Preferably, in immunotherapy, lentiviral vectors provide effective expression of the transgene that elicits a desired specific immune response. This requires that the expression is at a high level in APCs, such as dendritic cells.

It is also preferable that the cells transduced by the lentiviral vectors are eliminated by the immune response to provide a higher degree of safety. That is, the immune response generated against the transgene can elicit an immune response in the host sufficient to eliminate the cells that are transduced by the lentiviral vectors. The elimination of transduced cells eliminates the persistence of the lentiviral vector in the host, and possible secondary effects of the vector. In order for the transduced cells to be eliminated, expression is required in non-dendritic cells at a level that allows elimination by the immune response.

At the same time, the promoter should maximize immune stimulation through the key cells (i.e., dendritic cells) involved in the activation of naïve and memory T cells, and should minimize the risk of insertional mutagenesis and genotoxicity in stem cells, leading to malignancies. Thus, the promoter should have sufficiently high activity in dendritic and other cells, but not contain an enhancer. Based on these criteria, viral promoters, such as the CMV promoter, are not ideal because of the presence of strong enhancers. These criteria are summarized as follows:

1. high expression in dendritic cells to induce maximal immune responses;
2. expression in other transduced cell types sufficient for elimination by the induced immune response; and
3. lack of an enhancer element to avoid insertional effects.

The vector should be capable of being generated at high titers to maximize delivery and expression, while minimizing contaminants.

Thus, a need exists in the art for improved vectors. The present invention fulfils these needs in the art.

SUMMARY

The disclosure encompasses compositions comprising lentiviral vectors and methods of making and using the vectors. In one aspect, the present invention refers to a method for producing a lentiviral vector comprising a transgene sequence under control of an MHC class I or β2 microglobulin promoter, comprising inserting 300-1100 nucleotides of an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence into a lentiviral vector, the MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, or SEQ ID NO:36. In one example, disclosed is a method for producing a lentiviral vector comprising a transgene sequence under control of an MHC class I or β2 microglobulin promoter, comprising inserting 300-1100 nucleotides of an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence into a lentiviral vector, the MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence being selected from the group consisting of β2 microglobulin upstream promoter sequence, HLA-A2 upstream promoter sequence, HLA-B7 upstream promoter sequence, HLA-E upstream promoter sequence and HLA-DRα upstream promoter sequence. In one example, the disclosure encompasses a lentiviral vector comprising an MHC class I, MHC class II, or β2

microglobulin upstream promoter sequence, preferably further comprising an MHC class I or β2 microglobulin promoter.

The disclosure encompasses methods for producing a lentiviral vector comprising inserting at least 300 nucleotides, preferably 300-400, 300-600 or 300-1100 nucleotides, of an MHC class I, MHC class II, or β2 microglobulin upstream promoter sequence into a lentiviral vector. In one example, disclosed herein is a method for producing a lentiviral vector comprising inserting 300-1100 nucleotides of an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence into a lentiviral vector.

Preferably, the upstream promoter sequence is inserted upstream of an MHC class I or β2 microglobulin promoter. Most preferably, the upstream promoter sequence is inserted in the same orientation as the MHC class I or β2 microglobulin promoter.

In some examples, the upstream promoter sequence is an MHC class I upstream promoter sequence. In some examples, the upstream promoter sequence is a β2 microglobulin upstream promoter sequence, preferably comprising SEQ ID NO:1 or SEQ ID NO:27.

In some examples, the promoter is an MHC class I promoter. In some examples, the promoter is a β2 microglobulin promoter.

The disclosure encompasses a lentiviral vector comprising at least 300 nucleotides, preferably 300-400, 300-600 or 300-1100 nucleotides, of an MHC class I, MHC class II, or β2 microglobulin upstream promoter sequence. In one example, disclosed herein is a lentiviral vector comprising 300-1100 nucleotides of an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence, and a transgene sequence under control of an MHC class I or β2 microglobulin promoter, the MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence being selected from the group consisting of β2 microglobulin upstream promoter sequence, HLA-A2 upstream promoter sequence, HLA-B7 upstream promoter sequence, HLA-E upstream promoter sequence and HLA-DRα upstream promoter sequence. In another example, disclosed herein is a lentiviral vector comprising 300-1100 nucleotides of an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence. In yet another example, the present invention refers to a lentiviral vector as disclosed herein for use as a medicament or vaccine. In another aspect, the present invention refers to a lentiviral vector comprising 300-1100 nucleotides of an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence, and a transgene sequence under control of an MHC class I or β2 microglobulin promoter, the MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, or SEQ ID NO:36.

Preferably, the upstream promoter sequence is upstream of an MHC class I or β2 microglobulin promoter. Most preferably, the upstream promoter sequence is in the same orientation as the MHC class I or β2 microglobulin promoter.In some examples, the upstream promoter sequence is an MHC class I upstream promoter sequence. In some examples, the upstream promoter sequence is a β2 microglobulin upstream promoter sequence, preferably comprising SEQ ID NO:1 or SEQ ID NO:27.

In some examples, the promoter is an MHC class I promoter. In some examples, the promoter is a β2 microglobulin promoter. Preferably, the MHC class I promoter is an HLA-A2 promoter, HLA-B7 promoter, or an HLA-E promoter. Preferably, the upstream promoter sequence is an HLA-A2, HLA-B7, or an HLA-E, or HLA-DRα upstream promoter sequence.

Preferably, the lentiviral vector comprises a lentiviral cPPT/CTS sequence. Preferably, the lentiviral vector comprises a lentiviral Ψ (psi) sequence.

The disclosure encompasses an isolated host cell comprising a lentiviral vector as described herein. The disclosure encompasses a lentiviral vector as described herein for use as a medicament or vaccine, particularly for gene therapy. In a further aspect, the present invention refers to an isolated host cell comprising the lentiviral vector as described herein. In another aspect, the present invention refers to a lentiviral vector as disclosed herein for use as a medicament or vaccine.

In preferred examples, the upstream promoter sequence comprises the nucleotide sequence of any one of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, or SEQ ID NO:36.

BRIEF DESRIPTION OF THE DRAWINGS

Figure 1 A, B, and C depict schematic representations of (A) the β2m promoter (promoter region and upstream chromosomal region), (B) the MHC class I promoter, and (C) the MHC class II promoter.

Figure 2 depicts production yields of various lentiviral constructs, with or without the β2m upstream promoter sequence cloned upstream of various promoters. The β2m upstream promoter sequence was cloned upstream of various promoters by fusion PCR. The resulting lentiviral vectors were produced and used to transduce HEK-293T cells, and the percentage of transduced cells were evaluated by specific qPCR.

Figure 3 depicts production yields of various lentiviral constructs, with or without the β2m upstream promoter sequence cloned upstream of various promoters, in direct or reverse orientation. The β2m upstream promoter sequence was cloned upstream of various promoters by fusion PCR, either in direct (5'-3') or reverse (3'-5') orientation. The resulting lentiviral vectors were produced and used to transduce HEK-293T cells, and the percentage of transduced cells were evaluated by specific qPCR.

Figure 4 depicts production yields of various lentiviral constructs, with or without the β2m upstream promoter sequence cloned downstream of the transgene (GFP), in direct or reverse orientation. The β2m upstream promoter sequence was cloned downstream of the transgene (GFP) of lentiviral constructs harboring various promoters, either in direct (5'-3') or reverse (3'-5') orientation.

CLAIMS

We claim:

1. A method for producing a lentiviral vector comprising a transgene sequence under control of an MHC class I or β2 microglobulin promoter, comprising inserting 300-1100 nucleotides of an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence into a lentiviral vector,

the MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, or SEQ ID NO:36.

2. The method of claim 1, wherein the upstream promoter sequence is inserted in the same orientation as the MHC class I or β2 microglobulin promoter.

3. The method of claim 1 or 2, wherein the upstream promoter sequence is an MHC class I upstream promoter sequence selected from HLA-A2 upstream promoter sequence, HLA-B7 upstream promoter sequence and HLA-E upstream promoter sequence.

4. The method of claim 1 or 2, wherein the upstream promoter sequence is a β2 microglobulin upstream promoter sequence.

5. The method of claim 4, wherein the upstream promoter sequence comprises the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:27.

6. The method of any one of claims 1-5, wherein the promoter is an MHC class I promoter.

7. The method of any one of claims 1-5, wherein the promoter is a β2 microglobulin promoter.

8. A lentiviral vector comprising:

- 300-1100 nucleotides of an MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence, and
- a transgene sequence under control of an MHC class I or β2 microglobulin promoter,

the MHC class I, MHC Class II, or β2 microglobulin upstream promoter sequence comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4,

SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, or SEQ ID NO:36.

9. The lentiviral vector of claim 8, wherein the upstream promoter sequence is in the same orientation as the MHC class I or β2 microglobulin promoter.

10. The lentiviral vector of claim 8 or 9, wherein the upstream promoter sequence is an MHC class I upstream promoter sequence selected from HLA-A2 upstream promoter sequence, HLA-B7 upstream promoter sequence and HLA-E upstream promoter sequence.

11. The lentiviral vector of claim 8 or 9, wherein the upstream promoter sequence is a β2 microglobulin upstream promoter sequence.

12. The lentiviral vector of claim 11, wherein the upstream promoter sequence comprises the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:27.

13. The lentiviral vector of any one of claims 8-12, wherein the promoter is an MHC class I promoter.

14. The lentiviral vector of any one of claims 8-12, wherein the promoter is a β2 microglobulin promoter.

15. The lentiviral vector of claim 13, wherein the MHC class I promoter is an HLA-A2 promoter.

16. The lentiviral vector of claim 13, wherein the MHC class I promoter is an HLA-B7 promoter.

17. The lentiviral vector of claim 13, wherein the MHC class I promoter is an HLA-E promoter.

18. The lentiviral vector of claim 13, wherein the MHC class I promoter is an HLA-DRα promoter.

19. The lentiviral vector of any of claims 8-10 or 13-18, wherein the upstream promoter sequence is an HLA-E upstream promoter sequence.

20. The lentiviral vector of any one of claims 8-19, comprising a lentiviral cPPT/CTS sequence.

21. The lentiviral vector of any one of claims 8-19, comprising a lentiviral Ψ (psi) sequence.

22. The lentiviral vector according to claim 8, wherein the upstream promoter sequence has a nucleotide sequence selected from the group consisting of

SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, and SEQ ID NO:36.

23. The lentiviral vector according to claim 22, wherein the upstream promoter sequence has a nucleotide sequence selected from the group consisting of SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:35, and SEQ ID NO:36.

24. An isolated host cell comprising the lentiviral vector according to any one of claims8-23 and 26.

25. A lentiviral vector according to any one of claims 8-23 for use as a medicament or vaccine.

26. The method or lentiviral vector of claim 1 or 8, wherein the upstream promoter sequence comprises the nucleotide sequence of any one of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, or SEQ ID NO:36.

27. The method or lentiviral vector of claim 1 or 8, wherein the upstream promoter sequence comprises a nucleotide sequence selected from the group consisting of any one of SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, or SEQ ID NO:36.