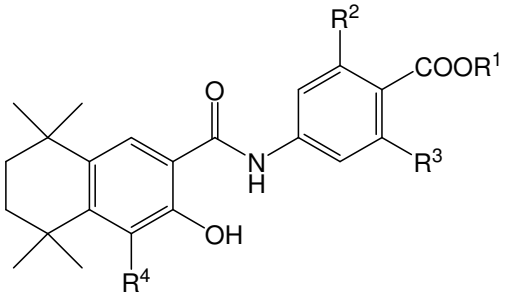
the bone marrow. However, the use of retinoid analogs which are not inactivated by CYP26 enables such retinoids to terminally differentiate, and thus eliminate, the cancer HSCs from the protective bone marrow niche. Since such differentiation is mediated by RARα and the use of RARα specific analogs, which are CYP26 resistant, enables the therapeutic differentiation-inducing activity without inactivation by the CYP26 enzyme.

[0005] Thus, the combination of RAR agonists, to induce differentiation of cancer stem cells, and targeted immunotherapy can be particularly useful in treating cancer.

SUMMARY

[0006] Disclosed herein are compounds for potentiation of targeted cancer immunotherapeutics. Compounds which act on retinoic acid receptors (RAR) are used in combination with chimeric antigen receptor (CAR)-modified immune cells (sometimes abbreviated as CAR-MIC) to potentiate the anti-cancer activity of the CAR-modified immune cells and/or immune checkpoint targeted therapeutics.

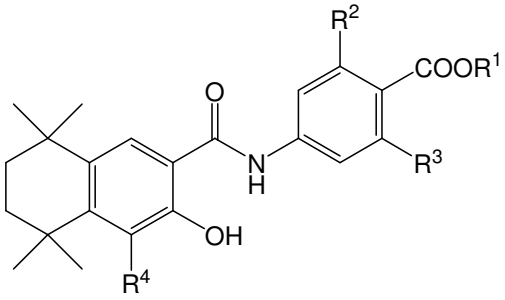
[0006a] In one aspect, provided herein is a use of at least one differentiating retinoic acid receptor (RAR) active agent in the manufacture of a medicament for potentiating chimeric antigen receptor-modified immune cells (CAR-MIC) cancer immunotherapy, wherein the differentiating RAR active agent is a RARα agonist, wherein the RARα agonist is a CYP26 metabolism-resistant, RARα selective agonist having a structure of general formula (I)



(I)

wherein R^1 is H or C_{1-6} alky, R^2 and R^3 are independently H or F, and R^4 is a halogen wherein the medicament is to be administered to a cancer patient, and wherein the cancer patient is receiving, has received, or is scheduled to receive, CAR-MIC.

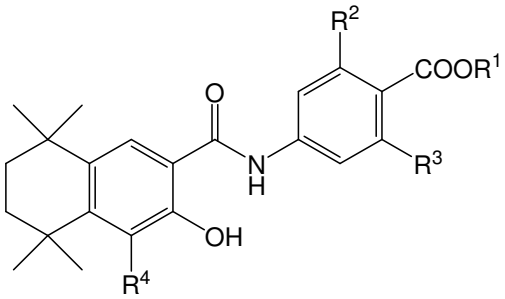
[0006b] In another aspect, provided herein is a use at least one differentiating RAR active agent in the manufacture of a medicament for cancer immunotherapy , wherein the differentiating RAR active agent is a RARα agonist, wherein the RARα agonist is a CYP26 metabolism-resistant, RARα selective agonist having a structure of general formula (I)



(I)

wherein R^1 is H or C_{1-6} alky, R^2 and R^3 are independently H or F, and R^4 is a halogen, wherein the medicament is to be administered to a subject in need thereof, and wherein the medicament is to be administered with CAR-MIC.

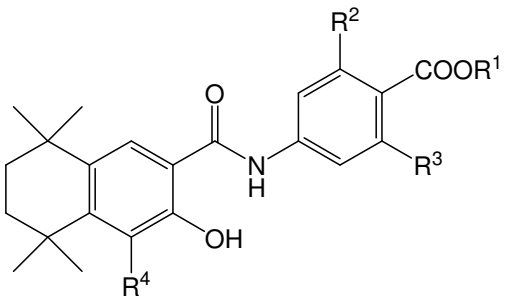
[0006c] In another aspect, provided herein is a use of at least one RARα agonist in the manufacture of a medicament for decreasing toxicity of CAR-modified immune cells, wherein the medicament is to be administered in combination with the CAR-modified immune cells such that as a result of the combination, a lower dose of CAR-modified immune cells is administered but the efficacy is not decreased, as compared to administration of the CAR-modified immune cells alone, wherein the RARα agonist is a CYP26 metabolism-resistant, RARα selective agonist having a structure of general formula (I)



(I)

wherein R^1 is H or C_{1-6} alky, R^2 and R^3 are independently H or F, and R^4 is a halogen.

[0006d] In another aspect, provided herein is a use of at least one differentiating retinoic acid receptor (RAR) active agent in the manufacture of a medicament for potentiating immune checkpoint inhibitor therapy, wherein the differentiating RAR active agent is a RARα agonist, wherein the RARα agonist is a CYP26 metabolism-resistant, RARα selective agonist having a structure of general formula (I)

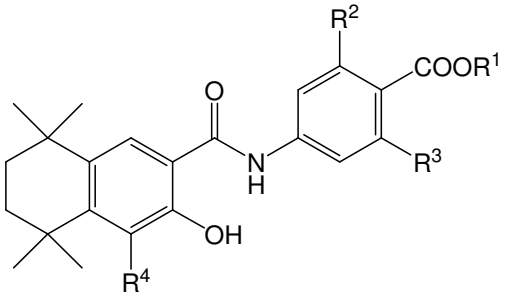


(I)

wherein R^1 is H or C_{1-6} alky, R^2 and R^3 are independently H or F, and R^4 is a halogen, wherein the medicament is to be administered to a cancer patient,

and wherein the cancer patient is receiving or is scheduled to receive, an immune checkpoint inhibitor antibody.

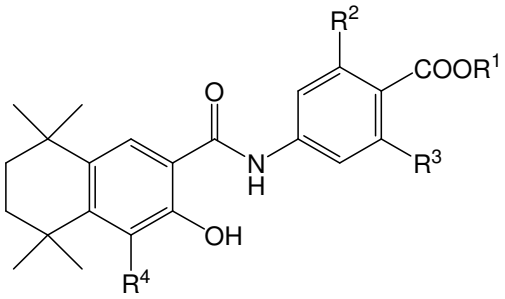
[0006e] In another aspect, provided herein is a method of augmenting the production of chimeric antigen receptor-modified immune cells (CAR-MIC) comprising culturing the CAR-MIC in a culture medium comprising at least one differentiating retinoic acid receptor (RAR) active agent, wherein the differentiating RAR active agent is a RARα agonist, wherein the RARα agonist is a CYP26 metabolism-resistant, RARα selective agonist having a structure of general formula (I)



(I)

wherein R^1 is H or C_{1-6} alky, R^2 and R^3 are independently H or F, and R^4 is a halogen.

[0006f] In another aspect, provided herein is a use of a culture of chimeric antigen receptor-modified immune cells (CAR-MIC) in the manufacture of a medicament for CAR-MIC cancer immunotherapy, wherein production of the culture of CAR-MIC is augmented by culturing CAR-MIC in a culture medium comprising at least one differentiating retinoic acid receptor (RAR) active agent, wherein the differentiating RAR active agent is a RARα agonist, wherein the RARα agonist is a CYP26 metabolism-resistant, RARα selective agonist having a structure of general formula (I)

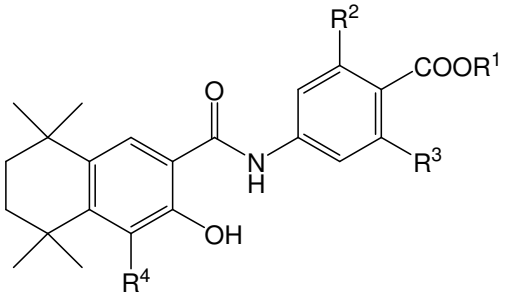


(I)

wherein R^1 is H or C_{1-6} alky, R^2 and R^3 are independently H or F, and R^4 is a halogen, and wherein the medicament is to be administered directly to a subject.

[0006g] In another aspect, provided herein is a use of a cryopreserved culture of chimeric antigen receptor-modified immune cells (CAR-MIC) in the manufacture of a medicament for CAR-MIC cancer immunotherapy, wherein the culture of CAR-MIC is cryopreserved for future administration to a subject, wherein production of the culture of CAR-MIC is augmented by culturing the CAR-MIC in a culture medium comprising at least one differentiating retinoic acid receptor (RAR) active agent, wherein the differentiating RAR

active agent is a RARα agonist, wherein the RARα agonist is a CYP26 metabolism-resistant, RARα selective agonist having a structure of general formula (I)



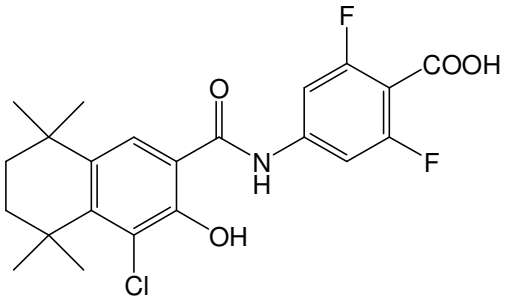
(I)

wherein R^1 is H or C_{1-6} alky, R^2 and R^3 are independently H or F, and R^4 is a halogen.

[0007] Retinoids and rexinoids have diverse activities. Particular retinoids have an anticancer effect based in terminally differentiating cancer stem cells. These differentiating retinoids, or differentiating RAR active agents, can be used in combination with CAR-MIC to increase the overall effectiveness of the immunotherapy. In some examples the differentiating retinoid is a RARα agonist. In some examples the RARα agonist is a RARα specific or selective agonist. In some examples the RARα agonists are CYP26 resistant.

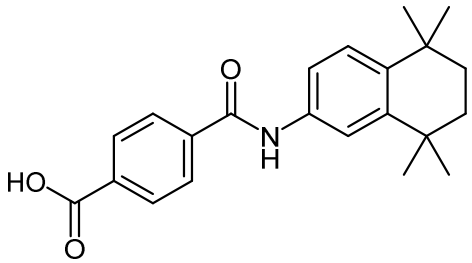
[0008] In some examples, the CAR-modified immune cells are, or comprise, CAR-modified T cells. In some examples, the CAR-modified immune cells are, or comprise, CAR-modified NK cells. In some examples, the CAR-modified immune cells are, or comprise, CAR-modified NKT cells. In some examples, the CAR-modified immune cells are, or comprise, CAR-modified macrophages. Further examples can comprise mixtures of these cell types. Most typically such cellular preparations are administered by infusion, for example intravenous infusion. In contrast, the RARα agonists are small molecules that can be administered orally, for example as pills or capsules and the like. Thus the RARα agonists and the CAR-modified immune cells may be administered on independent schedules.

[0009] In some examples, the RARα agonist is:

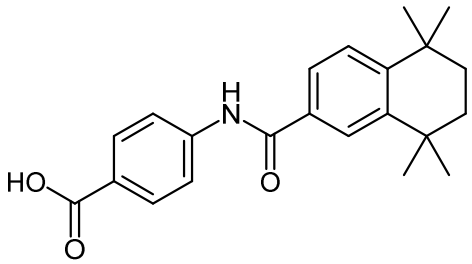


(III) (IRX5183).

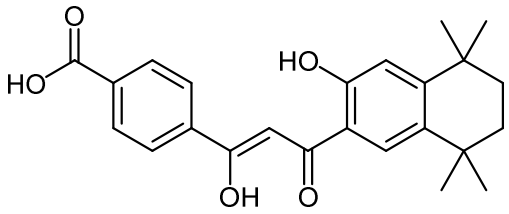
[0010] In other examples, the RARα agonist is tamibarotene (AM80), AM580, or Re 80.



Tamibarotene (AM80)



AM580



Re 80

Other examples specifically exclude one or more of these RARα agonists.

[0011] In some examples, the cancer is a hematologic malignancy, such as acute myeloid leukemia or multiple myeloma. In some examples, the cancer is a solid tumor.

[0012] In some examples, the methods further comprise administering to the subject at least one immune checkpoint inhibitor. In some examples, the immune checkpoint inhibitor is an inhibitor of at least one of CTLA-4, PD-1, TIM-3, LAG-3, PD-L1 ligand, B7-H3, B7-H4, BTLA, or is an ICOS or OX40 agonist. In some examples, the immune checkpoint inhibitor is an antibody specific for at least one of CTLA-4, PD-1, TIM-3, LAG-3, PD-L1 ligand, B7-H3, B7-H4, BTLA, ICOS, or OX40. Other examples specifically exclude one or more of these agents.

[0013] In some examples, the methods comprise additionally administering at least one antineoplastic agent such as a cancer chemotherapy agent or targeted therapy agent. In some examples the antineoplasitc agent is bortezomib.

[0014] Also disclosed herein are methods of prolonging the disease-free survival of a cancer patient comprising, administering CAR-modified immune cells and at least one RARα agonist as compared to patients receiving CAR-modified immune cells but not receiving the

RARα agonist. Other examples are methods of prolonging the progression-free survival or overall survival of a cancer patient comprising administering CAR-modified immune cells and at least one RARα agonist.

[0015] Also disclosed herein are methods of decreasing toxicity of CAR-modified immune cells comprising administering to a subject in need thereof at least one RARα agonist in combination with the CAR-modified immune cells such that as a result of the combination, a lower dose of CAR-modified immune cells is administered more safely and equally effectively than if the CAR-modified immune cells were administered alone; or that a higher dose of CAR-MIC can be administered with greater efficacy and equal safety.

[0016] Also disclosed herein are methods of treating cancer comprising administering to a subject in need thereof, chimeric antigen receptor (CAR)-modified immune cells, at least one RARα agonist, and at least one immune checkpoint inhibitor.

[0017] Also disclosed herein are methods of augmenting the production of CAR-modified immune cells by inclusion of an RARα agonist in the culture medium.

BRIEF DESCRIPTION OF THE DRAWINGS

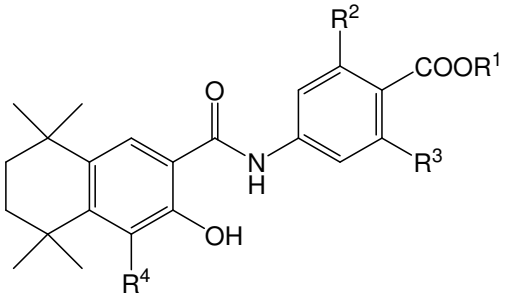
[0018] FIG. 1 depicts the extent to which compound IRX5183 binds to and activates transcription from RARα, RARβ, and RARγ using a transactivation assay.

[0019] FIG. 2A-C shows that RAR receptor specific agonists regulate FoxP3, α4β7, and CCR9 expression. Purified $CD4^+$ $CD25^-$ $FoxP3^-$ cells were cultured in media with the specified concentration of each RAR agonist and analyzed by flow cytometry for FoxP3 (FIG. 2A), α4β7 (FIG. 2B), and CCR9 (FIG. 2C) expression in total CD4 T cells. FoxP3 results are representative of three independent experiments. CCR9 and α4β7 results are representative of multiple experiments.

[0020] FIG. 3A-H depicts the relative concentration of plasma markers BCL6 (FIG. 3A and E), BLIMP-1 (FIG. 3B and F), XBPS-1 (FIG. 3C and G), and CHOP (FIG. 3D and H) in multiple myeloma (MM) cell lines H929 (FIG. 3A-D) or CD138+ MM cells from three different patient samples (FIG. 3E-H) incubated for 5 days either in the absence of stroma (Liquid), with or without AGN (RA receptor antagonist AGN194310, 1 μM), or co-cultured with BM

What is claimed is

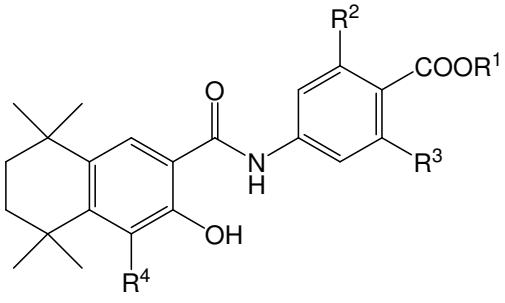
1. Use of at least one differentiating retinoic acid receptor (RAR) active agent in the manufacture of a medicament for potentiating chimeric antigen receptor-modified immune cells (CAR-MIC) cancer immunotherapy, wherein the differentiating RAR active agent is a RARα agonist, wherein the RARα agonist is a CYP26 metabolism-resistant, RARα selective agonist having a structure of general formula (I)



(I)

wherein R^1 is H or C_{1-6} alky, R^2 and R^3 are independently H or F, and R^4 is a halogen
wherein the medicament is to be administered to a cancer patient,
and wherein the cancer patient is receiving, has received, or is scheduled to receive, CAR-MIC.

2. Use of at least one differentiating RAR active agent in the manufacture of a medicament for cancer immunotherapy , wherein the differentiating RAR active agent is a RARα agonist, wherein the RARα agonist is a CYP26 metabolism-resistant, RARα selective agonist having a structure of general formula (I)

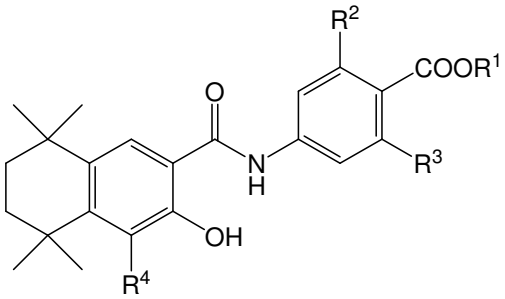


(I)

wherein R^1 is H or C_{1-6} alky, R^2 and R^3 are independently H or F, and R^4 is a halogen,
wherein the medicament is to be administered to a subject in need thereof,
and wherein the medicament is to be administered with CAR-MIC.

3. Use of at least one RARα agonist in the manufacture of a medicament for decreasing toxicity of CAR-modified immune cells, wherein the medicament is to be administered in combination with the CAR-modified immune cells such that as a result of the combination, a lower dose of CAR-modified immune cells is administered but the efficacy is not decreased, as compared to administration of the CAR-modified immune cells alone, wherein the RARα agonist is a CYP26 metabolism-resistant, RARα selective agonist having

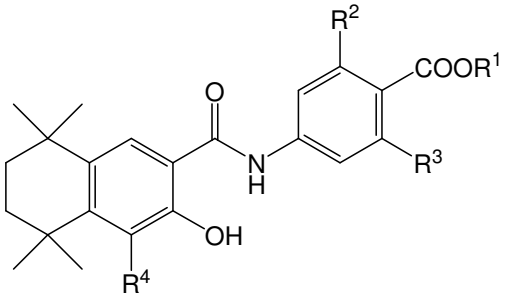
a structure of general formula (I)



(I)

wherein R^1 is H or C_{1-6} alky, R^2 and R^3 are independently H or F, and R^4 is a halogen.

4. Use of at least one differentiating retinoic acid receptor (RAR) active agent in the manufacture of a medicament for potentiating immune checkpoint inhibitor therapy, wherein the differentiating RAR active agent is a RARα agonist, wherein the RARα agonist is a CYP26 metabolism-resistant, RARα selective agonist having a structure of general formula (I)



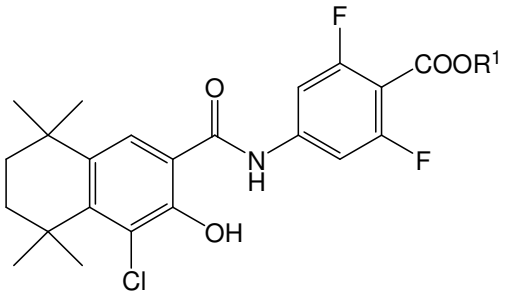
(I)

wherein R^1 is H or C_{1-6} alky, R^2 and R^3 are independently H or F, and R^4 is a halogen, wherein the medicament is to be administered to a cancer patient,
and wherein the cancer patient is receiving or is scheduled to receive, an immune checkpoint inhibitor antibody.

5. The use according to any one of claims 1-4, wherein an immune checkpoint inhibitor is to be further administered, wherein the immune checkpoint inhibitor is an inhibitor of at least one of CTLA-4, PD-1, TIM-3, LAG-3, PD-L1 ligand, B7-H3, B7-H4, BTLA, or is an ICOS, or OX40 agonist, and wherein the immune checkpoint inhibitor is an antibody or other immune checkpoint inhibitor.

6. The use according to any one of claims 1-4, wherein R^4 is F, Cl, Br or I.

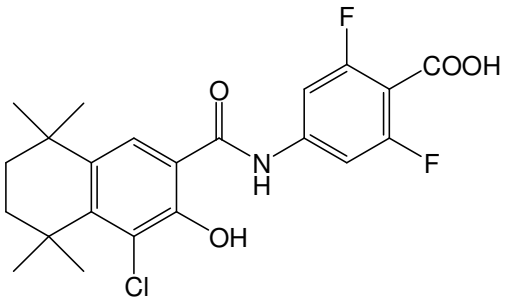
7. The use according to any one of claims 1-4, wherein the RARα agonist is a compound having a structure of formula (II):



(II)

wherein R^1 is H or C_{1-6} alkyl.

8. The use according to claim 7, wherein the RARα agonist is a compound having a structure of formula (III):



(III) (IRX5183).

9. The use according to any one of claims 1-4, wherein the CAR-modified immune cells are CAR-modified T cells.

10. The use according to any one of claims 1-4, wherein the CAR-modified immune cells are CAR-modified NK cells.

11. The use according to any one of claims 1-4, wherein the CAR-modified immune cells are CAR-modified macrophages.

12. The use according to any one of claims 1-4, wherein the cancer is a hematologic malignancy.

13. The use according to claim 12, wherein the hematologic malignancy is acute myeloid leukemia, chronic myelogenous leukemia (CML), accelerated CML, CML blast phase (CML-BP), acute lymphoblastic leukemia, chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, follicular lymphoma, mantle cell lymphoma, B-cell lymphoma, T-cell lymphoma, multiple myeloma, Waldenstrom's macroglobulinemia, myelodysplastic syndromes (MDS), refractory anemia (RA), RA with ringed sideroblasts, RA with excess blasts (RAEB), RAEB in transformation, or a myeloproliferative syndrome.

14. The use according to claim 12, wherein the hematologic malignancy is acute myeloid leukemia.

15. The use according to claim 12, wherein the hematologic malignancy is multiple myeloma.

16. The use according to any one of claims 1-4, wherein the cancer is a solid tumor.

17. The use according to claim 16, wherein the cancer is a solid tumor which can metastasize to the bone, wherein the solid tumor is pancreatic cancer; bladder cancer; colorectal cancer; breast cancer, including metastatic breast cancer; prostate cancer, including androgen-dependent and androgen-independent prostate cancer; renal cancer, metastatic renal cell carcinoma; hepatocellular cancer; lung cancer, non-small cell lung cancer (NSCLC), small cell lung cancer, bronchoalveolar carcinoma (BAC), and adenocarcinoma of the lung; ovarian cancer, progressive epithelial, primary peritoneal cancer; cervical cancer; gastric cancer; esophageal cancer; head and neck cancer, squamous cell carcinoma of the head and neck; melanoma; neuroendocrine cancer, metastatic neuroendocrine tumor; brain tumor, glioma, anaplastic oligodendroglioma, adult glioblastoma multiforme, adult anaplastic astrocytoma; bone cancer; and soft tissue sarcoma.

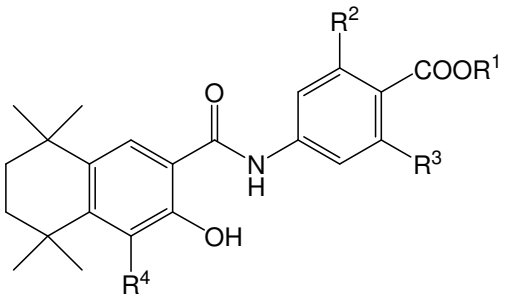
18. The use according to any one of claims 1-3, wherein the medicament is to be administered for at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 1 week, at least 2 weeks, at least 3 weeks, or at least one month before administration of the CAR-modified immune cells.

19. The use according to any one of claims 1-3, wherein administration of the medicament is to be discontinued before administration of CAR-modified immune cells.

20. The use according to claim 19, wherein bortezomib is to be further administered.

21. The use according to claim 20, wherein the cancer patient has multiple myeloma.

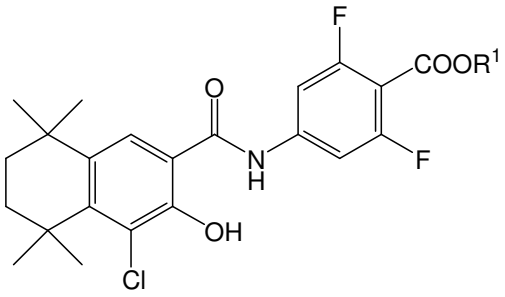
22. A method of augmenting the production of chimeric antigen receptor-modified immune cells (CAR-MIC) comprising culturing the CAR-MIC in a culture medium comprising at least one differentiating retinoic acid receptor (RAR) active agent, wherein the differentiating RAR active agent is a RARα agonist, wherein the RARα agonist is a CYP26 metabolism-resistant, RARα selective agonist having a structure of general formula (I)



(I)

wherein R^1 is H or C_{1-6} alky, R^2 and R^3 are independently H or F, and R^4 is a halogen.

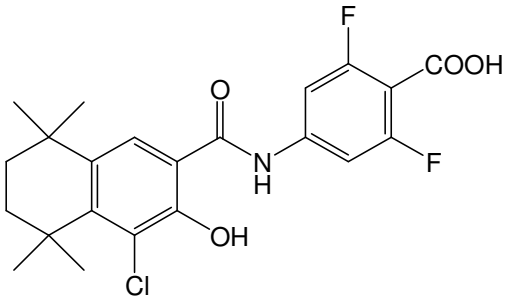
23. The method of claim 22, wherein the RARα agonist is a compound having a structure of formula (II):



(II)

wherein R^1 is H or C_{1-6} alkyl.

24. The method of claim 22, wherein the RARα agonist is a compound having a structure of formula (III):



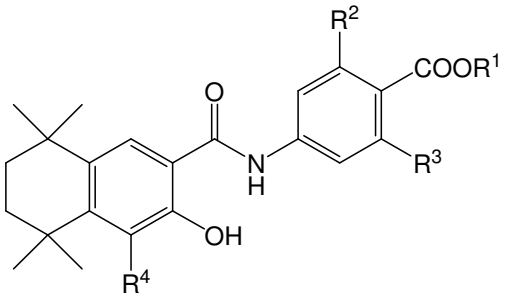
(III) (IRX5183).

25. The method of claim 22, wherein augmenting the production CAR-MIC comprises expansion of CAR-MIC numbers.

26. The method of claim 22, wherein augmenting the production CAR-MIC comprises maintenance of the CAR phenotype.

27. Use of a culture of chimeric antigen receptor-modified immune cells (CAR-MIC) in the manufacture of a medicament for CAR-MIC cancer immunotherapy, wherein production of the culture of CAR-MIC is augmented by culturing CAR-MIC in a culture medium comprising at least one differentiating retinoic acid receptor (RAR) active agent, wherein the differentiating RAR active agent is a RARα agonist, wherein the RARα agonist is a CYP26 metabolism-resistant, RARα selective agonist having a structure of general formula

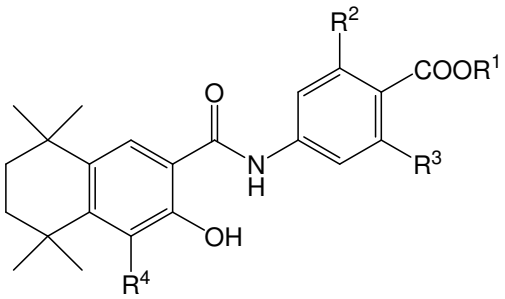
(I)



(I)

wherein R^1 is H or C_{1-6} alky, R^2 and R^3 are independently H or F, and R^4 is a halogen, and wherein the medicament is to be administered directly to a subject.

28. Use of a cryopreserved culture of chimeric antigen receptor-modified immune cells (CAR-MIC) in the manufacture of a medicament for CAR-MIC cancer immunotherapy, wherein the culture of CAR-MIC is cryopreserved for future administration to a subject, wherein production of the culture of CAR-MIC is augmented by culturing the CAR-MIC in a culture medium comprising at least one differentiating retinoic acid receptor (RAR) active agent, wherein the differentiating RAR active agent is a RARα agonist, wherein the RARα agonist is a CYP26 metabolism-resistant, RARα selective agonist having a structure of general formula (I)



(I)

wherein R^1 is H or C_{1-6} alky, R^2 and R^3 are independently H or F, and R^4 is a halogen.

29. The method of claim 22, wherein the CAR-MIC is a CAR-T cell.