highly specific targeting is the limited availability of truly tumor-restricted antigens. Accordingly, new methods of treating neoplasia are urgently required.

## Summary of the Invention

[0005] The present invention generally provides immunoresponsive cells, including T cells and Natural Killer (NK) cells, expressing an antigen binding receptor (e.g., CAR or TCR) having immune cell activating actvity and a chimeric co-stimulating receptor (CCR), and methods of use therefore for the treatment of neoplasia, infectious disease, and other pathologies.

[0005a] In one aspect, there is provided an immunoresponsive cell comprising:
a). an antigen recognizing receptor that binds to a first antigen, wherein binding of the receptor to the first antigen is capable of delivering an activation signal to the immunoresponsive cell, and
b). a chimeric co-stimulating receptor (CCR) that binds to a second antigen, wherein binding of the CCR to the second antigen is capable of delivering a costimulatory signal to the immunoresponsive cell, wherein the immunoresponsive cell exhibits a greater degree of cytolytic activity against cells that are positive for both the first antigen and the second antigen as compared to against cells that are singly positive for the first antigen, and the immunoresponsive cell exhibits substantially no or negligible cytolytic activity against cells that are singly positive for the first antigen.

[0005b] In one aspect, there is provided a method for producing an antigen-specific immunoresponsive cell, the method comprising:
introducing into the immunoresponsive cell a first nucleic acid sequence that encodes an antigen recognizing receptor that binds to a first antigen, wherein binding of the antigen recognizing receptor to the first antigen is capable of delivering an activation signal to the immunoresponsive cell; and introducing into the immunoresponsive cell a second nucleic acid sequence that encodes a chimeric co-stimulating receptor (CCR) that binds to a second antigen, wherein binding of the CCR to the second antigen is capable of delivering a costimulatory signal to the immunoresponsive cell, wherein the immunoresponsive cell exhibits a greater degree of cytolytic activity against cells that are positive for both the first and the second antigens as compared to against cells that are singly positive for the first antigen, and the immunoresponsive cell exhibits substantially no or negligible cytolytic activity against cells that are singly positive for the

first antigen.

[0005c] In one aspect, there is provided use of a T cell in the manufacture of a medicament for treating prostate cancer in a subject in need thereof, wherein the T cell comprises an antigen recognizing receptor that binds to a first antigen that is PSCA or CD19, wherein binding of the antigen recognizing receptor to the first antigen is capable of delivering an activation signal to the immunoresponsive cell, and a chimeric co-stimulating receptor (CCR) that binds to a second antigen that is PSMA, wherein binding of the CCR to the second antigen is capable of delivering a costimulatory signal to the immunoresponsive cell, wherein the immunoresponsive cell exhibits a greater degree of cytolytic activity against cells that are positive for both the first and the second antigens as compared to against cells that are singly positive for the first antigen, and the immunoresponsive cell exhibits substantially no or negligible cytolytic activity against cells that are singly positive for the first antigen.

[0005d] In one aspect, there is provided a kit comprising an immunoresponsive cell comprising an antigen recognizing receptor that binds to a first antigen, wherein binding of the receptor to the first antigen is capable of delivering an activation signal to the immunoresponsive cell, and a chimeric co-stimulating receptor (CCR) that binds to a second antigen, wherein binding of the CCR to the second antigen is capable of delivering a costimulatory signal to the immunoresponsive cell, wherein the immunoresponsive cell exhibits a greater degree of cytolytic activity against cells or that are positive for both the first and the second antigens as compared to against cells that are singly positive for the first antigen, and the immunoresponsive cell exhibits substantially no or negligible cytolytic activity against cells that are singly positive for the first antigen.

[0006] In one aspect, the invention provides an isolated immunoresponsive cell having an antigen recognizing receptor that binds a first antigen with low affinity, where the binding activates the immunoresponsive cell, and a chimeric co stimulating receptor (CCR) that binds a second antigen and stimulates the immunoresponsive cell.

[0006a] In one aspect, there is provided the use of an effective amount of an immunoresponsive cell comprising an antigen recognizing receptor that binds a first antigen with low affinity, wherein the binding activates the immunoresponsive cell, and a chimeric co-stimulating receptor (CCR) that binds a second antigen and

stimulates the immunoresponsive cell in the manufacture of a medicament for treating or preventing a neoplasia in a subject.

[0006b] In one aspect, there is provided the use of an effective amount of an immunoresponsive cell comprising an antigen recognizing receptor that binds a first antigen with low affinity, wherein the binding activates the immunoresponsive cell, and a chimeric co-stimulating receptor (CCR) that binds a second antigen and stimulates the immunoresponsive cell in the manufacture of a medicament for inducing tumor cell death.

[0006c] In one aspect, there is provided the use of a therapeutically effective amount of a T cell comprising an antigen recognizing receptor that binds PSCA or CD19 with low affinity, wherein the binding activates the immunoresponsive cell, and a chimeric co-stimulating receptor (CCR) that binds PSMA and stimulates the immunoresponsive cell in the manufacture of a medicament for treating prostate cancer in a subject in need thereof.

[0006d] In one aspect, there is provided an immunoresponsive cell comprising:

a). an antigen recognizing receptor that binds a first antigen with a low binding affinity, wherein binding of the receptor to the first antigen is capable of delivering an activation signal to the immunoresponsive cell, and

b). a chimeric co-stimulating receptor (CCR) that binds a second antigen, wherein binding of the CCR to the second antigen is capable of delivering a costimulatory signal to the immunoresponsive cell but does not alone deliver an activation signal,

wherein the immunoresponsive cell exhibits a greater degree of cytolytic activity against cells that are positive for both the first antigen and the second antigen as compared to against cells that are singly positive for the first antigen.

[0006e] In one aspect, there is provided the use of an immunoresponsive cell as described herein in the manufacture of a medicament for inducing tumor cell death in a tumor in a subject.

[0006f] In one aspect, there is provided the use of an immunoresponsive cell as described herein in the manufacture of a medicament for treating or preventing a neoplasia in a subject.

[0006g] In one aspect, there is provided a method for producing an antigen-specific immunoresponsive cell, the method comprising:

introducing into the immunoresponsive cell a first nucleic acid sequence that encodes an antigen recognizing receptor that binds a first antigen with a low binding affinity, wherein

binding of the antigen recognizing receptor to the first antigen is capable of delivering an activation signal to the immunoresponsive cell; and

introducing into the immunoresponsive cell a second nucleic acid sequence that encodes a chimeric co-stimulating receptor (CCR) that binds a second antigen, wherein binding of the CCR to the second antigen is capable of delivering a costimulatory signal to the immunoresponsive cell but does not alone deliver an activation signal,

wherein the immunoresponsive cell exhibits a greater degree of cytolytic activity against cells that are positive for both the first and the second antigens as compared to against cells that are singly positive for the first antigen.

[0006h] In one aspect, there is provided the use of a T cell in the manufacture of a medicament for treating prostate cancer in a subject in need thereof, wherein the T cell comprises an antigen recognizing receptor that binds PSCA or CD19 with a low binding affinity, wherein binding of the antigen recognizing receptor to the first antigen is capable of delivering an activation signal to the immunoresponsive cell, and a chimeric co-stimulating receptor (CCR) that binds PSMA, wherein binding of the CCR to the second antigen is capable of delivering a costimulatory signal to the immunoresponsive cell but does not alone deliver an activation signal, wherein the immunoresponsive cell exhibits a greater degree of cytolytic activity against cells that are positive for both the first and the second antigens as compared to against cells that are singly positive for the first antigen.

[0006i] In one aspect, there is provided a kit comprising an immunoresponsive cell comprising an antigen recognizing receptor that binds a first antigen, wherein binding of the receptor to the first antigen is capable of delivering an activation signal to the immunoresponsive cell, and a chimeric co-stimulating receptor (CCR) that binds a second antigen, wherein binding of the CCR to the second antigen is capable of delivering a costimulatory signal to the immunoresponsive cell but does not alone deliver an activation signal, wherein the immunoresponsive cell exhibits a greater degree of cytolytic activity against cells or that are positive for both the first and the second antigens as compared to against cells that are singly positive for the first antigen.

[0007] In another aspect, the invention provides a method of inducing tumor cell death in a subject, the method comprising administering an effective amount of an immunoresponsive cell comprising an antigen recognizing receptor that binds a first antigen with low affinity, where the binding activates the immunoresponsive cell, and a chimeric co-stimulating receptor (CCR) that binds a second antigen and stimulates the immunoresponsive cell, thereby inducing tumor cell death in the subject.

[0008] In still another aspect, the invention provides a method of treating or preventing a neoplasia in a subject, the method comprising administering an effective amount of an immunoresponsive cell comprising an antigen recognizing receptor that binds a first antigen with low affinity, where the binding activates the immunoresponsive cell, and a chimeric co-stimulating receptor (CCR) that binds a second antigen and stimulates the immunoresponsive cell, thereby treating or preventing a neoplasia in the subject.

[0009] In yet another aspect, the invention provides a method of treating prostate cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a T cell

**What is claimed is:**

1. An immunoresponsive cell comprising:

a). an antigen recognizing receptor that binds to a first antigen, wherein binding of the receptor to the first antigen is capable of delivering an activation signal to the immunoresponsive cell, and

b). a chimeric co-stimulating receptor (CCR) that binds to a second antigen, wherein binding of the CCR to the second antigen is capable of delivering a costimulatory signal to the immunoresponsive cell,

wherein the immunoresponsive cell exhibits a greater degree of cytolytic activity against cells that are positive for both the first antigen and the second antigen as compared to against cells that are singly positive for the first antigen, and the immunoresponsive cell exhibits substantially no or negligible cytolytic activity against cells that are singly positive for the first antigen.

2. The immunoresponsive cell of claim 1, wherein the antigen recognizing receptor binds to the first antigen with a binding affinity $K_d$ of from about $1 \times 10^{-9}$ to about $5 \times 10^{-8}$ M.

3. The immunoresponsive cell of claim 1, wherein the antigen recognizing receptor binds to the first antigen with a binding affinity $K_d$ of from about $1 \times 10^{-9}$ to about $1 \times 10^{-7}$ M.

4. The immunoresponsive cell of claim 1, wherein the antigen recognizing receptor binds to the first antigen with a binding affinity $K_d$ of from about $1 \times 10^{-9}$ to about $1 \times 10^{-6}$ M.

5. The immunoresponsive cell of claim 1, wherein the antigen recognizing receptor binds to the first antigen with a binding affinity that is lower compared to the binding affinity with which the CCR binds to the second antigen.

6. The immunoresponsive cell of any one of claims 1-5, wherein the immunoresponsive cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), and a regulatory T cell.

7. The immunoresponsive cell of any one of claims 1-6, wherein at least one of the first and second antigens is a tumor or pathogen antigen.

8. The immunoresponsive cell of any of claims 1-7, wherein said antigen recognizing receptor is a T cell receptor (TCR) or chimeric antigen receptor (CAR).

9. The immunoresponsive cell of any one of claims 1-8, wherein said antigen recognizing receptor is exogenous.

10. The immunoresponsive cell of any one of claims 1-9, wherein said antigen recognizing receptor is recombinantly expressed.

11. The immunoresponsive cell of any one of claims 1-10, wherein the antigen recognizing receptor is expressed from a vector.

12. The immunoresponsive cell of any one of claims 1-11, wherein the chimeric co-stimulating receptor (CCR) is expressed from a vector.

13. The immunoresponsive cell of any one of claims 1-12, wherein, following administration to a subject, the immunoresponsive cell is capable of destroying cells that are positive for both the first antigen and the second antigen without destroying or without substantially destroying cells that are singly positive for the first antigen.

14. The immunoresponsive cell of any one of claims 1-13, wherein said immunoresponsive cell is autologous to said subject.

15. The immunoresponsive cell of any one of claims 1-14, wherein said first and second antigens are selected from the group consisting of CAIX, CEA, CD5, CD7, CD10, CD19, CD20, CD22, CD30, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD74, CD133, CD138, a cytomegalovirus (CMV) infected cell antigen, EGP-2, EGP-40, EpCAM, erb-B2,3,4, FBP, Fetal acetylcholine receptor, folate receptor-a, GD2, GD3, HER-2, hTERT, IL-13R-a2, x-light chain, KDR, LeY, LI cell adhesion molecule, MAGE-AI , MUC1, Mesothelin, NKG2D ligands, NY-ESO-1, oncofetal antigen (h5T4), PSCA, PSMA, ROR1, TAG-72, VEGF-R2, and WT-1.

16. The immunoresponsive cell of any one of claims 1-15, wherein said first and second antigens are distinct antigens selected from the group consisting of CD133, a cytomegalovirus (CMV) infected cell antigen, erb-B2, KDR Mesothelin, NKG2D ligands, NY-ESO-1, oncofetal antigen (h5T4), PSCA, PSMA, CD19, VEGF-R2, and WT-1.

17. The immunoresponsive cell of any one of claims 1-15, wherein said first and second antigens are selected from the group consisting of HER-2, MUC1 , CD44, CD49f, EpCAM,

CEA, CD133, a cytomegalovirus (CMV) infected cell antigen, EGP-2, EGP-40, erb-B2,3,4, FBP, KDR, Mesothelin, NKG2D ligands, NY-ESO-1, oncofetal antigen (h5T4), PSCA, PSMA, VEGF-R2, and WT-1.

18. The immunoresponsive cell of any one of claims 1-17, wherein said first and second antigens are PSCA and PSMA, respectively.

19. The immunoresponsive cell of any one of claims 1-15, wherein said first and second antigens are CD10 and CD19.

20. The immunoresponsive cell of any one of claims 1-15, wherein said first and second antigens are CD56 and CD138.

21. The immunoresponsive cell of any one of claims 1-15, wherein said first and second antigens are distinct antigens selected from the group consisting of mesothelin, folate receptor-a, CD44, and CD133.

22. The immunoresponsive cell of any one of claims 1-21, wherein the intracellular signaling domain of said antigen recognizing receptor is the CD3ξ-chain signaling domain.

23. The immunoresponsive cell of any one of claims 1-22, wherein the intracellular signaling domain of the CCR is a CD97, CD1 la-CD18, CD2, ICOS, CD27, CD154, CD5, OX40, 4-1 BB or CD28 signaling domain.

24. The immunoresponsive cell of any one of claims 1-23, wherein said antigen recognizing receptor is a CAR.

25. The immunoresponsive cell of claim 24, wherein the CAR is 19z1, Pz1, or Lz1.

26. Use of an immunoresponsive cell of any one of claims 1-25 in the manufacture of a medicament for inducing tumor cell death in a tumor in a subject.

27. The use of claim 26, wherein administration of the medicament to the subject is capable of substantially reducing or eradicating cells in the subject that are positive for both the first antigen and the second antigen without destroying, or without substantially reducing or eradicating cells that are singly positive for the first antigen.

28. The use of claim 26 or 27, wherein the immunoresponsive cell reduces the number of tumor cells.

29. The use of any one of claims 26-28, wherein the immunoresponsive cell reduces tumor size.

30. The use of any one of claims 26-29, wherein the immunoresponsive cell eradicates the tumor.

31. Use of an immunoresponsive cell of any one of claims 1-25 in the manufacture of a medicament for treating or preventing a neoplasia in a subject.

32. The use of claim 31, wherein the neoplasia is selected from the group consisting of prostate cancer, breast cancer, B cell malignancies, B cell leukemia, multiple myeloma, and ovarian cancer.

33. The use of claim 32, wherein the neoplasia is prostate cancer.

34. The use of claim 33, wherein the first antigen is PSCA and the second antigen is PSMA.

35. The use of claim 31, wherein the neoplasia is breast cancer and the first and second tumor antigens are distinct antigens selected from the group consisting of HER2, MUC1 , CD44, CD49f, CEA, CD133, a cytomegalovirus (CMV) infected cell antigen, EGP-2, EGP-40, EpCAM, erb-B2,3,4, FBP, KDR, Mesothelin, NKG2D ligands, NY-ESO-1 , oncofetal antigen (h5T4), PSCA, PSMA, VEGF-R2, and WT-1.

36. The use of claim 31, wherein the neoplasia is B cell leukemia and the first and second tumor antigens are CD10 and CD19.

37. The use of claim 31, wherein the neoplasia is multiple myeloma and the first and second tumor antigens are CD56 and CD138.

38. The use of claim 31, wherein the neoplasia is ovarian cancer and the first and second tumor antigens are distinct antigens selected from the group consisting of mesothelin, folate receptor-a, CD44, and CD133.

39. The use of any one of claims 31-38, wherein the immunoresponsive cell reduces or eradicates the tumor burden in the subject.

40. A method for producing an antigen-specific immunoresponsive cell, the method comprising:

introducing into the immunoresponsive cell a first nucleic acid sequence that encodes an antigen recognizing receptor that binds to a first antigen, wherein binding of the antigen recognizing receptor to the first antigen is capable of delivering an activation signal to the immunoresponsive cell; and

introducing into the immunoresponsive cell a second nucleic acid sequence that encodes a chimeric co-stimulating receptor (CCR) that binds to a second antigen, wherein binding of the CCR to the second antigen is capable of delivering a costimulatory signal to the immunoresponsive cell,

wherein the immunoresponsive cell exhibits a greater degree of cytolytic activity against cells that are positive for both the first and the second antigens as compared to against cells that are singly positive for the first antigen, and the immunoresponsive cell exhibits substantially no or negligible cytolytic activity against cells that are singly positive for the first antigen.

41. Use of a T cell in the manufacture of a medicament for treating prostate cancer in a subject in need thereof, wherein the T cell comprises an antigen recognizing receptor that binds to a first antigen that is PSCA or CD19, wherein binding of the antigen recognizing receptor to the first antigen is capable of delivering an activation signal to the immunoresponsive cell, and a chimeric co-stimulating receptor (CCR) that binds to a second antigen that is PSMA, wherein binding of the CCR to the second antigen is capable of delivering a costimulatory signal to the immunoresponsive cell, wherein the immunoresponsive cell exhibits a greater degree of cytolytic activity against cells that are positive for both the first and the second antigens as compared to against cells that are singly positive for the first antigen, and the immunoresponsive cell exhibits substantially no or negligible cytolytic activity against cells that are singly positive for the first antigen.

42. A pharmaceutical composition comprising an immunoresponsive cell of any one of claims 1-25 and a pharmaceutically acceptable excipient.

43. A kit comprising an immunoresponsive cell comprising an antigen recognizing receptor that binds to a first antigen, wherein binding of the receptor to the first antigen is capable of delivering an activation signal to the immunoresponsive cell, and a chimeric co-stimulating receptor (CCR) that binds to a second antigen, wherein binding of the CCR to the second antigen is capable of delivering a costimulatory signal to the immunoresponsive cell, wherein the immunoresponsive cell exhibits a greater degree of cytolytic activity against cells or that are positive for both the first and the second antigens as compared to against cells that are singly positive for the first antigen, and the immunoresponsive cell exhibits substantially no or negligible cytolytic activity against cells that are singly positive for the first antigen.