GVHD often further worsened the outcomes.

[0006] There is a great need for new and improved methodologies aimed at increasing NK cell numbers.

[0007] Additional advantages of the disclosed method and compositions will be set forth in part in the description which follows, and in part will be understood from the description, or may be learned by practice of the disclosed method and compositions. The advantages of the disclosed method and compositions will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed.

BRIEF SUMMARY

[0008] In one aspect, the present disclosure refers to a composition comprising a plasma membrane vesicle purified from NK cell feeder cells transfected with at least one membrane-bound NK cell effector agent, wherein the at least one effector agent is IL-21. Disclosed are compositions comprising a plasma membrane vesicle, wherein the plasma membrane vesicle comprises an NK cell effector agent. NK cell effector agents can be a cytokine, an adhesion molecule, or an NK cell activating agent.

[0009] Disclosed are compositions comprising a plasma membrane vesicle, wherein the plasma membrane vesicle comprises an NK cell effector agent, wherein the NK cell effector agent can be IL-15, IL-21, IL-2, 41BBL, IL-12, IL-18, MICA, 2B4, LFA-1, or BCM1/SLAMF2.

[0010] Disclosed are compositions comprising a plasma membrane vesicle, wherein the plasma membrane vesicle comprises an NK cell effector agent, wherein the NK cell effector agent can be IL-15, IL-21, or 41BBL.

[0011] Disclosed are compositions comprising a plasma membrane vesicle, wherein the plasma membrane vesicle comprises an NK cell effector agent, wherein the plasma membrane can comprise two or more NK cell effector agents. At least one NK cell effector agent can be a cytokine. For example, disclosed are compositions comprising a plasma membrane vesicle, wherein the plasma membrane vesicle comprises an NK cell effector agent, wherein the plasma membrane can comprise IL-15 and 41BBL. In some aspects, the plasma membrane can further comprise IL-21. Disclosed are compositions comprising a plasma membrane vesicle, wherein the plasma membrane vesicle comprises an NK cell effector agent, wherein the plasma membrane can comprise IL-21 and 41BBL.

[0012] Disclosed are compositions comprising a plasma membrane vesicle, wherein the plasma membrane vesicle comprises an NK cell effector agent, wherein the plasma membrane vesicle surrounds a microparticle.

[0013] Disclosed are compositions comprising two or more plasma membrane vesicles, wherein the plasma membrane vesicles comprise an NK cell effector agent.

[0014] Disclosed are compositions comprising at least one composition comprising a plasma membrane vesicle comprising an NK cell effector agent and at least one composition comprising a membrane self-inserting peptide conjugated to an NK cell effector agent.

[0015] In another aspect, the present disclosure refers to a composition comprising a plasma membrane vesicle purified from NK cell feeder cells transfected with at least one membrane-bound NK cell effector agent conjugated to a membrane self-inserting peptide, wherein the at least one effector agent is IL-21. Disclosed are compositions comprising a membrane self-inserting peptide conjugated to an NK cell effector agent. NK cell effector agents can be a cytokine, an adhesion molecule, or an NK cell activating agent. Disclosed are compositions comprising a membrane self-inserting peptide conjugated to an NK cell effector agent, wherein the NK cell effector agent can be IL-15, IL-21, IL-2, 41BBL, IL-12, IL-18, MICA, 2B4, LFA-1, and BCM1/SLAMF2.

[0016] Disclosed are compositions comprising a membrane self-inserting peptide conjugated to an NK cell effector agent, wherein the NK cell effector agent is IL-15, IL-21, or 41BBL.

[0017] Disclosed are compositions comprising a membrane self-inserting peptide conjugated to an NK cell effector agent, wherein the membrane self-inserting peptide is a molecule that promotes insertion into a membrane and can be human Fc, GPI, transmembrane T cell receptor, or pHLIP.

[0018] Disclosed are compositions comprising two or more compositions comprising a membrane self-inserting peptide conjugated to an NK cell effector agent. In a further aspect, the present disclosure refers to a device or a kit comprising the composition as disclosed herein.

[0019] Disclosed are methods of treating cancer comprising administering to a subject an effective amount of a composition comprising a plasma membrane vesicle comprising at least one NK cell effector agent. At least one NK cell effector agent can be a cytokine, an adhesion molecule, or an NK cell activating agent. In some aspects, the NK cell effector agent can be IL-15, IL-21, or 41BBL.

[0020] Disclosed are methods of treating cancer comprising administering to a subject an effective amount of a composition comprising a plasma membrane vesicle comprising two or more NK cell effector agents. At least one NK cell effector agent can be a cytokine. Thus, disclosed are methods of treating cancer comprising administering to a subject an effective amount of a composition comprising a plasma membrane vesicle comprising two or more NK cell effector agents, wherein the plasma membrane can comprise IL-15 and 41BBL. In some aspects, the plasma membrane can further comprise IL-21. Also disclosed are methods of treating cancer comprising administering to a subject an effective amount of a composition comprising a plasma membrane vesicle comprising two or more NK cell effector agents, wherein the plasma membrane can comprise IL-21 and

41BBL. Also disclosed are methods of treating cancer comprising administering to a subject an effective amount of a composition comprising a plasma membrane vesicle comprising two or more NK cell effector agents, wherein the plasma membrane vesicle comprises IL-15 and IL-21.

[0021] Disclosed are methods of treating cancer comprising administering to a subject an effective amount of a composition comprising a plasma membrane vesicle comprising at least one NK cell effector agent, wherein the plasma membrane vesicle can be purified from NK cell feeder cells. NK cell feeder cells can be irradiated autologous or allogeneic peripheral blood mononuclear cells (PBMCs), RPMI8866, HFWT, K562, K562 cells transfected with membrane bound IL-15 and 41BBL), K562 cells transfected with membrane bound IL-21 and 41BBL, or EBV-LCL. In some aspects, the NK cell feeder cells can be K562 cells transfected with membrane bound IL-21 and 41BBL or K562 cells transfected with membrane bound IL-15 and 41BBL.

[0022] Disclosed are methods of treating cancer comprising administering to a subject an effective amount of a composition comprising a plasma membrane vesicle comprising at least one NK cell effector agent, wherein an effective amount of the composition stimulates expansion of NK cells. Any of the disclosed plasma membrane vesicle compositions can be used in these methods.

[0023] Disclosed are methods of treating cancer comprising administering to a subject an effective amount of a composition comprising a membrane self-inserting peptide conjugated to an NK cell effector agent. An effective amount of the composition can stimulate expansion of NK cells.

[0024] Disclosed are methods of treating cancer comprising administering to a subject an effective amount of a composition comprising a membrane self-inserting peptide conjugated to an NK cell effector agent, wherein the composition comprises a membrane self-inserting peptide conjugated to IL-15, IL-21, or 41BBL.

[0025] Disclosed are methods of treating cancer comprising administering to a subject an effective amount of a composition comprising a membrane self-inserting peptide conjugated to an NK cell effector agent, wherein the membrane self-inserting peptide can be human Fc, GPI, transmembrane T cell receptor, or pHLIP.

[0026] Disclosed are methods of treating cancer comprising administering to a subject an effective amount of a composition comprising a membrane self-inserting peptide conjugated to an NK cell effector agent, wherein the composition can comprise two or more membrane self-inserting peptides conjugated to an NK cell effector agent.

[0027] In another aspect, the present disclosure refers to a method of expanding an initial population of NK cells, the method comprising contacting the initial NK cell population *in vitro* with an effective amount of a composition comprising a plasma membrane vesicle purified from NK cell feeder cells transfected with at least one membrane bound NK cell effector agent, wherein at least one NK cell effector agent is IL-21. Disclosed are methods of expanding NK cells

comprising administering to a cell population an effective amount of a composition comprising a plasma membrane vesicle comprising at least one NK cell effector agent, wherein the cell population comprises NK cells. NK cell effector agents can be a cytokine, adhesion molecule or NK cell activating agent. In some aspects, the NK cell effector agent can be IL-15, IL-21 or 41BBL.

[0028] Disclosed are methods of expanding NK cells comprising administering to a cell population an effective amount of a composition comprising a plasma membrane vesicle comprising at least one NK cell effector agent, wherein the cell population comprises NK cells, wherein the plasma membrane vesicle comprises IL-15 and IL-21. In some aspects, the plasma membrane vesicle can further comprise 41BBL.

[0029] Disclosed are methods of expanding NK cells comprising administering to a cell population an effective amount of a composition comprising a plasma membrane vesicle comprising at least one NK cell effector agent, wherein the cell population comprises NK cells, wherein the plasma membrane vesicle comprises IL-15 and 41BBL. In some aspects, the plasma membrane vesicle can comprise IL-21 and 41BBL.

[0030] Disclosed are methods of expanding NK cells comprising administering to a cell population an effective amount of a composition comprising a plasma membrane vesicle comprising at least one NK cell effector agent, wherein the cell population comprises NK cells, wherein the plasma membrane vesicle can be purified from NK cell feeder cells. NK cell feeder cells can be irradiated autologous or allogeneic peripheral blood mononuclear cells (PBMCs), RPMI8866, HFWT, K562, K562 cells transfected with membrane bound IL-15 and 41BBL), K562 cells transfected with membrane bound IL-21 and 41BBL, or EBV-LCL. In some aspects, the NK cell feeder cells can be K562 cells transfected with membrane bound IL-21 and 41BBL or K562 cells transfected with membrane bound IL-15 and 41BBL.

[0031] Disclosed are methods of expanding NK cells comprising administering to a cell population an effective amount of a composition comprising a plasma membrane vesicle comprising at least one NK cell effector agent, wherein the cell population comprises NK cells, wherein an effective amount of the composition stimulates expansion of NK cells.

[0032] Disclosed are methods of expanding NK cells comprising administering to a cell population an effective amount of a composition comprising a plasma membrane vesicle comprising at least one NK cell effector agent, wherein the cell population comprises NK cells, wherein administering to a cell population comprises administering the composition to a subject, wherein the subject comprises the cell population.

[0033] Disclosed are methods of expanding NK cells comprising administering to a cell population an effective amount of the composition comprising a membrane self-inserting peptide conjugated

to an NK cell effector agent, wherein the cell population comprises NK cells. An effective amount of the composition can stimulate expansion of NK cells.

[0034] Disclosed are methods of expanding NK cells comprising administering to a cell population an effective amount of the composition comprising a membrane self-inserting peptide conjugated to an NK cell effector agent, wherein the cell population comprises NK cells, wherein the composition comprises a membrane self-inserting peptide conjugated to IL-15, IL-21, or 41BBL.

[0035] Disclosed are methods of expanding NK cells comprising administering to a cell population an effective amount of the composition comprising a membrane self-inserting peptide conjugated to an NK cell effector agent, wherein the cell population comprises NK cells, wherein the membrane self-inserting peptide can be human Fc, GPI, transmembrane T cell receptor, or pHLIP.

[0036] Disclosed are methods of expanding NK cells comprising administering to a cell population an effective amount of the composition comprising a membrane self-inserting peptide conjugated to an NK cell effector agent, wherein the cell population comprises NK cells, wherein administering to a cell population comprises administering the composition to a subject, wherein the subject comprises the cell population.

[0037] Disclosed are methods of expanding NK cells comprising administering to a cell population an effective amount of the composition comprising a membrane self-inserting peptide conjugated to an NK cell effector agent, wherein the cell population comprises NK cells, wherein the composition comprises at least two membrane self-inserting peptide conjugates, wherein the membrane self-inserting peptide conjugates are conjugated to an NK cell effector agent. At least two membrane self-inserting peptide conjugates can be conjugated to the same or different NK cell effector agents. In yet another aspect, the present disclosure refers to an expanded population of NK cells *in vitro*, in contact with a plasma membrane vesicle composition disclosed herein, wherein the plasma membrane composition selectively stimulates expansion of NK cells from PBMC's.

[0038] Disclosed are methods of modulating the immune system comprising administering to a subject one or more of the compositions comprising a membrane self-inserting peptide conjugated to an NK cell effector agent or a plasma membrane vesicle comprising an NK cell effector agent. Methods of modulating the immune system can comprise reducing the number of activated T cells, expanding the number of NK cells, or reducing the number of dendritic cells.

BRIEF DESCRIPTION OF THE DRAWINGS

[0039] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the disclosed method and compositions and together with the description, serve to explain the principles of the disclosed method and

CLAIMS

We claim:

1. A composition comprising a plasma membrane vesicle purified from NK cell feeder cells transfected with at least one membrane-bound NK cell effector agent, wherein the at least one effector agent is IL-21.

2. The composition of claim 1, further comprising at least one additional membrane bound NK cell effector agent, wherein the at least one additional NK cell effector agent is selected from a cytokine, an adhesion molecule, and an NK cell activating agent.

3. The composition of claim 2, wherein the at least one additional membrane bound NK cell effector agent is selected from the group consisting of IL-15, IL-2, 41BBL, IL-12, IL-18, MICA, 2B4, LFA-1, and BCM1/SLAMF2.

4. The composition of claim 3, wherein the at least one additional membrane bound NK cell effector agent is IL-15 or 41BBL.

5. The composition of any of claims 2-4, comprising at least two additional membrane bound NK cell effector agents.

6. The composition of claim 5, wherein the at least two additional membrane bound NKcell effector agents are IL-15 and 41BBL.

7. The composition of any of claims 2-6, wherein each NK cell effector agent is conjugated to a separate membrane self-inserting peptide.

8. The composition of claim 7, wherein the membrane self-inserting peptide is selected from human Fc, GPI, transmembrane T cell receptor, and pHLIP.

9. The composition of claim 1, comprising a plurality of the plasma membrane vesicles.

10. The composition of claim 1 or claim 9, wherein the plasma membrane vesicle or vesicles are purified from NK cell feeder cells transfected with at least one membrane-bound NK cell effector agent in addition to membrane-bound IL-21.

11. The composition of 10, wherein the at least one additional NK cell effector agent is selected from a cytokine, an adhesion molecule, and an NK cell activating agent.

12. The composition of claim 10, wherein the at least one additional cell effector agent is selected from IL-15, IL-2, 41BBL, IL-12, IL-18, MICA, 2B4, LFA-1, and BCM1/SLAMF2.

13. The composition of claim 1 or claim 10, wherein the plasma membrane vesicle or vesicles are purified from cells selected from the group consisting of K562 cells, peripheral blood mononuclear cells (PBMCs), RPMI8866, HFWT, K562 cells transfected with membrane bound IL-21 and 41BBL, K562 cells transfected with membrane bound IL-15 and 41BBL, and EBV-LCL.

14. A method of expanding an initial population of NK cells, the method comprising contacting the initial NK cell population *in vitro* with an effective amount of a composition comprising a plasma membrane vesicle purified from NK cell feeder cells transfected with at least one membrane bound NK cell effector agent, wherein at least one NK cell effector agent is IL-21.

15. The method of claim 14, wherein the membrane-bound NK cell effector agent in the plasma membrane vesicle is conjugated to a membrane self-inserting peptide.

16. The method of claim 14, wherein the plasma membrane vesicle comprises at least one membrane bound NK cell effector agent in addition to IL-21.

17. The method of claim 16, wherein IL-21 and the at least one additional NK cell effector agent are each conjugated to the same membrane self-inserting peptide selected from human Fc, GPI, transmembrane T cell receptor, and pHLIP.

18. The method of claim 16, wherein IL-21 and the at least one additional NK cell effector agent are each conjugated to a different membrane self-inserting peptide selected from human Fc, GPI, transmembrane T cell receptor, and pHLIP.

19. The method of claim 14, wherein the NK cell feeder cells are selected from K562 cells, irradiated autologous or allogeneic peripheral blood mononuclear cells (PBMCs), RPMI8866, HFWT, K562 cells transfected with membrane bound IL-15 and 41BBL, or EBV-LCL.

20. The method of claim 14, wherein the composition comprises a plurality of the plasma membrane vesicles.

21. An expanded population of NK cells *in vitro*, in contact with a plasma membrane vesicle composition of any one of claims 1-13, wherein the plasma membrane composition selectively stimulates expansion of NK cells from PBMC's.

22. A device or a kit comprising the composition of any of claims 1-13.

23. The expanded population of NK cells of claim 21, wherein the cells have increased cytotoxicity relative to NK cells not contacted by the composition of any of claims 1-13.

24. A composition comprising a plasma membrane vesicle purified from NK cell feeder cells transfected with at least one membrane-bound NK cell effector agent conjugated to a membrane self-inserting peptide, wherein the at least one effector agent is IL-21.