not require the addition of any prodrug, that also contributes to the ease of use and safety of the present invention.

Based on these results, one may anticipate that the oncolytic viruses of the invention may be successfully used as an alternative to the other existing oncolytic viruses, and may have a better efficiency profile. The oncolytic viruses of the invention can also be exploited in combination with additional anticancer therapy/ies.

SUMMARY OF THE INVENTION

In one aspect, the present disclosure refers to an oncolytic virus comprising a nucleotide sequence encoding a cytidine deaminase (CDAse) polypeptide. In another aspect, the present disclosure refers to a process for preparing an oncolytic virus, in which process: (i) An oncolytic virus as disclosed herein is introduced into a producer cell; (ii) said producer cell is cultured under conditions which are appropriate for enabling said oncolytic virus to be produced, and; (iii) said oncolytic virus is recovered from the cell culture. In another aspect, the present disclosure refers to a composition comprising the oncolytic virus as disclosed herein, or prepared as disclosed herein, which further comprises a pharmaceutically acceptable vehicle, wherein said composition preferably comprises a dose of oncolytic virus comprised between 10^6 and $5x10^9$ PFU, and wherein said oncolytic virus is preferably formulated for parenteral route administration with a preference for intravenous or intratumoral route. In yet another aspect, the present disclosure refers to the use of the oncolytic virus of the present disclosure, or prepared according to the present disclosure, or the composition as disclosed herein, in the manufacture of a medicament for the prophylaxis or the treatment of a proliferative disease.

One example of the disclosure relates to an oncolytic virus comprising a nucleotide sequence encoding a cytidine deaminase (CDAse) polypeptide.

In one example, the oncolytic virus of the disclosure is a poxvirus belonging to the Orthopoxvirus and especially a vaccinia virus or a cowpoxvirus.

In another example, the oncolytic virus of the disclosure is defective in the thymidine kinase (TK-) encoding J2R locus.

In yet another example, the oncolytic virus of the disclosure is defective in the I4L and/or F4L locus (alternatively or in combination with a defective TK- locus).

In a further example, said oncolytic virus further comprises a nucleic acid of interest, in particular a gene encoding a therapeutic molecule different from CDAse, such as an immunostimulatory polypeptide, an antigen, a permease or any other molecules of interest.

In another example, the present disclosure further provides a composition comprising the oncolytic virus of the present disclosure and a pharmaceutically acceptable vehicle. In one example, the oncolytic virus is preferably formulated for intra-venous or intra-tumoral administration.

In a further example, the present disclosure also concerns a process for preparing the oncolytic virus, comprising at least the steps of introducing said oncolytic virus into a producer cell, culturing the producer cell under conditions that are appropriate for enabling said oncolytic virus to be produced and recovering the produced virus from the cell culture. Optionally, the recovered oncolytic virus can be purified at least partially.

In still a further example, the present disclosure provides the oncolytic virus or the composition of the disclosure, for use for the prophylaxis and/or the treatment of a disease. In one example, said disease is a proliferative disease such as cancers, tumors and restenosis. Said cancer is preferably selected from the group consisting in renal cancer, bladder cancer, prostate cancer, breast cancer, colorectal cancer, lung cancer, hepatic cancer, gastric cancer, pancreatic cancer, melanoma, ovarian cancer and glioblastoma, and especially metastatic ones. In another example, said disease is a disease associated with an increased osteoclast activity like rheumatoid arthritis and osteoporosis.

In yet a further example, the present disclosure provides a method of treating a disease which comprises the administration into a host organism in need thereof of a therapeutically effective amount of the oncolytic virus or the composition of the disclosure. Said method of treatment may be used in conjunction with one or more additional therapies such as ones selected from the group consisting of surgery, radiotherapy, chemotherapy, cryotherapy, hormonal therapy, toxin therapy, immunotherapy or cytokine therapy.

In a particular example, cytidine deaminase is used as a therapeutic gene, and not as a suicide gene, insofar as no prodrug is required to obtain a therapeutic effect in the presence of cytidine deaminase. Specifically, capecitabine (Xeloda®) is transformed into 5'-DFCR (Deoxy-5-fluorocytidine), which is then transformed by cytidine deaminase into 5'-DFUR (5'-Deoxy-5-fluorouridine), which is finally transformed into toxic 5-FU (5-fluoro-uracile). This cascade of reaction is not required to obtain cytolytic activity of the oncolytic virus of the present disclosure. Accordingly, the oncolytic virus of the present disclosure is not used in combination with capecitabine and the method of treatment according to the present disclosure does not comprise any step of administering a precursor of a drug such as capecitabine. Usually, nucleoside pool modulators other than CDAse are used in combination with a prodrug which is transformed in a cytotoxic drug by the action of said nucleoside pool modulators. Such an enzymatic transformation generally contributes to the anti-tumor effect. For example, a method of treating relying on an oncolytic virus encoding a cytosine deaminase provides an effective anti-tumor effect when administered in conjunction with the 5-fluorocytosine (5-FC) which is transformed in cytotoxic 5-fluorouracile (5-FU) in the infected cells and contributes to their lysis.

CLAIMS

1. An oncolytic virus comprising a nucleotide sequence encoding a cytidine deaminase (CDAse) polypeptide.

2. The oncolytic virus of claim 1, wherein said CDAse polypeptide is selected from the group consisting of CDA, CDD, EC 3.5.4.5, APOBEC, AID and cytidine deaminase-like proteins.

3. The oncolytic virus of claim 2, wherein said APOBEC is selected from the group consisting of APOBEC1, APOBEC2, APOBEC3A, APOBEC3B, APOBEC3C, APOBEC3D, APOBEC3E, APOBEC3F, APOBEC3G, APOBEC3H and APOBEC4, and preferably wherein said APOBEC is APOBEC2.

4. The oncolytic virus of claim 1 or 2, wherein said CDAse is a yeast cytidine deaminase (CDD1), preferably a CDD1 comprising an amino acid sequence having at least 80%, preferably greater than 90%, and more preferably greater than 95% identity with SEQ ID NO:1, or a human cytidine deaminase (hCD), preferably a hCD comprising an amino acid sequence having at least 80%, preferably greater than 90%, and more preferably greater than 95% identity with SEQ ID NO:2.

5. The oncolytic virus of claim 3, wherein said APOBEC2 comprises an amino acid sequence having at least 80%, preferably greater than 90%, and more preferably greater than 95% identity with SEQ ID NO:3.

6. The oncolytic virus of any of claims 1 to 5, wherein said CDAse polypeptide catalyses the deamination of cytidine-based components, preferably cytidine-based components that are free or complexed, to uridine-based components, like cytidine to uridine and deoxycytidine (dC) to deoxyuridine (dU).

7. The oncolytic virus of any of claims 1 to 6, wherein said virus is selected from the group consisting of poxvirus, herpes simplex virus (HSV), reovirus, Seneca Valley virus (SVV), vesicular stomatitis virus (VSV), Newcastle disease virus (NDV), morbillivirus, retrovirus, adenovirus, measles virus, foamy virus, alpha virus, lentivirus, influenza virus, Sinbis virus, myxoma virus, rhabdovirus, picornavirus, coxsackievirus, parvovirus or the like.

8. The oncolytic virus of claim 7, wherein said virus is a poxvirus belonging to the Orthopoxvirus genus, wherein said poxvirus preferably belongs to the vaccinia virus (VV) species, preferably selected from the group consisting of Copenhagen, Wyeth and Western Reserve (WR), or to the cowpox virus species.

9. The oncolytic virus of claim 7 or 8, wherein said virus is defective in the J2R locus and/or wherein said virus is defective in the I4L and/or F4L locus, wherein said virus is optionally a vaccinia virus defective in viral TK and RR encoded activities which encodes a human or yeast CDAse, such as VVTK-RR-/CDD1, VVTK-RR-/hCD or VVTK-RR-/APOBEC2.

10. The oncolytic virus of any of claims 7 to 9, wherein said virus further comprises one or more nucleic acid(s) of interest, said nucleic acid of interest preferably encoding an immunostimulatory polypeptide, an antigen or a permease.

11. The oncolytic virus of any of claims 1 to 10, wherein said virus further comprises the elements necessary for the expression of said cytidine deaminase and said one or more nucleic acid(s) of interest.

12. A process for preparing an oncolytic virus, in which process:

    (i) An oncolytic virus according to anyone of claims 1 to 11 is introduced into a producer cell;

    (ii) said producer cell is cultured under conditions which are appropriate for enabling said oncolytic virus to be produced, and;

(iii) said oncolytic virus is recovered from the cell culture.

13. A composition comprising the oncolytic virus of any one of claims 1 to 11, or prepared according to claim 12, which further comprises a pharmaceutically acceptable vehicle, wherein said composition preferably comprises a dose of oncolytic virus comprised between 10^6 and $5x10^9$ PFU, and wherein said oncolytic virus is preferably formulated for parenteral route administration with a preference for intravenous or intratumoral route.

14. The oncolytic virus of any one of claims 1 to 11, or prepared according to claim 12, or the composition of claim 13, for use in the treatment of a disease or a pathologic condition in a subject in need thereof.

15. Use of the oncolytic virus of any one of claims 1 to 11, or prepared according to claim 12, or the composition of claim 13, in the manufacture of a medicament for the prophylaxis or the treatment of a proliferative disease.

16. The use of claim 15, wherein the proliferative disease is cancer, tumor and restenosis.

17. The use of claim 16, wherein the cancer is a gastro-intestinal cancer, preferably comprising a cancer of oesophagus, gallbladder, liver, pancreas, stomach, small intestine, bowel (large intestine or colon and rectum), or anus.

18. The use of any one of claims 15 to 17, further comprising that one or more substances effective in anticancer therapy are to be administered.