indicated the presence of at least the following markers: CD4, CD9, CD11a, CD13, CD14, CD15, CD32, CD33, CD64, CD65, CD87, CD92, CD93, CD116, CD118, and CD155. Matsuo, *et al.*, *Leukemia* **1997,** 11, 1469-77. Additional phenotypic characterization of MOLM-14 found higher levels of HLA-A/B/C, CD64, CD80, ICOS-L, CD58, and lower levels of CD86. MOLM-14 cells and the closely-related MOLM-13 cells have not been previously reported as useful aAPCs for the expansion of cells for tumor immunotherapy applications.

[006] The EM-3 human cell line was established from the bone marrow of a patient with Philadelphia chromosome-positive CML. Konopka, *et al.*, *Proc. Nat'l Acad. Sci. USA* **1985**, 82, 1810-4. EM-3 cells and the closely-related EM-2 cell line have not been previously reported as useful aAPCs for the expansion of cells for tumor immunotherapy applications. Phenotypic characterization for EM-3 cells indicates the presence of at least the following markers: CD13, CD15, and CD33.

[007] The present invention provides the unexpected finding that engineered myeloid lineage cells, including MOLM-13, MOLM-14, EM-3, and EM-2 cells, transduced with additional costimulatory molecules, including CD86 (B7-2), 4-1BBL (CD137L), and OX40L (CD134L), provide for superior and highly efficient expansions of TILs in large numbers with minimal variability, reduced cost, and no reliance on human blood samples as a source of PBMCs, with the benefit of using an aAPC which can be produced efficiently from a master cell bank. CD86 and 4-1BBL are costimulatory molecules that provide costimulatory signals for T cell activation. The MOLM-14, MOLM-13, EM-3, and/or EM-2 cells transduced with additional costimulatory molecules are useful, for example, in the expansion of TILs for use in cancer immunotherapy and other therapies.

SUMMARY

[007a] In one aspect, the disclosure provides an artificial antigen presenting cell (aAPC) comprising a myeloid cell transduced with one or more viral vectors, wherein the one or more viral vectors comprise (i) a nucleic acid encoding CD86; (ii) one or more nucleic acids encoding one or more costimulatory molecules selected from the group consisting of OX40L and 4-

1BBL, and (iii) a nucleic acid encoding SEQ ID NO:27 or SEQ ID NO:28; wherein the myeloid cell expresses a protein encoded by each of the nucleic acids of (i), (ii), and (iii).

[007b] In another aspect, the disclosure provides a method of expanding a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of: (a) transducing a myeloid cell with one or more viral vectors to obtain a population of artificial antigen presenting cells (aAPCs), wherein the one or more viral vectors comprise (i) a nucleic acid encoding CD86, (ii) one or more nucleic acids encoding one or more costimulatory molecules selected from the group consisting of OX40L and 4-1BBL, and (iii) a nucleic acid encoding SEQ ID NO:27 or SEQ ID NO:28; wherein the myeloid cell expresses a protein encoded by each of the nucleic acids of (i), (ii), and (iii), and (b) contacting the population of TILs with the population of aAPCs in a cell culture medium.

[007c] In yet another aspect, the disclosure provides the use of a population of tumor infiltrating lymphocytes (TILs) expanded by the method as described herein in the manufacture of a medicament for treating cancer in a patient.

[007d] In yet another aspect, the disclosure provides the use of a population of tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for treating a cancer, wherein the population of tumor infiltrating lymphocytes is a first population of TILs and is obtainable by a method comprising the steps of: (a) performing a rapid expansion of a first population of TILs using a population of myeloid artificial antigen presenting cells (myeloid aAPCs) in a cell culture medium to obtain the second population of TILs, wherein the first population of TILs are previously obtained from a tumor resected from a patient, and further wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs after 7 days from the start of the rapid expansion; and wherein the myeloid aAPCs are transduced to express a CD86 protein and a co-stimulatory molecule selected from the group consisting of a 4-1BBL protein, an OX40L protein, and a combination thereof.

[008] In an example, the disclosure provides an artificial antigen presenting cell (aAPC) comprising a myeloid cell transduced with one or more vectors, wherein the one or more viral

vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein.

[009] In an example, each of the CD86 protein and the 4-1BBL protein are human proteins.

[0010] In an example, the disclosure provides an aAPC comprising a myeloid cell transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, wherein the aAPC can stimulate and expand a tumor infiltrating lymphocyte (TIL) contacted with the aAPC.

[0011] It will be apparent that in certain examples of the disclosure, the nucleic acid molecule encoding CD86 may be comprised in a different viral vector to the nucleic acid molecule encoding 4-1BBL or the same viral vector.

[0012] In an example, the disclosure provides an aAPC comprising a myeloid cell transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, wherein the aAPC expands a population of TILs by at least 50-fold over a period of 7 days in a cell culture medium comprising IL-2 at a concentration of about 3000 IU/mL and OKT-3 antibody at a concentration of about 30 ng/mL.

[0013] In an example, the disclosure provides an aAPC comprising a myeloid cell transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, wherein the aAPC can stimulate and expand a T cell contacted with the aAPC.

[0014] In an example, the disclosure provides an aAPC comprising a myeloid cell transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, wherein the myeloid cell endogenously expresses HLA-A/B/C, ICOS-L, and CD58.

[0015] In an example, the disclosure provides an aAPC comprising a myeloid cell transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, wherein the myeloid cell is essentially devoid of membrane-bound IL-15.

[0016] In an example, the disclosure provides an aAPC comprising a myeloid cell transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, wherein the myeloid cell is a MOLM-14 cell.

[0017] In an example, the disclosure provides an aAPC comprising a myeloid cell transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, wherein the myeloid cell is a MOLM-13 cell.

[0018] In an example, the disclosure provides an aAPC comprising a myeloid cell transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, wherein the myeloid cell is a EM-3 cell.

[0019] In an example, the disclosure provides an aAPC comprising a myeloid cell transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, wherein the myeloid cell is a EM-2 cell.

[0020] In an example, the disclosure provides an aAPC comprising a myeloid cell transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, wherein the CD86 protein comprises an amino acid sequence as set forth in SEQ ID NO:8, or an amino acid sequence

comprising one or more conservative amino acid substitutions thereof, and the 4-1BBL protein comprises SEQ ID NO:9, or an amino acid sequence comprising one or more conservative amino acid substitutions thereof.

[0021] In an example, the disclosure provides an aAPC comprising a myeloid cell transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, wherein the nucleic acid molecule encoding CD86 comprises a nucleic acid sequence as set forth in SEQ ID NO:16 and the nucleic acid molecule encoding 4-1BBL comprises a nucleic acid sequence as set forth in SEQ ID NO:19.

[0022] In an example, the disclosure provides a method of expanding tumor infiltrating lymphocytes (TILs), the method comprising the step of contacting a population of TILs with an aAPC comprising a myeloid cell transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, and wherein the population of TILs is expanded. In an example, the method is an *in vitro* or an *ex vivo* method.

[0023] In an example, the disclosure provides a method of expanding a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) transducing a myeloid cell with one or more viral vectors to obtain a population of artificial antigen presenting cells (aAPCs), wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, and

(b) contacting the population of TILs with the population of aAPCs in a cell culture medium.

[0024] In an example, the foregoing method is an *in vitro* or an *ex vivo* method.

[0025] In an example, the disclosure provides a method of expanding a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) transducing a myeloid cell with one or more viral vectors to obtain a population of

artificial antigen presenting cells (aAPCs), wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, and

(b) contacting the population of TILs with the population of aAPCs in a cell culture medium, wherein the cell culture medium further comprises IL-2 at an initial concentration of about 3000 IU/mL and OKT-3 antibody at an initial concentration of about 30 ng/mL.

[0026] In an example, the foregoing method is an *in vitro* or an *ex vivo* method.

[0027] In an example, the disclosure provides a method of expanding a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) transducing a myeloid cell with one or more viral vectors to obtain a population of artificial antigen presenting cells (aAPCs), wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, and

(b) contacting the population of TILs with the population of aAPCs in a cell culture medium, wherein the population of APCs expands the population of TILs by at least 50-fold over a period of 7 days in a cell culture medium.

[0028] In an example, the foregoing method is an *in vitro* or an *ex vivo* method.

[0029] In an example, the disclosure provides a method of expanding a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) transducing a myeloid cell with one or more viral vectors to obtain a population of artificial antigen presenting cells (aAPCs), wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, and

(b) contacting the population of TILs with the population of aAPCs in a cell culture medium, wherein the myeloid cell endogenously expresses HLA-A/B/C, ICOS-L, and CD58.

[0030] In an example, the foregoing method is an *in vitro* or an *ex vivo* method.

[0031] In an example, the disclosure provides a method of expanding a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) transducing a myeloid cell with one or more viral vectors to obtain a population of artificial antigen presenting cells (aAPCs), wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, and

(b) contacting the population of TILs with the population of aAPCs in a cell culture medium, wherein the myeloid cell is a MOLM-14 cell.

[0032] In an example, the foregoing method is an *in vitro* or an *ex vivo* method.

[0033] In an example, the disclosure provides a method of expanding a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) transducing a myeloid cell with one or more viral vectors to obtain a population of artificial antigen presenting cells (aAPCs), wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, and

(b) contacting the population of TILs with the population of aAPCs in a cell culture medium, wherein the myeloid cell is a MOLM-13 cell.

[0034] In an example, the foregoing method is an *in vitro* or an *ex vivo* method.

[0035] In an example, the disclosure provides a method of expanding a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) transducing a myeloid cell with one or more viral vectors to obtain a population of artificial antigen presenting cells (aAPCs), wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, and

(b) contacting the population of TILs with the population of aAPCs in a cell culture medium, wherein the myeloid cell is a EM-3 cell.

[0036] In an example, the foregoing method is an *in vitro* or an *ex vivo* method.

[0037] In an example, the disclosure provides a method of expanding a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) transducing a myeloid cell with one or more viral vectors to obtain a population of artificial antigen presenting cells (aAPCs), wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, and

(b) contacting the population of TILs with the population of aAPCs in a cell culture medium, wherein the myeloid cell is a EM-2 cell.

[0038] In an example, the foregoing method is an *in vitro* or an *ex vivo* method.

[0039] In an example, the disclosure provides a method of expanding a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) transducing a myeloid cell with one or more viral vectors to obtain a population of artificial antigen presenting cells (aAPCs), wherein the one or more viral vectors comprise a nucleic acid molecule encoding CD86 and a nucleic acid molecule encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, and

(b) contacting the population of TILs with the population of aAPCs in a cell culture medium, wherein the CD86 protein comprises an amino acid sequence as set forth in SEQ ID NO:8, or comprises an amino acid sequence comprising one or more conservative amino acid substitutions thereof, and the 4-1BBL protein comprises an amino acid sequence as set forth in SEQ ID NO:9, or comprises an amino acid sequence comprising one or conservative amino acid substitutions thereof.

[0040] In an example, the disclosure provides a method of expanding a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) transducing a myeloid cell with one or more viral vectors to obtain a population of artificial antigen presenting cells (aAPCs), wherein the one or more viral vectors comprise a nucleic acid encoding CD86 and a nucleic acid encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, and

(b) contacting the population of TILs with the population of aAPCs in a cell culture medium, wherein the nucleic acid encoding CD86 comprises a nucleic acid sequence as set forth in SEQ ID NO:16 and the nucleic acid encoding 4-1BBL comprises a nucleic acid sequence as set forth in SEQ ID NO:19.

[0041] In an example, the disclosure provides a method of expanding a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) transducing a myeloid cell with one or more viral vectors to obtain a population of artificial antigen presenting cells (aAPCs), wherein the one or more viral vectors comprise a nucleic acid encoding CD86 and a nucleic acid encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, and

(b) contacting the population of TILs with the population of aAPCs in a cell culture medium, wherein the expansion is performed using a gas permeable container.

[0042] In an example, the disclosure provides a method of expanding a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) transducing a myeloid cell with one or more viral vectors to obtain a population of artificial antigen presenting cells (aAPCs), wherein the one or more viral vectors comprise a nucleic acid encoding CD86 and a nucleic acid encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, and

(b) contacting the population of TILs with the population of aAPCs in a cell culture medium, wherein the ratio of the population of TILs to the population of aAPCs is between 1 to 200 and 1 to 400.

[0043] In an example, the disclosure provides a method of expanding a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) transducing a myeloid cell with one or more viral vectors to obtain a population of artificial antigen presenting cells (aAPCs), wherein the one or more viral vectors

comprise a nucleic acid encoding CD86 and a nucleic acid encoding 4-1BBL, and wherein the myeloid cell expresses a CD86 protein and a 4-1BBL protein, and

(b) contacting the population of TILs with the population of aAPCs in a cell culture medium, wherein the ratio of the population of TILs to the population of aAPCs is about 1 to 300.

[0044] In an example, the disclosure provides a method of expanding tumor infiltrating lymphocytes (TILs), the method comprising contacting a population of TILs comprising a population of TILs with a myeloid artificial antigen presenting cell (aAPC), wherein the myeloid aAPC comprises at least two co-stimulatory ligands that specifically bind with at least two co-stimulatory molecules on the TILs, wherein binding of the co-stimulatory molecules with the co-stimulatory ligand induces proliferation of the TILs, thereby specifically expanding TILs, and wherein the at least two co-stimulatory ligands comprise CD86 and 4-1BBL. In an example, the foregoing method is an *in vitro* or *ex vivo* method.

[0045] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing a rapid expansion of the first population of TILs using a population of myeloid artificial antigen presenting cells (myeloid aAPCs) in a cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs after 7 days from the start of the rapid expansion; and

(c) administering a therapeutically effective portion of the second population of TILs to a patient with the cancer;

wherein the myeloid aAPCs endogenously expresses HLA-A/B/C, ICOS-L, and CD58, and wherein the myeloid aAPCs are transduced to express a CD86 protein and a 4-1BBL protein.

[0046] In an example, the disclosure provides a population of tumor infiltrating lymphocytes (TILs) for use in treating cancer, wherein the TILs are a second population of TILs and are obtainable from a method comprising the steps of:

(a) performing a rapid expansion of a first population of TILs using a population of myeloid artificial antigen presenting cells (myeloid aAPCs) in a cell culture medium to obtain the

second population of TILs, wherein the TILs are/ have been obtained from a tumor resected from a patient, and wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs after 7 days from the start of the rapid expansion; and

wherein the myeloid aAPCs endogenously expresses HLA-A/B/C, ICOS-L, and CD58, and wherein the myeloid aAPCs are transduced to express a CD86 protein and a 4-1BBL protein.

[0047] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing a rapid expansion of the first population of TILs using a population of myeloid artificial antigen presenting cells (myeloid aAPCs) in a cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs after 7 days from the start of the rapid expansion; and

(c) administering a therapeutically effective portion of the second population of TILs to a patient with the cancer;

wherein the myeloid aAPCs endogenously expresses HLA-A/B/C, ICOS-L, and CD58, wherein the myeloid aAPCs are transduced to express a CD86 protein and a 4-1BBL protein, wherein the myeloid aAPCs comprise MOLM-14 cells transduced with one or more viral vectors, and wherein the one or more viral vectors comprise a nucleic acid encoding CD86 and a nucleic acid encoding 4-1BBL, and wherein the MOLM-14 cells express a CD86 protein and a 4-1BBL protein.

[0048] In an example, the disclosure provides a population of tumor infiltrating cells (TILs) for use in treating a cancer, wherein the population of TILs is a second population of TILs and is obtainable by a process comprising:

(a) performing a rapid expansion of a first population of TILs using a population of myeloid artificial antigen presenting cells (myeloid aAPCs) in a cell culture medium to obtain the second population of TILs, wherein the first population of TILs are/have been obtained from a tumor resected from a patient, wherein the second population of TILs is at least

50-fold greater in number than the first population of TILs after 7 days from the start of the rapid expansion;

wherein the myeloid aAPCs endogenously expresses HLA-A/B/C, ICOS-L, and CD58, wherein the myeloid aAPCs are transduced to express a CD86 protein and a 4-1BBL protein, wherein the myeloid aAPCs comprise MOLM-14 cells transduced with one or more viral vectors, and wherein the one or more viral vectors comprise a nucleic acid encoding CD86 and a nucleic acid encoding 4-1BBL, and wherein the MOLM-14 cells express a CD86 protein and a 4-1BBL protein.

[0049] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing a rapid expansion of the first population of TILs using a population of myeloid artificial antigen presenting cells (myeloid aAPCs) in a cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs after 7 days from the start of the rapid expansion; and

(c) administering a therapeutically effective portion of the second population of TILs to a patient with the cancer;

wherein the myeloid aAPCs endogenously expresses HLA-A/B/C, ICOS-L, and CD58, wherein the myeloid aAPCs are transduced to express a CD86 protein and a 4-1BBL protein, wherein the myeloid aAPCs comprise EM-3 cells transduced with one or more viral vectors, and wherein the one or more viral vectors comprise a nucleic acid encoding CD86 and a nucleic acid encoding 4-1BBL, and wherein the EM-3 cells express a CD86 protein and a 4-1BBL protein.

[0050] In an example, the disclosure provides a population of tumor infiltrating lymphocytes (TILs) for use in treating a cancer, the population of TILs being a second population of TILs and obtainable by a process comprising:

(a) performing a rapid expansion of a first population of TILs using a population of myeloid artificial antigen presenting cells (myeloid aAPCs) in a cell culture medium to obtain the

second population of TILs, wherein the first population of TILs are/have been obtained from a tumor resected from a patient, and wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs after 7 days from the start of the rapid expansion; and

wherein the myeloid aAPCs endogenously expresses HLA-A/B/C, ICOS-L, and CD58, wherein the myeloid aAPCs are transduced to express a CD86 protein and a 4-1BBL protein, wherein the myeloid aAPCs comprise EM-3 cells transduced with one or more viral vectors, and wherein the one or more viral vectors comprise a nucleic acid encoding CD86 and a nucleic acid encoding 4-1BBL, and wherein the EM-3 cells express a CD86 protein and a 4-1BBL protein.

[0051] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing a rapid expansion of the first population of TILs using a population of myeloid artificial antigen presenting cells (myeloid aAPCs) in a cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs after 7 days from the start of the rapid expansion; and

(c) administering a therapeutically effective portion of the second population of TILs to a patient with the cancer;

wherein the myeloid aAPCs endogenously expresses HLA-A/B/C, ICOS-L, and CD58, wherein the myeloid aAPCs are transduced to express a CD86 protein and a 4-1BBL protein, and wherein the rapid expansion is performed over a period not greater than 14 days.

[0052] In an example, the disclosure provides a population of tumor infiltrating lymphocytes (TILs) for use in treating a cancer, wherein the population of TILs is a second population and is obtainable by a method comprising the steps of:

(a) performing a rapid expansion of the first population of TILs using a population of myeloid artificial antigen presenting cells (myeloid aAPCs) in a cell culture medium to obtain the second population of TILs, wherein the second population of TILs is at least

50-fold greater in number than the first population of TILs after 7 days from the start of the rapid expansion, wherein the myeloid aAPCs endogenously express HLA-A/B/C, ICOS-L and CD58, wherein the myeloid aAPCs are transduced to express a CD86 protein and a 4-1BBL protein, and wherein the rapid expansion is performed over a period not greater than 14 days.

[0053] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing a rapid expansion of the first population of TILs using a population of myeloid artificial antigen presenting cells (myeloid aAPCs) in a cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs after 7 days from the start of the rapid expansion; and

(c) administering a therapeutically effective portion of the second population of TILs to a patient with the cancer;

wherein the myeloid aAPCs endogenously expresses HLA-A/B/C, ICOS-L, and CD58, wherein the myeloid aAPCs are transduced to express a CD86 protein and a 4-1BBL protein, and wherein the cell culture medium further comprises IL-2 at an initial concentration of about 3000 IU/mL and OKT-3 antibody at an initial concentration of about 30 ng/mL.

[0054] In an example, the disclosure provides a population of tumor infiltrating lymphocytes (TILs) for use in treating a cancer, the population of TILs being a second population of TILs and obtainable by a process comprising:

(a) performing a rapid expansion of a first population of TILs using a population of myeloid artificial antigen presenting cells (myeloid aAPCs) in a cell culture medium to obtain the second population of TILs, wherein the first population of TILs are/have been obtained from a tumor resected from a patient, and wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs after 7 days from the start of the rapid expansion; and wherein the myeloid aAPCs endogenously express HLA-A/B/C, ICOS-L, and CD58, wherein the myeloid aAPCs are transduced to express

a CD86 protein and a 4-1BBL protein, and wherein the cell culture medium further comprises IL-2 at an initial concentration of about 3000 IU/mL and OKT-3 antibody at an initial concentration of about 30 ng/mL.

[0055] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing a rapid expansion of the first population of TILs using a population of myeloid artificial antigen presenting cells (myeloid aAPCs) in a cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs after 7 days from the start of the rapid expansion; and

(c) administering a therapeutically effective portion of the second population of TILs to a patient with the cancer;

wherein the myeloid aAPCs endogenously expresses HLA-A/B/C, ICOS-L, and CD58, wherein the myeloid aAPCs are transduced to express a CD86 protein and a 4-1BBL protein, and wherein the expansion is performed using a gas permeable container.

[0056] In an example, the disclosure provides a population of tumor infiltrating lymphocytes (TILs) for use in treating a cancer, the population of TILs being a second population of TILs and obtainable by a process comprising:

(a) performing a rapid expansion of a first population of TILs using a population of myeloid artificial antigen presenting cells (myeloid aAPCs) in a cell culture medium to obtain the second population of TILs, wherein the first population of TILs are/ have been obtained from a tumor resected from a patient, and wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs after 7 days from the start of the rapid expansion; and wherein the myeloid aAPCs endogenously express HLA-A/B/C, ICOS-L, and CD58, wherein the myeloid aAPCs are transduced to express a CD86 protein and a 4-1BBL protein, and wherein the expansion is performed using a gas permeable container.

[0057] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing a rapid expansion of the first population of TILs using a population of myeloid artificial antigen presenting cells (myeloid aAPCs) in a cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs after 7 days from the start of the rapid expansion; and

(c) administering a therapeutically effective portion of the second population of TILs to a patient with the cancer;

wherein the myeloid aAPCs endogenously expresses HLA-A/B/C, ICOS-L, and CD58, wherein the myeloid aAPCs are transduced to express a CD86 protein and a 4-1BBL protein, and wherein the ratio of the second population of TILs to the population of aAPCs is between 1 to 200 and 1 to 400.

[0058] In an example, the disclosure provides a population of tumor infiltrating cells (TILs) for use in treating a cancer, the population of TILs being a second population of TILs and obtainable by a process comprising the steps of:

(a) performing a rapid expansion of a first population of TILs using a population of myeloid artificial antigen presenting cells (myeloid aAPCs) in a cell culture medium to obtain the second population of TILs, wherein the first population of TILs is/ has been obtained from a tumor resected from a patient, and wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs after 7 days from the start of the rapid expansion; and

wherein the myeloid aAPCs endogenously expresses HLA-A/B/C, ICOS-L, and CD58, wherein the myeloid aAPCs are transduced to express a CD86 protein and a 4-1BBL protein, and wherein the ratio of the second population of TILs to the population of aAPCs is between 1 to 200 and 1 to 400. In certain examples, the ratio of the second population of TILs to the population of aAPCs is about 1 to 300.

[0059] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing a rapid expansion of the first population of TILs using a population of myeloid artificial antigen presenting cells (myeloid aAPCs) in a cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs after 7 days from the start of the rapid expansion; and

(c) administering a therapeutically effective portion of the second population of TILs to a patient with the cancer;

wherein the myeloid aAPCs endogenously expresses HLA-A/B/C, ICOS-L, and CD58, wherein the myeloid aAPCs are transduced to express a CD86 protein and a 4-1BBL protein, and wherein the ratio of the second population of TILs to the population of aAPCs is about 1 to 300.

[0060] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing a rapid expansion of the first population of TILs using a population of myeloid artificial antigen presenting cells (myeloid aAPCs) in a cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs after 7 days from the start of the rapid expansion; and

(c) administering a therapeutically effective portion of the second population of TILs to a patient with the cancer;

wherein the myeloid aAPCs endogenously expresses HLA-A/B/C, ICOS-L, and CD58, wherein the myeloid aAPCs are transduced to express a CD86 protein and a 4-1BBL protein, wherein the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, cancer caused by human papilloma virus, head and neck cancer, renal cancer, and

renal cell carcinoma.

[0061] In an example, the disclosure provides a population of tumor infiltrating lymphocytes (TILs) for use in treating a cancer, the population of TILs being a second population of TILs and obtainable by a method comprising the steps of:

(a) performing a rapid expansion of a first population of TILs using a population of myeloid artificial antigen presenting cells (myeloid aAPCs) in a cell culture medium to obtain the second population of TILs, wherein the first population of TILs is/has been obtained from a tumor resected from a patient, and wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs after 7 days from the start of the rapid expansion; and

wherein the myeloid aAPCs endogenously expresses HLA-A/B/C, ICOS-L, and CD58, wherein the myeloid aAPCs are transduced to express a CD86 protein and a 4-1BBL protein, wherein the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, cancer caused by human papilloma virus, head and neck cancer, renal cancer, and renal cell carcinoma.

[0062] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing an initial expansion of the first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, and wherein the first cell culture medium comprises IL-2;

(c) performing a rapid expansion of the second population of TILs using a population of myeloid artificial antigen presenting cells (aAPCs) in a second cell culture medium to obtain a third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; and wherein the second cell culture medium comprises IL-2 and OKT-3;

(d) administering a therapeutically effective portion of the third population of TILs to a

patient with the cancer.

[0063] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing an initial expansion of the first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, and wherein the first cell culture medium comprises IL-2;

(c) performing a rapid expansion of the second population of TILs using a population of myeloid artificial antigen presenting cells (aAPCs) in a second cell culture medium to obtain a third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; and wherein the second cell culture medium comprises IL-2 and OKT-3;

(d) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer,

wherein the myeloid aAPCs comprise MOLM-14 cells transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid encoding CD86 and a nucleic acid encoding 4-1BBL, and wherein the MOLM-14 cells express a CD86 protein and a 4-1BBL protein.

[0064] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing an initial expansion of the first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, and wherein the first cell culture medium comprises IL-2;

(c) performing a rapid expansion of the second population of TILs using a population of myeloid artificial antigen presenting cells (aAPCs) in a second cell culture medium to

obtain a third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; and wherein the second cell culture medium comprises IL-2 and OKT-3;

(d) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer,

wherein the myeloid aAPCs comprise EM-3 cells transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid encoding CD86 and a nucleic acid encoding 4-1BBL, and wherein the EM-3 cells express a CD86 protein and a 4-1BBL protein.

[0065] In an example, the disclosure provides a population of tumor infiltrating lymphocytes (TILs) for use in treating a cancer, wherein the population of TILs is a third population of TILs and obtainable by a method comprising the steps of:

(a) performing an initial expansion of a first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the first population of TILs is/ has been obtained from a tumor resected from a patient, and wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, and wherein the first cell culture medium comprises IL-2;

(b) performing a rapid expansion of the second population of TILs using a population of myeloid artificial antigen presenting cells (aAPCs) in a second cell culture medium to obtain the third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; and wherein the second cell culture medium comprises IL-2 and OKT-3.

[0066] In an example, the myeloid aAPCs comprise MOLM-14 cells transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid encoding CD86 and a nucleic acid encoding 4-1BBL, and wherein the MOLM-14 cells express a CD86 protein and a 4-1BBL protein. In an example, the myeloid cells comprise MOLM-13 cells transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid encoding CD86 and a nucleic acid encoding 4-1BBL, and wherein the MOLM-13 cells express a

CD86 protein and a 4-1BBL protein. In certain examples, the myeloid cells comprise EM-3 cells transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid encoding CD86 and a nucleic acid encoding 4-1BBL, and wherein the EM-3 cells express a CD86 protein and a 4-1BBL protein. In certain examples, the myeloid cells comprise EM-2 cells transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid encoding CD86 and a nucleic acid encoding 4-1BBL, and wherein the EM-2 cells express a CD86 protein and a 4-1BBL protein.

[0067] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing an initial expansion of the first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, and wherein the first cell culture medium comprises IL-2;

(c) performing a rapid expansion of the second population of TILs using a population of myeloid artificial antigen presenting cells (aAPCs) in a second cell culture medium to obtain a third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; and wherein the second cell culture medium comprises IL-2 and OKT-3;

(d) treating the patient with a non-myeloablative lymphodepletion regimen, wherein the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for five days;

(e) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer; and

(f) treating the patient with a high-dose IL-2 regimen, wherein the high-dose IL-2 regimen comprises 600,000 or 720,000 IU/kg of aldesleukin administered as a 15-minute bolus intravenous infusion every eight hours until tolerance;

wherein the myeloid aAPCs comprise MOLM-14 cells transduced with one or more viral

vectors, wherein the one or more viral vectors comprise a nucleic acid encoding CD86 and a nucleic acid encoding 4-1BBL, and wherein the MOLM-14 cells express a CD86 protein and a 4-1BBL protein.

[0068] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing an initial expansion of the first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, and wherein the first cell culture medium comprises IL-2;

(c) performing a rapid expansion of the second population of TILs using a population of myeloid artificial antigen presenting cells (aAPCs) in a second cell culture medium to obtain a third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; and wherein the second cell culture medium comprises IL-2 and OKT-3;

(d) treating the patient with a non-myeloablative lymphodepletion regimen, wherein the non-myeloablative lymphodepletion regimen comprises the steps of administration of cyclophosphamide at a dose of 60 mg/m^2/day for two days followed by administration of fludarabine at a dose of 25 mg/m^2/day for five days;

(e) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer; and

(f) treating the patient with a high-dose IL-2 regimen, wherein the high-dose IL-2 regimen comprises 600,000 or 720,000 IU/kg of aldesleukin administered as a 15-minute bolus intravenous infusion every eight hours until tolerance;

wherein the myeloid aAPCs comprise EM-3 cells transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid encoding CD86 and a nucleic acid encoding 4-1BBL, and wherein the EM-3 cells express a CD86 protein and a 4-1BBL protein.

[0069] In an example, the disclosure provides a population of tumor infiltrating lymphocytes (TILs) for use in treating a cancer, wherein the population of TILs are a third population of TILs and obtainable by a method comprising the steps of:

(a) an initial expansion of a first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the first population of TILs is/ has been obtained from a tumor resected from a patient, and wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, and wherein the first cell culture medium comprises IL-2; and

(b) performing a rapid expansion of the second population of TILs using a population of myeloid artificial antigen presenting cells (aAPCs) in a second cell culture medium to obtain the third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; and wherein the second cell culture medium comprises IL-2 and OKT-3;

and further wherein the population of TILs is for administration to a patient in combination with a non-myeloablative lymphodepletion regimen, wherein the non-myeloablative lymphodepletion regimen comprises cyclophosphamide which is for administration at a dose of 60 mg/m^2/day for two days followed by fludarabine which is for administration at a dose of 25 mg/m^2/day for five days and further wherein the population of TILs is for administration in combination with a high-dose IL-2 regimen, wherein the high-dose IL-2 regimen comprises 600,000 or 720,000 IU/kg of aldesleukin for administration as a 15-minute bolus intravenous infusion every eight hours until tolerance. In certain examples, the population of TILs is for administration prior to the high-dose IL-2 regimen and subsequent to the non-myeloablative lymphodepletion regimen.

[0070] In certain examples, the myeloid aAPCs comprise MOLM-14 cells transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid encoding CD86 and a nucleic acid encoding 4-1BBL, and wherein the MOLM-14 cells express a CD86 protein and a 4-1BBL protein. the myeloid aAPCs comprise MOLM-13 cells transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid encoding CD86 and a nucleic acid encoding 4-1BBL, and wherein the MOLM-13 cells express a CD86 protein and a 4-1BBL protein. In certain examples, the myeloid aAPCs comprise EM-3

cells transduced with one or more viral vectors, wherein the one or more viral vectors comprise a nucleic acid encoding CD86 and a nucleic acid encoding 4-1BBL, and wherein the EM-3 cells express a CD86 protein and a 4-1BBL protein.

[0071] In an example, the population of TILs is for use in the treating of a cancer selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, cancer caused by human papilloma virus, head and neck cancer, renal cancer, and renal cell carcinoma.

[0072] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing an initial expansion of the first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, and wherein the first cell culture medium comprises IL-2;

(c) performing a rapid expansion of the second population of TILs using a population of myeloid artificial antigen presenting cells (aAPCs) in a second cell culture medium to obtain a third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; and wherein the second cell culture medium comprises IL-2 and OKT-3; and

(d) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer,

wherein IL-2 is present at an initial concentration of about 3000 IU/mL and OKT-3 antibody is present at an initial concentration of about 30 ng/mL in the second cell culture medium.

[0073] In an example, the disclosure provides a population of tumor infiltrating lymphocytes (TILs) for use in treating a cancer, wherein the population of TILs is a third population of TILs and is obtainable by a method comprising the steps:

(a) performing an initial expansion of a first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the first population of TILs is/has been

obtained from a tumor resected from a patient, and wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, and wherein the first cell culture medium comprises IL-2; and

(b) performing a rapid expansion of the second population of TILs using a population of myeloid artificial antigen presenting cells (aAPCs) in a second cell culture medium to obtain the third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; and wherein the second cell culture medium comprises IL-2 and OKT-3; wherein IL-2 is present at an initial concentration of about 3000 IU/mL and OKT-3 antibody is present at an initial concentration of about 30 ng/mL in the second cell culture medium.

[0074] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing an initial expansion of the first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, and wherein the first cell culture medium comprises IL-2;

(c) performing a rapid expansion of the second population of TILs using a population of myeloid artificial antigen presenting cells (aAPCs) in a second cell culture medium to obtain a third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; and wherein the second cell culture medium comprises IL-2 and OKT-3; and

(d) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer,

wherein the rapid expansion is performed over a period not greater than 14 days.

[0075] In an example, the disclosure provides a population of tumor infiltrating lymphocytes (TILs) for use in treating a cancer, wherein the population of TILs is a third population of TILs and is obtainable by a method comprising the steps:

(a) performing an initial expansion of a first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the first population of TILs is/has been obtained from a tumor resected from a patient, and wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, and wherein the first cell culture medium comprises IL-2; and

(b) performing a rapid expansion of the second population of TILs using a population of myeloid artificial antigen presenting cells (aAPCs) in a second cell culture medium to obtain the third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; and wherein the second cell culture medium comprises IL-2 and OKT-3; wherein the rapid expansion is performed over a period not greater than 14 days.

[0076] In example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing an initial expansion of the first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, and wherein the first cell culture medium comprises IL-2;

(c) performing a rapid expansion of the second population of TILs using a population of myeloid artificial antigen presenting cells (aAPCs) in a second cell culture medium to obtain a third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; and wherein the second cell culture medium comprises IL-2 and OKT-3; and

(d) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer,

wherein the initial expansion is performed using a gas permeable container.

[0077] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing an initial expansion of the first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, and wherein the first cell culture medium comprises IL-2;

(c) performing a rapid expansion of the second population of TILs using a population of myeloid artificial antigen presenting cells (aAPCs) in a second cell culture medium to obtain a third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; and wherein the second cell culture medium comprises IL-2 and OKT-3; and

(d) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer,

wherein the rapid expansion is performed using a gas permeable container.

[0078] In an example, the disclosure provides a population of tumor infiltrating lymphocytes (TILs) for use in treating a cancer, wherein the population of TILs is a third population of TILs and is obtainable by a method comprising the steps:

(a) performing an initial expansion of a first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the first population of TILs is/has been obtained from a tumor resected from a patient, and wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, and wherein the first cell culture medium comprises IL-2;

(b) performing a rapid expansion of the second population of TILs using a population of myeloid artificial antigen presenting cells (aAPCs) in a second cell culture medium to obtain the third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of

the rapid expansion; and wherein the second cell culture medium comprises IL-2 and OKT-3; wherein the initial expansion and/or the rapid expansion is performed using a gas-permeable container.

[0079] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing an initial expansion of the first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, and wherein the first cell culture medium comprises IL-2;

(c) performing a rapid expansion of the second population of TILs using a population of myeloid artificial antigen presenting cells (aAPCs) in a second cell culture medium to obtain a third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; and wherein the second cell culture medium comprises IL-2 and OKT-3;

(d) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer,

wherein the ratio of the second population of TILs to the population of aAPCs in the rapid expansion is between 1 to 80 and 1 to 400.

[0080] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing an initial expansion of the first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, and wherein the first cell culture medium comprises IL-2;

(c) performing a rapid expansion of the second population of TILs using a population of myeloid artificial antigen presenting cells (aAPCs) in a second cell culture medium to

obtain a third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; and wherein the second cell culture medium comprises IL-2 and OKT-3;

(d) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer,

wherein the ratio of the second population of TILs to the population of aAPCs in the rapid expansion is about 1 to 300.

[0081] In an example, the disclosure provides a population of tumor infiltrating lymphocytes (TILs) for use in treating a cancer, wherein the population of TILs is a third population of TILs and is obtainable by a method comprising the steps:

(a) performing an initial expansion of a first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the first population of TILs is/has been obtained from a tumor resected from a patient, and wherein the second population of TILs is at least 5-fold greater in number than the first population of TILs, and wherein the first cell culture medium comprises IL-2;

(b) performing a rapid expansion of the second population of TILs using a population of myeloid artificial antigen presenting cells (aAPCs) in a second cell culture medium to obtain the third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; and wherein the second cell culture medium comprises IL-2 and OKT-3, and wherein the ratio of the second population of TILs to the population of aAPCs in the rapid expansion is between 1 to 80 and 1 to 400.

[0082] In an example, the the ratio of the second population of TILs to the population of aAPCs in the rapid expansion is about 1 to 300.

[0083] In an example, the disclosure provides a method of treating a cancer with a population of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) obtaining a first population of TILs from a tumor resected from a patient;

(b) performing an initial expansion of the first population of TILs in a first cell culture medium to obtain a second population of TILs, wherein the second population of TILs is

at least 5-fold greater in number than the first population of TILs, and wherein the first cell culture medium comprises IL-2;

(c) performing a rapid expansion of the second population of TILs using a population of myeloid artificial antigen presenting cells (aAPCs) in a second cell culture medium to obtain a third population of TILs, wherein the third population of TILs is at least 50-fold greater in number than the second population of TILs after 7 days from the start of the rapid expansion; and wherein the second cell culture medium comprises IL-2 and OKT-3;

(d) administering a therapeutically effective portion of the third population of TILs to a patient with the cancer,

wherein the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, cancer caused by human papilloma virus, head and neck cancer, renal cancer, and renal cell carcinoma.

[0084] In an example, the disclosure provides a kit for specifically inducing proliferation of a tumor infiltrating lymphocyte expressing a known co-stimulatory molecule, the kit comprising an effective amount of an aAPC, wherein said aAPC comprises a MOLM-14 cell or a EM-3 cell transduced using a lentiviral vector (LV), wherein the LV comprises a nucleic acid encoding at least one co-stimulatory ligand that specifically binds said known co-stimulatory molecule, wherein binding of the known co-stimulatory molecule with said co-stimulatory ligand stimulates and expands said T cell, the kit further comprising an applicator and an instructional material for the use of said kit.

[0085] In an example, the disclosure provides a method for assessing the potency of tumor infiltrating lymphocytes (TILs) comprising the steps of:

(a) providing a plurality of mouse mastocytoma P815 cells expressing the endogenous CD16 Fc receptor, wherein the P815 cells are transduced with a lentiviral vector based on enhanced green fluorescent protein (GFP) and Firefly Luciferase;

(b) co-culturing the plurality of P815 cells TILs with and without OKT-3 to assess T cell receptor (TCR) activation (specific killing) or lymphokine activated killing (LAK, non-specific killing), respectively;

(c) incubating for four hours;

(d) adding Luciferin and incubating for 5 minutes;

(e) reading bioluminescence intensity using a luminometer; and

(f) and calculating percent cytotoxicity and survival.

BRIEF DESCRIPTION OF THE DRAWINGS

[0086] The foregoing summary, as well as the following detailed description of the invention, will be better understood when read in conjunction with the appended drawings.

[0087] FIG. 1 illustrates the results of rapid expansion of TILs using irradiated allogeneic PBMC feeder cells. Each TIL line (M1015T and M1016T) (1.3×10^5 cells) was co-cultured with 46 different irradiated feeders (1.3×10^7 cells), IL-2 (3000 IU/mL) and OKT-3 (30 ng/mL) in a T25 flask for 7 days. The fold expansion value for TILs was calculated on Day 7. The figure shows the number of fold expansions for two TIL lines in separate stimulation

Claims

1. An artificial antigen presenting cell (aAPC) comprising a myeloid cell transduced with one or more viral vectors, wherein the one or more viral vectors comprise (i) a nucleic acid encoding CD86; (ii) one or more nucleic acids encoding one or more costimulatory molecules selected from the group consisting of OX40L and 4-1BBL, and (iii) a nucleic acid encoding SEQ ID NO:27 or SEQ ID NO:28; wherein the myeloid cell expresses a protein encoded by each of the nucleic acids of (i), (ii), and (iii).

2. The aAPC of Claim 1, wherein the aAPC can stimulate and expand tumor infiltrating lymphocytes (TILs) contacted with the aAPC.

3. The aAPC of any one of Claims 1 to 2, wherein the aAPC expands a population of TILs by at least 50-fold over a period of 7 days in a cell culture medium comprising IL-2 at a concentration of about 3000 IU/mL and OKT-3 antibody at a concentration of about 30 ng/mL.

4. The aAPC of any one of Claims 1 to 2, wherein the aAPC can stimulate and expand a T cell contacted with the aAPC.

5. The aAPC of any one of Claims 1 to 4, wherein the myeloid cell endogenously expresses HLA-A/B/C, ICOS-L, and CD58.

6. The aAPC of any one of Claims 1 to 5, wherein the myeloid cell is selected from the group consisting of a MOLM-14 cell, an EM-3 cell, a K562 cell, a MOLM-13 cell, a KG1-246 cell, a KG1-8031 cell, and an EM-2 cell.

7. The aAPC of Claim 6, wherein the EM-3 cell is further transduced to express a single chain fragment variable (scFv) binding domain capable of binding the Fc domain of OKT-3 antibody.

8. The aAPC of any one of Claims 1 to 7, wherein the CD86 protein comprises a sequence selected from the group consisting of SEQ ID NO:8 and SEQ ID NO:19, or a sequence comprising one or more conservative amino acid substitutions thereof.

9. The aAPC of Claim 1, wherein the 4-1BBL protein comprises a sequence as set forth in SEQ ID NO:9 or SEQ ID NO:16, or a sequence comprising one or more conservative amino acid substitutions thereof.

10. The aAPC of Claim 1, wherein the OX40L protein comprises a sequence as set forth in SEQ ID NO: 10, or a sequence comprising one or more conservative amino acid substitutions thereof.

11. A method of expanding a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) transducing a myeloid cell with one or more viral vectors to obtain a population of artificial antigen presenting cells (aAPCs), wherein the one or more viral vectors comprise (i) a nucleic acid encoding CD86, (ii) one or more nucleic acids encoding one or more costimulatory molecules selected from the group consisting of OX40L and 4-1BBL, and (iii) a nucleic acid encoding SEQ ID NO:27 or SEQ ID NO:28; wherein the myeloid cell expresses a protein encoded by each of the nucleic acids of (i), (ii), and (iii), and

(b) contacting the population of TILs with the population of aAPCs in a cell culture medium.

12. The method of Claim 11, wherein the cell culture medium further comprises IL-2 at an initial concentration of about 3000 IU/mL and OKT-3 antibody at an initial concentration of about 30 ng/mL.

13. The method of any one of Claims 11 to 12, wherein the myeloid cell endogenously expresses HLA-A/B/C, ICOS-L, and CD58.

14. The method of Claim 11, wherein the myeloid cell is a K562 cell.

15. The method of Claim 11, wherein the expansion is performed using a gas permeable container.

16. The method of Claim 11, wherein the ratio of the population of TILs to the population of aAPCs is between 1 to 200 and 1 to 400.

17. Use of a population of tumor infiltrating lymphocytes (TILs) expanded by the method of claim 11 in the manufacture of a medicament for treating cancer in a patient.

18. The use of Claim 17, wherein the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, cancer caused by human papilloma virus, head and neck cancer, renal cancer, renal cell carcinoma, pancreatic cancer, and glioblastoma.

19. The use of Claim 17, wherein the patient has received a non-myeloablative lymphodepletion regimen.

20. Use of a population of tumor infiltrating lymphocytes (TILs) in the manufacture of a medicament for treating a cancer, wherein the population of tumor infiltrating lymphocytes is a first population of TILs and is obtainable by a method comprising the steps of:

(a) performing a rapid expansion of a first population of TILs using a population of myeloid artificial antigen presenting cells (myeloid aAPCs) in a cell culture medium to obtain the second population of TILs, wherein the first population of TILs are previously obtained from a tumor resected from a patient, and further wherein the second population of TILs is at least 50-fold greater in number than the first population of TILs after 7 days from the start of the rapid expansion; and

wherein the myeloid aAPCs are transduced to express a CD86 protein and a co-stimulatory molecule selected from the group consisting of a 4-1BBL protein, an OX40L protein, and a combination thereof.

21. The use described in Claim 20, wherein the rapid expansion is performed over a period not greater than 14 days.

22. The use described in Claim 20, wherein the cell culture medium further comprises IL-2 at an initial concentration of about 3000 IU/mL and OKT-3 antibody at an initial concentration of about 30 ng/mL.

23. The use described in Claim 20, wherein the expansion is performed using a gas permeable container.

24. The use described in Claim 20, wherein the ratio of the second population of TILs to the population of aAPCs is between 1 to 200 and 1 to 400.

25. The use according to Claim 20, wherein the cancer is selected from the group consisting of melanoma, ovarian cancer, cervical cancer, non-small-cell lung cancer (NSCLC), lung cancer, bladder cancer, breast cancer, cancer caused by human papilloma virus, head and neck cancer, renal cancer, renal cell carcinoma, pancreatic cancer, and glioblastoma.