the method comprising:

a) obtaining, providing, or generating a library comprising bacterial cells or beads comprising a plurality of tumor antigens, wherein each bacterial cell or bead of the library comprises a different tumor antigen;

b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from a subject, wherein the APCs internalize the bacterial cells or beads;

c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a tumor antigen presented by one or more APCs;

d) determining whether one or more lymphocytes are activated by one or more tumor antigens presented by one or more APCs by assessing a level of expression and/or secretion of one or more immune mediators;

e) identifying each activating tumor antigen as (i) an antigen that stimulates the level of expression and/or secretion of one or more immune mediators, or (ii) an antigen that inhibits and/or suppresses the level of expression and/or secretion of one or more immune mediators; and
f) selecting from among the identified tumor antigens (i) one or more antigens that increase a level of expression and/or secretion of one or more immune mediators associated with at least one beneficial response to cancer, (ii) one or more tumor antigens that inhibit and/or suppress a level of expression and/or secretion of one or more immune mediators associated with at least one deleterious and/or non-beneficial response to cancer, (iii) one or more antigens that increase a level of expression and/or secretion of one or more immune mediators associated with at least one deleterious and/or non-beneficial response to cancer, and/or (iv) one or more tumor antigens that inhibit and/or suppress a level of expression and/or secretion of one or more immune mediators associated with at least one beneficial response to cancer.

[002b] In another aspect of the present disclosure, there is provided a method of selecting tumor antigens, the method comprising:
a) providing a library comprising bacterial cells or beads, wherein each bacterial cell or bead of the library comprises a different heterologous polypeptide comprising one or more mutations, splice variants, or translocations expressed in a cancer or tumor cell of a subject;
b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from the subject, wherein the APCs internalize the bacterial cells or beads;
c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a polypeptide presented by one or more APCs;
d) determining whether one or more lymphocytes are activated by, or not responsive to, one or more polypeptides presented by one or more APCs, by assessing a level of expression and/or secretion of one or more immune mediators;
e) identifying each activating tumor antigen as (i) an antigen that stimulates the level of expression and/or secretion of one or more immune mediators, or (ii) an antigen that inhibits and/or suppresses the level of expression and/or secretion of one or more immune mediators, wherein stimulation, inhibition and/or suppression indicate that the polypeptide is a tumor antigen; and
f) selecting from among the identified tumor antigens (i) one or more tumor antigens that increase level of expression and/or secretion of one or more immune mediators associated with at

least one beneficial response to cancer, (ii) one or more tumor antigens that inhibit and/or suppress level of expression and/or secretion of one or more immune mediators associated with at least one deleterious and/or non-beneficial response to cancer, (iii) one or more tumor antigens that increase level of expression and/or secretion of one or more immune mediators associated with at least one deleterious and/or non-beneficial response to cancer, and/or (iv) one or more tumor antigens that inhibit and/or suppress level of expression and/or secretion of one or more immune mediators associated with at least one beneficial response to cancer.

[003] The present disclosure features, *inter alia*, methods of identifying tumor antigens and potential tumor antigens of human lymphocytes, methods of selecting tumor antigens and potential tumor antigens, as well as compositions including the tumor antigens and potential tumor antigens, methods of making such compositions, and methods of using the tumor antigens and potential tumor antigens. The present disclosure also features methods of evaluating an immune response in a cancer subject, *e.g.*, for identifying and/or selecting a cancer subject for initiation, continuation, modification, and/or discontinuation of a cancer therapy

[004] Accordingly, in one example the present disclosure features a method of obtaining or generating a subject response profile. In some examples, the method comprises: a) obtaining, providing, or generating a library comprising bacterial cells or beads comprising a plurality of tumor antigens, wherein each bacterial cell or bead of the library comprises a different tumor antigen; b) contacting the bacterial cells or beads of the library with antigen presenting cells (APCs) from a subject, wherein the APCs internalize the bacterial cells or beads;

c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a tumor antigen presented by one or more APCs; d) determining whether one or more lymphocytes are activated by, or not responsive to, one or more tumor antigens presented by one or more APCs, e.g., by assessing (e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control) of expression and/or secretion of one or more immune mediators; and e) identifying one or more tumor antigens that stimulate, inhibit and/or suppress, and/or have minimal effect on a level of expression and/or secretion of one or more immune mediators, to obtain or generate a subject response profile.

[005] In some examples, the subject response profile comprises a representation of the level of expression and/or secretion of the one or more immune mediators associated with the plurality of tumor antigens.

[006] In some examples, the APCs are human APCs isolated from the subject; and/or the bacterial cells further comprise a cytolysin polypeptide; and/or the cytolysin polypeptide is listeriolysin O (LLO); and/or the APCs are provided in an array, and/or the APCs in each location of the array are contacted with a set of bacterial cells, each set comprising a different tumor antigen; and/or the APCs and lymphocytes are isolated from peripheral blood; and/or the APCs comprise immortalized cells; and/or the lymphocytes are derived from a cancer or tumor.

[007] In some examples, the tumor antigens comprise full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoding autoantigens associated with a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding autoantigens associated with a cancer or tumor.

[008] In another example, the present disclosure features a method of obtaining or generating a target response profile. In some examples, the method comprises: a) obtaining, providing, or generating a library comprising bacterial cells or beads comprising a plurality of tumor antigens, wherein each bacterial cell or bead of the library comprises a different tumor antigen; b)

contacting the bacterial cells or beads of the library with antigen presenting cells (APCs) from a subject who exhibits or previously exhibited a response to cancer, wherein the APCs internalize the bacterial cells or beads; c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a tumor antigen presented by one or more APCs; d) determining whether one or more lymphocytes are activated by, or not responsive to, one or more tumor antigens presented by one or more APCs, e.g., by assessing (e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control) of expression and/or secretion of one or more immune mediators; and e) identifying one or more tumor antigens that stimulate, inhibit and/or suppress, and/or have a minimal effect on a level of expression and/or secretion of one or more immune mediators, to obtain or generate a target response profile.

[009] In some examples, the subject exhibits or previously exhibited at least one beneficial response to cancer. In some examples, the beneficial response comprises a positive clinical response, e.g., one or more positive clinical endpoints, to a cancer therapy or combination of therapies. In some examples, the beneficial response comprises a spontaneous response to a cancer. In some examples, the beneficial response comprises clearance of a cancer, e.g., a level of one or more clinical measures associated with clearance of a cancer. In some examples, the beneficial response comprises a lack of a relapse, recurrence, and/or metastasis of a cancer, e.g., over a defined period of time (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 weeks, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 years). In some examples, the beneficial response comprises a positive cancer prognosis. In some examples, the beneficial response comprises a lack of measurable toxic responses or side effects to a cancer therapy or combination of therapies.

[0010] In some examples, the subject exhibits or previously exhibited at least one deleterious or non-beneficial response to cancer. In some examples, the deleterious response comprises a negative clinical response and/or a failure to respond, to a cancer therapy or combination of therapies. In some examples, the deleterious response comprises a lack of clearance of a cancer, e.g., a level of one or more clinical measures associated with lack of clearance of a cancer. In some examples, the deleterious response comprises at least one relapse, recurrence, and/or metastasis of a cancer. In some

examples, the deleterious response comprises a negative cancer prognosis. In some examples, the deleterious response

comprises one or more toxic responses or side effects (e.g., one or more measurable toxic responses or side effects) to a cancer therapy or combination of therapies.

[0011] In some examples, the library used to obtain the target response profile is the same library used to obtain a subject response profile.

[0012] In some examples, the method further comprises the step of repeating steps a) through e) with antigen presenting cells (APCs) and/or lymphocytes from additional subjects, to obtain a population-based or composite target response profile.

[0013] In some examples, the target response profile comprises a representation of the level of expression and/or secretion of the one or more immune mediators associated with the plurality of tumor antigens.

[0014] In some examples, the APCs are human APCs isolated from the subject; and/or the bacterial cells further comprise a cytolysin polypeptide; and/or the cytolysin polypeptide is listeriolysin O (LLO); and/or the APCs are provided in an array, and/or the APCs in each location of the array are contacted with a set of bacterial cells, each set comprising a different tumor antigen; and/or the APCs and lymphocytes are isolated from peripheral blood; and/or the APCs comprise immortalized cells; and/or the lymphocytes are derived from a cancer or tumor.

[0015] In some examples, the tumor antigens comprise full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoding autoantigens associated with a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding autoantigens associated with a cancer or tumor.

[0016] In another example, the present disclosure features a method of identifying a subject as a candidate for cancer therapy. In some examples,, the method comprises: a) obtaining, providing, or generating a library comprising bacterial cells or beads comprising a plurality of tumor antigens, wherein each bacterial cell or bead of the library comprises a different tumor antigen; b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from the

subject, wherein the APCs internalize the bacterial cells or beads; c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a tumor antigen presented by one or more APCs; d) determining whether one or more lymphocytes are activated by, or not responsive to, one or more tumor antigens presented by one or more APCs, e.g., by assessing (e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control), of expression and/or secretion of one or more immune mediators; e) identifying one or more tumor antigens that stimulate, inhibit and/or suppress, and/or have a minimal effect on a level of expression and/or secretion of one or more immune mediators, to obtain or generate a subject response profile; and f) comparing the subject response profile to a target response profile to select the subject as a candidate subject for initiation, continuation, modification, discontinuation or non-initiation of a cancer therapy. In some examples, the subject response profile comprises a representation of the level of expression and/or secretion of the one or more immune mediators associated with the plurality of tumor antigens.

[0017] In some examples, the method further comprises generating the target response profile by a method comprising: g) contacting the bacterial cells or beads with antigen presenting cells (APCs) from a target subject, wherein the APCs internalize the bacterial cells or beads; h) contacting the APCs with lymphocytes from the target subject, under conditions suitable for activation of lymphocytes by a tumor antigen presented by one or more APCs; i) determining whether one or more lymphocytes are activated by, or not responsive to, one or more tumor antigens presented by one or more APCs, e.g., by assessing (e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control), of expression and/or secretion of one or more immune mediators; andj) identifying one or more tumor antigens that stimulate, inhibit and/or suppress, and/or have a minimal effect on a level of expression and/or secretion of one or more immune mediators, to obtain or generate the target response profile. In some examples, the target response profile comprises a representation of the level of expression and/or secretion of the one or more immune mediators associated with the plurality of tumor antigens.

[0018] In some examples, the target response profile is from one or more target subjects who exhibit or previously exhibited at least one beneficial response to cancer. In some examples, the beneficial response comprises a positive clinical response to a cancer therapy or combination of therapies. In some examples, the beneficial response comprises a

spontaneous response to a cancer. In some examples, the beneficial response comprises clearance of a cancer, e.g., a level of one or more clinical measures associated with clearance of a cancer. In some examples, the beneficial response comprises a lack of a relapse, recurrence, and/or metastasis of a cancer, e.g., over a defined period of time (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 weeks, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 years). In some examples, the beneficial response comprises a positive cancer prognosis. In some examples, the beneficial response comprises a lack of one or more toxic responses and/or side effects (e.g., one or more measurable toxic responses or side effects) to a cancer therapy or combination of therapies.

[0019] In some examples, the target response profile is from one or more target subjects who exhibit or previously exhibited one or more deleterious and/or non-beneficial response to cancer. In some examples, the deleterious and/or non-beneficial response comprises a negative clinical response and/or a failure to respond, to a cancer therapy or combination of therapies. In some examples, the deleterious and/or non-beneficial response comprises a lack of clearance of a cancer, e.g., a level of one or more clinical measures associated with lack of clearance of a cancer. In some examples, the deleterious and/or non-beneficial response comprises at least one relapse, recurrence, and/or metastasis of a cancer. In some examples, the deleterious and/or non-beneficial response comprises a negative cancer prognosis. In some examples, the deleterious and/or non-beneficial response comprises one or more toxic responses and/or side effects (e.g., one or more measurable toxic responses and/or side effects) to a cancer therapy or combination of therapies.

[0020] In some examples, the method further comprises selecting the candidate subject for initiation of a cancer therapy or combination of cancer therapies. In some examples, the method further comprises selecting the candidate subject for continuation of a cancer therapy or combination of cancer therapies. In some examples, the method comprises selecting the subject as a candidate subject (i) if the subject response profile is similar to the target response profile from a target subject who exhibits or previously exhibited one or more beneficial responses to the cancer therapy or combination, and/or (ii) if the subject response profile is dissimilar to the target response profile

from a target subject who exhibits or previously exhibited one or more deleterious responses to the cancer therapy or combination. In some

examples, the method further comprises administering the cancer therapy or combination of cancer therapies to the candidate subject.

[0021] In some examples, the method further comprises selecting the candidate subject for modification of a cancer therapy. In some examples, the method further comprises selecting the candidate subject for discontinuation or non-initiation of a cancer therapy. In some examples, the method further comprises selecting the subject as a candidate subject for modification, discontinuation, and/or non-initiation of a cancer therapy (i) if the subject response profile is similar to the target response profile from a target subject who exhibits or previously exhibited one or more deleterious responses to the cancer therapy, and/or (ii) if the subject response profile is dissimilar to the target response profile from a target subject who exhibits or previously exhibited one or more beneficial responses to the cancer therapy. In some examples, the method further comprises modifying the cancer therapy administered to the candidate subject. In some examples, the method further comprises discontinuing or not initiating the cancer therapy to the candidate subject.

[0022] In some examples, the APCs are human APCs isolated from the subject; and/or the bacterial cells further comprise a cytolysin polypeptide; and/or the cytolysin polypeptide is listeriolysin O (LLO); and/or the APCs are provided in an array, and/or the APCs in each location of the array are contacted with a set of bacterial cells, each set comprising a different tumor antigen; and/or the APCs and lymphocytes are isolated from peripheral blood; and/or the APCs comprise immortalized cells; and/or the lymphocytes are derived from a cancer or tumor.

[0023] In some examples, the tumor antigens comprise full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoding autoantigens associated with a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding autoantigens associated with a cancer or tumor.

[0024] In another example, the present disclosure features a method of selecting tumor antigens. In some examples, the, method comprises: a) obtaining, providing, or generating a library comprising bacterial cells or beads comprising a plurality of tumor antigens, wherein each bacterial cell or bead of the library comprises a different tumor antigen; b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from a subject, wherein the APCs internalize the bacterial cells or beads;c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a tumor antigen presented by one or more APCs; d) determining whether one or more lymphocytes are activated by, or not responsive to, one or more tumor antigens presented by one or more APCs, e.g., by assessing (e.g., detecting or measuring) a level)e.g., an increased or decreased level, relative to a control), of expression and/or secretion of one or more immune mediators; e) identifying one or more tumor antigens that stimulate, inhibit and/or suppress, and/or have minimal effect on a level of expression and/or secretion of one or more immune mediators, to obtain or generate a subject response profile; f) comparing the subject response profile to a target response profile; and g) selecting one or more tumor antigens based on the comparison. In some examples, the subject response profile comprises a representation of the level of expression and/or secretion of the one or more immune mediators associated with the plurality of tumor antigens.

[0025] In some examples, the method further comprises generating the target response profile by a method comprising: h) contacting the bacterial cells or beads with antigen presenting cells (APCs) from a target subject, wherein the APCs internalize the bacterial cells or beads; i) contacting the APCs with lymphocytes from the target subject, under conditions suitable for activation of lymphocytes by a tumor antigen presented by one or more APCs; j) determining whether one or more lymphocytes are activated by, or not responsive to, one or more tumor antigens presented by one or more APCs, e.g., by assessing (e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control), of expression and/or secretion of one or more immune mediators; andk) identifying one or more tumor antigens that stimulate, inhibit and/or suppress, and/or have a minimal effect on a level of expression and/or secretion of one or more immune mediators, to obtain or generate the target response profile. In some examples, the target response profile comprises a
representation of the level of expression and/or secretion of the one or more immune mediators associated with the plurality of tumor antigens.

[0026] In some examples, the target response profile is from one or more target subjects who exhibit or previously exhibited one or more beneficial response to cancer. In some examples, the beneficial response comprises a positive clinical response to a cancer therapy or combination of therapies. In some examples, the beneficial response comprises a spontaneous response to a cancer. In some examples, the beneficial response comprises clearance of a cancer, e.g., a level of one or more clinical measures associated with clearance of a cancer. In some examples, the beneficial response comprises a relapse, recurrence, and/or metastasis of a cancer e.g., over a defined period of time (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 weeks, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 years). In some examples, the beneficial response comprises a positive cancer prognosis. In some examples, the beneficial response comprises a lack of one or more toxic responses and/or side effects (e.g., one or more measurable toxic responses and/or side effects) to a cancer therapy or combination of therapies.

[0027] In some examples, the target response profile is from one or more target subjects who exhibit or previously exhibited one or more deleterious or non-beneficial response to cancer. In some examples, the deleterious and/or non-beneficial response comprises a negative clinical response and/or a failure to respond, to a cancer therapy or combination of therapies. In some examples, the deleterious and/or non-beneficial response comprises a lack of clearance of a cancer, e.g., a level of one or more clinical measures associated with lack of clearance of a cancer. In some examples, the deleterious and/or non-beneficial response comprises at least one relapse, recurrence, and/or metastasis of a cancer. In some examples, the deleterious and/or non-beneficial response comprises a negative cancer prognosis. In some examples, the deleterious and/or non-beneficial response comprises one or more toxic responses and/or side effects (e.g., one or more measurable toxic responses and/or side effects) to a cancer therapy or combination of therapies.

[0028] In some examples, the method further comprises selecting (i) one or more tumor antigens that increase level of expression and/or secretion of one or more immune mediators associated with a beneficial response to cancer, and/or (ii) one or more tumor antigens that inhibit and/or suppress level of expression and/or secretion of one or more immune mediators associated with deleterious or not beneficial responses to cancer. In some examples, the method further comprises administering to the subject an immunogenic composition comprising

one or more of the selected antigens or immunogenic fragments thereof. In some examples, the method further comprises administering to the subject a cancer therapy or combination of therapies.

[0029] In some examples, the method further comprises selecting (i) one or more tumor antigens that increase level of expression and/or secretion of one or more immune mediators associated with deleterious or not beneficial responses to cancer, and/or (ii) one or more tumor antigens that inhibit and/or suppress level of expression and/or secretion of one or more immune mediators associated with beneficial responses to cancer. In some examples, the method further comprises administering to the subject an immunogenic composition that does not comprise one or more of the selected antigens or immunogenic fragments thereof. In some examples, the method further comprises administering to the subject a cancer therapy or combination of therapies.

[0030] In some examples, the APCs are human APCs isolated from the subject; and/or the bacterial cells further comprise a cytolysin polypeptide; and/or the cytolysin polypeptide is listeriolysin O (LLO); and/or the APCs are provided in an array, and/or the APCs in each location of the array are contacted with a set of bacterial cells, each set comprising a different tumor antigen; and/or the APCs and lymphocytes are isolated from peripheral blood; and/or the APCs comprise immortalized cells; and/or the lymphocytes are derived from a cancer or tumor.

[0031] In some examples, the tumor antigens comprise full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoding autoantigens associated with a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding autoantigens associated with a cancer or tumor.

[0032] In another example, the present disclosure features a method of inducing an immune response in a subject. In some examples, the method comprises: a)
obtaining, providing, or generating a library comprising bacterial cells or beads comprising a plurality of tumor antigens, wherein

each bacterial cell or bead of the library comprises a different tumor antigen; b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from a subject, wherein the APCs internalize the bacterial cells or beads; c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a tumor antigen presented by one or more APCs; d) determining whether one or more lymphocytes are activated by, or not responsive to, one or more tumor antigens presented by one or more APCs, e.g., by assessing (e.g., detecting or measuring) a level (e.g., an increased level or decreased level, relative to a control) of expression and/or secretion of one or more immune mediators; e) identifying one or more tumor antigens that stimulate, inhibit and/or suppress, and/or have a minimal effect on a level of expression and/or secretion of one or more immune mediators, to obtain or generate a subject response profile; f) comparing the subject response profile to a target response profile; g) selecting one or more tumor antigens based on the comparison; and h) administering to the subject an immunogenic composition comprising one or more of the selected antigens or immunogenic fragment thereof. In some examples, the subject response profile comprises a representation of the level of expression and/or secretion of the one or more immune mediators associated with the plurality of tumor antigens.

[0033] In some examples, the method further comprises generating the target response profile by a method comprising: i) contacting the bacterial cells or beads with antigen presenting cells (APCs) from a target subject, wherein the APCs internalize the bacterial cells or beads; j) contacting the APCs with lymphocytes from the target subject, under conditions suitable for activation of lymphocytes by a tumor antigen presented by one or more APCs; k) determining whether one or more lymphocytes are activated by, or not responsive to, one or more tumor antigens presented by one or more APCs, e.g., by assessing (e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control), of expression and/or secretion of one or more immune mediators; andl) identifying one or more tumor antigens that stimulate, inhibit and/or suppress, and/or have a minimal effect on a level of expression and/or secretion of one or more immune mediators, to obtain or generate the target response profile. In some examples, the target response profile comprises a representation of the level of expression and/or secretion of the one or more immune mediators associated with the plurality of tumor antigens.

[0034] In some examples, the target response profile is from one or more target subjects who exhibit or previously exhibited at least one beneficial response to cancer. In some examples, the beneficial response comprises a positive clinical response to a cancer therapy or combination of therapies. In some examples, the beneficial response comprises a spontaneous response to a cancer. In some examples, the beneficial response comprises clearance of a cancer, e.g., a level of one or more clinical measures associated with clearance of a cancer. In some examples, the beneficial response comprises a lack of a relapse, recurrence, and/or metastasis of a cancer, e.g., over a defined period of time (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 weeks, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 years). In some examples, the beneficial response comprises a positive cancer prognosis. In some examples, the beneficial response comprises a lack of one or more toxic responses and/or side effects (e.g., one or more measurable toxic responses or side effects) to a cancer therapy or combination of therapies.

[0035] In some examples, the method further comprises selecting and administering to the subject (i) one or more tumor antigens that increase level of expression and/or secretion of one or more immune mediators associated with one or more beneficial responses to cancer, and/or (i) one or more tumor antigens that inhibit and/or suppress level of expression and/or secretion of one or more immune mediators associated with one or more deleterious or not beneficial responses to cancer.

[0036] In some examples, the method further comprises administering to the subject a cancer therapy or combination of therapies.

[0037] In some examples, the APCs are human APCs isolated from the subject; and/or the bacterial cells further comprise a cytolysin polypeptide; and/or the cytolysin polypeptide is listeriolysin O (LLO); and/or the APCs are provided in an array, and/or the APCs in each location of the array are contacted with a set of bacterial cells, each set comprising a different tumor antigen; and/or the APCs and lymphocytes are isolated from peripheral blood; and/or the APCs comprise immortalized cells; and/or the lymphocytes are derived from a cancer or tumor.

[0038] In some examples, the tumor antigens comprise full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor

antigens comprise full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoding autoantigens associated with a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding autoantigens associated with a cancer or tumor.

[0039] In another example, the present disclosure features a method of inducing an immune response in a subject. In some examples, the method comprises: a) obtaining, providing, or generating a library comprising bacterial cells or beads comprising a plurality of tumor antigens, wherein each bacterial cell or bead of the library comprises a different tumor antigen; b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from a subject, wherein the APCs internalize the bacterial cells or beads; c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a tumor antigen presented by one or more APCs; d) determining whether one or more lymphocytes are activated by, or not responsive to, one or more tumor antigens presented by one or more APCs, e.g., by assessing (e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control) of expression and/or secretion of one or more immune mediators; e) identifying one or more tumor antigens that stimulate, inhibit and/or suppress, and/or have a minimal effect on a level of expression and/or secretion of one or more immune mediators, to obtain or generate a subject response profile; f) comparing the subject response profile to a target response profile; g) selecting one or more tumor antigens based on the comparison; and h) administering to the subject an immunogenic composition that does not comprise one or more of the selected antigens or immunogenic fragment thereof. In some examples, the subject response profile comprises a representation of the level of expression and/or secretion of the one or more immune mediators associated with the plurality of tumor antigens.

[0040] In some examples, the method further comprises generating the target response profile by a method comprising: i) contacting the bacterial cells or beads with antigen presenting cells (APCs) from a target subject, wherein the APCs internalize the bacterial cells or beads; j) contacting the APCs with lymphocytes from the target subject, under conditions suitable for activation of lymphocytes by a tumor antigen presented by one or more APCs; k) determining whether one or more lymphocytes are activated by, or not responsive to, one or more tumor

antigens presented by one or more APCs, e.g., by assessing (e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control), of expression and/or secretion of one or more immune mediators; and l) identifying one or more tumor antigens that stimulate, inhibit and/or suppress, and/or have a minimal effect on a level of expression and/or secretion of one or more immune mediators, to obtain or generate the target response profile. In some examples, the target response profile comprises a representation of the level of expression and/or secretion of the one or more immune mediators associated with the plurality of tumor antigens.

[0041] In some examples, the target response profile is from one or more target subjects who exhibit or previously exhibited one or more deleterious and/or non-beneficial response to cancer. In some examples, the deleterious and/or non-beneficial response comprises a negative clinical response and/or a failure to respond, to a cancer therapy or combination of therapies. In some examples, the deleterious and/or non-beneficial response comprises a lack of clearance of a cancer, e.g., a level of one or more clinical measures associated with lack of clearance of a cancer. In some examples, the deleterious and/or non-beneficial response comprises at least one relapse, recurrence, and/or metastasis of a cancer. In some examples, the deleterious and/or non-beneficial response comprises a negative cancer prognosis. In some examples, the deleterious and/or non-beneficial response comprises one or more toxic responses and/or side effects (e.g., one or more measurable toxic responses and/or side effects) to a cancer therapy or combination of therapies.

[0042] In some examples, the method further comprises selecting one or more tumor antigens that increase expression or secretion of immune mediators associated with deleterious or not beneficial responses to cancer, and/or one or more tumor antigens that inhibit and/or suppress expression or secretion of immune mediators associated with beneficial responses to cancer.

[0043] In some examples, the method further comprises administering to the subject a cancer therapy or combination of therapies.

[0044] In some examples, the APCs are human APCs isolated from the subject; and/or the bacterial cells further comprise a cytolysin polypeptide; and/or the cytolysin polypeptide is listeriolysin O (LLO); and/or the APCs are provided in an array, and/or the APCs in each location of the array are contacted with a set of bacterial cells, each set comprising a different

tumor antigen; and/or the APCs and lymphocytes are isolated from peripheral blood; and/or the APCs comprise immortalized cells; and/or the lymphocytes are derived from a cancer or tumor.

[0045] In some examples, the tumor antigens comprise full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoding autoantigens associated with a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding autoantigens associated with a cancer or tumor.

[0046] In another example, the present disclosure features a method of selecting tumor antigens. In some examples, the method comprises: a) obtaining, providing, or generating a library comprising bacterial cells or beads comprising a plurality of tumor antigens, wherein each bacterial cell or bead of the library comprises a different tumor antigen; b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from a subject, wherein the APCs internalize the bacterial cells or beads; c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a tumor antigen presented by one or more APCs; d) determining whether one or more lymphocytes are activated by one or more tumor antigens presented by one or more APCs by assessing (e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control) of expression and/or secretion of one or more immune mediators; e) identifying one or more tumor antigens that stimulate, inhibit and/or suppress, and/or have a minimal effect on a level of expression and/or secretion of one or more immune mediators, to obtain a subject response profile; and f) selecting from among the identified tumor antigens one or more antigens that increase a level of expression and/or secretion of one or more immune mediators associated with at least one beneficial response to cancer, and/or selecting one or more tumor antigens that inhibit and/or suppress a level of expression and/or secretion of one or more immune mediators associated with at least one deleterious and/or non-beneficial response to cancer.

[0047] In some examples, the method further comprises comparing the subject response profile to a target response profile, e.g., a target response profile generated using a method described herein, and selecting one or more tumor antigens based on the comparison.

[0048] In some examples, the target response profile is from one or more target subjects who exhibit or previously exhibited at least one beneficial response to cancer. In some examples, the beneficial response comprises a positive clinical response to a cancer therapy or combination of therapies. In some examples, the beneficial response comprises a spontaneous response to a cancer. In some examples, the beneficial response comprises clearance of a cancer, e.g., a level of one or more clinical measures associated with clearance of a cancer. In some examples, the beneficial response comprises a lack of a relapse, recurrence, and/or metastasis of a cancer, e.g., over a defined period of time (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 weeks, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 years). In some examples, the beneficial response comprises a positive cancer prognosis. In some examples, the beneficial response comprises a lack of one or more toxic responses and/or side effects (e.g., one or more measurable toxic responses or side effects) to a cancer therapy or combination of therapies.

[0049] In some examples, the method further comprises administering to the subject an immunogenic composition comprising one or more of the selected antigens or immunogenic fragments thereof.

[0050] In some examples, the method further comprises administering to the subject a cancer therapy or combination of therapies.

[0051] In some examples, the APCs are human APCs isolated from the subject; and/or the bacterial cells further comprise a cytolysin polypeptide; and/or the cytolysin polypeptide is listeriolysin O (LLO); and/or the APCs are provided in an array, and/or the APCs in each location of the array are contacted with a set of bacterial cells, each set comprising a different tumor antigen; and/or the APCs and lymphocytes are isolated from peripheral blood; and/or the APCs comprise immortalized cells; and/or the lymphocytes are derived from a cancer or tumor.

[0052] In some examples, the tumor antigens comprise full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor

antigens comprise full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoding autoantigens associated with a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding autoantigens associated with a cancer or tumor.

[0053] In another example, the present disclosure features a method of selecting tumor antigens. In some examples, the method comprises a) obtaining, providing, or generating a library comprising bacterial cells or beads comprising a plurality of tumor antigens, wherein each bacterial cell or bead of the library comprises a different tumor antigen; b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from a subject, wherein the APCs internalize the bacterial cells or beads; c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a tumor antigen presented by one or more APCs; d) determining whether one or more lymphocytes are activated by one or more tumor antigens presented by one or more APCs by assessing (e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control) of expression and/or secretion of one or more immune mediators; e) identifying one or more tumor antigens that stimulate, inhibit and/or suppress, and/or have a minimal effect on a level of expression and/or secretion of one or more immune mediators, to obtain a subject response profile; and f) selecting from among the identified tumor antigens (i) one or more antigens that increase a level of expression and/or secretion of one or more immune mediators associated with at least one deleterious and/or non-beneficial response to cancer, and/or (ii) one or more tumor antigens that inhibit and/or suppress a level of expression and/or secretion of one or more immune mediators associated with at least one beneficial response to cancer.

[0054] In some examples, the method further comprises comparing the subject response profile to a target response profile, e.g., a target response profile generated using a method described herein, and selecting one or more tumor antigens based on the comparison.

[0055] In some examples, the target response profile is from one or more target subjects who exhibit or previously exhibited one or more deleterious and/or non-beneficial response to cancer. In some examples, the deleterious and/or non-beneficial response comprises a negative clinical response and/or a failure to respond, to a cancer therapy or combination of

therapies. In some examples, the deleterious and/or non-beneficial response comprises a lack of clearance of a cancer, e.g., a level of one or more clinical measures associated with lack of clearance of a cancer. In some examples, the deleterious and/or non-beneficial response comprises at least one relapse, recurrence, and/or metastasis of a cancer. In some examples, the deleterious and/or non-beneficial response comprises a negative cancer prognosis. In some examples, the deleterious and/or non-beneficial response comprises one or more toxic responses and/or side effects (e.g., one or more measurable toxic responses and/or side effects) to a cancer therapy or combination of therapies.

[0056] In some examples, the method further comprises administering to the subject an immunogenic composition that does not comprise one or more of the selected antigens or immunogenic fragments thereof.

[0057] In some examples, the method further comprises administering to the subject a cancer therapy or combination of therapies.

[0058] In some examples, the APCs are human APCs isolated from the subject; and/or the bacterial cells further comprise a cytolysin polypeptide; and/or the cytolysin polypeptide is listeriolysin O (LLO); and/or the APCs are provided in an array, and/or the APCs in each location of the array are contacted with a set of bacterial cells, each set comprising a different tumor antigen; and/or the APCs and lymphocytes are isolated from peripheral blood; and/or the APCs comprise immortalized cells; and/or the lymphocytes are derived from a cancer or tumor.

[0059] In some examples, the tumor antigens comprise full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoding autoantigens associated with a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding autoantigens associated with a cancer or tumor.

[0060] In another example, the present disclosure features a method of inducing an immune response in a subject. In some examples, the method comprises: a) obtaining, providing, or generating

a library comprising bacterial cells or beads comprising a plurality of tumor antigens, wherein each bacterial cell or bead of the library comprises a different tumor antigen; b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from a subject, wherein the APCs internalize the bacterial cells or beads; c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a tumor antigen presented by one or more APCs; d) determining whether one or more lymphocytes are activated by, or not responsive to, one or more tumor antigens presented by one or more APCs, e.g., by assessing (e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control), of expression and/or secretion of one or more immune mediators; e) identifying one or more tumor antigens that stimulate, inhibit and/or suppress, and/or have a minimal effect on a level of expression and/or secretion of one or more immune mediators, to obtain a subject response profile; f) selecting from among the identified tumor antigens (i) one or more antigens that increase level of expression and/or secretion of one or more immune mediators associated with at least one beneficial response to cancer, and/or (ii) one or more tumor antigens that inhibit and/or suppress level of expression and/or secretion of one or more immune mediators associated with at least one deleterious or non-beneficial response to cancer; and g) administering to the subject an immunogenic composition comprising one or more of the selected antigens or immunogenic fragment thereof.

[0061] In some examples, the method further comprises comparing the subject response profile to a target response profile, e.g., a target response profile generated using a method described herein, and selecting one or more tumor antigens based on the comparison, prior to administration of the immunogenic composition.

[0062] In some examples, the target response profile is from one or more target subjects who exhibit or previously exhibited at least one beneficial response to cancer. In some examples, the beneficial response comprises a positive clinical response to a cancer therapy or combination of therapies. In some examples, the beneficial response comprises a spontaneous response to a cancer. In some examples, the beneficial response comprises clearance of a cancer, e.g., a level of one or more clinical measures associated with clearance of a cancer. In some examples, the beneficial response comprises a lack of a relapse, recurrence, and/or metastasis of a cancer, e.g., over a defined period of time (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 weeks, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months, or at least 1,

2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 years). In some examples, the beneficial response comprises a positive cancer prognosis. In some examples, the beneficial response comprises a lack of one or more toxic responses and/or side effects (e.g., one or more measurable toxic responses or side effects) to a cancer therapy or combination of therapies.

[0063] In some examples, the method further comprises administering to the subject a cancer therapy or combination of therapies.

[0064] In some examples, the APCs are human APCs isolated from the subject; and/or the bacterial cells further comprise a cytolysin polypeptide; and/or the cytolysin polypeptide is listeriolysin O (LLO); and/or the APCs are provided in an array, and/or the APCs in each location of the array are contacted with a set of bacterial cells, each set comprising a different tumor antigen; and/or the APCs and lymphocytes are isolated from peripheral blood; and/or the APCs comprise immortalized cells; and/or the lymphocytes are derived from a cancer or tumor.

[0065] In some examples, the tumor antigens comprise full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoding autoantigens associated with a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding autoantigens associated with a cancer or tumor.

[0066] In another example, the present disclosure features a method of inducing an immune response in a subject. In some examples, the method comprises: a) obtaining, providing, or generating a library comprising bacterial cells or beads comprising a plurality of tumor antigens, wherein each bacterial cell or bead of the library comprises a different tumor antigen; b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from a subject, wherein the APCs internalize the bacterial cells or beads; c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a tumor antigen presented by one or more APCs; d) determining whether one or more lymphocytes are activated by, or not responsive to, one or more tumor antigens presented by one or more

APCs, e.g., by assessing e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control). of expression and/or secretion of one or more immune mediators; e) identifying one or more tumor antigens that stimulate, inhibit and/or suppress, and/or have a minimal effect on level of expression and/or secretion of one or more immune mediators, to obtain a subject response profile; f) comparing the subject response profile to a target response profile, e.g., a target response profile generated using a method described herein; g) selecting from among the identified tumor antigens (i) one or more antigens that increase level of expression and/or secretion of one or more immune mediators associated with at least one deleterious and/or non-beneficial response to cancer, and/or (ii) one or more tumor antigens that inhibit and/or suppress level of expression and/or secretion of one or more immune mediators associated with at least one beneficial response to cancer; and h) administering to the subject an immunogenic composition that does not comprise one or more of the selected antigens or immunogenic fragment thereof.

[0067] In some examples, the method further comprises comparing the subject response profile to a target response profile e.g., a target response profile generated using a method described herein, and selecting one or more tumor antigens based on the comparison.

[0068] In some examples, the target response profile is from one or more target subjects who exhibit or previously exhibited one or more deleterious and/or non-beneficial response to cancer. In some examples, the deleterious and/or non-beneficial response comprises a negative clinical response and/or a failure to respond, to a cancer therapy or combination of therapies. In some examples, the deleterious and/or non-beneficial response comprises a lack of clearance of a cancer, e.g., a level of one or more clinical measures associated with lack of clearance of a cancer. In some examples, the deleterious and/or non-beneficial response comprises at least one relapse, recurrence, and/or metastasis of a cancer. In some examples, the deleterious and/or non-beneficial response comprises a negative cancer prognosis. In some examples, the deleterious and/or non-beneficial response comprises one or more toxic responses and/or side effects (e.g., one or more measurable toxic responses and/or side effects) to a cancer therapy or combination of therapies.

[0069] In some examples, the method further comprises administering to the subject a cancer therapy or combination of therapies.

[0070] In some examples, the APCs are human APCs isolated from the subject; and/or the bacterial cells further comprise a cytolysin polypeptide; and/or the cytolysin polypeptide is listeriolysin O (LLO); and/or the APCs are provided in an array, and/or the APCs in each location of the array are contacted with a set of bacterial cells, each set comprising a different tumor antigen; and/or the APCs and lymphocytes are isolated from peripheral blood; and/or the APCs comprise immortalized cells; and/or the lymphocytes are derived from a cancer or tumor.

[0071] In some examples, the tumor antigens comprise full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoding autoantigens associated with a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding autoantigens associated with a cancer or tumor.

[0072] In another example, the present disclosure features a method of identifying tumor antigens. In some examples, the method comprises: a) obtaining, providing, or generating a library comprising bacterial cells or beads, wherein each bacterial cell or bead of the library comprises a different heterologous polypeptide comprising one or more mutations, splice variants, or translocations expressed in a cancer or tumor cell of a subject; b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from the subject, wherein the APCs internalize the bacterial cells or beads; c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a polypeptide presented by one or more APCs; d) determining whether one or more lymphocytes are activated by, or not responsive to, one or more polypeptides presented by one or more APCs, e.g., by assessing (e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control) of expression and/or secretion of one or more immune mediators; and e) identifying one or more polypeptides that stimulate, inhibit and/or suppress, and/or have a minimal effect on level of expression and/or secretion of one or more immune mediators, wherein stimulation, inhibition and/or suppression indicate that the polypeptide is a tumor antigen.

[0073] In another example, the present disclosure features a method of selecting tumor antigens. In some examples, the method comprises: a) providing a library comprising bacterial cells or beads, wherein each bacterial cell or bead of the library comprises a different heterologous polypeptide comprising one or more mutations, splice variants, or translocations expressed in a cancer or tumor cell expressed in a cancer or tumor cell of a subject; b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from the subject, wherein the APCs internalize the bacterial cells or beads; c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a polypeptide presented by one or more APCs; d) determining whether one or more lymphocytes are activated by, or not responsive to, one or more polypeptides presented by one or more APCs, e.g., by assessing (e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control), of expression and/or secretion of one or more immune mediators; e) identifying one or more polypeptides that stimulate, inhibit and/or suppress, and/or have a minimal effect on level of expression and/or secretion of one or more immune mediators, wherein stimulation, inhibition and/or suppression indicate that the polypeptide is a tumor antigen; and f) selecting from among the identified tumor antigens (i) one or more tumor antigens that increase level of expression and/or secretion of one or more immune mediators associated with at least one beneficial response to cancer, and/or (ii) one or more tumor antigens that inhibit and/or suppress level of expression and/or secretion of one or more immune mediators associated with at least one deleterious and/or non-beneficial response to cancer.

[0074] In some examples, the method further comprises selecting from among the identified polypeptides one of more polypeptides that have a minimal effect on level of expression and/or secretion of one of more immune mediators.

[0075] In some examples, the APCs are human APCs isolated from the subject; and/or the bacterial cells further comprise a cytolysin polypeptide; and/or the cytolysin polypeptide is listeriolysin O (LLO); and/or the APCs are provided in an array, and/or the APCs in each location of the array are contacted with a set of bacterial cells, each set comprising a different tumor antigen; and/or the APCs and lymphocytes are isolated from peripheral blood; and/or the APCs comprise immortalized cells; and/or the lymphocytes are derived from a cancer or tumor.

[0076] In some examples, the tumor antigens comprise full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor

antigens comprise polypeptides that are fragments of full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoding autoantigens associated with a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding autoantigens associated with a cancer or tumor.

[0077] In another example, the present disclosure features a method of selecting potential tumor antigens. In some examples, the method comprises: a) obtaining, providing, or generating a library comprising bacterial cells or beads, wherein each bacterial cell or bead of the library comprises a heterologous polypeptide comprising one or more mutations, splice variants, or translocations expressed in a cancer or tumor cell expressed in a cancer or tumor cell of a subject; b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from the subject, wherein the APCs internalize the bacterial cells or beads; c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a polypeptide presented by one or more APCs; d) determining whether one or more lymphocytes are activated by, or not responsive to, one or more polypeptides presented by one or more APCs, e.g., by assessing (e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control) of expression and/or secretion of one or more immune mediators; e) identifying one or more polypeptides that stimulate, inhibit and/or suppress, and/or have a minimal effect on level of expression and/or secretion of one or more immune mediators, and identifying one or more polypeptides that stimulate, inhibit, and/or suppress as a tumor antigen; and f) selecting from among the identified polypeptides one or more polypeptides that have a minimal effect on level of expression and/or secretion of one or more immune mediators.

[0078] In some examples, the method further comprises repeating steps b) through e), or steps c) through e), with lymphocytes from the subject that have undergone one or more previous rounds of exposure to APCs.

[0079] In some examples, the method further comprises selecting from among the identified tumor antigens (i) one or more tumor antigens that increase level of expression and/or secretion of one or more immune mediators associated with at least one beneficial response to

cancer, and/or (ii) one or more tumor antigens that inhibit and/or suppress level of expression and/or secretion of one or more immune mediators associated with at least one deleterious and/or non-beneficial responses to cancer.

[0080] In some examples, the method further comprises administering to the subject an immunogenic composition comprising one or more of the selected tumor antigens or selected polypeptides, or immunogenic fragments thereof. In some examples, the method further comprises administering to the subject an immunogenic composition comprising a combination of one or more of the selected tumor antigens and selected polypeptides, or immunogenic fragments thereof. In some examples, the method further comprises administering to the subject a cancer therapy or combination of therapies.

[0081] In some examples, the APCs are human APCs isolated from the subject; and/or the bacterial cells further comprise a cytolysin polypeptide; and/or the cytolysin polypeptide is listeriolysin O (LLO); and/or the APCs are provided in an array, and/or the APCs in each location of the array are contacted with a set of bacterial cells, each set comprising a different tumor antigen; and/or the APCs and lymphocytes are isolated from peripheral blood; and/or the APCs comprise immortalized cells; and/or the lymphocytes are derived from a cancer or tumor.

[0082] In some examples, the tumor antigens comprise full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoding autoantigens associated with a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding autoantigens associated with a cancer or tumor.

[0083] In another example, the present disclosure features a method of selecting tumor antigens. In some examples, the method comprises: a) obtaining, providing, or generating a library comprising bacterial cells or beads, wherein each bacterial cell or bead of the library comprises a different heterologous polypeptide comprising one or more mutations, splice variants, or translocations expressed in a cancer or tumor cell expressed in a cancer or tumor cell of a

subject; b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from the subject, wherein the APCs internalize the bacterial cells or beads; c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a polypeptide presented by one or more APCs; d) determining whether one or more lymphocytes are activated by, or not responsive to, one or more polypeptides presented by one or more APCs, e.g., by assessing (e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control), of expression and/or secretion of one or more immune mediators; e) identifying one or more polypeptides that stimulate, inhibit and/or suppress, and/or have a minimal effect on level of expression and/or secretion of one or more immune mediators, and identifying one or more polypeptides that stimulate, inhibit, and/or suppress as a tumor antigen; and f) selecting from among the identified tumor antigens (i) one or more tumor antigens that increase level of expression and/or secretion of one or more immune mediators associated with at least one deleterious and/or non-beneficial response to cancer, and/or (ii) one or more tumor antigens that inhibit and/or suppress level of expression and/or secretion of one or more immune mediators associated with at least one beneficial response to cancer.

[0084] In some examples, the method further comprises administering to the subject an immunogenic composition that does not comprise one or more of the selected tumor antigens or immunogenic fragments thereof. In some examples, the method further comprises administering to the subject a cancer therapy or combination of therapies.

[0085] In some examples, the APCs are human APCs isolated from the subject; and/or the bacterial cells further comprise a cytolysin polypeptide; and/or the cytolysin polypeptide is listeriolysin O (LLO); and/or the APCs are provided in an array, and/or the APCs in each location of the array are contacted with a set of bacterial cells, each set comprising a different tumor antigen; and/or the APCs and lymphocytes are isolated from peripheral blood; and/or the APCs comprise immortalized cells; and/or the lymphocytes are derived from a cancer or tumor.

[0086] In some examples, the tumor antigens comprise full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding mutations, splice variants, or translocations present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full

length polypeptides encoded by a virus or other infectious agent present in a cancer or tumor; and/or the tumor antigens comprise full length polypeptides encoding autoantigens associated with a cancer or tumor; and/or the tumor antigens comprise polypeptides that are fragments of full length polypeptides encoding autoantigens associated with a cancer or tumor.

[0087] In another example, the present disclosure features a method of inducing an immune response in a subject. In some examples, the method comprises: a) obtaining, providing, or generating a library comprising bacterial cells or beads, wherein each bacterial cell or bead of the library comprises a different heterologous polypeptide comprising one or more mutations, splice variants, or translocations expressed in a cancer or tumor cell of a subject; b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from the subject, wherein the APCs internalize the bacterial cells or beads; c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a polypeptide presented by one or more APCs; d) determining whether one or more lymphocytes are activated by, or not responsive to, one or more polypeptides presented by one or more APCs, e.g., by assessing (e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control) of expression and/or secretion of one or more immune mediators; e) identifying one or more polypeptides that stimulate, inhibit and/or suppress, and/or have a minimal effect on level of expression and/or secretion of one or more immune mediators, and identifying a polypeptide that stimulates, inhibits and/or suppresses as a tumor antigen; f) selecting from among the identified tumor antigens (i) one or more tumor antigens that increase level of expression and/or secretion of one or more immune mediators associated with at least one beneficial response to cancer, and/or (ii) one or more tumor antigens that inhibit and/or suppress level of expression and/or secretion of one or more immune mediators associated with at least one deleterious and/or non-beneficial responses to cancer; and g) administering to the subject an immunogenic composition comprising one or more of the selected antigens or immunogenic fragment thereof.

[0088] In another example, the present disclosure features a method of inducing an immune response in a subject. In some examples, the method comprises: a) obtaining, providing, or generating a library comprising bacterial cells or beads, wherein each bacterial cell or bead of the library comprises a different heterologous polypeptide comprising one or more mutations, splice variants, or translocations expressed in a cancer or tumor cell of a subject; b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from the subject, wherein the APCs

internalize the bacterial cells or beads; c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a polypeptide presented by one or more APCs; d) determining whether one or more lymphocytes are activated by, or not responsive to, one or more polypeptides presented by one or more APCs, e.g., by assessing (e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control), of expression and/or secretion of one or more immune mediators; e) identifying one or more polypeptides that stimulate, inhibit and/or suppress, and/or have a minimal effect on level of expression and/or secretion of one or more immune mediators, wherein stimulation, inhibition and/or suppression indicate that the polypeptide is a tumor antigen; f) selecting from among the identified polypeptides one or more polypeptides that have a minimal effect on level of expression and/or secretion of one or more immune mediators; and g) administering to the subject an immunogenic composition comprising one or more of the selected polypeptides or immunogenic fragment thereof.

[0089] In another example, the present disclosure features a method of inducing an immune response in a subject. In some examples, the method comprises: a) obtaining, providing, or generating a library comprising bacterial cells or beads, wherein each bacterial cell or bead of the library comprises a different heterologous polypeptide comprising one or more mutations, splice variants, or translocations expressed in a cancer or tumor cell of a subject; b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from the subject, wherein the APCs internalize the bacterial cells or beads; c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a polypeptide presented by one or more APCs; d) determining whether one or more lymphocytes are activated by, or not responsive to, one or more polypeptides presented by one or more APCs, e.g., by assessing (e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control), of expression and/or secretion of one or more immune mediators; e) identifying one or more polypeptides that stimulate, inhibit and/or suppress, and/or have a minimal effect on level of expression and/or secretion of one or more immune mediators, wherein stimulation, inhibition and/or suppression indicate that the polypeptide is a tumor antigen; f) selecting from among the identified tumor antigens and polypeptides (i) one or more polypeptides that have a minimal effect on level of expression and/or secretion of one or more immune mediators, (ii) one or more tumor antigens that increase level of expression and/or secretion of one or more immune

mediators associated with at least one beneficial response to cancer; and/or (iii) one or more tumor antigens that inhibit and/or suppress level of expression and/or secretion of one or more immune mediators associated with at least one deleterious and/or non-beneficial response to cancer; and g) administering to the subject an immunogenic composition comprising one or more of the selected tumor antigens and polypeptides, or immunogenic fragments thereof.

[0090] In some examples, the method further comprises administering to the subject a cancer therapy or combination of therapies.

[0091] In another example, the present disclosure features a method of inducing an immune response in a subject. In some examples, the method comprises: a) obtaining, providing, or generating a library comprising bacterial cells or beads, wherein each bacterial cell or bead of the library comprises a different heterologous polypeptide comprising one or more mutations, splice variants, or translocations expressed in a cancer or tumor cell of a subject; b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from the subject, wherein the APCs internalize the bacterial cells or beads; c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a polypeptide presented by one or more APCs; d) determining whether one or more lymphocytes are activated by, or not responsive to, one or more polypeptides presented by one or more APCs, e.g., by assessing (e.g., detecting or measuring) a level (e.g., an increased or decreased level, relative to a control) of expression and/or secretion of one or more immune mediators; e) identifying one or more polypeptides that stimulate, inhibit and/or suppress, and/or have a minimal effect on level of expression and/or secretion of one or more immune mediators, and identifying a polypeptide that stimulates, inhibits and/or suppresses as a tumor antigen; f) selecting from among the identified tumor antigens (i) one or more tumor antigens that increase level of expression and/or secretion of one or more immune mediators associated with at least one deleterious and/or non-beneficial response to cancer, and/or (ii) one or more tumor antigens that inhibit and/or suppress level of expression and/or secretion of one or more immune mediators associated with at least one beneficial response to cancer; and g) administering to the subject an immunogenic composition that does not comprise one or more of the selected antigens or immunogenic fragment thereof.

[0092] In some examples, the method further comprises administering to the subject a cancer therapy or combination of therapies.

[0093] In any of the examples described herein, the plurality of tumor antigens comprises at least 1, 3, 5, 10, 15, 20, 25, 30, 50, 100, 150, 250, 500, 750, 1000 or more different tumor antigens, or portions thereof; and/or determining whether one or more lymphocytes are activated by, or not responsive to, one or more tumor antigens comprises measuring a level of one or more immune mediators; and/or the one or more immune mediators are selected from the group consisting of cytokines, soluble mediators, and cell surface markers expressed by the lymphocytes; and/or the one or more immune mediators are cytokines; and/or the one or more cytokines are selected from the group consisting of TRAIL, IFN-gamma, IL-12p70, IL-2, TNF-alpha, MIP1-alpha, MIP1-beta, CXCL9, CXCL10, MCP1, RANTES, IL-1 beta, IL-4, IL-6, IL-8, IL-9, IL-10, IL-13, IL-15, CXCL11, IL-3, IL-5, IL-17, IL-18, IL-21, IL-22, IL-23A, IL-24, IL-27, IL-31, IL-32, TGF-beta, CSF, GM-CSF, TRANCE (also known as RANK L), MIP3-alpha, and fractalkine; and/or the one or more immune mediators are soluble mediators; and/or the one or more soluble mediators are selected from the group consisting of granzyme A, granzyme B, sFas, sFasL, perforin, and granulysin; and/or the one or more immune mediators are cell surface markers; and/or the one or more cell surface markers are selected from the group consisting of CD107a, CD107b, CD25, CD69, CD45RA, CD45RO, CD137 (4-1BB), CD44, CD62L, CD27, CCR7, CD154 (CD40L), KLRG-1, CD71, HLA-DR, CD122 (IL-2RB), CD28, IL7Ra (CD127), CD38, CD26, CD134 (OX-40), CTLA-4 (CD152), LAG-3, TIM-3 (CD366), CD39, PD1 (CD279), FoxP3, TIGIT, CD160, BTLA, 2B4 (CD244), and KLRG1; and/or the lymphocytes comprise CD4+ T cells; and/or the lymphocytes comprise CD8+ T cells; and/or the lymphocytes comprise NKT cells, gamma-delta T cells, or NK cells; and/or the lymphocytes comprise any combination of CD4+ T cells, CD8+ T cells, NKT cells, gamma-delta T cells, and NK cells; and/or lymphocyte activation is determined by assessing a level of one or more expressed or secreted immune mediators that is at least 20%, 40%, 60%, 80%, 100%, 120%, 140%, 160%, 180%, or 200% higher or lower than a control level; and/or lymphocyte activation is determined by assessing a level of one or more expressed or secreted immune mediators that is at least one, two, or three standard deviations greater or lower than the mean of a control level; and/or lymphocyte activating is determined by assessing a level of one or more expressed or secreted immune mediators that is at least 1, 2, 3, 4 or 5 median absolute deviations (MADs) greater or lower than a median response level to a control; and/or lymphocyte non-responsiveness is determined by assessing a level of one or more expressed or secreted immune mediators that is

or more immune checkpoint inhibitors; and/or the cancer therapy comprises immune suppression blockade therapy; and/or the immune suppression blockade therapy comprises administration of Vista (B7-H5, v-domain Ig suppressor of T cell activation) inhibitors, Lag-3 (lymphocyte-activation gene 3, CD223) inhibitors, IDO (indolemamine-pyrrole-2,3,-dioxygenase-1,2) inhibitors, or KIR receptor family (killer cell immunoglobulin-like receptor) inhibitors, CD47 inhibitors, or Tigit (T cell immunoreceptor with Ig and ITIM domain) inhibitors; and/or the immune suppression blockade therapy comprises administration of two or more immune suppression inhibitors; and/or the cancer therapy comprises immune activation therapy; and/or the immune activation therapy comprises administration of CD40 agonists, GITR (glucocorticoid-induced TNF-R-related protein, CD357) agonists, OX40 (CD134) agonists, 4-1BB (CD137) agonists, ICOS (inducible T cell stimulator, CD278) agonists, IL-2 (interleukin 2) agonists, or interferon agonists; and/or immune activation comprises administration of two or more immune activators; and/or the cancer therapy comprises adjuvant therapy; and/or the adjuvant therapy comprises administration of a TLR agonist (e.g., CpG or Poly I:C), STING agonist, non-specific stimulus of innate immunity, dendritic cells, GM-CSF, IL-12, IL-7, Flt-3, or other cytokines; and/or the cancer therapy comprises oncolytic virus therapy; and/or the oncolytic viral therapy comprises administration of talimogene leherparepvec; and/or the cancer therapy comprises administration of one or more chemotherapeutic agents; and/or the cancer therapy comprises radiation; and/or the cancer therapy comprises surgical excision; and/or the cancer therapy comprises cell-based therapy; and/or the cell-based therapy comprises administration of dendritic cells, chimeric antigen receptor T (CAR-T) cells, T cell receptor-transduced cells, tumor infiltrating lymphocytes (TIL), or natural killer (NK) cells; and/or the cancer therapy comprises localized hyperthermia or hypothermia; and/or the cancer therapy comprises administration of one or more anti-tumor antibodies; and/or the anti-tumor antibodies comprise bi-specific antibodies; and/or the cancer therapy comprises administration of one or more anti-angiogenic agents; and/or the cancer therapy comprises any combination of immune checkpoint blockade, immune suppression blockade, immune activation, adjuvant, oncolytic virus, chemotherapeutic, radiation, surgical, cell-based, hyperthermia, hypothermia, anti-tumor antibody, and anti-angiogenic therapies.

[0094] In another example, the present disclosure features a method of inducing an immune response in a subject with one or more selected antigens, the method comprising: a) obtaining, providing

or generating a library comprising bacterial cells or beads comprising a plurality of tumor antigens, wherein each bacterial cell or bead of the library comprises a different tumor antigen; b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from a first subject, wherein the APCs internalize the bacterial cells or beads; c) contacting the APCs with lymphocytes from the first subject, under conditions suitable for stimulation or inhibition and/or suppression of lymphocytes by a tumor antigen presented by one or more APCs; d) identifying one or more stimulatory tumor antigens that stimulate lymphocytes and identifying one or more non-stimulatory tumor antigens that do not stimulate lymphocytes, to produce a subject response profile; e) comparing the subject response profile to a target response profile, wherein the target response profile is from a second subject who responds clinically to a cancer therapy, and wherein the target response profile comprises one or more identified stimulatory tumor antigens that stimulate lymphocytes and comprises one or more identified non-stimulatory tumor antigens that do not stimulate lymphocytes; f) selecting one or more antigens, wherein the one or more antigens are identified as non-stimulatory in the subject response profile and the same one or more antigens are identified as stimulatory in the target response profile; and g) administering to the first subject an immunogenic composition comprising one or more of the selected antigens.

[0095] In some examples, the method further comprises administering a cancer therapy to the subject. In some examples, the subject response profile comprises a representation of the level of expression and/or secretion of the one or more immune mediators associated with the plurality of tumor antigens.

[0096] In another example, the present disclosure features an immunogenic composition, comprising one or more antigens of the target response profile obtained or generated according to any of the methods described herein.

[0097] In another example, the present disclosure features an immunogenic composition, comprising one or more antigens selected according to any of the methods described herein.

[0098] In another example, the present disclosure features an immunogenic composition comprising (i) one or more heparanase polypeptides or immunogenic fragments thereof and (ii) a SMAD4 polypeptide or immunogenic fragment thereof.

[0099] In some examples, the one or more heparanase polypeptides or fragments and the SMAD4 polypeptide or fragment are each 8-29 amino acids in length. In some examples,

the heparanase polypeptides comprise the amino acid sequence of SEQ ID NO:6 or SEQ ID NO:7. In some examples, the SMAD4 polypeptide comprises the amino acid sequence of SEQ ID NO:8. In some examples, the one or more immunogenic fragments consist of about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the total number of amino acids of SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8. In some examples, the one or more immunogenic fragments consist of SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8 lacking about 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, or more amino acids. In some examples, the one or more heparanse polypeptides comprise an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical to SEQ ID NO:6 or SEQ ID NO:7. In some examples, the SMAD4 polypeptide comprises an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical to SEQ ID NO:8.

[00100] In another example, the present disclosure features an immunogenic composition comprising a heparanase isoform 1 polypeptide or immunogenic fragment, a heparanase isoform 2 polypeptide or immunogenic fragment, and a SMAD4 polypeptide or immunogenic fragment.

[00101] In some examples, the heparanase isoform 1 polypeptide or immunogenic fragment, the heparanase isoform 2 polypeptide or immunogenic fragment and the SMAD4 polypeptide or immunogenic fragment are each 8-29 amino acids in length. In some examples, the heparanase isoform 1 polypeptide comprises the amino acid sequence of SEQ ID NO:1 and the heparanase isoform 2 polypeptide comprises the amino acid sequence of SEQ ID NO:2. In some examples, the SMAD4 polypeptide comprises the amino acid sequence of SEQ ID NO:3. In some examples, the one or more immunogenic fragments consist of about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the total number of amino acids of SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8. In some examples, one or more immunogenic fragments consist of SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8 lacking about 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, or more amino acids. In some examples, the heparanase isoform 1 polypeptide comprises an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical to SEQ ID NO:6 and wherein the heparanase isoform 1 polypeptide comprises an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical to SEQ ID NO:7. In some examples, the SMAD4 polypeptide comprises an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical to SEQ ID NO:8. In some examples, the compositions further comprises an adjuvant.

[00102] In another example of the present disclosure, methods of treating cancer comprises administering to a subject the immunogenic compositions described herein. In some examples, the subject has or is at risk of cancer, and/or exhibits one or more signs or symptoms of cancer, and/or exhibits one or more risk factors for cancer. In some examples, the cancer is colorectal cancer, melanoma, or lung cancer.

[00103] In another example of the present disclosure, methods of inducing an immune response in a subject, comprise administering to a subject the immunogenic compositions described herein. In some examples, the immune response comprises activation of one or more lymphocytes. In some examples, the one or more lymphocytes comprise CD4+ T cells. In some examples, the one or more lymphocytes comprise CD8+ T cells. In some examples, the one or more lymphocytes comprise NKT cells, gamma-delta T cells, or NK cells. In some examples, the one or more lymphocytes comprise any combination of CD4+ T cells, CD8+ T cells, NKT cells, gamma-delta T cells, and NK cells. In some examples, the immune response comprises an increased expression and/or secretion of one or more immune mediators relative to a control. In some examples, the lymphocyte signaling molecule is selected from among immune mediators. In some examples, the one or more immune mediators are cytokines. In some examples, the one or more cytokines are selected from TRAIL, IFN-gamma, IL-12p70, IL-2, TNF-alpha, MIP1-alpha, MIP1-beta, CXCL9, CXCL10, MCP1, RANTES, IL-1 beta, IL-4, IL-6, IL-8, IL-9, IL-10, IL-13, IL-15, CXCL11, IL-3, IL-5, IL-17, IL-18, IL-21, IL-22, IL-23A, IL-24, IL-27, IL-31, IL-32, TGF-beta, CSF, GM-CSF, TRANCE (also known as RANK L), MIP3-alpha, MCP1, and fractalkine. In some examples, the one or more immune mediators are soluble mediators. In some examples, the one or more soluble mediators are selected from granzyme A, granzyme B, sFas, sFasL, perforin, and granulysin. In some examples, the one or more immune mediators are cell surface markers. In some examples, the one or more cell surface markers are selected from CD107a, CD107b, CD25, CD69, CD45RA, CD45RO, CD137 (4-1BB), CD44, CD62L, CD27, CCR7, CD154 (CD40L), KLRG-1, CD71, HLA-DR, CD122 (IL-2RB), CD28, IL7Ra (CD127), CD38, CD26, CD134 (OX-40), CTLA-4 (CD152), LAG-3, TIM-3 (CD366), CD39, PD1 (CD279), FoxP3, TIGIT, CD160, BTLA, 2B4 (CD244), and KLRG1. In some examples, a level of one or more expressed or secreted immune mediators that is at least 20%, 40%, 60%, 80%, 100%, 120%, 140%, 160%, 180%, or 200% higher than a control level. In some examples, a level of one

or more expressed or secreted immune mediators that is at least one, two, or three standard deviations higher than the mean of a control level indicates lymphocyte activation. In some examples, a level of one or more expressed or secreted immune mediators that is at least 1, 2, 3, 4 or 5 median absolute deviations (MADs) higher or lower than a median response level to a control indicates lymphocyte activation. In some examples, the immune response comprises a humoral response and/or a cellular response. In some examples, humoral response comprises an increase in magnitude of response or fold rise from baseline of antigen specific immunoglobulin G (IgG) levels and/or of antigen specific neutralizing antibody levels. In some examples, the humoral response comprises a 4-fold or greater rise in IgG titer from baseline. In some examples, the humoral response comprises a 2-fold or greater rise in 50% neutralizing antibody titer from baseline. In some examples, the cellular response comprises secretion of granzyme B (GrB). In some examples, the cellular response comprises an increase in magnitude of response or fold rise from baseline of granzyme B (GrB) levels. In some examples, the cellular response comprises an increase in IFN-gamma secretion for T cells. In some examples, the subject has or is at risk of cancer, and/or exhibits one or more signs or symptoms of cancer, and/or exhibits one or more risk factors for cancer. In some examples, the cancer is colorectal cancer, melanoma, or lung cancer.

[00104] In another example, the present disclosure features a method for manufacturing an immunogenic composition, the method comprising combining one or more antigens identified by any method described herein and a carrier.

[00105] In some examples, the antigen is produced using recombinant DNA technology in a suitable host cell. In some examples, the method comprises formulating the immunogenic composition as a pharmaceutical composition.

[00106] In another example, the present disclosure features a method for manufacturing an immunogenic composition for administration to a subject in need thereof, the method comprising: a. providing, preparing, or obtaining a plurality of antigenic compositions comprising a plurality of antigens, each composition comprising a different antigen; b. providing, preparing, or obtaining a target response profile, wherein the target response profile comprises a representation of the level of expression and/or secretion of one or more immune mediators associated (e.g., determined, measured, observed) with the plurality of antigens; c. providing, preparing, or obtaining a subject response profile, wherein the subject response profile

comprises a representation of the level of expression and/or secretion of one or more immune mediators associated (e.g., determined, measured, observed) with the plurality of antigens; d. comparing the target response profile to the subject response profile; e. selecting one or more antigens based on the comparison; and f. formulating at least a portion of one or more antigenic compositions comprising the one or more selected antigens as a pharmaceutical composition.

[00107] In some examples, the selecting step comprises selecting one or more antigens that increase expression or secretion of immune mediators associated with a beneficial response to cancer, and/or one or more antigens that inhibit and/or suppress expression or secretion of immune mediators associated with deleterious or not beneficial responses to cancer. In some examples, the plurality of antigenic compositions are in solution, lyophilized, or on a synthetic matrix.

[00108] In another example, the present disclosure features a method of manufacturing an immunogenic composition for administration to a subject in need thereof, the method comprising: preparing one or more antigens, or fragments thereof, identified by any of the method described herein; combining one or more antigens, or fragments thereof, wherein the one or more antigens or fragments thereof are selected according to the subject's response profile; and formulating the immunogenic composition as a pharmaceutical composition.

[00109] In another example, the present disclosure features a method of manufacturing an immunogenic composition for administration to a subject in need thereof, the method comprising: preparing one or more antigens, or fragments thereof, identified by any method described herein; combining one or more antigens, or fragments thereof, wherein the one or more antigens or fragments thereof are selected according to whether or not the one or more antigens have been shown to stimulate, inhibit and/or suppress and/or have minimal effect on level of expression and/or secretion of one or more immune mediators by the subject's lymphocytes; and formulating the immunogenic composition as a pharmaceutical composition.

[00110] In another example, the present disclosure features a method of manufacturing an immunogenic composition for administration to a subject in need thereof, the method comprising: preparing one or more antigens, or fragments thereof, identified by any method described herein;

[00111] combining one or more antigens, or fragments thereof, wherein the one or more antigens or fragments thereof are selected according to the subject's response profile; and formulating the immunogenic composition as a pharmaceutical composition.

CLAIMS

We claim:

1. A method of selecting tumor antigens, the method comprising:

a) obtaining, providing, or generating a library comprising bacterial cells or beads comprising a plurality of tumor antigens, wherein each bacterial cell or bead of the library comprises a different tumor antigen;

b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from a subject, wherein the APCs internalize the bacterial cells or beads;

c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a tumor antigen presented by one or more APCs;

d) determining whether one or more lymphocytes are activated by one or more tumor antigens presented by one or more APCs by assessing a level of expression and/or secretion of one or more immune mediators;

e) identifying each activating tumor antigen as (i) an antigen that stimulates the level of expression and/or secretion of one or more immune mediators, or (ii) an antigen that inhibits and/or suppresses the level of expression and/or secretion of one or more immune mediators; and

f) selecting from among the identified tumor antigens (i) one or more antigens that increase a level of expression and/or secretion of one or more immune mediators associated with at least one beneficial response to cancer, (ii) one or more tumor antigens that inhibit and/or suppress a level of expression and/or secretion of one or more immune mediators associated with at least one deleterious and/or non-beneficial response to cancer, (iii) one or more antigens that increase a level of expression and/or secretion of one or more immune mediators associated with at least one deleterious and/or non-beneficial response to cancer, and/or (iv) one or more tumor antigens that inhibit and/or suppress a level of expression and/or secretion of one or more immune mediators associated with at least one beneficial response to cancer.

2. A method of selecting tumor antigens, the method comprising:

a) providing a library comprising bacterial cells or beads, wherein each bacterial cell or bead of the library comprises a different heterologous polypeptide comprising one or more mutations, splice variants, or translocations expressed in a cancer or tumor cell of a subject;

b) contacting the bacterial cells or beads with antigen presenting cells (APCs) from the subject, wherein the APCs internalize the bacterial cells or beads;

c) contacting the APCs with lymphocytes from the subject, under conditions suitable for activation of lymphocytes by a polypeptide presented by one or more APCs;

d) determining whether one or more lymphocytes are activated by, or not responsive to, one or more polypeptides presented by one or more APCs, by assessing a level of expression and/or secretion of one or more immune mediators;

e) identifying each activating tumor antigen as (i) an antigen that stimulates the level of expression and/or secretion of one or more immune mediators, or (ii) an antigen that inhibits and/or suppresses the level of expression and/or secretion of one or more immune mediators, wherein stimulation, inhibition and/or suppression indicate that the polypeptide is a tumor antigen; and

f) selecting from among the identified tumor antigens (i) one or more tumor antigens that increase level of expression and/or secretion of one or more immune mediators associated with at least one beneficial response to cancer, (ii) one or more tumor antigens that inhibit and/or suppress level of expression and/or secretion of one or more immune mediators associated with at least one deleterious and/or non-beneficial response to cancer, (iii) one or more tumor antigens that increase level of expression and/or secretion of one or more immune mediators associated with at least one deleterious and/or non-beneficial response to cancer, and/or (iv) one or more tumor antigens that inhibit and/or suppress level of expression and/or secretion of one or more immune mediators associated with at least one beneficial response to cancer.

3. The method of claim 2, further comprising repeating steps b) through e), or steps c) through e), with lymphocytes from the subject that has undergone one or more previous rounds of exposure to APCs.

4. The method of claim 2, wherein the bacterial cells further comprise a cytolysin polypeptide.

5. The method of claim 4, wherein the cytolysin polypeptide is listeriolysin O (LLO).

6. The method of claim 2, wherein the APCs are provided in an array, and wherein the APCs in each location of the array are contacted with a set of bacterial cells, each set comprising a different tumor antigen.

7. The method of claim 2, wherein the APCs and lymphocytes are isolated from peripheral blood.

8. The method of claim 2, wherein the lymphocytes are derived from a cancer or tumor.

9. The method of claim 2, wherein lymphocyte activation is determined by assessing a level of one or more expressed or secreted immune mediators that is at least about 20% higher or lower than a control level.

10. The method of claim 2, wherein lymphocyte activation is determined by assessing a level of one or more expressed or secreted immune mediators that is at least two standard deviations greater or lower than the mean of a control level.

11. The method of claim 2, wherein lymphocyte activation is determined by assessing a level of one or more expressed or secreted immune mediators that is at least 2 median absolute deviations (MADs) greater or lower than a median response level to a control.

12. The method of claim 2, wherein lymphocyte non-responsiveness is determined by assessing a level of one or more expressed or secreted immune mediators that is within about 20% of a control level.

13. The method of claim 2, wherein lymphocyte non-responsiveness is determined by assessing a level of one or more expressed or secreted immune mediators that is less than one standard deviation higher or lower than the mean of a control level.

14. The method of claim 2, wherein lymphocyte non-responsiveness is determined by assessing a level of one or more expressed or secreted immune mediators that is less than one median absolute deviation (MAD) higher or lower than a median response level to a control.

15. The method of claim 1, wherein lymphocyte activation is determined by assessing a level of one or more expressed or secreted immune mediators that is at least about 60% higher or lower than a control level.

16. The method of claim 1, wherein lymphocyte activation is determined by assessing a level of one or more expressed or secreted immune mediators that is at least two standard deviations greater or lower than the mean of a control level.

17. The method of claim 1, wherein lymphocyte activation is determined by assessing a level of one or more expressed or secreted immune mediators that is at least 2 median absolute deviations (MADs) greater or lower than a median response level to a control.

18. The method of claim 1, wherein lymphocyte non-responsiveness is determined by assessing a level of one or more expressed or secreted immune mediators that is within about 20% of a control level.

19. The method of claim 1, wherein lymphocyte non-responsiveness is determined by assessing a level of one or more expressed or secreted immune mediators that is less than one standard deviation higher or lower than the mean of a control level.

20. The method of claim 1, wherein lymphocyte non-responsiveness is determined by assessing a level of one or more expressed or secreted immune mediators that is less than one median absolute deviation (MAD) higher or lower than a median response level to a control.