METHODS AND COMPOSITIONS FOR TREATING DISEASE

Background of the Invention

Field of the Invention

[0001] The present invention relates to methods and compositions for treating a subject comprising destroying diseased cells in the subject by causing the selective expression of a lethal polypeptide in cells that are expressing at least one disease marker gene.

Background of the Invention

[0002] Cancer is a set of diseases resulting from uncontrolled cell growth, which causes intractable pain and death for more than 300,000 people per year in the United States alone. Oncogenes are genes that, generally speaking, promote cancer cell growth. The development of cancer is believed to depend on the activation of oncogenes and the coincident inactivation of growth suppressor genes (Park, M., "Oncogenes" in The Genetic Basis of Human Cancer (B. Vogelstein et al., eds.) pp. 205-228 (1998)). Oncogenes are mutated, dominant forms of cellular proto-oncogenes that stimulate cell proliferation, while tumor suppressor genes are recessive and normally inhibit cell proliferation.

[0003] Treatment of cancer patients with chemotherapeutic agents remains the primary method of treating systemic disease and there is a direct association between chemotherapeutic dose intensity and clinical response rate. Increasing doses of chemotherapy, however, have significant side effects including the widespread destruction of bone marrow hematopoietic progenitor cells with concomitant destruction of peripheral myeloid and lymphoid cellularity. Stem cell transplantation is often used in conjunction with high dose chemotherapy to facilitate the recovery of the hematopoietic system following chemotherapy.

[0004] Allogeneic stem cell transplantation, which is transplantation of stem cells from a donor other than the patient is often used. The allogeneic transplant protocol, however,

carries a high mortality rate due primarily to graft-versus-host disease (GVD), wherein the transplanted cells attack the patient's own tissues.

[0005] Autologous stem cell transplantation is a protocol wherein the patient's stem cells are isolated prior to the high dose chemotherapy and subsequently reinfused. Autologous transplantation avoids complications associated with GVD, but may result in the reinfusion of tumor cells from within the stem cell product. Reinfusion of tumor cells is important because gene marking studies have demonstrated that reinfused tumor cells can directly contribute to disease relapse and a poor clinical outcome. For example, in the cases of lymphoma, leukemia, breast cancer and neuroblastoma, at least some of the contaminating tumor cells in a peripheral blood stem cells transplantation protocol have the capacity to grow clonogenically *in vitro* (Ross, *et al., Blood* 82:2605-2610 (1993)) as well in the patient.

[0006] To avoid reinfusing the cancer cells into the patient undergoing autologous stem cell transplantation, practitioners have attempted to "purge" bone marrow cells of their contaminating tumor cells. Various approaches for the *ex vivo* purging of tumor cell contaminating stem cell populations have been developed. For example, the use of monoclonal antibodies against membrane antigens with cytotoxic drugs, toxins, phototherapy, and biological modifiers or cytotoxic drugs can reduce tumor contamination by 1 to 3 orders of magnitude (Seiden, *et al., J. Infusional Chemotherapy* 6:17-22 (1996), incorporated by reference). In another protocol, antibodies that are directed towards tumor cells are conjugated to radioisotopes have been used in an attempt to purge tumor cells. The use of cytotoxic drugs and/or radioactivity may not be specific as tumor cells and progenitor cells often display a similar phenotype of cell surface proteins and the use of such techniques may delay engraftment. The selection of $CD34^+$ hematopoietic progenitor cells has also been used to reduce tumor cell reinfusion, although at a much lower purging efficacy. Again, these methods of CD34-based selection may not be specific enough as tumor cells may often display the CD34 antigen.

[0007] Thus, there is a need in the art for new methods that specifically remove diseased cells from a cell population that is targeted for autologous transplantation.

WHAT IS CLAIMED IS:

1. An ex-vivo method of destroying diseased cells, said method comprising

   (a) determining the activity of at least one disease marker gene within a population of cells;

   (b) introducing into said cells a polynucleotide that encodes a selectable marker and a polypeptide that is lethal to said cells, wherein the expression of said lethal polypeptide is directly or indirectly controlled by the promoter of said at least one disease marker gene;

   (c) exposing said cells to selection conditions to obtain cells comprising said polynucleotide;

   (d) treating said cells with conditions to induce expression of said lethal polypeptide, wherein said expression of said lethal polypeptide kills said cells expressing said at least one disease marker gene; and

   (e) separating said killed cells from the remaining live, non-diseased cells, wherein said live cells do not express said lethal polypeptide to an extent sufficient to kill said non-diseased cells.

2. The method of claim 1, wherein said promoter is operably linked to said polynucleotide encoding said lethal polypeptide.

3. The method of claim 1, wherein said cells are selected from the group consisting of hematopoietic stem cells, liver stem cells, mammary stem cells, pancreatic stem cells, and neuronal stem cells.

4. The method of claim 3, wherein said cells are hematopoietic stem cells.

5. The method of claim 1, wherein said introducing said polynucleotide comprises transient transfection of said polynucleotide into said cells.

6. The method of claim 1, wherein said introducing said polynucleotide comprises stable transfection of said polynucleotide into said cells.

7. The method of claim 1, wherein said polynucleotide comprises at least two gene programs.

8. The method of claim 7, wherein said promoter of said disease marker gene is ligated between a first and a second molecular insertion pivot.

9. The method of claim 7, wherein said polynucleotide encoding said lethal polypeptide is ligated between a second and a third molecular insertion pivot.

10. The method of claim 8 or 9, wherein said molecular insertion pivots are comprised of three or four rare or uncommon restriction sites in a contiguous arrangement, said rare or uncommon restriction sites being selected from the group consisting of the restriction sites correlating to the AsiS I, Pac I, Sbf I, Fse I, Asc I, Mlu I, SnaB I, Not I, Sal I, Swa I, Rsr II, BSiW I, Sfo I, Sgr AI, Afl III, Pvu I, Ngo MIV, Ase I, Flp I, Pme I, Sda I, Sgf I, Srf I and Sse878 I restriction enzymes.

11. The method of claim 7, wherein polynucleotide further comprises at least one chromatin modification domain.

12. The method of claim 1, wherein said introducing said polynucleotide comprises locus-specific insertion of said polynucleotide.

13. The method of claim 12, wherein said locus-specific insertion is selected from the group consisting of homologous recombination and recombinase mediated genome insertion.

14. The method of claim 12, wherein said polynucleotide comprises at least two genome integration sites.

15. The method of claim 13, wherein said polynucleotide comprises at least two genome integration sites.

16. The method of claim 1, further comprising the step of determining if the polynucleotide was inserted into a bio-neutral site in the genome.

17. The method of claim 1, further comprising the step of excising said polynucleotide from the genome of said cells.

18. The method of claim 17, wherein said excising comprises site-specific recombinase activity.

19. The method of claim 18, wherein said polynucleotide further comprises at least two recombinase sites.

20. The method of claim 1, wherein said polynucleotide is not excised from said remaining live, non-diseased cells.

21. The method of claim 1, further comprising the step of inducing expression of said lethal polypeptide.

22. An ex-vivo method of treating cells, said method comprising

(a) determining the activity of at least one disease marker gene within a population of cells;

(b) isolating at least one promoter of said at least one disease marker gene;

(c) directly or indirectly linking said promoter to a polynucleotide encoding a polypeptide that is lethal to said cells;

(d) placing said linked polynucleotide into a vector that comprises a selectable marker;

(e) introducing said polynucleotide into said cells;

(f) exposing said cells to selection conditions to obtain cells comprising said polynucleotide;

(g) treating said cells with conditions to induce expression of said lethal polypeptide, wherein said expression of said lethal polypeptide kills said cells expressing said at least one disease marker gene; and

(h) separating said killed cells from the remaining live, non-diseased cells, wherein said live cells do not express said lethal polypeptide to an extent sufficient to kill said non-diseased cells.

23. The method of claim 22, wherein said promoter is operably linked to said polynucleotide encoding said lethal polypeptide.

24. The method of claim 22, wherein said cells are selected from the group consisting of hematopoietic stem cells, liver stem cells, mammary stem cells, pancreatic stem cells, and neuronal stem cells.

25. The method of claim 24, wherein said cells are hematopoietic stem cells.

26. The method of claim 22, further comprising the step of determining if the polynucleotide was inserted into a bio-neutral site in the genome.

27. The method of claim 23, wherein said promoter is operably linked to said polynucleotide encoding said lethal polypeptide by ligating said promoter between a first and second molecular insertion pivot, said second molecular insertion pivot located at the 3' terminus of said promoter.

28. The method of claim 27, wherein said molecular insertion pivots are comprised of three or four rare or uncommon restriction sites in a contiguous arrangement, said rare or uncommon restriction sites being selected from the group consisting of the restriction sites correlating to the AsiS I, Pac I, Sbf I, Fse I, Asc I, Mlu I, SnaB I, Not I, Sal I, Swa I, Rsr II, BSiW I, Sfo I, Sgr AI, Afl III, Pvu I, Ngo MIV, Ase I, Flp I, Pme I, Sda I, Sgf I, Srf I and Sse878 I restriction enzymes.

29. An ex-vivo method of destroying diseased cells, said method comprising

(a) determining the activity of at least one disease marker gene within a population of cells;

(b) introducing into said cells a polynucleotide molecule that encodes a polypeptide that is lethal to said cells, wherein the expression of said lethal polypeptide is controlled by the promoter of said at least one disease marker gene, and wherein said promoter is operably linked to said polynucleotide encoding said lethal polypeptide;

(c) treating said cells with conditions to induce expression of said lethal polypeptide, wherein said expression of said lethal polypeptide kills said cells expressing said at least one disease marker gene; and

(d) separating said killed cells from the remaining live, non-diseased cells, wherein said live cells do not express said lethal polypeptide to an extent sufficient to kill said non-diseased cells.

30. The method of claim 29, wherein said cells are selected from the group consisting of hematopoietic stem cells, liver stem cells, mammary stem cells, pancreatic stem cells, neuronal stem cells.

31. The method of claim 30, wherein said cells are hematopoietic stem cells.

32. The method of claim 30, wherein said introducing said polynucleotide comprises transient transfection of said polynucleotide into said cells.

33. The method of claim 30, wherein said introducing said polynucleotide comprises stable transfection of said polynucleotide into said cells.

34. The method of claim 30, wherein said polynucleotide comprises at least two gene programs.

35. The method of claim 34, wherein said promoter of said disease marker gene is ligated between the first and second of said molecular insertion pivots.

36. The method of claim 35, wherein said polynucleotide encoding said lethal polypeptide is ligated between the second molecular insertion pivots and the third molecular insertion points.

37. The method of claim 36, wherein said polynucleotide further comprises at least one selectable marker.

38. The method of claim 37, wherein polynucleotide further comprises at least one chromatin modification domain.

39. The method of claim 38, wherein said introducing said polynucleotide comprises locus-specific insertion of said polynucleotide.

40. The method of claim 39, wherein said locus-specific insertion is selected from the groups consisting of homologous recombination and recombinase mediated genome insertion.

41. The method of claim 40, wherein said polynucleotide comprises at least two genome integration sites.

42. The method of claim 41, wherein said polynucleotide comprises at least two genome integration sites.

43. The method of claim 42, further comprising excision of said polynucleotide from the genome of said cells.

44. The method of claim 43, wherein said excision comprises site-specific recombinase activity.

45. The method of claim 44, wherein said polynucleotide further comprises at least two recombinase sites.

46. An ex-vivo method of treating cells, said method comprising

(a) determining the activity of at least one disease marker gene within a population of cells;

(b) isolating at least one promoter of said at least one disease marker gene;

(c) generating a therapeutic polynucleotide by operably linking said promoter to a polynucleotide encoding a polypeptide that is lethal to said cells;

(d) introducing said therapeutic polynucleotide into said cells;

(e) treating said cells with conditions to induce expression of said lethal polypeptide, wherein said expression of said lethal polypeptide kills said cells expressing said at least one disease marker gene; and

(f) separating said killed cells from the remaining live, non-diseased cells, wherein said live cells do not express said lethal polypeptide to an extent sufficient to kill said non-diseased cells.

47. The method of claim 46, wherein said cells are selected from the group consisting of hematopoietic stem cells, liver stem cells, mammary stem cells, pancreatic stem cells, neuronal stem cells.

48. The method of claim 47, wherein said cells are hematopoietic stem cells.

49. The method of claim 48, wherein said operably linking said promoter to said polynucleotide encoding said lethal polypeptide comprises ligating said promoter between a first and second molecular insertion pivot, said second molecular insertion pivot located at the 3' terminus of said promoter.

50. The method of claim 49, wherein said molecular insertion pivots are comprised of three or four rare or uncommon restriction sites in a contiguous arrangement, said rare or uncommon restriction sites being selected from the group consisting of the restriction sites correlating to the AsiS I, Pac I, Sbf I, Fse I, Asc I, Mlu I, SnaB I, Not I, Sal I, Swa I, Rsr II, BSiW I, Sfo I, Sgr AI, Afl III, Pvu I, Ngo MIV, Ase I, Flp I, Pme I, Sda I, Sgf I, Srf I and Sse878 I restriction enzymes.