RECOMBINANT VECTORS COMPRISING 2A PEPTIDE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Serial No. 62/214,884, filed September 4, 2015, the disclosures of which are incorporated herein by reference.

REFERENCE TO SEQUENCE LISTING

[0002] The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled Sequence-Listing_ST25.txt, created September 1, 2016, which is 245,703 bytes (239 Kb) in size. The information in the electronic format of the Sequence Listing is incorporated herein by reference in its entirety.

TECHNICAL FIELD

[0003] This disclosure relates to replication competent retroviral vectors. The disclosure further relates to the use of such replication competent retroviral vectors for delivery and expression of heterologous nucleic acids in cells.

BACKGROUND

[0004] Effective methods of delivering genes and heterologous nucleic acids to cells and subjects has been a goal of researchers for scientific development and for possible treatments of diseases and disorders.

SUMMARY

[0004a] In one aspect, the present invention provides a recombinant replication competent retrovirus comprising: a retroviral GAG protein; a retroviral POL protein; a retroviral envelope; a retroviral polynucleotide comprising Long-Terminal Repeat (LTR) sequences at the 3' end of the retroviral polynucleotide sequence, a promoter sequence at the 5' end of the retroviral polynucleotide, said promoter being suitable for expression in a mammalian cell, a gag nucleic acid domain, a pol nucleic acid domain and an env nucleic acid domain; a cassette comprising a GSG-2A peptide or GSG-2A peptide-like coding sequence operably linked to a heterologous polynucleotide, wherein the cassette is positioned 5' to the 3' LTR and is operably linked and 3' to the env nucleic acid domain encoding the retroviral envelope;

and cis-acting sequences necessary for reverse transcription, packaging and integration in a target cell.

[0004b] In another aspect, the present invention provides a recombinant replication competent retrovirus comprising: a retroviral GAG protein; a retroviral POL protein; a retroviral envelope; a retroviral polynucleotide comprising Long-Terminal Repeat (LTR) sequences at the 3' end of the retroviral polynucleotide sequence, a promoter sequence at the 5' end of the retroviral polynucleotide, said promoter being suitable for expression in a mammalian cell, a gag nucleic acid domain, a pol nucleic acid domain and an env nucleic acid domain; a cassette comprising a regulatory domain operably linked to a first heterologous polynucleotide operably linked to at least one 2A cassette comprising a GSG-2A peptide or GSG-2A peptide-like coding sequence operably linked to a second heterologous polynucleotide, wherein the cassette is positioned 5' to the 3' LTR and 3' to the env nucleic acid domain encoding the retroviral envelope and wherein the 2A cassette is downstream and operably linked to first heterologous polynucleotide; and cis-acting sequences necessary for reverse transcription, packaging and integration in a target cell.

[0004c] In yet another aspect, the present invention provides recombinant retroviral polynucleotide genome for producing a retrovirus disclosed herein.

[0004d] In yet another aspect, the present invention provides a recombinant replication competent retrovirus that is resistant to inactivation by human APOBEC by engineering codons in a retroviral polynucleotide susceptible to APOBEC hypermutation to a non-susceptible codon.

[0004e] In yet another aspect, the present invention provides use of a retrovirus disclosed herein in the manufacture of a medicament for treating a cell proliferative disorder, wherein the subject is to be contacted with said retrovirus under conditions such that the heterologous polynucleotide is expressed and wherein the heterologous polynucleotide encodes a protein the converts a prodrug to a cytotoxic drug.

[0005] The disclosure provides a recombinant replication competent retrovirus comprising a retroviral GAG protein; a retroviral POL

protein; a retroviral envelope; a retroviral polynucleotide comprising Long-Terminal Repeat (LTR) sequences at the 3' end of the retroviral polynucleotide sequence, a promoter sequence at the 5' end of the retroviral polynucleotide, said promoter being suitable for expression in a mammalian cell, a gag nucleic acid domain, a pol nucleic acid domain and an env nucleic acid domain; a cassette comprising a 2A peptide or peptide-like coding sequence operably linked to a heterologous polynucleotide, wherein the cassette is positioned 5' to the 3' LTR and is operably linked and 3' to the env nucleic acid domain encoding the retroviral envelope; and cis-acting sequences necessary for reverse transcription,

packaging and integration in a target cell. In one example, the envelope is chosen from one of amphotropic, polytropic, xenotropic, 10A1, GALV, Baboon endogenous virus, RD114, rhabdovirus, alphavirus, measles or influenza virus envelopes. In another example, the retroviral polynucleotide sequence is derived from a virus selected from the group consisting of murine leukemia virus (MLV), Moloney murine leukemia virus (MoMLV), Feline leukemia virus (FeLV), Baboon endogenous retrovirus (BEV), porcine endogenous virus (PERV), the cat derived retrovirus RD114, squirrel monkey retrovirus, Xenotropic murine leukemia virus-related virus (XMRV), avian reticuloendotheliosis virus (REV), or Gibbon ape leukemia virus (GALV). In yet another example, the retrovirus is a gammaretrovirus. In another example, the target cell is a mammalian cell. In yet another example, the 2A peptide or peptide like coding sequence encodes a peptide containing the sequence of SEQ ID NO:1. In still another example, the 2A peptide or peptide-like coding sequence encodes a peptide set forth in any one of SEQ ID Nos:55-125. In another example, the 2A peptide or peptide-like coding sequence comprises a sequence as set forth in SEQ ID Nos: 8-19. In still another example of any of the foregoing examples, the heterologous polynucleotide is > 500 bp, > 1000bp, > 1100bp, >1200bp >1300 bp, >1400bp or > 1500bp in length. In another example, the heterologous polynucleotide comprises at least 2 coding sequences. In yet another example, the retrovirus further comprises a second cassette comprising a 2A peptide or peptide-like coding sequence downstream of the cassette. In yet another example, the retrovirus further comprises a second cassette downstream of the cassette, wherein the second cassette comprises an internal ribosome entry site (IRES) or a minipromoter or a polIII promoter operably linked to a second heterologous polynucleotide.

[0006] The disclosure also provides a recombinant replication competent retrovirus comprising: a retroviral GAG protein; a retroviral POL protein; a retroviral envelope; a retroviral polynucleotide comprising Long-Terminal Repeat (LTR) sequences at the 3' end of the retroviral polynucleotide sequence, a promoter sequence at the 5' end of the retroviral polynucleotide, said

promoter being suitable for expression in a mammalian cell, a gag nucleic acid domain, a pol nucleic acid domain and an env nucleic acid domain; a cassette comprising a regulatory domain operably linked to a first heterologous polynucleotide operably linked to at least one 2A cassette comprising a 2A peptide or peptide-like coding sequence operably linked to a second heterologous polynucleotide, wherein the cassette is positioned 5' to the 3' LTR and 3' to the env nucleic acid domain encoding the retroviral envelope and wherein the 2A cassette is downstream and operably linked to first heterologous polynucleotide; and cis-acting sequences necessary for reverse transcription, packaging and integration in a target cell. In one example, the envelope is chosen from one of amphotropic, polytropic, xenotropic, 10A1, GALV, Baboon endogenous virus, RD114, rhabdovirus, alphavirus, measles or influenza virus envelopes. In another example, the retroviral polynucleotide sequence is derived from a virus selected from the group consisting of murine leukemia virus (MLV), Moloney murine leukemia virus (MoMLV), Feline leukemia virus (FeLV), Baboon endogenous retrovirus (BEV), porcine endogenous virus (PERV), the cat derived retrovirus RD114, squirrel monkey retrovirus, Xenotropic murine leukemia virus-related virus (XMRV), avian reticuloendotheliosis virus (REV), or Gibbon ape leukemia virus (GALV). In yet another example, the retrovirus is a gammaretrovirus. In yet another example, the target cell is a mammalian cell. In another example, the 2A peptide or peptide like coding sequence encodes a peptide containing the sequence of SEQ ID NO:1. In another example, the 2A peptide or peptide-like coding sequence encodes a peptide set forth in any one of SEQ ID Nos: 55-125. In still another example, the 2A peptide or peptide-like coding sequence comprises a sequence as set forth in any one of SEQ ID Nos:8-19. In any of the foregoing examples, the target cell is selected from the group consisting of lung cancer cell, colon-rectum cancer cell, breast cancer cell, prostate cancer cell, urinary tract cancer cell, uterine cancer cell, brain cancer cell, head and neck cancer cell, pancreatic cancer cell, melanoma cell, stomach cancer and ovarian cancer cell. In still any of the foregoing examples, the promoter sequence is

associated with a growth regulatory gene. In yet a further example of any of the foregoing examples, the promoter sequence comprises a tissue-specific promoter sequence. In a further example, the tissue-specific promoter sequence comprises at least one androgen response element (ARE). In a further example of any of the foregoing, the promoter comprises a CMV promoter having a sequence as set forth in SEQ ID NO:2 from nucleotide 1 to about nucleotide 582 and may include modification to one or more nucleic acid bases and which is capable of directing and initiating transcription. In a further example of any of the foregoing, the promoter comprises a CMV-R-U5 domain polynucleotide. In further example, the CMV-R-U5 domain comprises the immediately early promoter from human cytomegalovirus linked to an MLV R-U5 region. In still a further example, the CMV-R-U5 domain polynucleotide comprises a sequence as set forth in SEQ ID NO:2 from about nucleotide 1 to about nucleotide 1202 or sequences that are at least 95% identical to a sequence as set forth in SEQ ID NO:2 from nucleotde 1 to about 1202, wherein the polynucleotide promotes transcription of a nucleic acid molecule operably linked thereto. In a further example of any of the foregoing, the gag polynucleotide is derived from a gammaretrovirus. In a further example, the gag nucleic acid domain comprises a sequence from about nucleotide number 1203 to about nucleotide 2819 of SEQ ID NO: 2 or a sequence having at least 95%, 98%, 99% or 99.8% identity thereto. In a further example of any of the foregoing, the pol domain of the polynucleotide is derived from a gammaretrovirus. In a further example, the pol domain comprises a sequence from about nucleotide number 2820 to about nucleotide 6358 of SEQ ID NO:2 or a sequence having at least 95%, 98%, 99% or 99.9% identity thereto. In a further example of any of the foregoing, the env domain comprises a sequence from about nucleotide number 6359 to about nucleotide 8323 of SEQ ID NO:2 or a sequence having at least 95%, 98%, 99% or 99.8% identity thereto. In a further example of any of the foregoing, the 3' LTR is derived from a gammaretrovirus. In a further example, the 3' LTR comprises a U3-R-U5 domain. In still a further example, the 3' LTR comprises a sequence as set forth in SEQ ID

NO:2 from about nucleotide 9111 to about 11654 or a sequence that is at least 95%, 98% or 99.5% identical thereto. In a further example of any of the foregoing, the heterologous nucleic acid sequence encodes a biological response modifier or an immunopotentiating cytokine. In a further example, the immunopotentiating cytokine is selected from the group consisting of interleukins 1 through 38, interferon, tumor necrosis factor (TNF), and granulocyte-macrophage-colony stimulating factor (GM-CSF). In a further example, the immunopotentiating cytokine is interferon gamma. In a further example of any of the foregoing, the heterologous nucleic acid encodes a polypeptide that converts a nontoxic prodrug in to a toxic drug. In a further example, the polypeptide that converts a nontoxic prodrug in to a toxic drug is thymidine kinase, purine nucleoside phosphorylase (PNP), or cytosine deaminase. In a further example of any of the foregoing, the heterologous nucleic acid sequence encodes a receptor domain, an antibody, or antibody fragment. In one example, the second cassette comprises an inhibitory polynucleotide. In a further example, the inhibitory polynucleotide comprises an miRNA, RNAi or siRNA sequence.

[0007] The disclosure also provides a recombinant retroviral polynucleotide genome for producing a retrovirus as describe in any of the examples above. In one example, the polynucleotide comprises an MLV 4070A envelope protein gene in-frame with 2A peptide or peptide-like coding sequence with or without a GSG linker coding sequence, and a second gene in-frame with the 2A peptide or 2A-like coding sequence. In another example, the polynucleotide comprises an MLV 10A1 envelope protein gene in-frame with 2A peptide or peptide-like coding sequence with or without a GSG linker coding sequence, and a second gene in-frame with the 2A peptide or 2A-like coding sequence. In another example, the polynucleotide comprises an XMRV envelope protein gene in-frame with 2A peptide or peptide-like coding sequence with or without a GSG linker coding sequence, and a second gene in-frame with the 2A peptide or 2A-like coding sequence. In another example, the polynucleotide comprises a non-Friend MLV envelope protein gene in-frame with 2A peptide or peptide-like coding sequence with or

without a GSG linker coding sequence, and a second gene in-frame with the 2A peptide or 2A-like coding sequence. In another example of any of the foregoing, the heterologous polynucleotide is a secretory, membrane, cytoplasmic, nuclear, or cellular-compartment-specific proteins.

[0008] In any of the foregoing examples, the recombinant retrovirus and/or the heterologous polynucleotide are engineered to remove tryptophan codons susceptible to human APOBEC hypermutations. In one example, the heterologous polynucleotide encodes a polypeptide having cytosine deaminase activity. In a further example, the polypeptide having cytosine deaminase activity encodes a polypeptide of SEQ ID NO:29, wherein X is any amino acid except tryptophan.

[0009] The disclosure also provides a recombinant replication competent retrovirus that is resistant to inactivation by human APOBEC by engineering codons in a retroviral polynucleotide susceptible to APOBEC hypermutation to a non-susceptible codon. In one example, a codon susceptible to APOBEC hypermutation encodes a tryptophan amino acid. In yet another example, the recombinant retrovirus comprises an IRES cassette, promoter cassette and/or 2A peptide cassette downstream of the env gene.

[0010] The disclosure also provides a method of treating a cell proliferative disorder comprising contacting the subject with a retrovirus as described in any of the foregoing examples, under conditions such that the heterologous polynucleotide is expressed and wherein the heterologous polynucleotide encodes a protein the converts a prodrug to a cytotoxic drug.

[0011] The details of one or more examples of the disclosure are set forth in the accompanying drawings and the description below. Other features, objects, and advantages will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF DRAWINGS

[0012] **Figure 1** shows a sequence alignment of amino acid sequence of the 2A regions of foot-and-mouth disease virus (F2A), equine rhinitis A virus (E2A), *Thosea asigna* virus (T2A) and porcine teschovirus-1 (P2A) (SEQ ID Nos: 55 to 58).

WHAT IS CLAIMED IS:

1. A recombinant replication competent retrovirus comprising:

a retroviral GAG protein;

a retroviral POL protein;

a retroviral envelope;

a retroviral polynucleotide comprising Long-Terminal Repeat (LTR) sequences at the 3' end of the retroviral polynucleotide sequence, a promoter sequence at the 5' end of the retroviral polynucleotide, said promoter being suitable for expression in a mammalian cell, a gag nucleic acid domain, a pol nucleic acid domain and an env nucleic acid domain;

a cassette comprising a GSG-2A peptide or GSG-2A peptide-like coding sequence operably linked to a heterologous polynucleotide, wherein the cassette is positioned 5' to the 3' LTR and is operably linked and 3' to the env nucleic acid domain encoding the retroviral envelope; and

cis-acting sequences necessary for reverse transcription, packaging and integration in a target cell.

2. The recombinant replication competent retrovirus of claim 1, wherein the envelope is chosen from one of amphotropic, polytropic, xenotropic, 10A1, GALV, Baboon endogenous virus, RD114, rhabdovirus, alphavirus, measles or influenza virus envelopes.

3. The retrovirus of claim 1, wherein the retroviral polynucleotide sequence is engineered from a virus selected from the group consisting of murine leukemia virus (MLV), Moloney murine leukemia virus (MoMLV), Feline leukemia virus (FeLV), Baboon endogenous retrovirus (BEV), porcine endogenous virus (PERV), the cat derived retrovirus RD114, squirrel monkey retrovirus, Xenotropic murine leukemia virus-related virus (XMRV), avian reticuloendotheliosis virus(REV), or Gibbon ape leukemia virus (GALV).

4. The retrovirus of claim 1, wherein the retrovirus is a gammaretrovirus.

5. The retrovirus of claim 1, wherein the target cell is a mammalian cell.

6. The retrovirus of claim 1, wherein the GSG-2A peptide or GSG-2A peptide like coding sequence encodes a peptide containing the sequence of SEQ ID NO:1.

7. The retrovirus of claim 1, wherein the GSG-2A peptide or GSG-2A peptide-like coding sequence encodes a peptide of any one of SEQ ID Nos: 55-125.

8. The retrovirus of claim 1, wherein the GSG-2A peptide or GSG-2A peptide-like coding sequence comprises a sequence as set forth in any one of SEQ ID Nos: 8-19.

9. The retrovirus of any one of claims claims 1 through 8, wherein the heterologous polynucleotide is > 500 bp.

10. The retrovirus of claim 1, wherein the heterologous polynucleotide comprises at least 2 coding sequences.

11. The retrovirus of claim 1, further comprising a second cassette comprising a GSG-2A peptide or GSG-2A peptide-like coding sequence downstream of the cassette.

12. The retrovirus of claim 1, further comprising a second cassette downstream of the cassette, wherein the second cassette comprises an internal ribosome entry site (IRES) or a minipromoter or a polIII promoter operably linked to a second heterologous polynucleotide.

13. A recombinant replication competent retrovirus comprising:

a retroviral GAG protein;

a retroviral POL protein;

a retroviral envelope;

a retroviral polynucleotide comprising Long-Terminal Repeat (LTR) sequences at the 3' end of the retroviral polynucleotide sequence, a promoter sequence at the 5' end of the retroviral polynucleotide, said promoter being suitable for expression in a mammalian cell, a gag nucleic acid domain, a pol nucleic acid domain and an env nucleic acid domain;

a cassette comprising a regulatory domain operably linked to a first heterologous polynucleotide operably linked to at least one 2A cassette comprising a GSG-2A peptide or GSG-2A peptide-like coding sequence operably linked to a second heterologous polynucleotide, wherein the cassette is positioned 5' to the 3' LTR and 3' to the env nucleic acid domain encoding the retroviral envelope and wherein the 2A cassette is downstream and operably linked to first heterologous polynucleotide; and

cis-acting sequences necessary for reverse transcription, packaging and integration in a target cell.

14. The retrovirus of claim 13, wherein the envelope is chosen from one of amphotropic, polytropic, xenotropic, 10A1, GALV, Baboon endogenous virus, RD114, rhabdovirus, alphavirus, measles or influenza virus envelopes.

15. The retrovirus of claim 13, wherein the retroviral polynucleotide sequence is derived from a virus selected from the group consisting of murine leukemia virus (MLV), Moloney murine leukemia virus (MoMLV), Feline leukemia virus (FeLV), Baboon endogenous retrovirus (BEV), porcine endogenous virus (PERV), the cat derived retrovirus RD114, squirrel monkey retrovirus, Xenotropic murine leukemia virus-related virus (XMRV), avian reticuloendotheliosis virus(REV), or Gibbon ape leukemia virus (GALV).

16. The retrovirus of claim 13, wherein the retrovirus is a gammaretrovirus.

17. The retrovirus of claim 13, wherein the target cell is a mammalian cell.

18. The retrovirus of claim 13, wherein the GSG-2A peptide or GSG-2A peptide like coding sequence encodes a peptide containing the sequence of SEQ ID NO:1.

19. The retrovirus of claim 13, wherein the GSG-2A peptide or GSG-2A peptide-like coding sequence encodes a peptide set forth in Figure 1 or 2.

20. The retrovirus of claim 13, wherein the GSG-2A peptide or GSG-2A peptide-like coding sequence comprises a sequence as set forth in any one of SEQ ID Nos: 8-19.

21. The retrovirus of any one of claims 1 to 20, wherein the target cell is selected from the group consisting of lung cancer cell, colon-rectum cancer cell, breast cancer cell, prostate cancer cell, urinary tract cancer cell, uterine cancer cell, brain cancer cell, head and neck cancer cell, pancreatic cancer cell, melanoma cell, stomach cancer and ovarian cancer cell.

22. The retrovirus of any one of claims 1-21, wherein the promoter sequence is associated with a growth regulatory gene.

23. The retrovirus of any one of claims 1-21, wherein the promoter sequence comprises a tissue-specific promoter sequence.

24. The retrovirus of claim 23, wherein the tissue-specific promoter sequence comprises at least one androgen response element (ARE).

25. The retrovirus of claim 1 or 13, wherein the gag polynucleotide is derived from a gammaretrovirus.

26. The retrovirus of claim 1 or 13, wherein the pol domain of the polynucleotide is derived from a gammaretrovirus.

27. The retrovirus of claim 1 or 13, wherein the 3' LTR is derived from a gammaretrovirus.

28. The retrovirus of claim 34, wherein the 3' LTR comprises a U3-R-U5 domain.

29. The retrovirus of claim 1 or 13, wherein the heterologous nucleic acid sequence encodes a biological response modifier or an immunopotentiating cytokine.

30. The retrovirus according to claim 29, wherein the immunopotentiating cytokine is selected from the group consisting of interleukins 1 through 38, interferon, tumor necrosis factor (TNF), and granulocyte-macrophage-colony stimulating factor (GM-CSF).

31. The retrovirus according to claim 29, wherein the immunopotentiating cytokine is interferon gamma.

32. The retrovirus according to claim 1 or 13, wherein the heterologous nucleic acid encodes a polypeptide that converts a nontoxic prodrug in to a toxic drug.

33. The retrovirus according to claim 32, wherein the polypeptide that converts a nontoxic prodrug in to a toxic drug is thymidine kinase, purine nucleoside phosphorylase (PNP), or cytosine deaminase.

34. The retrovirus according to claim 1 or 13, wherein the heterologous nucleic acid sequence encodes a receptor domain, an antibody, or antibody fragment.

35. The retrovirus according to claim 12, wherein the second cassette comprises an inhibitory polynucleotide.

36. The retrovirus according to claim 35, wherein the inhibitory polynucleotide comprises an miRNA, RNAi or siRNA sequence.

37. A recombinant retroviral polynucleotide genome for producing a retrovirus of claim 1 or 13.

38. The polynucleotide of claim 37, comprising an MLV 4070A envelope protein gene in-frame with GSG-2A peptide or GSG-2A peptide-like coding sequence and a second gene in-frame with the GSG-2A peptide or GSG-2A-like coding sequence.

39. The polynucleotide of claim 37, comprising an MLV 10A1 envelope protein gene in-frame with GSG-2A peptide or GSG-2A peptide-like coding sequence and a second gene in-frame with the GSG-2A peptide or GSG-2A-like coding sequence.

40. The polynucleotide of claim 37, comprising an XMRV envelope protein gene in-frame with GSG-2A peptide or GSG-2A peptide-like coding sequence and a second gene in-frame with the GSG-2A peptide or GSG-2A-like coding sequence.

41. The polynucleotide of claim 37, comprising a non-Friend MLV envelope protein gene in-frame with GSG-2A peptide or GSG-2A peptide-like coding sequence and a second gene in-frame with the GSG-2A peptide or GSG-2A-like coding sequence.

42. The polynucleotide of any one of claims 37-41, wherein the heterologous is a secretory, membrane, cytoplasmic, nuclear, or cellular-compartment-specific proteins.

43. The recombinant replication competent retrovirus of claim 1, wherein the retrovirus and/or the heterologous polynucleotide have been engineered to remove tryptophan codons susceptible to human APOBEC hypermutations.

44. The recombinant replication competent retrovirus of claim 13, wherein the retrovirus and/or the first and/or second heterologous polynucleotide have been engineered to remove tryptophan codons susceptible to human APOBEC hypermutation.

45. The recombinant replication competent retrovirus of claim 44, wherein the heterologous polynucleotide encodes a polypeptide having cytosine deaminase activity.

46. The recombinant replication competent retrovirus of claim 45, wherein the polypeptide having cytosine deaminase activity encodes a polypeptide of SEQ ID NO:29, wherein X is any amino acid except tryptophan.

47. A recombinant replication competent retrovirus that is resistant to inactivation by human APOBEC by engineering codons in a retroviral polynucleotide susceptible to APOBEC hypermutation to a non-susceptible codon.

48. The recombinant replication competent retrovirus of claim 47, wherein a codon susceptible to APOBEC hypermutation encodes a tryptophan amino acid.

49. The recombinant replication competent retrovirus of claim 47 or 48, wherein the retrovirus comprises an IRES cassette, promoter cassette and/or 2A peptide cassette downstream of the env gene.

50. Use of a retrovirus of claim 1 or 13 in the manufacture of a medicament for treating a cell proliferative disorder, wherein the subject is to be contacted with said retrovirus under conditions such that the heterologous polynucleotide is expressed and wherein the heterologous polynucleotide encodes a protein the converts a prodrug to a cytotoxic drug.