**[0005]** Despite the efforts to date, there is a need for improved oncolytic viruses that, in particular, exhibit tumor-selective replication, viral mediated lysis, and/or therapeutic transgene expression for treating cancers and hyperproliferative disorders in human patients.

## SUMMARY

**[0006]** The present disclosure is based, in part, upon the discovery that, for certain viral promoters, the TATA and/or CAAT box, while necessary to drive transcription in normal, healthy cells, is dispensable for active transcription in cancerous cells.

**[0006a]** In one aspect, the present disclosure refers to a recombinant virus comprising: (i) a modified TATA box-based promoter operably linked to a gene, wherein the modified TATA box-based promoter lacks a functional TATA box and permits selective expression of the gene in a hyperproliferative cell; and/or (ii) a modified CAAT box-based promoter operably linked to a gene, wherein the modified CAAT box-based promoter lacks a functional CAAT box and permits selective expression of the gene in a hyperproliferative cell; the modified TATA box-based promoter is an early gene promoter; the modified CAAT box-based promoter is an early gene promoter; and the modification included in the modified TATA box-based promoter or CAAT box-based promoter does not comprise an addition, of or a substitution with, a separate, functional promoter sequence.

**[0006b]** In another aspect, the present disclosure refers to a pharmaceutical composition comprising the recombinant virus disclosed herein and at least one pharmaceutically acceptable carrier or diluent.

**[0006c]** In a further aspect, the present disclosure refers to a method of expressing a therapeutic transgene in a target cell comprising exposing the cell to an effective amount of the recombinant virus disclosed herein to express the target transgene.

**[0006d]** In another aspect, the present disclosure refers to a use of a recombinant virus disclosed herein in the manufacture of a medicament for treating cancer.

**[0006e]** In a further aspect, the present disclosure refers to a use of a recombinant virus disclosed herein in the manufacture of a medicament for treating hyperproliferative disease.

**[0006f]** In another aspect, the present disclosure refers to a method of engineering an oncolytic virus, the method comprising modifying a viral TATA box-based promoter operably linked to a gene such that the modified TATA box-based promoter lacks a functional TATA box and permits selective expression of the gene in a hyperproliferative cell and/or modifying a viral CAAT box-based promoter operably linked to a gene such that the modified CAAT box-

based promoter lacks a functional CAAT box and permits selective expression of the gene in a hyperproliferative cell; the modified TATA box-based promoter is an early gene promoter; the modified CAAT box-based promoter is an early gene promoter; and the modification included in the modified TATA box-based promoter or CAAT box-based promoter does not comprise an addition, of or a substitution with, a separate, functional promoter sequence.

**[0006g]** In a further aspect, the present disclosure refers to an isolated nucleic acid comprising a nucleotide sequence selected from SEQ ID NO: 3, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22 and SEQ ID NO: 23.

**[0006h]** In another aspect, the present disclosure refers to a recombinant virus disclosed herein for use in treating cancer or hyperproliferative disease.

**[0007]** In one example, the present disclosure provides a recombinant virus comprising: (i) a modified TATA box-based promoter operably linked to a gene, wherein the modified TATA box-based promoter lacks a functional TATA box and permits selective expression of the gene in a hyperproliferative and/or non-growth arrested cell; and/or (ii) a modified CAAT box-based promoter operably linked to a gene, wherein the modified CAAT box-based promoter lacks a functional CAAT box and permits selective expression of the gene in a hyperproliferative and/or non-growth arrested cell.

**[0008]** In another example, the present disclosure provides a recombinant virus comprising a modified TATA box-based promoter operably linked to a gene, wherein the modified TATA box-based promoter lacks a functional TATA box and permits selective expression of the gene in a hyperproliferative and/or non-growth arrested cell.

**[0009]** In another example, the present disclosure provides a recombinant virus comprising a modified CAAT box-based promoter operably linked to a gene, wherein the modified CAAT box-based promoter lacks a functional CAAT box and permits selective expression of the gene in a hyperproliferative and/or non-growth arrested cell.

**[0010]** In certain examples of any of the foregoing recombinant viruses, the recombinant virus is selected from a recombinant vaccinia virus, adenovirus, adeno-associated virus (AAV), herpes simplex virus 1 (HSV1), myxoma virus, reovirus, poliovirus, vesicular stomatitis virus (VSV), measles virus (MV), and Newcastle disease virus (NDV). In certain examples, the recombinant virus is an adenovirus, *e.g.*, a type 5 adenovirus (Ad5) or a type 35 adenovirus (Ad35), *e.g.,* a type 5 adenovirus. In certain examples, the modified TATA

What is claimed is:

1. A recombinant virus comprising:

(i) a modified TATA box-based promoter operably linked to a gene, wherein the modified TATA box-based promoter lacks a functional TATA box and permits selective expression of the gene in a hyperproliferative cell; and/or

(ii) a modified CAAT box-based promoter operably linked to a gene, wherein the modified CAAT box-based promoter lacks a functional CAAT box and permits selective expression of the gene in a hyperproliferative cell, wherein the recombinant virus is selected from a recombinant vaccinia virus, adenovirus, adeno-associated virus (AAV), herpes simplex virus 1 (HSV1), myxoma virus, reovirus, poliovirus, vesicular stomatitis virus (VSV), measles virus (MV), and Newcastle disease virus (NDV); the modified TATA box-based promoter is an early gene promoter; the modified CAAT box-based promoter is an early gene promoter; and the modification included in the modified TATA box-based promoter or CAAT box-based promoter does not comprise an addition, of or a substitution with, a separate, functional promoter sequence.

2. The recombinant virus of claim 1, wherein the recombinant virus is a recombinant adenovirus.

3. The recombinant virus of claim 2, wherein the recombinant virus is selected from a type 5 adenovirus and a type 35 adenovirus.

4. The recombinant virus of any one of claims 1-3, wherein the modified TATA box-based promoter is an E1a promoter, E1b promoter, or E4 promoter.

5. The recombinant virus of any one of claims 1-4, wherein the modification included in the modified TATA box-based promoter comprises a deletion of the entire TATA box.

6. The recombinant virus of any one of claims 1-5, wherein the virus comprises a deletion of nucleotides corresponding to -27 to -24 of the E1a promoter.

7. The recombinant virus of claim 6, wherein the virus comprises a deletion of nucleotides corresponding to -31 to -24 of the E1a promoter.

8. The recombinant virus of claim 7, wherein the virus comprises a deletion of nucleotides corresponding to -44 to +54 of the E1a promoter.

9. The recombinant virus of claim 8, wherein the virus comprises a deletion of nucleotides corresponding to -146 to +54 of the E1a promoter.

10. The recombinant virus of any one of claims 1-9, wherein the virus comprises a deletion of nucleotides corresponding to 472 to 475 of the Ad5 genome (SEQ ID NO: 8).

11. The recombinant virus of claim 10, wherein the virus comprises a deletion of nucleotides corresponding to 468 to 475 of the Ad5 genome (SEQ ID NO: 8).

12. The recombinant virus of claim 11, wherein the virus comprises a deletion of nucleotides corresponding to 455 to 552 of the Ad5 genome (SEQ ID NO: 8).

13. The recombinant virus of claim 12, wherein the virus comprises a deletion of nucleotides corresponding to 353 to 552 of the Ad5 genome (SEQ ID NO: 8).

14. The recombinant virus of any one of claims 1-13, wherein the virus comprises a polynucleotide deletion that results in the virus comprising the sequence CTAGGACTG (SEQ ID NO: 7), AGTGCCCG (SEQ ID NO: 12) and/or TATTCCCG (SEQ ID NO: 13).

15. The recombinant virus of any one of claims 1-14, wherein the modified CAAT box-based promoter is an E1a promoter, E1b promoter, or E4 promoter.

16. The recombinant virus of any one of claims 1-15, wherein the modification included in the modified CAAT box-based promoter comprises a deletion of the entire CAAT box.

17. The recombinant virus of any one of claims 1-16, wherein the virus comprises a deletion of nucleotides corresponding to -76 to -68 of the E1a promoter.

18. The recombinant virus of any one of claims 1-17, wherein the virus comprises a deletion of nucleotides corresponding to 423 to 431 of the Ad5 genome (SEQ ID NO: 8).

19. The recombinant virus of any one of claims 1-18, wherein the virus comprises a polynucleotide deletion that results in the virus comprising the sequence TTCCGTGGCG (SEQ ID NO: 14).

20. The recombinant virus of any one of claims 1-19, wherein the virus comprises a deletion of nucleotides corresponding to 477 to 484 of the Ad35 genome (SEQ ID NO: 24).

21. The recombinant virus of any one of claims 1-20, further comprising a nucleotide sequence encoding a therapeutic transgene.

22. The recombinant virus of claim 21, wherein the adenovirus comprises an E1b-19K and an E1b-55K start site, and wherein the nucleotide sequence encoding the therapeutic transgene is inserted between the start site of E1b-19K and the start site of E1b-55K.

23. The recombinant virus of any one of claims 1-22, wherein the recombinant virus selectively replicates in a hyperproliferative cell.

24. The recombinant virus of any one of claims 1-23, wherein the recombinant virus selectively has cytolytic activity in a hyperproliferative cell.

25. The recombinant virus of any one of claims 1-24, wherein the recombinant virus selectively expresses E1a and/or E1b in a hyperproliferative cell.

26. The recombinant virus of any one of claims 21-25, wherein the recombinant virus selectively expresses the therapeutic transgene in a hyperproliferative cell.

27. The recombinant virus of any one of claims 1-25, wherein the hyperproliferative cell is a cancer cell, endothelial cell, epidermal cell, fibroblast, and/or immune cell.

28. A pharmaceutical composition comprising the recombinant virus of any one of claims 1-27 and at least one pharmaceutically acceptable carrier or diluent.

29. A method of expressing a therapeutic transgene in a target cell comprising exposing the cell to an effective amount of the recombinant virus of any one of claims 21-27 to express the target transgene.

30. Use of a recombinant virus of any one of claims 1-27 in the manufacture of a medicament for treating cancer.

31. Use of a recombinant virus of any one of claims 1-27 in the manufacture of a medicament for treating hyperproliferative disease.

32. A method of engineering an oncolytic virus, the method comprising modifying a viral TATA box-based promoter operably linked to a gene such that the modified TATA box-based promoter lacks a functional TATA box and permits selective expression of the gene in a hyperproliferative cell and/or modifying a viral CAAT box-based promoter operably linked to a gene such that the modified CAAT box-based promoter lacks a functional CAAT box and permits selective expression of the gene in a hyperproliferative cell, wherein the recombinant virus is selected from a recombinant vaccinia virus, adenovirus, adeno-associated virus (AAV), herpes simplex virus 1 (HSV1), myxoma virus, reovirus, poliovirus, vesicular stomatitis virus (VSV), measles virus (MV), and Newcastle disease virus (NDV); the modified TATA box-based promoter is an early gene promoter; the modified CAAT box-based promoter is an early gene promoter; and the modification included in the modified TATA box-based promoter or CAAT box-based promoter does not comprise an addition, of or a substitution with, a separate, functional promoter sequence.

33. An isolated nucleic acid comprising a nucleotide sequence selected from SEQ ID NO: 3, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22 and SEQ ID NO: 23.

34. A recombinant virus of any one of claims 1-27 for use in treating cancer or hyperproliferative disease.