2000). Thorne et al. showed that compared with other vaccinia strains, WR strain has the highest burst ratio in tumor cell lines relative to normal cells (Thorne et al., 2007). The inventors selected a derivative of this strain, vaccinia E3LΔ83N WR strain as their vector for further modification.

Human Flt3L (Fms-like tyrosine kinase 3 ligand), a type I transmembrane protein that stimulates the proliferation of bone marrow cells, was cloned for in 1994 (Lyman et al., 1994). The use of hFlt3L has been explored in various preclinical and clinical settings including stem cell mobilization in preparation for bone marrow transplantation, cancer immunotherapy such as expansion of dendritic cells, as well as an vaccine adjuvant. Recombinant human Flt3L (rhuFlt3L) has been tested in more than 500 human subjects and is bioactive, safe, and well tolerated (Fong et al., 1998; Maraskovsky et al., 2000; Shackleton et al., 2004; He et al., 2014; Anandasabapathy et al., 2015). Much progress has been recently made in the understanding of the critical role of the growth factor Flt3L in the development of DC subsets, including $CD8\alpha^+/CD103^+$ DCs and pDCs (McKenna et al., 2000; Waskow et al., 2008; Liu et al., 2007; 2009; Naik et al., 2006; Ginhoux et al., 2009).

SUMMARY OF THE DISCLOSURE

In one aspect, there is provided a use of a composition comprising one or more of a thymidine kinase-deficient vaccinia virus or virus construct lacking Z-DNA-binding domain of E3L gene (E3LΔ83N-TK^-), a recombinant E3LΔ83N-TK^- virus or viral construct expressing human Fms-like tyrosine kinase 3 ligand (E3LΔ83N-TK^--hFlt3L), and a recombinant E3LΔ83N-TK^- virus or viral construct expressing granulocyte macrophage colony stimulating factor (E3LΔ83N-TK^--GM-CSF), wherein each is replication competent or in inactivated form, and a pharmaceutically acceptable excipient, in the manufacture of a medicament, for treating a malignant solid tumor in a subject in need thereof.

In another aspect, there is provided a E3LΔ83N-TK^--hFlt3L virus which is replication competent or in inactivated form.

In the present disclosure, the inventors generated recombinant E3LΔ83N-TK^- virus and also built a construct of the same virus expressing human Flt3L, with the goal of

delivering this growth factor to the tumor microenvironment to facilitate recruitment, differentiation and function of immune cells, including $CD103^+/CD8\alpha$ dendritic cells (DCs). A similar goal was pursued with E3LΔ83N-TK^- expressing GM-CSF. However, experiments were also conducted with "naked" E3LΔ83N-TK^- and with inactivated (specifically heat-inactivated) virus and viral constructs with favorable results, especially when administered in conjunction with checkpoint blockade inhibition therapy.

The present disclosure concerns methods and compositions for the treatment of solid tumors using a replication competent attenuated vaccinia virus either alone or in combination with immune checkpoint blocking agents. In some embodiments, methods and compositions involve deletion of Z-DNA-binding domain of E3Land thymidine kinase (TK) gene from wild-type vaccinia (Western Reserve strain, WR) and the expression of GM-CSF or Flt3L under vaccinia promoter.

CLAIMS

1. Use of a composition comprising one or more of:

a thymidine kinase-deficient vaccinia virus or virus construct lacking Z-DNA-binding domain of E3L gene (E3LΔ83N-TK$^-$),

a recombinant E3LΔ83N-TK$^-$ virus or viral construct expressing human Fms-like tyrosine kinase 3 ligand (E3LΔ83N-TK$^-$-hFlt3L), and

a recombinant E3LΔ83N-TK$^-$ virus or viral construct expressing granulocyte macrophage colony stimulating factor (E3LΔ83N-TK$^-$-GM-CSF),

wherein each is replication competent or in inactivated form, and a pharmaceutically acceptable excipient, in the manufacture of a medicament, for treating a malignant solid tumor in a subject in need thereof.

2. The use according to claim 1, wherein said composition comprises two or more of the E3LΔ83N-TK$^-$-hFlt3L, E3LΔ83N-TK$^-$, and E3LΔ83N-TK$^-$-GM-CSF viruses or viral constructs.

3. The use according to claim 1, wherein said composition comprises a E3LΔ83N-TK$^-$-hFlt3L virus which is replication competent or in inactivated form.

4. The use according to any one of the preceding claims, wherein the composition is intended for delivery to the cells of the tumor.

5. The use according to any one of the preceding claims, wherein the composition is to be administered intratumorally, intravenously, intraperitoneally, intracranially or via a combination of localized injection and a systemic injection.

6. The use according to any one of the preceding claims, wherein the tumor is primary or metastatic melanoma, breast carcinoma, or colon carcinoma.

7. The use according to any one of the preceding claims, wherein the virus is heat-inactivated.

8. The use according to any one of the preceding claims, wherein said composition is to be administered in an amount effective to accomplish one or more of the following: reduce the size of the tumor, delay growth of the tumor, inhibit growth of the tumor, inhibit metastasis or metastatic growth of the tumor, wherein the tumor includes tumor located at the site of administration and/or tumor located both at the site of administration and elsewhere in the body of the subject.

9. The use according to any one of the preceding claims, wherein said composition is to be administered in an amount effective to eradicate the tumor, wherein the tumor is located at the site of administration.

10. The use according to any one of the preceding claims, wherein the composition is to be administered in an amount effective to elicit in the subject an immune response against the tumor and any metastases thereof, upon local delivery to tumor cells of the subject.

11. The use according to claim 10, wherein the immune response includes one or more of the following:

oncolysis of tumor cells and release of tumor antigen;

an increase in cytotoxic CD8+ T cells within the tumor and/or in tumor-draining lymph nodes;

induction of maturation of dendritic cells infiltrating said tumor or circulating in remote locations within the patient's body through induction of type I IFN;

induction of effector CD4+ T cells in the subject recognizing tumor cells within the tumor and/or in tumor draining lymph nodes; and

increase of CD103+ dendritic cells in noninjected tumors of the subject.

12. The use according to any one of the preceding claims, wherein the composition is to be used in combination with other treatment modalities comprising surgery, chemotherapy, targeted therapy, or radiation.

13. The use according to any one of the preceding claims, wherein the composition further comprises an immune checkpoint blocking agent.

14. The use according to claim 13, wherein the immune checkpoint blocking agent comprises CTLA-4, CD80, CD86, PD-1, PDL1, PDL2, LAG3, B7-H3, B7-H4, TIM3, ICOS, II DLBCL inhibitors, BTLA, or any combination thereof.

15. The use according to claim 13, wherein the immune checkpoint blocking agent comprises ipilimumab, nivolumab, pembrolizumab, pidilizumab, AMP-224, MPDL3280A, BMS-936559, MEDI4736, MSB 00107180, or any combination thereof.

16. A E3LΔ83N-TK$^-$-hFlt3L virus which is replication competent or in inactivated form.