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 current disclosure refers to an artificial antigen presenting cell (aAPC) comprising an EM-3 or MOLM-14 myeloid cell transduced with one or more viral vectors, wherein the one or more viral vectors comprise

(i) a nucleic acid encoding CD86;

(ii) (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 wherein the myeloid cell endogenously expresses ICOS-L, CD58, HLA-A, HLA-B and HLA-C.

[007b] In another aspect, the present disclosure refers to a method of expanding a population of tumor infiltrating lymphocytes (TILs), the method comprising the steps of:

(a) transducing an EM-3 or MOLM-14 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 endogenously expresses ICOS-L, CD58, HLA-A, HLA-B and HLA-C; and

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

[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

Claims

1. An artificial antigen presenting cell (aAPC) comprising an EM-3 or MOLM-14 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); and wherein the myeloid cell endogenously expresses ICOS-L, CD58, HLA-A, HLA-B and HLA-C.

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 is 4 a MOLM-14 cell4.

6. The aAPC of any one of Claims 1 to 4, wherein the myeloid cell is an EM-3 cell.

7. The aAPC of any one of Claims 1 to 6, wherein the CD86 protein comprises a sequence as set forth in SEQ ID NO:8, or a sequence comprising one or more conservative amino acid substitutions thereof.

8. The aAPC of any one of Claims 1 to 6, wherein the nucleic acid encoding CD86 comprises SEQ ID NO:19.

9. The aAPC of any one of Claims 1 to 8, wherein the 4-1BBL protein comprises a sequence as set forth in SEQ ID NO:9, or a sequence comprising one or more conservative amino acid substitutions thereof.

10. The aAPC of any one of Claims 1 to 8, wherein the one or more nucleic acids encoding the one or more costimulatory molecules comprises SEQ ID NO:16.

11. 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.

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

(a) transducing an EM-3 or MOLM-14 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 endogenously expresses ICOS-L, CD58, HLA-A, HLA-B and HLA-C; and

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

13. The method of Claim 12, 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.

14. The method of any one of Claims 12 to 13, wherein the myeloid cell is a MOLM-14 cell.

15. The method of any one of Claims 12 to 13, wherein the myeloid cell is an EM-3 cell.

16. The method of any one of Claims 12 to 15, wherein the expansion is performed using a gas permeable container.

17. The method of any one of Claims 12 to 16, wherein the ratio of the population of TILs to the population of aAPCs is between 1 to 200 and 1 to 400.