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(54) **Title:** COMBINATION THERAPY FOR TREATING CANCER WITH A POXVIRUS EXPRESSING A TUMOR ANTIGEN AND AN ANTAGONIST AND/OR AGONIST OF AN IMMUNE CHECKPOINT INHIBITOR

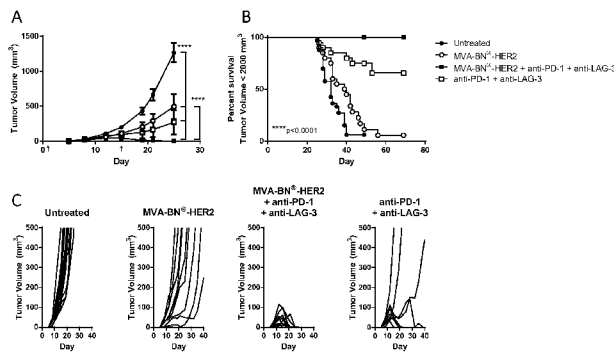
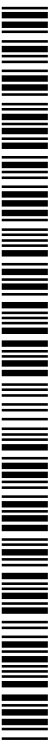


Figure 12

(57) **Abstract:** The present disclosure encompasses therapies, compositions, and methods for treatment of a human cancer patient using a recombinant poxvirus encoding a tumor-associated antigen in combination with one or more agonists or antagonists of immune checkpoint inhibitors.



UNITED STATES PATENT APPLICATION

FOR

**COMBINATION THERAPY FOR TREATING CANCER WITH
A POXVIRUS EXPRESSING A TUMOR ANTIGEN AND AN ANTAGONIST AND/OR
AGONIST OF AN IMMUNE CHECKPOINT INHIBITOR**

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**COMBINATION THERAPY FOR TREATING CANCER WITH
A POXVIRUS EXPRESSING A TUMOR ANTIGEN AND AN ANTAGONIST AND/OR
AGONIST OF AN IMMUNE CHECKPOINT INHIBITOR**

FIELD OF THE INVENTION

[001] The invention relates to the treatment of cancers using recombinant poxviruses encoding a tumor antigen. More particularly, the present invention is directed to the treatment of cancers using one or more recombinant poxviruses encoding a tumor antigen in combination with one or more agonists and/or antagonists of an immune checkpoint inhibitor.

BACKGROUND OF THE INVENTION

[002] Recombinant poxviruses have been used as vaccines for infectious organisms and, more recently, for tumors. Mastrangelo et al. *J Clin Invest.* 2000;105(8):1031–1034. Two of these poxvirus groups, avipoxvirus and orthopoxvirus, have been shown to be effective at battling tumors and have been involved with potential cancer treatments.

[003] One exemplary avipoxvirus species, fowlpox, has been shown to be a safe vehicle for human administrations as fowlpox virus enters mammalian cells and expresses proteins, but replicates abortively. Skinner et al. *Expert Rev Vaccines.* 2005 Feb;4(1):63-76. Additionally, the use of fowlpox virus as a vehicle for expression is being evaluated in numerous clinical trials of vaccines against cancer, malaria, tuberculosis, and AIDS. *Id.*

[004] Orthopoxviruses have been shown to be useful vectors for the administration of antigens to patients to induce an immune response against the antigen. Vaccinia, the most well-known of the orthopoxviruses, was used in the world-wide eradication of smallpox and has shown usefulness as a vector and/or vaccine. Recombinant Vaccinia Vector has been engineered

to express a wide range of inserted genes, including several tumor associated genes such as p97, HER-2/neu, p53 and ETA (Paoletti, et al., 1993).

[005] Another known orthopoxvirus is Modified Vaccinia Ankara (MVA) virus. MVA is related to vaccinia virus, a member of the genera Orthopoxvirus, in the family of Poxviridae. MVA was generated by 516 serial passages on chicken embryo fibroblasts of the Ankara strain of vaccinia virus (CVA) (for review see Mayr, A., et al. *Infection* 3, 6-14 (1975)). As a consequence of these long-term passages, the genome of the resulting MVA virus had about 31 kilobases of its genomic sequence deleted and, therefore, was described as highly host cell restricted for replication to avian cells (Meyer, H. et al., *J. Gen. Virol.* 72, 1031-1038 (1991)). It was shown in a variety of animal models that the resulting MVA was significantly avirulent (Mayr, A. & Danner, K., *Dev. Biol. Stand.* 41: 225-34 (1978)). Additionally, this MVA strain has been tested in clinical trials as a vaccine to immunize against the human smallpox disease (Mayr et al., *Zbl. Bakt. Hyg. I, Abt. Org. B* 167, 375-390 (1987); Stickl et al., *Dtsch. med. Wschr.* 99, 2386-2392 (1974)). In these human studies, MVA had diminished virulence or infectiousness as compared to vaccinia-based vaccines, while MVA still induced a good specific immune response.

[006] In the following decades, MVA was engineered for use as a viral vector for recombinant gene expression or as a recombinant vaccine (Sutter, G. et al., *Vaccine* 12: 1032-40 (1994)).

[007] Even though Mayr et al. demonstrated during the 1970s that MVA is highly attenuated and avirulent in humans and mammals, certain investigators have reported that MVA is not fully attenuated in mammalian and human cell lines since residual replication might occur in these cells. (Blanchard et al., *J Gen Virol* 79, 1159-1167 (1998); Carroll & Moss, *Virology*

238, 198-211 (1997); Altenberger, U.S. Pat. No. 5,185,146; Ambrosini et al., J Neurosci Res 55(5), 569 (1999)). It is assumed that the results reported in these publications have been obtained with various known strains of MVA, since the viruses used essentially differ in their properties, particularly in their growth behavior in various cell lines. Such residual replication is undesirable for various reasons, including safety concerns in connection with use in humans.

[008] Strains of MVA having enhanced safety profiles for the development of safer products, such as vaccines or pharmaceuticals, have been described. *See* U.S. Pat. Nos. 6,761,893 and 6,193,752. Such strains are capable of reproductive replication in non-human cells and cell lines, especially in chicken embryo fibroblasts (CEF), but are not capable of significant reproductive replication in certain human cell lines known to permit replication with known vaccinia strains. Such cell lines include a human keratinocyte cell line, HaCat (Boukamp et al. J Cell Biol 106(3): 761-71 (1988)), a human cervix adenocarcinoma cell line, HeLa (ATCC No. CCL-2), a human embryo kidney cell line, 293 (ECACC No. 85120602), and a human bone osteosarcoma cell line, 143B (ECACC No. 91112502). Such strains are also not capable of significant reproductive replication *in vivo*, for example, in certain mouse strains, such as the transgenic mouse model AGR 129, which is severely immune-compromised and highly susceptible to a replicating virus. *See* U.S. Pat. Nos. 6,761,893. One such MVA strain and its derivatives and recombinants, referred to as "MVA-BN," has been described. *See* U.S. Pat. Nos. 6,761,893 and 6,193,752, which are hereby incorporated by reference.

[009] MVA and MVA-BN have each been engineered for use as a viral vector for recombinant gene expression or as a recombinant vaccine. *See, e.g.*, Sutter, G. et al., Vaccine 12: 1032-40 (1994), U.S. Pat. Nos. 6,761,893 and 6,193,752.

[010] Certain approaches to cancer immunotherapy have included vaccination with tumor-associated antigens. In certain instances, such approaches have included use of a delivery system to promote host immune responses to tumor-associated antigens. In certain instances, such delivery systems have included recombinant viral vectors. *See, e.g.*, Harrop et al., *Front. Biosci.* 11:804-817 (2006); Arlen et al., *Semin. Oncol.* 32:549-555 (2005); Liu et al., *Proc. Natl. Acad. Sci. USA* 101 (suppl. 2):14567-14571 (2004).

[011] HER-2 is a tumor-associated antigen that is over-expressed in tumor cells of a number of cancer patients. Immunization with various HER-2 polypeptides has been used to generate an immune response against tumor cells expressing this antigen. *See, e.g.*, Renard et al., *J. Immunology* 171:1588-1595 (2003); Mittendorf et al., *Cancer* 106:2309-2317 (2006).

[012] An MVA encoding a HER-2 antigen, MVA-BN-HER2, has been shown to exert potent anti-tumor efficacy in a murine model of experimental pulmonary metastasis, despite a strong tumor-mediated immunosuppressive environment characterized by a high frequency of regulatory T cells (T_{reg}) in the lungs. Mandl et al., *Cancer Immunol Immunother* (2012) 61:19–29. The recombinant MVA was reported to induce strongly Th1-dominated HER-2-specific antibody and T-cell responses. *Id.* The anti-tumor activity was characterized by an increased infiltration of lungs with highly activated, HER-2-specific, CD8+CD11c+ T cells, and was accompanied by a decrease in the frequency of T_{reg} cells in the lung, resulting in a significantly increased ratio of effector T cells to T_{reg} cells. *Id.*

[013] MVA-BN-HER2 has also been shown to be safe and break tolerance to induce specific T and B cell responses in human clinical studies in a metastatic setting. Guardino et al., *Cancer Research*: December 15, 2009; Volume 69, Issue 24, Supplement 3.

[014] Trastuzumab (Herceptin) is a humanized monoclonal antibody (mAb) targeting the extra-cellular domain of HER2, and has shown clinical efficacy in HER2-positive breast cancer. Wang et al., *Cancer Res.* 2012 September 1; 72(17): 4417–4428. However, a significant number of patients fail to respond to initial trastuzumab treatment and many trastuzumab-responsive tumors develop resistance after continuous treatment. *Id.*

[015] Inhibitory receptors on immune cells are pivotal regulators of immune escape in cancer. Woo et al., *Cancer Res.*; 72(4); 917–27, 2011. Among these inhibitory receptors, CTLA-4 (cytotoxic T-lymphocyte-associated protein 4) serves as a dominant off-switch while other receptors such as PD-1 (programmed death 1, CD279) and LAG-3 (lymphocyte activation gene, CD223) seem to serve more subtle rheostat functions. *Id.*

[016] CTLA-4 is an immune checkpoint molecule, which is up-regulated on activated T-cells. Mackiewicz, *Wspolczesna onkol* 2012; 16 (5):363-370. An anti-CTLA4 mAb can block the interaction of CTLA-4 with CD80/86 and switch off the mechanism of immune suppression and enable continuous stimulation of T-cells by DCs. Two IgG mAb directed against CTLA-4, ipilimumab and tremelimumab, have been used in clinical trials in patients with melanoma. However, treatments with anti-CTLA-4 antibodies have shown high levels of immune-related adverse events. *Id.*

[017] Another human mAb modulating the immune system is BMS-936558 (MDX-1106) directed against the death-1 receptor (PD-1R), the ligand of which (PD-1L) can be directly expressed on melanoma cells. *Id.* PD-1R is a part of the B7:CD28 family of co-stimulatory molecules that regulate T-cell activation and tolerance, and thus anti-PD-1R can play a role in breaking tolerance. *Id.*

[018] Engagement of the PD-1/PD-L1 pathway results in inhibition of T-cell effector function, cytokine secretion and proliferation. Turnis et al., *OncoImmunology* 1:7, 1172-1174; 2012. High levels of PD-1 are associated with exhausted or chronically stimulated T cells. *Id.* Moreover, increased PD-1 expression correlates with reduced survival in cancer patients. *Id.*

[019] While these recent studies have suggested that PD-1 expression can be linked to survival rates in cancer, early studies with inhibition of PD-1 in treating cancers have shown a wide variety of adverse side effects. Mellman et al. *Nature* 2011; 480(7378): 480-489; see also Chow, *Am Soc Clin Oncol Educ Book*, 2013, "Exploring novel immune-related toxicities and endpoints with immune-checkpoint inhibitors in non-small cell lung cancer".

[020] LAG-3 is a negative regulatory molecule expressed upon activation of various lymphoid cell types. *Id.* LAG-3 is required for the optimal function of both natural and induced immunosuppressive Treg cells. *Id.*

[021] Combinatorial blockade of PD-1 and LAG-3 with monoclonal antibodies synergistically limited the growth of established tumors. Woo et al., *Cancer Res*; 72(4); 917-27, 2011. Although anti-LAG-3/anti-PD-1 combinatorial immunotherapy effectively cleared established Sa1N and MC38 tumors, this therapy was not effective against established B16 tumors. *Id.* Turnis et al. reported that their study, "highlighted the difficulty in predicting the outcome of combination treatments." Turnis et al., *OncoImmunology* 1:7, 1172-1174; 2012.

[022] The inducible co-stimulatory molecule (ICOS) has been reported to be highly expressed on Tregs infiltrating various tumors, including melanoma and ovarian cancers. Faget et al., *OncoImmunology* 2:3, e23185; March 2013. It has also been reported that the ICOS/ICOSL interaction occurs during the interaction of TA-T_{regs} with TA-pDCs in breast carcinoma. *Id.* Antagonist antibodies against ICOS have been used to inhibit ICOS:ICOS-L

interaction and abrogate proliferation of T_{reg} induced by pDC. WO 2012/131004. An antagonist antibody was used in a murine model of mammary tumor to reduce tumor progression. *Id.*

[023] An agonist antibody directed against ICOS has been suggested as being useful in combination with a blocking anti-CTLA-4 antibody and a blocking anti-PD-1 antibody for the treatment of tumors. WO 2011/041613.

[024] There is clearly a substantial unmet medical need for additional cancer treatments, including active immunotherapies and cancer vaccines. In many aspects, the embodiments of the present disclosure address these needs by providing combination therapies that increase and improve the cancer treatments currently available.

BRIEF SUMMARY OF THE INVENTION

[025] In one general aspect, the present invention encompasses therapies, compositions, and methods for treating cancer patients using recombinant poxviruses encoding at least one tumor antigen in combination with one or more antagonists or agonists of an immune checkpoint inhibitor.

[026] In a more particular aspect, the present invention encompasses uses, methods, and compositions utilizing a combination of an orthopoxvirus and/or an avipoxvirus expressing a tumor antigen with one or more combinations of antagonists of PD-1, LAG-3, and/or agonists of ICOS.

[027] In one embodiment, the present invention includes a therapy for the treatment of a human cancer patient. The therapy comprises: (a) a recombinant orthopoxvirus comprising a nucleic acid encoding a polypeptide of at least one tumor antigen; and (b) at least one of an anti-PD-1 antagonist, an anti-LAG-3 antagonist, or an anti-ICOS agonist. It is contemplated by the present invention that (a) and (b) are administered as a combination.

[028] In another embodiment, the present invention includes a method for treating a human cancer patient, the method comprising: (a) administering to the patient a recombinant orthopoxvirus comprising a nucleic acid encoding a polypeptide of at least one tumor antigen; and (b) administering to the patient at least one of an anti-PD-1 antagonist, an anti-LAG-3 antagonist, or an anti-ICOS agonist.

[029] In yet another embodiment, the therapy and method for treating a human cancer patient further comprises a recombinant avipoxvirus comprising a nucleic acid encoding a polypeptide of at least one tumor antigen and the administration thereof to a human cancer patient in combination with at least one of: an anti-PD-1 antagonist, an anti-LAG-3 antagonist, or an anti-ICOS agonist.

[030] In still another embodiment, the therapy and method for treating a human cancer patient further comprises two or more recombinant orthopoxviruses comprising a nucleic acid encoding a polypeptide of at least one tumor antigen and the administration thereof to a human cancer patient in combination with at least one of an anti-PD-1 antagonist, an anti-LAG-3 antagonist, or an anti-ICOS agonist.

[031] In an additional embodiment, the present invention encompasses a medicament or a composition for use in the treatment of a human cancer patient. The medicament or composition comprises: (a) a recombinant orthopoxvirus comprising a nucleic acid encoding a polypeptide of at least one tumor antigen; and (b) at least one of an anti-PD-1 antagonist, an anti-LAG-3 antagonist, or an anti-ICOS agonist. In additional embodiments, the medicament or composition additionally includes: (a) a recombinant avipoxvirus comprising a nucleic acid encoding a polypeptide of at least one tumor antigen; and (b) at least one of an anti-PD-1 antagonist, an anti-LAG-3 antagonist, or an anti-ICOS agonist.

[032] In yet another embodiment, the present invention includes the use of a composition in the preparation of a pharmaceutical composition or medicament for the treatment of a human cancer patient, the composition comprising: (a) a recombinant orthopoxvirus comprising a nucleic acid encoding a polypeptide of at least one tumor antigen; and (b) at least one of an anti-PD-1 antagonist, an anti-LAG-3 antagonist, or an anti-ICOS agonist. In additional embodiments, the medicament or composition additionally includes: (a) a recombinant avipoxvirus comprising a nucleic acid encoding a polypeptide of at least one tumor antigen; and (b) at least one of an anti-PD-1 antagonist, an anti-LAG-3 antagonist, or an anti-ICOS agonist.

[033] In certain embodiments, as described herein, the recombinant orthopoxvirus is selected from a vaccinia virus, a modified vaccinia Ankara (MVA) virus, and/or MVA-BN. In certain additional embodiments, the recombinant avipoxvirus is a fowlpox virus.

[034] In certain embodiments, as described herein, the anti-PD-1 antagonist, anti-LAG-3 antagonist, and the anti-ICOS agonist each comprises an antibody.

[035] In certain embodiments, as described herein, the at least one tumor antigen encoded by the recombinant orthopoxvirus and recombinant avipoxvirus can be selected from a CEA, MUC-1, PAP, PSA, and a HER-2 antigen. In preferred embodiments, the at least one tumor antigen is selected from PAP and PSA. In another preferred embodiment, the at least one tumor antigen is selected from CEA and MUC-1. In yet another preferred embodiment, the at least one tumor antigen is a HER-2 antigen.

[036] In certain additional embodiments, the treatment of a human cancer patient encompasses a cancer selected from breast cancer, colorectal cancer, lung cancer, gastric cancer, pancreatic cancer, prostate cancer, bladder cancer, and/or ovarian cancer.

[037] Additional objects and advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description or may be learned by practice of the invention. The objects and advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

[038] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

BRIEF DESCRIPTION OF THE DRAWINGS

[039] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate one or more embodiments of the invention and together with the description, serve to explain the principles of the invention.

[040] Figure 1. Shows ICOS expression in the lungs, spleen, and blood on T cells after treatment with MVA-BN-HER2. Naïve (tumor-free) mice were treated on day 1 (A and B) or days 1 and 15 (C and D) with MVA-BN-HER2. ICOS expression on CD8+T cells (A and C) and CD4+ T cells (B and D). Data shown as mean \pm SEM, three mice per group at each time point.

[041] Figure 2. Shows tumor volume after administration of MVA-BN-HER2 in combination with anti-ICOS agonist. Mice with solid CT26-HER-2 tumors were treated with MVA-BN-HER2 on days 1 and 15, and anti-ICOS on days 1, 4, 8, 11, 15, 18, 22, 25 (i.p.). A) Average tumor growth. B) Tumor growth in individual mice.

[042] Figure 3. Shows MVA-BN-HER2 synergizes with anti-CTLA-4 to eliminate tumors and increase survival in an experimental lung metastasis model. Mice with CT26-HER-2 tumors in the lung were treated with MVA-BN-HER2 on days 4 and 18, and anti-CTLA-4 on days 3 and 17.

[043] Figure 4. Shows MVA-BN-HER2 alone and in combination with anti-CTLA-4 reduces CT26-HER2 lung tumor burden 25 days after tumor implant. Mice with CT26-HER-2 tumors in the lung were treated with MVA-BN-HER2 on days 4 and 18, and anti-CTLA-4 on days 3 and 17; tumor burden analyzed on day 25. A) Representative lungs from each group show tumors visible as small masses in Untreated and anti-CTLA-4 treated lungs. There were no visible tumors in mice treated with MVA-BN-HER2. Scale bar equals 1 cm. B) Mice treated with MVA-BN-HER2 have similar lung weight to Naïve mice on day 25, while lung weight is significantly greater in Untreated and anti-CTLA-4 treated mice on day 25.

[044] Figure 5. Shows ICOS expression increased on T cells from the tumor/lungs and in the periphery in mice treated with MVA-BN-HER2, and in the tumor/lungs of mice with high tumor burden. Mice with CT26-HER-2 tumors in the lung were treated with MVA-BN-HER2 on days 4 and 18, and anti-CTLA-4 on days 3 and 17. A) ICOS expression on CD4+ T cells at day 11 and 25. B) ICOS expression on CD8+ T cells at day 11 and 25. C) Average ICOS expression on CD4+ T cells at day 25 from three independent experiments with 3-4 mice per group. D) Average ICOS expression on CD8+ T cells at day 25 from three independent experiments with 3-4 mice per group.

[045] Figure 6. Shows ICOS+ CD4+ T Cells are approximately equal proportions of FoxP3+ in tumor bearing mice, but are primarily FoxP3- in mice responding to treatment. Mice with CT26-HER-2 tumors in the lung were treated with MVA-BN-HER2 on days 4 and 18, and

anti-CTLA-4 on days 3 and 17 and FACS analysis performed on day 24 or 25 after tumor implant. A) ICOS expression on FoxP3+ Tregs. B) ICOS expression on FoxP3- CD4 T cells. C) Average ICOS expression on FoxP3+ Tregs from three independent experiments with 3-4 mice per group. D) ICOS expression on FoxP3- CD4 T from three independent experiments with 3-4 mice per group.

[046] Figure 7. Shows MVA-BN-HER2 and combination treatment with anti-CTLA-4 increased the effector to regulatory T cell ratio. Mice with CT26-HER-2 tumors in the lung were treated with MVA-BN-HER2 on days 4 and 18, and anti-CTLA-4 on days 3 and 17 and FACS analysis performed on day 24 or 25 after tumor implant. A) CD8 Teff:Treg ratio ($CD8+ICOS+FoxP3-/CD4+ICOS+FoxP3+$) and B) CD4 Teff:Treg ratio ($CD4+ICOS+FoxP3-/CD4+ICOS+FoxP3+$) from a single experiment with 3-4 mice per group. C) Average CD8 Teff:Treg ratio and D) Average CD4 Teff:Treg ratio from three independent experiments with 3-4 mice per group.

[047] Figure 8. Shows PD-1 expression in the lungs, spleen, and blood on T cells after treatment with MVA-BN-HER2. Naïve (tumor-free) mice were treated on day 1 (A and B) or days 1 and 15 (C and D) with MVA-BN-HER2. PD-1 expression on CD8+ T cells (A and C) and CD4+ T cells (B and D). Data shown as mean \pm SEM, three mice per group at each time point.

[048] Figure 9. Shows MVA-BN-HER2 treatment and anti-PD-1 slow tumor growth and increase survival. Mice were implanted with solid CT26-HER-2 solid tumors and treated on days 1 and 15 with MVA-BN-HER2 and anti-PD-1. A) Average tumor volume in mice. B) Percent survival in mice based on tumor volume $<2000 \text{ mm}^3$. C) Individual tumor growth in mice.

[049] Figure 10. Shows LAG-3 expression in the lungs, spleen, and blood on T cells after treatment with MVA-BN-HER2. Naïve (tumor-free) mice were treated on day 1 (A and B) or days 1 and 15 (C and D) with MVA-BN-HER2. LAG-3 expression on CD8+ T cells (A and C) and CD4+ T cells (B and D). Data shown as mean \pm SEM, three mice per group at each time point.

[050] Figure 11. Shows MVA-BN-HER2 and anti-LAG-3 slow tumor growth and increase survival. Mice were implanted with solid CT26-HER-2 tumors on day 1 and treated on days 1 and 15 with MVA-BN-HER2 and anti-LAG-3. A) Average tumor volume in mice. B) Percent Survival in mice based on tumor volume $<2000 \text{ mm}^3$. C) Individual tumor growth in mice.

[051] Figure 12. Shows MVA-BN-HER2, anti-PD-1, and anti-LAG-3 treatment leads to complete tumor regression in mice. Mice were implanted with solid CT26-HER-2 tumors on day 1 and treated on days 1 and 15 with MVA-BN-HER2, anti-PD-1, and anti-LAG-3. A) Average tumor volume in mice. B) Percent Survival in mice based on tumor volume $<2000 \text{ mm}^3$. C) Individual tumor growth in mice.

[052] Figure 13. Shows MVA-BN-HER2, anti-PD-1, and anti-LAG-3 treatment leads to tumor regression in mice. Mice were implanted with solid CT26-HER-2 tumors on day 1 and treated on days 4 and 18 with MVA-BN-HER2, anti-PD-1, and anti-LAG-3. (As compared to Figure 12, treatment was delayed to days 4 and 18 (Figure 12, on days 1 and 15). (A) Average tumor volume in mice. B) Percent Survival in mice based on tumor volume $<2000 \text{ mm}^3$. C) Individual tumor growth in mice.

[053] Figure 14. Shows MVA-BN-HER2 alone and in combination with anti-PD-1 and anti-LAG-3 increase survival in an experimental lung metastasis model. Mice with CT26-HER-2

tumors in the lung were treated on day 4 and 18 with MVA-BN-HER2, anti-PD-1, and anti-LAG-3.

[054] Figure 15. Shows the HER2 specific T-cell responses of mice treated with MVA-BN-HER2, anti-PD-1, and anti-LAG-3 antibodies were higher with triple combination therapy. Mice were implanted with solid CT26-HER-2 tumors on day 1 and treated on days 4 and 18 with MVA-BN-HER2, anti-PD-1, and anti-LAG-3 and IFN- γ was measured by ELISPOT four weeks after the last treatment. Splenocytes from tumor free mice were re-stimulated with HER-2 ECD overlapping peptide library (A, 166 overlapping 15mers) or the K^d binding HER-2 peptide p63 (B).

[055] Figure 16. Shows the growing CT26-HER-2 tumor induced HER2 specific antibodies that are similar among all treatment groups. Mice were implanted with solid CT26-HER-2 tumors on day 1 and treated on days 4 and 18 with MVA-BN-HER2, anti-PD-1, and anti-LAG-3. Serum was collected on day 25 from mice and HER-2 titers measured by ELISA.

[056] Figure 17. Shows MVA-BN-CV301 and anti-PD-1 slow tumor growth. Mice were implanted with MC38-CEA solid tumors and treated on days 1 and 15 with MVA-BN-CV301 and anti-PD-1. A) Average tumor volume in mice. B) Individual tumor growth in mice.

[057] Figure 18. Shows tumor volume after administration of MVA-BN-CV301 and anti-LAG-3. Mice were implanted with MC38-CEA solid tumors and treated on days 1 and 15 with MVA-BN-CV301 and anti-LAG-3. A) Average tumor volume in mice. B) Individual tumor growth in mice.

[058] Figure 19. Shows tumor volume after administration MVA-BN-CV301 in combination with anti-PD-1 and anti-LAG-3. Mice were implanted with MC38-CEA solid

tumors and treated on days 1 and 15 with MVA-BN-CV301, anti-PD-1, and anti-LAG-3. A) Average tumor volume in mice. B) Individual tumor growth in mice.

[059] Figure 20. Shows tumor volume after administration of PROSTVAC and anti-PD-1. Mice were implanted with E6 (RM11-PSA) solid tumors and treated on day 1 with PROSTVAC-V, and days 8 and 15 with PROSTVAC-F. Anti-PD-1 was given on days 1 and 15. A) Average tumor volume in mice. B) Individual tumor growth in mice.

[060] Figure 21. Shows tumor volume after administration of PROSTVAC and anti-LAG-3. Mice were implanted with E6 (RM11-PSA) solid tumors and treated on day 1 with PROSTVAC-V and days 8 and 15 with PROSTVAC-F. Anti-LAG-3 was given on days 1 and 15. A) Average tumor volume in mice. B) Individual tumor growth in mice.

[061] Figure 22. Shows tumor volume after administration of PROSTVAC, anti-PD-1, and anti-LAG-3. Mice were implanted with E6 (RM11-PSA) solid tumors and treated on day 1 with PROSTVAC-V and days 8 and 15 with PROSTVAC-F. Anti-PD-1 and anti-LAG-3 were given on days 1 and 15. A) Average tumor volume in mice. B) Individual tumor growth in mice.

[062] Figure 23. Shows tumor volume after administration of CV301 and anti-PD-1. CEA transgenic mice were implanted with MC38-CEA solid tumors and treated with CV301-V on day 4 CV301-F on days 11 and 18. Fowlpox GM-CSF (admixed with CV301-V/F) and anti-PD-1 were given on days 4, 11, and 18. A) Average tumor volume in mice. B) Individual tumor growth in mice.

BRIEF DESCRIPTION OF THE SEQUENCES

[063] SEQ ID NO: 1 is the nucleotide sequence of a construct encoding a HER2 protein including two T_H-cell epitopes derived from tetanus toxin.

[064] SEQ ID NO: 2 is an amino acid sequence of the modified HER2 protein encoded by the nucleotide sequence of SEQ ID NO: 1.

DETAILED DESCRIPTION OF THE INVENTION

[065] A number of current clinical trials involve therapies employing vaccinia, Modified Vaccinia Ankara (MVA), and fowlpox-based vectors that were engineered to express one or more tumor-associated antigens (TAA). These vectors are used alone or in prime-boost strategies to generate an active immune response against a variety of cancers. PROSTVAC® employs a heterologous prime-boost strategy using vaccinia and fowlpox expressing PSA and TRICOMTM and is currently in a global Phase III clinical trial (PROSPECT) for castration-resistant metastatic prostate cancer.

[066] MVA-BN-HER2 (Mandl et al, 2012) is in Phase I clinical trials for the treatment of HER-2⁺-breast cancer. This recombinant vector is derived from the highly attenuated Modified Vaccinia Ankara (MVA) virus stock known as MVA-BN. It expresses a modified form of HER-2 (designated HER2) consisting of the extracellular domain of HER-2 that has been engineered to include two universal T cell epitopes from tetanus toxin (TTP2 and TTP30) to facilitate the induction of effective immune responses against HER-2.

[067] To further enhance the anti-tumor efficacy of the poxvirus-based immunotherapy, MVA-BN-HER2 was combined with a monoclonal antibody that blocks the activity of CTLA-4, an immune checkpoint protein that down-regulates T cell activation. In the CT26-HER-2 experimental lung metastasis model, the median survival time increased from 30 days in untreated mice to 49.5 days with MVA-BN-HER2 treatment while anti-CTLA-4 treatment by itself showed little survival benefit (median survival 35 days). In contrast, MVA-BN-HER2 in

combination with anti-CTLA-4 significantly increased the survival to greater than 100 days ($p < 0.0001$) in more than 50% of the mice. At 100 days, the lungs of the surviving mice were examined and there were no visible tumors. In separate experiments, phenotypic analysis was performed. MVA-BN-HER2 therapy led to a dramatic increase in the inducible co-stimulatory molecule (ICOS) on CD8⁺ T cells in the lungs of naïve mice (no tumors). In tumor bearing mice at day 25, there was an increase in the number of regulatory T cells (CD4⁺FoxP3⁺) in the lungs of untreated and anti-CTLA-4 treated mice which correlated with increased pulmonary tumor burden. The regulatory T cells were positive for ICOS. Mice treated with MVA-BN-HER2 had an increase in ICOS⁺ CD4⁺ T-cells that were FoxP3 negative.

[068] MVA-BN-HER2 was tested in combination with various agonist and antagonist antibodies directed against PD-1, LAG-3, and ICOS in various tumor models. Combinations were found to enhance the effects of MVA-BN-HER2.

[069] Additionally, various antagonist antibodies directed against PD-1 and LAG-3 were tested in combination with heterologous-prime boost dosing regimens utilizing a recombinant orthopoxvirus and a recombinant avipoxvirus. For example, PROSTVAC®, which includes a Vaccinia virus and a Fowlpox virus, each expressing PSA and TRICOM, was tested in combination with the various antagonist antibodies. CV301 (also known as PANVAC), which includes a Vaccinia virus and a Fowlpox Virus, each expressing CEA, MUC-1, and TRICOM, was also tested in combination with the various antagonist antibodies. The effectiveness of the heterologous prime-boost dosing regimens increased when administered in combination with the various antagonist antibodies.

[070] Various antagonist antibodies directed against PD-1 and LAG-3 were additionally tested in the homologous prime-boost regimen of MVA-CV301. The effectiveness of MVA-

CV301, which includes a prime and boosting dosing of an MVA virus expressing CEA, MUC-1, and TRICOM, was found to be improved when tested in combination with the antibodies directed against PD-1 and/or LAG-3.

Orthopoxvirus and/or Avipoxvirus encoding a polypeptide comprising a tumor antigen

[071] In various aspects, the present disclosure includes a recombinant orthopoxvirus and/or a recombinant avipoxvirus each encoding and/or expressing a tumor antigen. In one or more preferred aspects, the orthopoxvirus and the avipoxvirus are a vaccinia virus and a fowlpox virus, respectively.

[072] The term "avipoxvirus" refers to any avipoxvirus, such as Fowlpoxvirus, Canarypoxvirus, Uncopoxvirus, Mynahpoxvirus, Pigeonpoxvirus, Psittacinepoxvirus, Quailpoxvirus, Peacockpoxvirus, Penguinpoxvirus, Sparrowpoxvirus, Starlingpoxvirus and Turkeypoxvirus. Preferred avipoxviruses are Canarypoxvirus and Fowlpoxvirus.

[073] An example of a canarypox virus is strain Rentschler. A plaque purified Canarypox strain termed ALVAC (U.S. Pat. No. 5,766,598) was deposited under the terms of the Budapest treaty with the American Type Culture Collection (ATCC), accession number VR-2547. Another Canarypox strain is the commercial canarypox vaccine strain designated LF2 CEP 524 24 10 75, available from Institute Merieux, Inc.

[074] Examples of a Fowlpox virus include, but are not limited to, strains FP-1, FP-5, TROVAC (U.S. Pat. No. 5,766,598), and POXVAC-TC (U.S. Patent 7,410,644). FP-1 is a Duvette strain modified to be used as a vaccine in one-day old chickens. The strain is a commercial fowlpox virus vaccine strain designated O DCEP 25/CEP67/2309 October 1980 and is available from Institute Merieux, Inc. FP-5 is a commercial fowlpox virus vaccine strain of

chicken embryo origin available from American Scientific Laboratories (Division of Schering Corp.) Madison, Wis., United States Veterinary License No. 165, serial No. 30321.

[075] In one or more embodiments, the recombinant orthopoxvirus is preferably selected from a vaccinia virus, a modified vaccinia Ankara (MVA) virus, and MVA-BN.

[076] Examples of vaccinia virus strains include, but are not limited to, the strains Temple of Heaven, Copenhagen, Paris, Budapest, Dairen, Gam, MRIVP, Per, Tashkent, TBK, Tom, Bern, Patwadangar, BIEM, B-15, Lister, EM-63, New York City Board of Health, Elstree, Ikeda and WR. A preferred vaccinia virus (VV) strain is the Wyeth (DRYVAX) strain (U.S. Patent 7,410,644).

[077] Another preferred VV strain is a modified vaccinia Ankara (MVA) virus (Sutter, G. et al. [1994], Vaccine 12: 1032-40). Examples of MVA virus strains that are useful in the practice of the present invention and that have been deposited in compliance with the requirements of the Budapest Treaty include, but are not limited to, strains MVA 572, deposited at the European Collection of Animal Cell Cultures (ECACC), Vaccine Research and Production Laboratory, Public Health Laboratory Service, Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 0JG, United Kingdom, with the deposition number ECACC 94012707 on January 27, 1994, and MVA 575, deposited under ECACC 00120707 on December 7, 2000. MVA-BN, deposited on Aug. 30, 2000 at the European Collection of Cell Cultures (ECACC) under number V00083008, and its derivatives, are additional exemplary strains.

[078] Although MVA-BN is preferred for its higher safety (less replication competent), all MVAs are suitable for this invention. According to an embodiment of the present invention,

the MVA strain is MVA-BN and its derivatives. A definition of MVA-BN and its derivatives is given in PCT/EP01/13628, which is incorporated by reference herein.

[079] In certain embodiments, the MVA is MVA-BN, deposited on Aug. 30, 2000, at the European Collection of Cell Cultures (ECACC) under number V00083008, and described in International PCT publication WO2002042480 (see also e.g. U.S. Pat. Nos. 6,761,893 and 6,913,752), all of which are incorporated by reference herein. As described in those patent publications, MVA-BN does not reproductively replicate in cell lines 293, 143B, HeLa and HaCat. In particular, MVA-BN exhibits an amplification ratio of 0.05 to 0.2 in the human embryo kidney cell line 293. In the human bone osteosarcoma cell line 143B, MVA-BN exhibits an amplification ratio of 0.0 to 0.6. MVA-BN exhibits an amplification ratio of 0.04 to 0.8 in the human cervix adenocarcinoma cell line HeLa, and 0.02 to 0.8 in the human keratinocyte cell line HaCat. MVA-BN has an amplification ratio of 0.01 to 0.06 in African green monkey kidney cells (CV1: ATCC No. CCL-70).

[080] The amplification ratio of MVA-BN is above 1 in chicken embryo fibroblasts (CEF: primary cultures) as described in PCT publication WO2002042480 (see also e.g. U.S. Pat. Nos. 6,761,893 and 6,913,752). The virus can be easily propagated and amplified in CEF primary cultures with a ratio above 500.

[081] In certain embodiments, a recombinant MVA is a derivative of MVA-BN. Such "derivatives" include viruses exhibiting essentially the same replication characteristics as the deposited strain (ECACC No. V00083008), but exhibiting differences in one or more parts of its genome. Viruses having the same "replication characteristics" as the deposited virus are viruses that replicate with similar amplification ratios as the deposited strain in CEF cells and the cell

lines, HeLa, HaCat and 143B; and that show similar replication characteristics *in vivo*, as determined, for example, in the AGR129 transgenic mouse model.

[082] In certain embodiments, the orthopoxvirus is a recombinant vaccinia virus that contains additional nucleotide sequences that are heterologous to the orthopoxvirus. In certain such embodiments, the heterologous sequences code for epitopes that induce a response by the immune system. Thus, in certain embodiments, the recombinant orthopoxvirus is used to vaccinate against the proteins or agents comprising the epitope.

[083] In certain embodiments, the orthopoxvirus and avipoxvirus in accordance with the present disclosure comprise at least one tumor-associated antigen. In a preferred embodiment, the tumor-associated antigen includes, but is not limited to a HER-2, PSA, PAP, CEA, or MUC-1 antigen alone or in combinations (e.g., CEA and MUC-1 or PAP and PSA),.

[084] In further embodiments, the tumor-associated antigen is modified to include one or more foreign T_H epitopes. Such a cancer immunotherapeutic agent is described herein in a non-limiting example and is referred to as “MVA-BN-mHER2.” As described herein, such cancer immunotherapeutic agents, including, but not limited to MVA-BN-mHER2, are useful for the treatment of cancer. The invention allows for the use of such agents in prime/boost vaccination regimens of humans and other mammals, including immunocompromised patients; and inducing both humoral and cellular immune responses, such as inducing a Th1 immune response in a pre-existing Th2 environment.

[085] In certain embodiments, the tumor associated antigen is embodied in a heterologous nucleic acid sequence that is inserted into a non-essential region of the virus genome. In certain of those embodiments, the heterologous nucleic acid sequence is inserted at a naturally occurring deletion site of the MVA genome as described in PCT/EP96/02926.

Methods for inserting heterologous sequences into the poxviral genome are known to a person skilled in the art.

[086] In certain embodiments, pharmaceutical compositions comprise one or more pharmaceutically acceptable and/or approved carriers, additives, antibiotics, preservatives, adjuvants, diluents and/or stabilizers. Such additives include, for example, but not limited to, water, saline, glycerol, ethanol, wetting or emulsifying agents, and pH buffering substances. Exemplary carriers are typically large, slowly metabolized molecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates, or the like.

[087] For the preparation of vaccines, the orthopoxvirus can be converted into a physiologically acceptable form. In certain embodiments, such preparation is based on experience in the preparation of poxvirus vaccines used for vaccination against smallpox, as described, for example, in Stickl, H. et al., Dtsch. med. Wschr. 99, 2386-2392 (1974).

[088] An exemplary preparation follows. Purified virus is stored at -80°C with a titer of 5×10^8 TCID₅₀/ml formulated in 10 mM Tris, 140 mM NaCl, pH 7.4. For the preparation of vaccine shots, e.g., 10^2 - 10^8 particles of the virus can be lyophilized in phosphate-buffered saline (PBS) in the presence of 2% peptone and 1% human albumin in an ampoule, preferably a glass ampoule. Alternatively, the vaccine shots can be prepared by stepwise, freeze-drying of the virus in a formulation. In certain embodiments, the formulation contains additional additives such as mannitol, dextran, sugar, glycine, lactose, polyvinylpyrrolidone, or other additives, such as, including, but not limited to, antioxidants or inert gas, stabilizers or recombinant proteins (e.g. human serum albumin) suitable for *in vivo* administration. The ampoule is then sealed and can be stored at a suitable temperature, for example, between 4°C and room temperature for

several months. However, as long as no need exists, the ampoule is stored preferably at temperatures below -20°C.

[089] In various embodiments involving vaccination or therapy, the lyophilisate is dissolved in 0.1 to 0.5 ml of an aqueous solution, preferably physiological saline or Tris buffer, and administered either systemically or locally, i.e., by parenteral, subcutaneous, intravenous, intramuscular, intranasal, intradermal, or any other path of administration known to a skilled practitioner. Optimization of the mode of administration, dose, and number of administrations is within the skill and knowledge of one skilled in the art.

[090] In certain embodiments, attenuated vaccinia virus strains are useful to induce immune responses in immune-compromised animals, e.g., monkeys ($CD4 < 400/\mu\text{l}$ of blood) infected with SIV, or immune-compromised humans. The term "immune-compromised" describes the status of the immune system of an individual that exhibits only incomplete immune responses or has a reduced efficiency in the defense against infectious agents.

Certain Exemplary Tumor-Associated Antigens

[091] In certain embodiments, an immune response is produced in a subject against a cell-associated polypeptide antigen. In certain such embodiments, a cell-associated polypeptide antigen is a tumor-associated antigen.

[092] The term "polypeptide" refers to a polymer of two or more amino acids joined to each other by peptide bonds or modified peptide bonds. The amino acids may be naturally occurring as well as non-naturally occurring, or a chemical analogue of a naturally occurring amino acid. The term also refers to proteins, i.e. functional biomolecules comprising at least one polypeptide; when comprising at least two polypeptides, these may form complexes, be

covalently linked, or may be non-covalently linked. The polypeptide(s) in a protein can be glycosylated and/or lipidated and/or comprise prosthetic groups.

[093] As described herein, preferably, the tumor-associated antigen is HER-2, PSA, PAP, CEA, or MUC-1, alone or in combinations (e.g., CEA and MUC-1 or PAP and PSA).

[094] Numerous tumor-associated antigens are known in the art. Exemplary tumor-associated antigens include, but are not limited to, 5 alpha reductase, alpha-fetoprotein, AM-1, APC, April, BAGE, beta-catenin, Bcl12, bcr-abl, CA-125, CASP-8/FLICE, Cathepsins, CD19, CD20, CD21, CD23, CD22, CD33 CD35, CD44, CD45, CD46, CD5, CD52, CD55, CD59, CDC27, CDK4, CEA, c-myc, Cox-2, DCC, DcR3, E6/E7, CGFR, EMBP, Dna78, farnesyl transferase, FGF8b, FGF8a, FLK-1/KDR, folic acid receptor, G250, GAGE-family, gastrin 17, gastrin-releasing hormone, GD2/GD3/GM2, GnRH, GnTV, GP1, gp100/Pmel17, gp-100-in4, gp15, gp75/TRP-1, hCG, heparanase, Her2/neu, HMTV, Hsp70, hTERT, IGFR1, IL-13R, iNOS, Ki67, KIAA0205, K-ras, H-ras, N-ras, KSA, LKLR-FUT, MAGE-family, mammaglobin, MAP17, melan-A/MART-1, mesothelin, MIC A/B, MT-MMPs, mucin, NY-ESO-1, osteonectin, p15, P170/MDR1, p53, p97/melanotransferrin, PAI-1, PDGF, uPA, PRAME, probasin, progenipointin, PSA, PSM, RAGE-1, Rb, RCAS1, SART-1, SSX-family, STAT3, STn, TAG-72, TGF-alpha, TGF-beta, Thymosin-beta-15, TNF-alpha, TRP-1, TRP-2, tyrosinase, VEGF, ZAG, p16INK4, and glutathione-S-transferase.

[095] A preferred PSA antigen comprises the amino acid change of isoleucine to leucine at position 155, as described in U.S. Patent 7,247,615, which is incorporated herein by reference.

[096] One or more preferred CEA antigens include, but are not limited to, CEA antigens described in US Patent 7,723,096 and PCT application Nos. PCT/US2004/037810 and PCT/US2004/038643; all of which are incorporated by reference herein.

[097] One or more preferred MUC-1 antigens include, but are not limited to, MUC-1 antigens described in US Patent 7,118,738 and PCT application Nos. PCT/US2013/020058, PCT/US2004/037810, and PCT/US2004/038643; all of which are incorporated by reference herein.

[098] Another exemplary tumor-associated antigen is HER-2. HER-2 is a member of the epidermal growth factor receptor family (c-erbB) which consists of four different receptors to date: c-erbB-1 (EGFr), c-erbB-2 (HER-2, c-Neu), c-erbB-3 and c-erbB-4 (Salomon et al, 1995). C-erbB-3 and c-erbB-4 are less well characterized than EGFr and HER-2. HER-2 is an integral membrane glycoprotein. The mature protein has a molecular weight of 185 kD with structural features that closely resemble the EGFr receptor (Prigent et al, 1992). EGFr is also an integral membrane receptor consisting of one subunit. It has an apparent molecular weight of 170 kD and consists of a surface ligand-binding domain of 621 amino acids, a single hydrophobic transmembrane domain of 23 amino acids, and a highly conserved cytoplasmic tyrosine kinase domain of 542 amino acids. The protein is N-glycosylated (Prigent et al, 1994).

[099] All proteins in this family are tyrosine kinases. Interaction with the ligand leads to receptor dimerization, which increases the catalytic action of the tyrosine kinase (Bernard, 1995, Chantry 1995). The proteins within the family are able to homo- and heterodimerise, which is important for their activity. The EGFr conveys growth promoting effects and stimulates uptake of glucose and amino acids by cells (Prigent et al 1992). HER-2 also conveys growth promoting signals.

[0100] The epidermal growth factor receptor is expressed on normal tissues in low amounts, but it is overexpressed in many types of cancers. EGFr is overexpressed in breast cancers (Earp et al, 1993, Eppenberger 1994), gliomas (Schlegel et al, 1994), gastric cancer

(Tkunaga et al, 1995), cutaneous squamous carcinoma (Fujii 1995), ovarian cancer (van Dam et al, 1994) and others. HER-2 is also expressed on few normal human tissues in low amount, most characteristically on secretory epithelia. Over-expression of HER-2 occurs in about 30% of breast, gastric, pancreatic, bladder and ovarian cancers.

[0101] The expression of these receptors varies depending on the degree of differentiation of the tumors and the cancer type, e.g., in breast cancer, primary tumors overexpress both receptors; whereas in gastric cancer, the overexpression occurs at a later stage in metastatic tumours (Salomon et al, 1995). The number of overexpressed receptors on carcinoma cells is greater than 10^6 /cell for several head and neck cancers, vulva, breast and ovarian cancer lines isolated from patients (Dean et al, 1994).

[0102] There are several reasons why the EGFr family of receptors constitutes suitable targets for tumor immunotherapy. First, they are overexpressed in many types of cancers, which should direct the immune response towards the tumor. Second, the tumors often express or overexpress the ligands for this family of receptors and some are hypersensitive to the proliferative effects mediated by the ligands. Third, patients with tumors that overexpress growth factor receptors often have a poor prognosis. The overexpression has been closely linked with poor prognosis especially in breast cancer, lung cancer, and bladder cancer and can be associated with invasive/metastatic phenotypes, which are rather insensitive to conventional therapies (Eccles et al, 1994).

[0103] It is contemplated that the nucleic acids encoding the tumor antigen (or tumor associated antigen) can be operatively linked to expression control sequences. An expression control sequence operatively linked to a coding sequence is joined such that expression of the coding sequence is achieved under conditions compatible with the expression control sequences.

The expression control sequences include, but are not limited to, appropriate promoters, enhancers, transcription terminators, a start codon at the beginning a protein-encoding open reading frame, splicing signals for introns, and in-frame stop codons. Suitable promoters include, but are not limited to, the SV40 early promoter, an RSV promoter, the retrovirus LTR, the adenovirus major late promoter, the human CMV immediate early I promoter, and various poxvirus promoters including, but not limited to the following vaccinia virus or MVA-derived promoters: the ATI promoter, the 30K promoter, the I3 promoter, the PrS promoter, the Pr7.5K, the 40K promoter, the PrSynIIIm promoter, the PrLE1 promoter, and the PrSSL promoter (as described in PCT Application PCT/EP2009/008459).

[0104] Additional expression control sequences include, but are not limited to, leader sequences, termination codons, polyadenylation signals and any other sequences necessary for the appropriate transcription and subsequent translation of the nucleic acid sequence encoding the desired recombinant protein (e.g., HER-2, PSA, PAP, CEA, or MUC-1) in the desired host system. The poxvirus vector may also contain additional elements necessary for the transfer and subsequent replication of the expression vector containing the nucleic acid sequence in the desired host system. It will further be understood by one skilled in the art that such vectors are easily constructed using conventional methods (Ausubel et al., (1987) in "Current Protocols in Molecular Biology," John Wiley and Sons, New York, N.Y.) and are commercially available.

Modified Tumor-Associated Antigens

[0105] In certain embodiments, a cell-associated polypeptide antigen is modified such that a CTL response is induced against a cell which presents epitopes derived from a polypeptide antigen on its surface, when presented in association with an MHC Class I molecule on the

surface of an APC. In certain such embodiments, at least one first foreign T_H epitope, when presented, is associated with an MHC Class II molecule on the surface of the APC. In certain such embodiments, a cell-associated antigen is a tumor-associated antigen (TAA).

[0106] Exemplary APCs capable of presenting epitopes include dendritic cells and macrophages. Additional exemplary APCs include any pinocytotic or phagocytizing APC, which is capable of simultaneously presenting 1) CTL epitopes bound to MHC class I molecules and 2) T_H epitopes bound to MHC class II molecules.

[0107] In certain embodiments, modifications to one or more of the tumor-associated antigens (TAA) presented herein, such as, but not limited to, CEA, MUC-1, PAP, PSA, HER2 are made such that, after administration to a subject, polyclonal antibodies are elicited that predominantly react with the one or more of the TAAs described herein. Such antibodies could attack and eliminate tumor cells as well as prevent metastatic cells from developing into metastases. The effector mechanism of this anti-tumor effect would be mediated via complement and antibody dependent cellular cytotoxicity. In addition, the induced antibodies could also inhibit cancer cell growth through inhibition of growth factor dependent oligodimerisation and internalisation of the receptors. In certain embodiments, such modified polypeptide antigens could induce CTL responses directed against known and/or predicted TAA epitopes displayed by the tumor cells.

[0108] In certain embodiments, a modified TAA polypeptide antigen comprises a CTL epitope of the cell-associated polypeptide antigen and a variation, wherein the variation comprises at least one CTL epitope of a foreign T_H epitope. Certain such modified TAAs can include, in one non-limiting example, one or more modified HER-2 polypeptide antigens comprising at least one CTL epitope and a variation comprising at least one CTL epitope of a

foreign T_H epitope. Methods of producing the same are described in U.S. Patent No. 7,005,498 and U.S. Patent Pub. Nos. 2004/0141958 and 2006/0008465.

[0109] In certain embodiments, a foreign T_H epitope is a naturally-occurring "promiscuous" T-cell epitope. Such promiscuous T-cell epitopes are active in a large proportion of individuals of an animal species or an animal population. In certain embodiments, a vaccine comprises such promiscuous T-cell epitopes. In certain such embodiments, use of promiscuous T-cell epitopes reduces the need for a very large number of different CTL epitopes in the same vaccine. Exemplary promiscuous T-cell epitopes include, but are not limited to, epitopes from tetanus toxin, including but not limited to, the P2 and P30 epitopes (Panina-Bordignon et al., 1989), diphtheria toxin, Influenza virus hemagglutinin (HA), and *P. falciparum* CS antigen.

[0110] Additional promiscuous T-cell epitopes include peptides capable of binding a large proportion of HLA-DR molecules encoded by the different HLA-DR. See, e.g., WO 98/23635 (Frazer IH et al., assigned to The University of Queensland); Southwood S et. al, 1998, *J. Immunol.* 160: 3363 3373; Sinigaglia F et al., 1988, *Nature* 336: 778 780; Rammensee HG et al., 1995, *Immunogenetics* 41: 4 178 228; Chicz RM et al., 1993, *J. Exp. Med* 178: 27 47; Hammer J et al., 1993, *Cell* 74: 197 203; and Falk K et al., 1994, *Immunogenetics* 39: 230 242. The latter reference also deals with HLA-DQ and -DP ligands. All epitopes listed in these references are relevant as candidate natural epitopes as described herein, as are epitopes which share common motifs with these.

[0111] In certain other embodiments, the promiscuous T-cell epitope is an artificial T-cell epitope which is capable of binding a large proportion of haplotypes. In certain such embodiments, the artificial T-cell epitope is a pan DR epitope peptide ("PADRE") as described in WO 95/07707 and in the corresponding paper Alexander J et al., 1994, *Immunity* 1: 751 761.

mHER2

[0112] Various modified HER-2 polypeptide antigens and methods for producing the same are described in U.S. Patent No. 7,005,498 and U.S. Patent Pub. Nos. 2004/0141958 and 2006/0008465, which are hereby incorporated by reference. Those documents describe various modified HER-2 polypeptide antigens comprising promiscuous T-cell epitopes at different positions in the HER-2 polypeptide.

[0113] The human HER-2 sequence can be divided into a number of domains based solely on the primary structure of the protein. Those domains are as follows. The extracellular (receptor) domain extends from amino acids 1-654 and contains several subdomains as follows: Domain I (N-terminal domain of mature polypeptide) extends from amino acids 1-173; Domain II (Cysteine rich domain, 24 cysteine residues) extends from amino acids 174-323; Domain III (ligand binding domain in the homologous EGF receptor) extends from amino acids 324-483; and Domain IV (Cysteine rich domain, 20 cysteine residues) extends from amino acids 484-623. The transmembrane residues extend from amino acids 654-675. The intracellular (Kinase) domain extends from amino acids 655-1235 and contains the tyrosine kinase domain, which extends from amino acids 655-1010 (core TK domain extends from 725-992); and the C-terminal domain, which extends from amino acids 1011-1235.

[0114] Selection of sites in the amino acid sequence of HER-2 to be displaced by either the P2 or P30 human T helper epitopes is described in U.S. Patent No. 7,005,498 and U.S. Patent Pub. Nos. 2004/0141958 and 2006/0008465. To summarize, the following parameters were considered:

1. Known and predicted CTL epitopes;
2. Homology to related receptors (EGFR in particular);

3. Conservation of cysteine residues;
4. Predicted loop, α -helix and β -sheet structures;
5. Potential N-glycosylation sites;
6. Prediction of exposed and buried amino acid residues;
7. Domain organization.

[0115] The CTL epitopes appear to be clustered in domain I, domain III, the TM domain and in two or three "hot spots" in the TK domain. As described in U.S. Patent No. 7,005,498 and U.S. Patent Pub. Nos. 2004/0141958 and 2006/0008465, these should be largely conserved.

[0116] Regions with a high degree of homology with other receptors are likely to be structurally important for the "overall" tertiary structure of HER-2, and hence for antibody recognition, whereas regions with low homology possibly can be exchanged with only local alterations of the structure as the consequence.

[0117] Cysteine residues are often involved in intramolecular disulphide bridge formation and are thus involved in the tertiary structure and should not be changed. Regions predicted to form alpha-helix or beta-sheet structures should be avoided as insertion points of foreign epitopes, as these regions are thought to be involved in folding of the protein.

[0118] Potential N-glycosylation sites should be conserved if mannosylation of the protein is desired.

[0119] Regions predicted (by their hydrophobic properties) to be interior in the molecule preferably should be conserved as these could be involved in the folding. In contrast, solvent exposed regions could serve as candidate positions for insertion of the model T_H epitopes P2 and P30.

[0120] Finally, the domain organization of the protein should be taken into consideration because of its relevance for protein structure and function.

[0121] As described in U.S. Patent No. 7,005,498 and U.S. Patent Pub. Nos. 2004/0141958 and 2006/0008465, the focus of the strategy has been to conserve the structure of the extracellular part of HER-2 as much as possible, because this is the part of the protein which is relevant as a target for neutralizing antibodies. By contrast, the intracellular part of native membrane bound HER-2 on the surface of cancer cells is inaccessible for the humoral immune system.

[0122] Various exemplary constructs using the P2 and P30 epitopes of tetanus toxin inserted in various domains of HER-2 are provided in U.S. Patent No. 7,005,498 and U.S. Patent Pub. Nos. 2004/0141958 and 2006/0008465. One exemplary modified HER-2 polypeptide antigen, referred to as "mHER2," comprises the extracellular domains and nine amino acids of the transmembrane domain; the P2 epitope inserted in Domain II between amino acid residues 273 to 287 of the modified HER-2 polypeptide; and the P30 epitope inserted in Domain IV between amino acid residues 655 to 675 of the modified HER-2 polypeptide.

Recombinant MVA-BN-mHER2

[0123] In a non-limiting embodiment, recombinant MVA comprising a tumor-associated antigen, e.g., MVA-BN-mHER2, is constructed as follows. The initial virus stock is generated by recombination in cell culture using a cell type permissive for replication, e.g., CEF cells. Cells are both inoculated with an attenuated vaccinia virus, e.g., MVA-BN, and transfected with a recombination plasmid (e.g., pBN279) that encodes the tumor-associated antigen, e.g., mHER2, sequence and flanking regions of the virus genome. In one non-limiting embodiment, the plasmid pBN279 contains sequences which are also present in MVA-BN (the flanking Intergenic

Region between ORF 64 and 65, IGR 64/65). The mHER2 sequence is inserted between the MVA-BN sequences to allow for recombination into the MVA-BN viral genome. In certain embodiments, the plasmid also contains a selection cassette comprising one or more selection genes to allow for selection of recombinant constructs in CEF cells. In a preferred embodiment, the recombinant MVA encodes a polypeptide comprising SEQ ID NO:2.

[0124] Simultaneous infection and transfection of cultures allows homologous recombination to occur between the viral genome and the recombination plasmid. Insert-carrying virus is then isolated, characterized, and virus stocks prepared. In certain embodiments, virus is passaged in CEF cell cultures in the absence of selection to allow for loss of the region encoding the selection genes, GPT and mRFP1.

Antagonists of PD-1 and LAG-3

[0125] In certain embodiments, the invention encompasses antagonists of PD-1 and LAG-3. An antagonist of PD-1 and LAG-3 interferes with PD-1 and LAG-3, respectively.

[0126] Such antagonists include: antibodies which specifically bind to PD-1 or LAG-3 and inhibit PD-1 or LAG-3 biological activity; antisense nucleic acids RNAs that interfere with the expression of PD-1 or LAG-3; small interfering RNAs that interfere with the expression of PD-1, LAG-3; and small molecule inhibitors of PD-1 or LAG-3.

[0127] Candidate antagonists of PD-1 or LAG-3 can be screened for function by a variety of techniques known in the art and/or disclosed within the instant application, such as ability to interfere with inhibition by PD-1 or LAG-3 function in an in vitro or mouse model.

Agonists of ICOS

[0128] The invention further encompasses agonists of ICOS. An agonist of ICOS activates ICOS. In one embodiment, the agonist is ICOS-L, an ICOS natural ligand. The agonist

can be a mutated form of ICOS-L that retains binding and activation properties. Mutated forms of ICOS-L can be screened for activity in stimulating ICOS in vitro.

[0129] Preferably, the agonist of ICOS is an antibody.

Antibodies

[0130] In one embodiment, the antagonist of PD-1 or LAG-3 or the agonist of ICOS is an antibody. Antibodies can be synthetic, monoclonal, or polyclonal and can be made by techniques well known in the art. Such antibodies specifically bind to PD-1, LAG-3, or ICOS via the antigen-binding sites of the antibody (as opposed to non-specific binding). PD-1, LAG-3, or ICOS polypeptides, fragments, variants, fusion proteins, etc., can be employed as immunogens in producing antibodies immunoreactive therewith. More specifically, the polypeptides, fragment, variants, fusion proteins, etc. contain antigenic determinants or epitopes that elicit the formation of antibodies.

[0131] These antigenic determinants or epitopes can be either linear or conformational (discontinuous). Linear epitopes are composed of a single section of amino acids of the polypeptide, while conformational or discontinuous epitopes are composed of amino acids sections from different regions of the polypeptide chain that are brought into close proximity upon protein folding (C. A. Janeway, Jr. and P. Travers, *Immuno Biology* 3:9 (Garland Publishing Inc., 2nd ed. 1996)). Because folded proteins have complex surfaces, the number of epitopes available is quite numerous; however, due to the conformation of the protein and steric hinderances, the number of antibodies that actually bind to the epitopes is less than the number of available epitopes (C. A. Janeway, Jr. and P. Travers, *Immuno Biology* 2:14 (Garland Publishing Inc., 2nd ed. 1996)). Epitopes can be identified by any of the methods known in the art.

[0132] Antibodies, including scFV fragments, which bind specifically to PD-1, LAG-3, or ICOS and either block its function (“antagonist antibodies”) or activate its function (agonist antibodies) are encompassed by the invention. Such antibodies can be generated by conventional means.

[0133] The invention encompasses monoclonal antibodies against PD-1, LAG-3, or ICOS that block its function (“antagonist antibodies”) or activate its function (agonist antibodies). Exemplary blocking monoclonal antibodies against PD-1, LAG-3, and ICOS are described in WO 2012/131004 and WO 2011/041613, which are hereby incorporated by reference.

[0134] Antibodies are capable of binding to their targets with both high avidity and specificity. They are relatively large molecules (~150kDa), which can sterically inhibit interactions between two proteins (e.g. PD-1, LAG-3, or ICOS and its target ligand) when the antibody binding site falls within proximity of the protein-protein interaction site. The invention further encompasses antibodies that bind to epitopes within close proximity to a PD-1, LAG-3, or ICOS -ligand binding site.

[0135] In various embodiments, the invention encompasses antibodies that interfere with intermolecular interactions (e.g. protein-protein interactions), as well as antibodies that perturb intramolecular interactions (e.g. conformational changes within a molecule). Antibodies can be screened for the ability to block the biological activity of PD-1 or LAG-3, or the binding of PD-1 or LAG-3 to a ligand, and/or for other properties. Agonist antibodies can further be screened for the ability to activate the biological activity of ICOS.

[0136] Both polyclonal and monoclonal antibodies can be prepared by conventional techniques.

[0137] PD-1, LAG-3, and ICOS and peptides based on the amino acid sequence of PD-1, LAG-3, and ICOS, can be utilized to prepare antibodies that specifically bind to PD-1, LAG-3, or ICOS. The term "antibodies" is meant to include polyclonal antibodies, monoclonal antibodies, fragments thereof, such as F(ab')₂ and Fab fragments, single-chain variable fragments (scFvs), single-domain antibody fragments (VHHs or Nanobodies), bivalent antibody fragments (diabodies), as well as any recombinantly and synthetically produced binding partners.

[0138] Antibodies are defined to be specifically binding if they bind PD-1, LAG-3, or ICOS polypeptide with a K_a of greater than or equal to about 10^7 M^{-1} . Affinities of binding partners or antibodies can be readily determined using conventional techniques, for example those described by Scatchard et al., *Ann. N.Y. Acad. Sci.*, 51:660 (1949).

[0139] Polyclonal antibodies can be readily generated from a variety of sources, for example, horses, cows, goats, sheep, dogs, chickens, rabbits, mice, or rats, using procedures that are well known in the art. In general, purified PD-1, LAG-3, or ICOS or a peptide based on the amino acid sequence of PD-1, LAG-3, or ICOS that is appropriately conjugated is administered to the host animal typically through parenteral injection. The immunogenicity of PD-1, LAG-3, or ICOS can be enhanced through the use of an adjuvant, for example, Freund's complete or incomplete adjuvant. Following booster immunizations, small samples of serum are collected and tested for reactivity to PD-1, LAG-3, or ICOS polypeptide. Examples of various assays useful for such determination include those described in *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988; as well as procedures, such as countercurrent immuno-electrophoresis (CIEP), radioimmunoassay, radio-immunoprecipitation, enzyme-linked immunosorbent assays (ELISA), dot blot assays, and sandwich assays. See U.S. Pat. Nos. 4,376,110 and 4,486,530.

[0140] Monoclonal antibodies can be readily prepared using well known procedures. See, for example, the procedures described in U.S. Pat. Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993; *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Plenum Press, Kennett, McKeam, and Bechtol (eds.), 1980.

[0141] For example, the host animals, such as mice, can be injected intraperitoneally at least once and preferably at least twice at about 3 week intervals with isolated and purified PD-1, LAG-3, or ICOS or conjugated PD-1, LAG-3, or ICOS peptide, optionally in the presence of adjuvant. Mouse sera are then assayed by conventional dot blot technique or antibody capture (ABC) to determine which animal is best to fuse. Approximately two to three weeks later, the mice are given an intravenous boost of PD-1, LAG-3, or ICOS or conjugated PD-1, LAG-3, or ICOS peptide. Mice are later sacrificed and spleen cells fused with commercially available myeloma cells, such as Ag8.653 (ATCC), following established protocols. Briefly, the myeloma cells are washed several times in media and fused to mouse spleen cells at a ratio of about three spleen cells to one myeloma cell. The fusing agent can be any suitable agent used in the art, for example, polyethylene glycol (PEG). Fusion is plated out into plates containing media that allows for the selective growth of the fused cells. The fused cells can then be allowed to grow for approximately eight days. Supernatants from resultant hybridomas are collected and added to a plate that is first coated with goat anti-mouse Ig. Following washes, a label, such as a labeled PD-1, LAG-3, or ICOS polypeptide, is added to each well followed by incubation. Positive wells can be subsequently detected. Positive clones can be grown in bulk culture and supernatants are subsequently purified over a Protein A column (Pharmacia).

[0142] The monoclonal antibodies of the invention can be produced using alternative techniques, such as those described by Alting-Mees et al., "Monoclonal Antibody Expression

Libraries: A Rapid Alternative to Hybridomas", *Strategies in Molecular Biology* 3:1-9 (1990), which is incorporated herein by reference. Similarly, binding partners can be constructed using recombinant DNA techniques to incorporate the variable regions of a gene that encodes a specific binding antibody. Such a technique is described in Larrick et al., *Biotechnology*, 7:394 (1989).

[0143] Antigen-binding fragments of such antibodies, which can be produced by conventional techniques, are also encompassed by the present invention. Examples of such fragments include, but are not limited to, Fab and F(ab')₂ fragments. Antibody fragments and derivatives produced by genetic engineering techniques are also provided.

[0144] The monoclonal antibodies of the present invention include chimeric antibodies, e.g., humanized versions of murine monoclonal antibodies. Such humanized antibodies can be prepared by known techniques, and offer the advantage of reduced immunogenicity when the antibodies are administered to humans. In one embodiment, a humanized monoclonal antibody comprises the variable region of a murine antibody (or just the antigen binding site thereof) and a constant region derived from a human antibody. Alternatively, a humanized antibody fragment can comprise the antigen binding site of a murine monoclonal antibody and a variable region fragment (lacking the antigen-binding site) derived from a human antibody. Procedures for the production of chimeric and further engineered monoclonal antibodies include those described in Riechmann et al. (*Nature* 332:323, 1988), Liu et al. (*PNAS* 84:3439, 1987), Larrick et al. (*Bio/Technology* 7:934, 1989), and Winter and Harris (*TIPS* 14:139, May, 1993). Procedures to generate antibodies transgenically can be found in GB 2,272,440, U.S. Pat. Nos. 5,569,825 and 5,545,806.

[0145] Antibodies produced by genetic engineering methods, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, can be used. Such chimeric and humanized monoclonal antibodies can be produced by genetic engineering using standard DNA techniques known in the art, for example using methods described in Robinson et al. International Publication No. WO 87/02671; Akira, et al. European Patent Application 0184187; Taniguchi, M., European Patent Application 0171496; Morrison et al. European Patent Application 0173494; Neuberger et al. PCT International Publication No. WO 86/01533; Cabilly et al. U.S. Pat. No. 4,816,567; Cabilly et al. European Patent Application 0125023; Better et al., Science 240:1041 1043, 1988; Liu et al., PNAS 84:3439 3443, 1987; Liu et al., J. Immunol. 139:3521 3526, 1987; Sun et al. PNAS 84:214 218, 1987; Nishimura et al., Canc. Res. 47:999 1005, 1987; Wood et al., Nature 314:446 449, 1985; and Shaw et al., J. Natl. Cancer Inst. 80:1553 1559, 1988); Morrison, S. L., Science 229:1202 1207, 1985; Oi et al., BioTechniques 4:214, 1986; Winter U.S. Pat. No. 5,225,539; Jones et al., Nature 321:552 525, 1986; Verhoeyan et al., Science 239:1534, 1988; and Beidler et al., J. Immunol. 141:4053 4060, 1988.

[0146] In connection with synthetic and semi-synthetic antibodies, such terms are intended to cover but are not limited to antibody fragments, isotype switched antibodies, humanized antibodies (e.g., mouse-human, human-mouse), hybrids, antibodies having plural specificities, and fully synthetic antibody-like molecules.

[0147] For therapeutic applications, "human" monoclonal antibodies having human constant and variable regions are often preferred so as to minimize the immune response of a patient against the antibody. Such antibodies can be generated by immunizing transgenic

animals which contain human immunoglobulin genes. See Jakobovits et al. *Ann NY Acad Sci* 764:525-535 (1995).

[0148] Human monoclonal antibodies against PD-1, LAG-3, or ICOS polypeptides can also be prepared by constructing a combinatorial immunoglobulin library, such as a Fab phage display library or a scFv phage display library, using immunoglobulin light chain and heavy chain cDNAs prepared from mRNA derived from lymphocytes of a subject. See, e.g., McCafferty et al. PCT publication WO 92/01047; Marks et al. (1991) *J. Mol. Biol.* 222:581-597; and Griffiths et al. (1993) *EMBO J* 12:725-734. In addition, a combinatorial library of antibody variable regions can be generated by mutating a known human antibody. For example, a variable region of a human antibody known to bind PD-1, LAG-3, or ICOS, can be mutated, by for example using randomly altered mutagenized oligonucleotides, to generate a library of mutated variable regions which can then be screened to bind to PD-1, LAG-3, or ICOS. Methods of inducing random mutagenesis within the CDR regions of immunoglobulin heavy and/or light chains, methods of crossing randomized heavy and light chains to form pairings and screening methods can be found in, for example, Barbas et al. PCT publication WO 96/07754; Barbas et al. (1992) *Proc. Nat'l Acad. Sci. USA* 89:4457-4461.

[0149] An immunoglobulin library can be expressed by a population of display packages, preferably derived from filamentous phage, to form an antibody display library. Examples of methods and reagents particularly amenable for use in generating antibody display library can be found in, for example, Ladner et al. U.S. Pat. No. 5,223,409; Kang et al. PCT publication WO 92/18619; Dower et al. PCT publication WO 91/17271; Winter et al. PCT publication WO 92/20791; Markland et al. PCT publication WO 92/15679; Breitling et al. PCT publication WO 93/01288; McCafferty et al. PCT publication WO 92/01047; Garrard et al. PCT publication WO

92/09690; Ladner et al. PCT publication WO 90/02809; Fuchs et al. (1991) *Bio/Technology* 9:1370 1372; Hay et al. (1992) *Hum Antibod Hybridomas* 3:81 85; Huse et al. (1989) *Science* 246:1275 1281; Griffiths et al. (1993) *supra*; Hawkins et al. (1992) *J Mol Biol* 226:889 896; Clackson et al. (1991) *Nature* 352:624 628; Gram et al. (1992) *PNAS* 89:3576 3580; Garrad et al. (1991) *Bio/Technology* 9:1373 1377; Hoogenboom et al. (1991) *Nuc Acid Res* 19:4133 4137; and Barbas et al. (1991) *PNAS* 88:7978 7982. Once displayed on the surface of a display package (e.g., filamentous phage), the antibody library is screened to identify and isolate packages that express an antibody that binds a PD-1, LAG-3, or ICOS polypeptide. In a preferred embodiment, the primary screening of the library involves panning with an immobilized PD-1, LAG-3, or ICOS polypeptide and display packages expressing antibodies that bind immobilized PD-1, LAG-3, or ICOS polypeptide are selected.

Exemplary Combination therapies with recombinant orthopoxvirus-expressing a tumor antigen and agonists/antagonists of PD-1, LAG-3, and ICOS

[0150] In at least one aspect, the present invention encompasses methods of treatment employing combinations of a recombinant orthopoxvirus and/or avipoxvirus each comprising a nucleic acid encoding a tumor antigen with one or more antibodies, agonists, or antagonists according to the invention.

[0151] In one exemplary embodiment, patients with a cancer mediated by cells over-expressing the tumor-associated antigen HER-2 (e.g., breast cancer) can be treated by the combination of: 1) one or more recombinant orthopoxviruses, for example a vaccinia virus (e.g., Wyeth or MVA) encoding a HER-2 antigen or 2) the combination of a recombinant orthopoxvirus and a recombinant an avipoxvirus (e.g., fowlpoxvirus, POXVAC-TC), encoding a HER-2 antigen; with combination 1) or 2) being administered with one or more antibodies,

agonists, and/or antagonists according to the invention. In a preferred embodiment, the MVA is MVA-BN. In a particularly preferred embodiment, the MVA encodes a polypeptide comprising SEQ ID NO:2.

[0152] In an additional exemplary embodiments, patients with prostate cancer can be treated by the combination of a recombinant orthopoxvirus, for example a vaccinia virus (e.g., Wyeth or MVA) and a recombinant avipoxvirus (e.g., fowlpoxvirus, POXVAC-TC), encoding a PSA and/or PAP antigen, with one or more antibodies, agonists, or antagonists according to the invention. In a particularly preferred embodiment, the combination of the recombinant vaccinia virus and fowlpox virus is PROSTVAC[®] (vaccinia virus and fowlpox virus, each expressing PSA and TRICOM) and is administered with one or more antibodies, agonists, and/or antagonists according to the invention.

[0153] In still an additional embodiment, patients with cancer mediated by cells over-expressing the tumor-associated antigen CEA and/or MUC-1 can be treated by the combination of a recombinant orthopoxvirus, for example a vaccinia virus (e.g., Wyeth, MVA, or MVA-BN) and a recombinant avipoxvirus (e.g., fowlpoxvirus, POXVAC-TC), each encoding CEA and MUC-1 antigen, with one or more antibodies, agonists, or antagonists according to the invention. Some non-limiting examples of cancers mediated by cells overexpressing CEA and MUC-1 include, but are not limited to, breast cancer, colorectal cancer, lung cancer, gastric cancer, pancreatic cancer, bladder cancer, and ovarian cancer. In one or more preferred embodiments, the combination therapy includes CV301 (vaccinia virus and fowlpox virus, each expressing CEA, MUC-1, and TRICOM). In another preferred embodiment, the combination therapy includes MVA/Fowlpox-CV301, a heterologous prime-boost that includes MVA (or MVA-BN) and Fowlpox each expressing CEA, MUC-1, and TRICOM.

[0154] In yet another embodiment, patients with cancer mediated by cells over-expressing the tumor-associated antigen CEA and/or MUC-1 can be treated by a homologous prime-boost encoding CEA and/or MUC-1 antigens in combination with one or more antibodies, agonists, or antagonists according to the invention. It is contemplated that the two or more recombinant orthopoxviruses can be a vaccinia virus (e.g., Wyeth, MVA, or MVA-BN). Some non-limiting examples of cancers mediated by cells overexpressing CEA and MUC-1 include, but are not limited to, breast cancer, colorectal cancer, lung cancer, gastric cancer, pancreatic cancer, bladder cancer, and ovarian cancer. In one or more preferred embodiments, the treatment can be MVA-CV301, which includes two or more MVA (or MVA-BN) viruses each expressing CEA, MUC-1, and TRICOM) administered as a homologous prime-boost.

[0155] The recombinant orthopoxvirus and/or avipoxvirus can be administered either systemically or locally, i.e., by parenteral, subcutaneous, intravenous, intramuscular, intranasal, intradermal, scarification, or any other path of administration known to a skilled practitioner. Preferably, the administration is via scarification. More preferably, the administration is via subcutaneous or intramuscular. In one embodiment, 10^5 - 10^{10} TCID₅₀ of the recombinant orthopoxvirus and/or avipoxvirus are administered to the patient. Preferably, 10^7 - 10^{10} TCID₅₀ of the recombinant orthopoxvirus and/or avipoxvirus are administered to the patient. More preferably, 10^8 - 10^{10} TCID₅₀ of the recombinant orthopoxvirus and/or avipoxvirus are administered to the patient. Most preferably, 10^8 - 10^9 TCID₅₀ of the recombinant orthopoxvirus and/or avipoxvirus are administered to the patient.

[0156] It is possible to induce an immune response with a single administration of the recombinant orthopoxvirus and/or avipoxvirus as defined above. The orthopoxvirus and/or avipoxvirus according to the present invention may also be used for a first vaccination and to

boost the immune response generated in the first vaccination by administration of the same or a related recombinant orthopoxvirus and/or avipoxvirus than the one used in the first vaccination. The recombinant orthopoxvirus and/or avipoxvirus according to the present invention may also be used in heterologous prime-boost regimes in which one or more of the priming vaccinations is done with an orthopoxvirus as defined above and in which one or more of the boosting vaccinations is done with another type of vaccine, e.g. an avipoxvirus as defined herein or another virus vaccine, a protein or a nucleic acid vaccine.

[0157] The cancer preferably includes, but is not limited to, breast cancer, colorectal cancer, lung cancer, gastric cancer, pancreatic cancer, bladder cancer, prostate cancer, and ovarian cancer.

[0158] The cancer patient can be any mammal, including a mouse or rat. Preferably, the cancer patient is a primate, most preferably, a human.

[0159] In one embodiment, one or more antibodies, agonists or antagonists, according to the invention and the orthopoxvirus encoding a polypeptide comprising a tumor antigen are administered at the same time. The combination treatment is superior to either treatment alone.

[0160] In preferred embodiments, the orthopoxvirus is for administration within 1, 2, 3, 4, 5, 6, or 7, days of agonist and/or antagonist administration. The orthopoxvirus can be administered before or after the agonist and/or antagonist.

[0161] The dosage agonist or antagonist administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight, most preferably 3 mg/kg to 10 mg/kg of the patient's body weight. Generally, human and humanized antibodies have a longer half-life within the human

body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible.

[0162] The quantities of active ingredient necessary for effective therapy will depend on many different factors, including means of administration, target site, physiological state of the patient, and other medicaments administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used in vitro can provide useful guidance in the amounts useful for in situ administration of the active ingredients. Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, for example, in Goodman and Gilman's the Pharmacological Basis of Therapeutics, 7th Edition (1985), MacMillan Publishing Company, New York, and Remington's Pharmaceutical Sciences 18th Edition, (1990) Mack Publishing Co, Easton Pa. Methods for administration are discussed therein, including oral, intravenous, intraperitoneal, intramuscular, transdermal, nasal, iontophoretic administration, and the like.

[0163] The compositions of the invention can be administered in a variety of unit dosage forms depending on the method of administration. For example, unit dosage forms suitable for oral administration include solid dosage forms such as powder, tablets, pills, capsules, and dragees, and liquid dosage forms, such as elixirs, syrups, and suspensions. The active ingredients can also be administered parenterally in sterile liquid dosage forms. Gelatin capsules contain the active ingredient and as inactive ingredients powdered carriers, such as glucose, lactose, sucrose, mannitol, starch, cellulose or cellulose derivatives, magnesium stearate, stearic acid, sodium saccharin, talcum, magnesium carbonate and the like. Examples of additional inactive ingredients that can be added to provide desirable color, taste, stability, buffering capacity, dispersion or other known desirable features are red iron oxide, silica gel, sodium

lauryl sulfate, titanium dioxide, edible white ink and the like. Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric-coated for selective disintegration in the gastrointestinal tract. Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

[0164] The concentration of the compositions of the invention in the pharmaceutical formulations can vary widely, i.e., from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

[0165] For solid compositions, conventional nontoxic solid carriers can be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more compositions of the invention of the invention, and more preferably at a concentration of 25%-75%.

[0166] For aerosol administration, the compositions of the invention are preferably supplied in finely divided form along with a surfactant and propellant. Preferred percentages of compositions of the invention are 0.01%-20% by weight, preferably 1-10%. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as

c-aproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides can be employed. The surfactant can constitute 0.1%-20% by weight of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included, as desired, as with, e.g., lecithin for intranasal delivery.

[0167] The constructs of the invention can additionally be delivered in a depot-type system, an encapsulated form, or an implant by techniques well-known in the art. Similarly, the constructs can be delivered via a pump to a tissue of interest.

[0168] Any of the foregoing formulations can be appropriate in treatments and therapies in accordance with the present invention, provided that the active agent in the formulation is not inactivated by the formulation and the formulation is physiologically compatible.

Therapeutic Compositions and Uses

[0169] The present invention further relates to the use of the orthopoxvirus vectors and avipoxvirus vectors according to the invention for the preparation of therapeutic compositions or vaccines which are capable of inducing or contributing to the occurrence or improvement of an immunological reaction against tumor epitopes. The present invention thus provides viruses or vectors that are useful as a medicament or vaccine.

[0170] Accordingly, the invention relates to an immunogenic composition comprising a recombinant orthopoxvirus vector (such as vaccinia, MVA, or MVA-BN) and/or a recombinant avipoxvirus vector according to the invention in combination with one or more antibodies, agonists, or antagonists according to the invention.

[0171] Thus, the recombinant orthopoxvirus vector and recombinant avipoxvirus vectors according to the invention can be used for the preparation of therapeutic composition or medicament for the treatment of cancer.

[0172] The invention encompasses a composition for use in prophylactic and/or therapeutic vaccination protocols, for the treatment of tumors and especially as anti-cancer treatment.

[0173] In one embodiment, the invention encompasses a composition for administration to or treatment of a cancer patient, such as but not limited to, a prostate cancer, a breast cancer, a colorectal cancer, a lung cancer, a gastric cancer, a pancreatic cancer, a bladder cancer, or an ovarian cancer patient.

[0174] In one or more preferred embodiments, the invention encompasses use of a composition for administration to or treatment of a cancer patient, particularly a breast cancer patient or a prostate cancer patient.

[0175] In one embodiment the composition for simultaneous or sequential administration comprising an MVA vector according to the invention and one or more antibody, agonist, or antagonist according to the invention.

[0176] The compositions and methods described herein additionally or alternatively can comprise one or more immunostimulatory/regulatory molecules. Any suitable immunostimulatory/regulatory molecule can be used, such as interleukin (IL)-2, IL-4, IL-6, IL-12, IL-15, IL-15/IL-15Ra, IL-15/IL-15Ra-Fc, interferon (IFN)- γ , tumor necrosis factor (TNF)- α , B7.1, B7.2, ICAM-1, ICAM-2, LFA-1, LFA-2, LFA-3, CD70, CD-72, RANTES, G-CSF, GM-CSF, OX-40L, 41 BBL, anti-CTLA-4, IDO inhibitor, anti-PDL1, anti-PD1, and combinations thereof. Preferably, the composition comprises a combination of B7.1, ICAM-1, and LFA-3 (also referred to as TRICOM). The one or more immunostimulatory/regulatory molecules can be administered in the form of a vector (e.g., a recombinant viral vector, such as a poxvirus vector) comprising a nucleic acid encoding one or more immunostimulatory/regulatory molecules. For

example, the one or more immunostimulatory/regulatory molecules (e.g., IL-12) can be administered in the form of a DNA plasmid with or without chitosan.

[0177] In a more preferred embodiment, in addition to comprising at least one tumor antigen, the recombinant orthopoxvirus and/or the recombinant avipoxvirus comprise one or more nucleic acids encoding for the combination of B7.1, ICAM-1, and/or LFA-3 (also referred to as TRICOM).

EXAMPLES

Example 1

Construction of MVA-BN-mHER2

[0179] Simultaneous infection and transfection of cultures allowed homologous recombination to occur between the viral genome and the recombination plasmid. Insert-carrying virus was isolated, characterized, and virus stocks were prepared.

[0180] Plasmid pBN279 contains sequences which are also present in MVA-BN (the Intergenic Region between ORF 64 and 65, IGR 64/65). The mHER2 sequence was inserted between the MVA-BN sequences to allow for recombination into the MVA-BN viral genome. Thus, a plasmid was constructed that contained the mHER2 sequence downstream of a poxvirus promoter, specifically the synthetic vaccinia virus promoter (PrS). The plasmid also contained a selection cassette comprising the PrS promoter upstream of a drug resistance gene (guanine-xanthine phosphoribosyltransferase; EcoGPT) and a PrS promoter upstream of monomeric Red Fluorescence Protein 1 (mRFP1).

[0181] The HER-2 sequence was modified by addition of nucleotides sequences encoding tetanus toxin epitopes of p2 and p30 to increase the immune response against it (mHER2). After mHER2 was inserted into the MVA-BN genome, and the selection cassette was removed, the virus “insert region” had the following structure (shown in the opposite orientation compared to the surrounding viral reading frame):

PrS promoter - mHER2 sequence. The insert region was flanked by MVA-BN intergenic region sequences 64/65 (Flank 1(64) and Flank 2 (65)) as in the bacterial recombination plasmid pBN279. The nucleotide sequence of the construct is shown below.

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1      AAAAAAATAA TAATTAACCA ATACCAACCC CAACAACCGG TATTATTAGT TGATGTGACT GTTTTCTCAT
71     CACTTAGAAC AGATTTAACA ATTTCTATAA AGTCTGTCAA ATCATCTTCC GGAGACCCCA TAAATACACC
141    AAATATAGCG GCGTACAAC TATCCATTTA TACATTGAAT ATTGGCTTTT CTTTATCGCT ATCTTCATCA
211    TATTCATCAT CAATATCAAC AAGTCCCAGA TTACGAGCCA GATCTTCTTC TACATTTTCA GTCATTGATA

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281	CACGTTCACT	ATCTCCAGAG	AGTCCGATAA	CGTTAGCCAC	CACTTCTCTA	TCAATGATTA	GTTTCTTGAG
351	TGCGAATGTA	ATTTTTGTTT	CCGTCCGGGA	TCATAGAAG	ACGATAGGTG	TGATAATTGC	CTTGGCCAAT
421	TGTCTTTCTC	TTTTACTGAG	TGATTCTAGT	TCACCTTCTA	TAGATCTGAG	AATGGATGAT	TCTCCAGTCG
491	AAACATATTC	TACCATGGAT	CCGTTAATTT	TGTTGATGAA	GATGGATTCA	TCCTTAAATG	TTTCTCTGT
561	AATAGTTTCC	ACCGAAAGAC	TATGCAAAGA	ATTTGGAATG	CGTTCCTTGT	GCTTAAATGTT	TCCATAGACG
631	GCTTCTAGAA	GTTGATACAA	CATAGGACTA	GCCGCGGTAA	CTTTTATTTT	TAGAAAAGTAT	CCATCGCTTC
701	TATCTTGTTT	AGATTTATTT	TTATAAAGTT	TAGTCTCTCC	TTCCAACATA	ATAAAAGTGG	AAGTCATTTG
771	ACTAGATAAA	CTATCAGTAA	GTTTTATAGA	GATAGACGAA	CAATTAGCGT	ATTGAGAAGC	ATTTAGTGTA
841	ACGTATTCGA	TACATTTTGC	ATTAGATTTA	CTAATCGATT	TTGCATACTC	TATAACACCC	GCACAAGTCT
911	GTAGAGAATC	GCTAGATGCA	GTAGGCTTGG	GTGAAGTTTC	AACTCTCTTC	TTGATTACCT	TACTCATGAT
981	TAAACCTAAA	TAATTGTA	TTGTAATATA	ATGATATATA	TTTTCACTTT	ATCTCATTTG	AGAATAAAAA
1051	TGGAATTCCT	GCAGCCCGGG	GGATCCTTAA	TTAACTGGGT	ACCCAAGGCC	TTGGGTTTGG	GGGATCCTTA
1121	ATTAACTGGG	TACCGGGCCC	CCCCTCGAGG	TCGACGGTAT	CGATAAGCTT	GATGGCCCGC	ACTGTGCTGG
1191	ATATCTGCAG	AAATCCACCA	ACTGGACTA	TGGATCCGA	GCTCGGTACC	AAGCTTCTAC	AGAACTGCCAA
1261	CCACCGCAGA	GACGATCTCC	AGGTGGCTGG	CGCTCACCTT	GGGCACGCCC	AGCCAGAAGC	TCACGGTGAA
1331	GTTGTTGAAG	GACGTCAGAG	GGCTGGCTCT	CTGCTCGGCG	GGGCAGCCCT	TGTCATCCAG	GTCCACACAG
1401	GAGTGGGTGC	AGTTGATGGG	GCAAGGCTGG	CATGCGCCCT	CCTCATCTGG	AAACTTCCAG	ATGGGCATGT
1471	AGGAGAGGTC	AGGTTTCA	CCGCTGGGGC	AGCGGGCCAC	GCAGAAGGGA	GGGTCTTAT	AGTGGGCACA
1541	GGCCACACAC	TGGTCAGCCT	CCGGTCCAAA	ACAGGTCACT	GAGCCATTC	GGGGCTGACA	CTCAGGTTGG
1611	CACGGCAAAC	AGTGCCCTGG	ATTCACATA	TCCTGCGGGA	GCCCTGCAG	TACTCCGCAT	TTCCCTCACGC
1681	ACTCCTGGCC	CCGAAGGAAC	TGGCTGCAGT	TGACACACTG	GGTGGGCCCT	GGACCCAGC	AGTGCCTCG
1751	GGCGCACAGC	TGGTGGCAGG	CCAGGCCCTC	GCCACACAC	TCGTCTCTG	GCCGGTTGGC	AGTGTGGAGC
1821	AGAGCTTGGT	GCGGGTCCG	AAAGAGCTGG	TCCAGGGCA	CCGTGTGCAC	GAAGCAGAGG	TGGGTGTTAT
1891	GGTGGATGAG	GGCCAGTCCA	CTGCCAGTT	CCCTCAGTGA	GCGCAGCCCC	AGCCAGCTGA	TGCCAGCC
1961	TTGCAGGGT	AGCGAGTAGG	CGCCATFTG	CAGAATTCGT	CCCCGGATTA	CTTGCAGGTT	CTGGAAGACG
2031	CTGAGGTCAG	GCAAGGCTGC	CGGCCATGCT	GAGATGTATA	GGTAACCTGT	GATCTCTTCC	AGACTCTCAA
2101	ACACTTGGAG	CTGCTCTGGC	TGGAGCGGG	CAGTGTGGA	GGCTGGGTCC	CCATCAAAGC	TCTCCGGCAG
2171	AAATGCCAGG	CTCCCAAAGA	TCTTCTTGCA	GCCAGCAAAC	TCCTGGATAT	TGGCACTGGT	AACTGCCCTC
2241	ACCTCTCGCA	AGTGCTCCAT	GCCCAGACCA	TAGCACACTC	GGGCACAGGG	CTTGTGCAC	TTCTCACACC
2311	GCTGTGTTCC	ATCCTCTGCT	GTCACCTCTT	GGTTGTGCA	GGGGCAGACG	AGGGTGCAGG	ATCCCACGTC
2381	CGTAGAAAG	TAGTTGTAGG	GACAGGCAGT	CACACAGCTG	GCGCCGAATG	TATACCAGCAG	CTCGGTGATA
2451	CCGATGAATT	TGGAGTTAGC	TTTGATGTAC	TGGACAGGG	CTGGGCAGTG	CAGCTCACAG	ATGCCACTGT
2521	GGTTGAAGTG	GAGGCAGGCC	AGGCAGTCAG	AGTGCTTGGG	GCCCCTGCAG	CCGGCAGCAC	ACTGCTCATG
2591	GCAGCAGTCA	GTGGGCAGTG	GCCCCTTGCA	GCGGGCACAG	CCACCGGCAC	AGACAGTGGC	CGTCAGGCTC
2661	TGACAATCCT	CAGAACTCTC	TCCCAGCAG	CGGGAGCCCT	TACACATCGG	AGAACAGGGG	TGGCAGGCCC
2731	GAGAGCCGTT	GGTGTCTATC	AGTGTGAGAG	CCAGCTGGTT	GTTCTTGTGG	AAGATGTCTT	TCCACAAAAT
2801	CGTGTCTGAG	TAGCAGAGCT	GGGGTTCFCC	CTGAGTCAAG	ACCCCTCCTT	TCAAGATCTC	TTGAGGCTTT
2871	CGAAGCTGCA	GCTCCCGCAG	GCCTCCTGGG	GAGGCCCTTG	TGACAGGGGT	GGTATFTGTT	AGCGGGTCTC
2941	CATTGTCTAG	CACGGCCAGG	GCATAGTTGT	CCTCAAAGAG	CTGGGTGCC	CGCACAAATC	GCAGCCTCTG
3011	CAGTGGGACC	TGCCTCACCT	GGTTGTGAGC	GATGAGCAGC	TAGCCCTGCA	CCTCCTGGAT	ATCCTGCAGG
3081	AAACTTAAGC	TGGCATTGGT	GGGCAGGTAG	GTGAGTTCCA	GGTTTCCCTG	CACCACCTGG	CAGCCCTGGT
3151	AGAGGTGGCG	GAGCATGTCC	AGGTGGGTCT	CGGGACTGGC	AGGGAGCCGC	AGCTTTCATG	CTGTGCCGGT
3221	GCACACTTGG	GTGCTCGCGG	CTCCGGGGGG	CAAGAGGGCG	AGGAGGAGCC	CCCAGCGGCA	CAAGGCCGCC
3291	AGCTCCATGG	TGGCGGCTAG	ATCGAATTCC	TGCAGCCCAA	ACCCGATTTA	AATTGGCGCC	CGTACGGGAAG
3361	ATCTTCGACG	TCTAAGCGGC	CGCAATAGCT	AGCTAGTCCG	GATATTTATA	TTCCAAAAAA	AAAAAATAAA
3431	ATTTCAATTT	TTGTTTTAAC	ACGCGTTCTA	GATTTTGT	AACCACTGCA	TGATGTACAG	ATTTCGGAAAT
3501	CGCAAACCAC	CAGTGGTTTT	ATTTTATCCT	TGTTCAATGT	GAATTGAATG	GGAGCGGATG	CGGGTTTCTGT
3571	ACGTAGATAG	TACATTCCCG	TTTTTAGACC	GAGACTCCAT	CCGTAATAAT	GCATACCTGT	TAGTTTGGAA
3641	TAACTCGGAT	CTGCTATATG	GATATPCATA	GATPGACTTT	GATCGATGAA	GGCTCCCTGT	TCTGCAGCCA
3711	TTTTTATGAT	CGTCTTTTGT	GGAATTTCCC	AAATAGTTTT	ATAAACTCGC	TTAATATCTT	CTGGAAGGTT
3781	TGTATTCTGA	ATGGATCCAC	CATCTGCCAT	AATCCTATTC	TTGATCTCAT	CATTCCATAA	TTTTCTCTCG
3851	GTTAAAACCT	TAAGGAGATG	CGGATTAAC	ACTTGAAAT	CTCCAGACAA	TACTCTCCGA	GTGTAATAAT
3921	TACTGGTATA	CGGTTCCACC	GACTCATAT	TTCCCAAAAT	TTGAGCAGTT	GATGCAGTCG	GCATAGGTGC
3991	CACCAATAAA	CTATTTCTAA	GACCGTATG	TCGTATTTA	TCTTTTAGAG	GTTCCCAATT	CCAAAGATCC
4061	GACGGTACAA	CATTCCAAAG	ATCATATTGT	AGAATACCGT	TACTGGCGTA	CGATCTTACA	TATGTATCGT
4131	ATGGTCTTTC	CTTCTCAGCT	AGTTCACAAC	TCGCCTCTAA	TGCACCGTAA	TAAATGGTTT	CGAAGATCTT
4201	CTATTTTAGA	TCTTGTGCTT	CCAGGCTATC	AAATGGATAA	TTTAAGAGAA	TAAACGGGTC	CGCTAATCCT
4271	TGAACACCAA	TACCGATAGG	TCTATGTCTC	TTATTAGAGA	TTTCAGCTTC	TGGAATFAGGA	TAATAATTTAA
4341	TATCTATAAT	TTTATTGAGA	TTTCTGACAA	TTACTTTGAC	CACATCCTTC	AGTTTGGAGAA	AATCAAAATCG
4411	CCATCTATT	ACAAACATGT	TCAAGGCAAC	AGATGCCAGA	TTACAAACGG	CTACCTCATT	AGCATCCGCA
4481	TATTGTA						

(SEQ ID NO:1).

[0182] HER2 start and stop codons are indicated in bold. Flanking sequences are indicated in italics. Flank 1 (64): 451-1052, underlined HER2: 3298-1247 (bold), start: 3298-3296 (bold + underlined), stop: 1249-1247 (bold + underlined), PrS promoter: 3442-3403 (italic + underlined), Flank (65): 3463-4065 (underlined).

[0183] Translation of the encoded mHER2 polypeptide is shown below:

MELAAALCRWGLLLALLPPGAASTQVCTGTDMKLRLPASPETHLDMLRHLIYQGCQVVQGNL
ELTYLPTNASLSFLQDIQEVQGYVLIAHNQVRQVPLQRLRIVRGTQLFEDNYALAVLDNG
DPLNNTTPVTGASPGGLRELQLRSLTEILKGGVLIQRNPQLCYQDTILWKDIFHKNNQLA
LTLIDTNRSRACHPCSPMCKGSRGWGESSEDCQSLTRTVCAAGGCARCKGPLPTDCCHEQC
AAGCTGPKHSDCLACLHFNHSGICELHCPALV**QYIKANSKFIGITEL**RYTFGASCVTACP
YNYLSTDVGSCTLVCPHNLQEVTAEDGTQRCCKSKPCARVCYGLGMEHLREVRVAVTSAN
IQEFAGCKKIFGSLAFLPESFDGDPASNTAPLQPEQLQVFETLEEITGYLYISAWPDSL
DLVVFQNLQVIRGRILHNGAYSLTLQGLGISWLGLRSLRELGSGLALIHHTHLFCFVHTV
PWDQLFRNPHQALLHTANRPEDECVGEGLACHQLCARGHCWGPPTQCVNCSQFLRGQEC
VEECRVLQGLPREYVNARHCLPCHPECQPQNGSVTCFGPEADQCVACAHYKDPPFCVARC
PSGVKPDLSYMPIWKFPDEEGACQPCPINCTHSCVDLDDKGCPAEQRASPLTS**FNNFTVS**
FWLRVPKVSASHLEIVSAVVGIL.

(SEQ ID NO:2).

[0184] The tetanus toxin epitopes of p2 and p30 sequences are indicated in bold.

[0185] CEF cultures were inoculated with MVA-BN and also transfected with pBN279 plasmid DNA. In turn, samples from these cell cultures were inoculated into CEF cultures in medium containing selection drugs, and mRFP1-expressing viral clones were isolated by plaque purification. Virus stocks which grew in the presence of the selection drugs and expressed mRFP1 were designated MVA-BN-mHER2. Generation of MVA-BN-mHER2 and preparation of the virus stock involved nine (9) sequential passages, including four (4) plaque purifications.

[0186] MVA-BN-mHER2 was passaged in CEF cell cultures in the absence of selection drugs. The absence of selection drugs allowed loss of the region encoding the selection genes, gpt and mRFP1 and the associated promoters (the selection cassette) from the inserted sequence.

Recombination resulting in loss of the selection cassette is mediated by the Flank 1 (F1) region and a subsection of that region, the F1 repeat (F1 rpt), which flank the selection cassette in plasmid pBN279. These duplicated sequences were included to mediate recombination that results in loss of the selection cassette, leaving only the mHER2 sequence inserted in the 64/65 intergenic region.

[0187] Plaque-purified virus lacking the selection cassette was prepared. Such preparation involved fifteen (15) passages including five (5) plaque purifications.

[0188] The presence of the mHER2 sequence and absence of parental MVA-BN virus in MVA-BN-mHER2 stocks was confirmed by PCR analysis, and nested PCR was used to verify the absence of the selection cassette (the gpt and mRFP1 genes).

[0189] Expression of the mHER2 protein was demonstrated in cells inoculated with MVA-BN-mHER2 *in vitro*.

Example 2

Induction of an anti-tumor response in mice treated with MVA-BN-HER2 and antibodies

[0190] Female BALB/c mice (6-8 weeks old, ~ 20 g) were purchased from Simonsen Laboratories, Gilroy, CA. For the experimental lung metastasis model, mice were implanted i.v. with 5.0×10^4 CT26-HER-2 cells in 300 μ L DPBS which forms tumors in the lungs. In the solid tumor model, mice were implanted i.d. in the back with 1.0×10^5 CT26-HER-2 cells in 100 μ L DPBS. Tumors were measured twice weekly and the tumor volume calculated according to the formula: tumor volume (mm^3) = (length \times width²)/2.

[0191] The following antibodies were purchased from Bio X Cell (West, Lebanon, NH): anti-ICOS (Clone 17G9), anti-CTLA-4 (9D9), anti-PD-1 (RMP1-14), and anti-LAG-3 (C9B7W). All antibodies were injected i.p. at 200 μ g per mouse in 100 μ L PBS on the days indicated in the

figure legends. For virus treatments, mice were treated with 7.1 μL of 1.0×10^7 Inf. U. MVA-BN-mHER2 by tail scarification (t.s., produced by Bavarian Nordic [BN], Martinsried, Germany).

[0192] Serum antibody titers were determined by ELISA, and IFN- γ by ELISPOT as described in Mandl et al., *Cancer Immunol Immunother* (2012) 61:19–29. Whole blood, spleens, and lungs were collected for FACS analysis. Lungs were cut to 1-2 mm pieces and incubated for 1 h at 37 °C in DMEM with 10% FBS, 50 U/mL DNase I and 250 U/mL Collagenase I (Worthington Biochemical Corporation, Lakewood, NJ). Red blood cells from the lungs, splenocytes, and whole blood were lysed and single cell suspensions were stained according to standard protocols with antibodies purchased from Biolegend (San Diego, CA): CD3e (145-2c11 or 500A2), CD4 (RM4-5), CD8 (53-6.7), CD278 (ICOS, 7E.17G9), CD279 (PD-1, 29F.1AA12), and CD223 (LAG-3, C9B7W). Regulatory T-cells were identified using the FoxP3/Transcription Factor Staining Buffer Set and FoxP3 antibody (FJK-16s) according to the manufacturer's instructions (eBioscience, San Diego, CA).

[0193] All statistical analysis was performed as described in the figure legends using GraphPad Prism version 6.01 for Windows (GraphPad Software, La Jolla, CA).

Example 3

MVA-BN-HER2 Treatment Increases ICOS on CD8⁺ and CD4⁺ T Cells

[0194] Naïve, tumor free mice were treated with MVA-BN-mHER2 (1×10^7 Inf.U. t.s.) on day 1 or days 1 and 15. Organs from 3 mice at each time point. Shown in Figure 1, treatment with MV-BN-mHER2 increased ICOS expression on CD8⁺ and CD4⁺ T Cells.

Example 4

Induction of an anti-tumor response in mice treated with MVA-BN-HER2 with anti-ICOS.

[0195] Balb/c mice were implanted on day 1 with 1E5 CT26-HER2 cells i.d. Mice were treated with 1E7 Inf.U. MVA-BN-mHER2 by tail scarification (t.s.) on days 1 and 15, and anti-ICOS on days 1, 4, 8, 11, 15, 18, 22, and 25 (200, µg i.p.). **, p<0.01, **** p<0.0001, Repeated measures Two-way ANOVA with Tukey's Multiple Comparisons post-test. Shown in Figure 2, tumor volume was significantly decreased with MVA-BN-mHER2 plus anti-ICOS as compared to MVA-BN-mHER2 alone.

Example 5

Induction of an anti-tumor response in mice treated with MVA-BN-HER2 with anti-CTLA-4

[0196] In a CT26-HER-2 experimental lung metastasis model, mice were implanted on day 1 with 5E4 CT26-HER-2 cells i.v. which forms tumors in the lungs. Mice were treated with MVA-BN-mHER2 (1E7 Inf.U. t.s.) on days 4 and 18 and anti-CTLA-4 (200 µg, i.p.) on days 3 and 17. **** p<0.0001, Log-Rank Test. Shown in Figure 3, treatment with MVA-BN-mHER2 and anti-CTLA-4 was effective at increasing the overall survival rate.

Example 6

MVA-BN-HER2 Significantly Reduces Pulmonary Tumor Burden by Day 25

[0197] Mice were implanted i.v. on day 1 with 5E4 CT26-HER-2 cells i.v. which forms tumors in the lungs. Mice were treated with MVA-BN-mHER2 (1E7 Inf.U. t.s.) on day 4 and 18 and anti-CTLA-4 (200 µg, i.p.) on days 3 and 17. A) On day 25, mice were euthanized and perfused through the trachea with Trypan Blue. Lungs were removed and briefly submerged in Hydrogen Peroxide and washed in PBS. Tumors visible as small masses in Untreated and anti-CTLA-4 treated mice. There were no visible tumors in the lungs of mice treated with MVA-BN-

mHER2. Scale bar equals 1 cm. B) Lung weight on day 25. **** p<0.0001, One-Way ANOVA with Dunnett's Multiple Comparisons test. The results are shown in Figure 4.

Example 7

Pulmonary ICOS increases with MVA-BN-HER2 Treatment or Pulmonary Tumors

[0198] Mice were implanted i.v. on day 1 with 5E4 CT26-HER-2 cells i.v. which forms tumors in the lungs. Mice were treated with MVA-BN-mHER2 (1E7 Inf.U. t.s.) on day 4 and 18 and anti-CTLA-4 (200 µg, i.p.) on days 3 and 17. Organs from 3 mice at each time point were pooled for analysis (A and B). Data shown as mean ± SEM from three independent experiments with 3-4 mice per group (C and D). Shown in Figure 5, Pulmonary ICOS increased upon treatment with MVA-BN-mHER2.

Example 8

In Tumor Bearing Mice, ICOS⁺ CD4⁺ T Cells are FoxP3⁺

[0199] Mice were implanted i.v. on day 1 with 5E4 CT26-HER-2 cells i.v. which forms tumors in the lungs. Mice were treated with MVA-BN-mHER2 (1E7 Inf.U. t.s.) on day 4 and 18 and anti-CTLA-4 (200 µg, i.p.) on days 3 and 17. Organs from 3 mice at each time point were pooled for analysis (A and B). Data shown as mean ± SEM from three independent experiments with 3-4 mice per group (C and D). Shown in Figure 6, ICOS expression increased in both FoxP3⁺ Tregs and FoxP3⁻ Teff cells in tumor bearing control and anti-CTLA-4 treated mice where the tumor burden was high. ICOS increased only on FoxP3⁻ Teff cells following MVA-BN-mHER2 treatment and was more pronounced following combination with anti-CTLA-4.

Example 9

MVA-BN-HER2 with anti-CTLA-4 Increases the Effector to Regulatory T Cell Ratio

[0200] Mice were implanted i.v. on day 1 with 5E4 CT26-HER-2 cells i.v. which forms tumors in the lungs. Mice were treated with MVA-BN-mHER2 (1E7 Inf.U. t.s.) on days 4 and 18 and anti-CTLA-4 (200 µg, i.p.) on days 3 and 17. Organs from 3 mice at each time point were pooled for analysis (A and B). Data shown as mean ± SEM from three independent experiments with 3-4 mice per group (C and D). Shown in Figure 7, treatment with MVA-BN-mHER2 and anti-CTLA-4 increased both the CD8 and CD4 Effector to Regulatory T cell ratio in the tumor site, as well as the spleen and blood.

Example 10

PD-1 Expression Increases with MVA-BN-HER2 Treatment

[0201] Naïve, tumor free mice were treated with MVA-BN-HER2 (1E7 Inf.U., t.s.) on day 1 or days 1 and 15. Organs from 3 mice at each time point. Shown in Figure 8, treatment with MV-BN-mHER2 increased PD-1 expression on CD8⁺ and CD4⁺ T Cells.

Example 11

Induction of an anti-tumor response in mice treated with MVA-BN-HER2 and anti-PD1

[0202] Balb/c mice were implanted on day 1 with 1E5 CT26-HER-2 cells i.d. Mice were treated with 1E7 Inf.U. MVA-BN-mHER2 by tail scarification (t.s.) on days 1 and 15, and anti-PD1 on days 1 and 15 (200 µg i.p.). **** p<0.0001, Repeated measures Two-way ANOVA with Tukey's Multiple Comparisons post-test. Shown in Figure 9, tumor volume was significantly decreased with MVA-BN-mHER2 plus anti-PD1 as compared treatment with antiPD-1 alone, and survival was significantly increased compared to MVA-BN-mHER2 alone.

Example 12

LAG-3 Immune Response to MVA-BN-HER2

[0203] Naïve, tumor free mice were treated with MVA-BN-mHER2 (1E7 Inf.U. t.s.) on day 1 or days 1 and 15. Organs from 3 mice at each time point. Shown in Figure 10, LAG-3 expression on CD8⁺ and CD4⁺ T Cells.

Example 13

Induction of an anti-tumor response in mice treated with MVA-BN-HER2 and anti-LAG-3

[0204] Balb/c mice were implanted on day 1 with 1E5 CT26-HER-2 cells i.d. Mice were treated with 1E7 Inf.U. MVA-BN-mHER2 by tail scarification (t.s.) on days 1 and 15, and anti-LAG-3 on days 1 and 15 (200 µg i.p.). **** p<0.0001, Repeated measures Two-way ANOVA with Tukey's Multiple Comparisons post-test. Shown in Figure 11, treatment with MVA-BN-mHER2 in combination with anti-LAG3 increased the overall survival rate as compared to MVA-BN-mHER2 alone and anti-LAG3 alone.

Example 14

Induction of an anti-tumor response in mice treated with MVA-BN-HER2 and anti-PD-1 and anti-LAG-3

[0205] Balb/c mice were implanted on day 1 with 1E5 CT26-HER-2 cells i.d. In Figure 12, mice were treated with 1E7 Inf.U. MVA-BN-mHER2 by tail scarification (t.s.) on days 1 and 15, and anti-PD1 and anti-LAG-3 on days 1 and 15 (200 µg each, i.p.). In Figure 13, mice were treated with 1E7 Inf.U. MVA-BN-mHER2 by t.s. on days 4 and 18, and anti-PD-1 and anti-LAG-3 on days 4 and 18 (200 µg i.p.). **** p<0.0001, Repeated measures Two-way ANOVA with Tukey's Multiple Comparisons post-test. Shown in Figure 12 and 13, treatment with MVA-BN-mHER2 in combination with both anti-PD1 and anti-LAG3, decreased the tumor volume (Figure 12A and 13A) and increased the overall survival rate (Figure 12B and 13B) as compared to MVA-BN-mHER2 alone and anti-PD1 and anti-LAG3 alone.

Example 15

Induction of an anti-tumor response in mice treated with MVA-BN-HER2 and anti-PD-1 and anti-LAG-3

[0206] In a CT26-HER-2 experimental lung metastasis model, mice were implanted on day 1 with 5E4 CT26-HER-2 cells i.v. which form tumors in the lungs. Mice were treated with MVA-BN-mHER2 (1E7 Inf.U. t.s.) on day 4 and 18 and anti-PD-1 and anti-LAG-3 (200 µg each, i.p.) on days 4 and 18. * P<0.05, *** p<0.001, Log-Rank Test. Shown in Figure 14, treatment with MVA-BN-mHER2 in combination with anti-PD-1 and anti-LAG3 increased the overall survival rate as compared to or anti-PD1 and anti-LAG3 alone.

Example 16

ELISPOT MVA response

[0207] Balb/c mice were implanted on day 1 with 1E5 CT26-HER-2 cells i.d. Mice were treated with 1E7 Inf.U. MVA-BN-mHER2 by tail scarification (t.s.) on days 4 and 18, and anti-PD1 and anti-LAG-3 on days 4 and 18 (200 µg i.p.). Four weeks after the last treatment, specific T-cell responses were determined by IFN-γ ELISPOT as described in Mandl et al., Cancer Immunol Immunother (2012) 61:19–29. Shown in Figure 15, treatment with MVA-BN-mHER2 in combination with anti-PD-1 and anti-LAG3 increased tumor antigen specific IFN-γ levels as compared to anti-PD1 and anti-LAG3 alone.

Example 17

Antibody Titers

[0208] Balb/c mice were implanted on day 1 with 1E5 CT26-HER-2 cells i.d. Mice were treated with 1E7 Inf.U. MVA-BN-mHER2 by tail scarification (t.s.) on days 4 and 18, and anti-PD1 and anti-LAG-3 on days 4 and 18 (200 µg i.p.). Serum antibody titers were determined by

ELISA as described in Mandl et al., Cancer Immunol Immunother (2012) 61:19–29. 2. Results are shown in Figure 16.

Example 18

Induction of an anti-tumor response in mice treated with MVA-BN-CV301 and anti-PD-1

[0209] Female C57/BL6 mice (6-8 weeks old, ~20 g, Simonsen Laboratories, Gilroy CA) were implanted on day 1 with MC38-CEA cells (2×10^5 , i.d. in the back flank). Mice were treated on days 1 and 15 with MVA-BN-CV301 (1×10^7 Inf.U., s.c. above the tail base). Mice were treated with anti-PD-1 on days 1 and 15 (200 μ g i.p.). The results are shown in Figure 17. * $p < 0.05$, **** $p < 0.0001$, Repeated measures Two-way ANOVA with Tukey's Multiple Comparisons post-test.

Example 19

Induction of an anti-tumor response in mice treated with MVA-BN-CV301 and anti-LAG-3

[0210] Female C57BL/6 mice (6-8 weeks old, ~20 g, Simonsen Laboratories, Gilroy CA) were implanted on day 1 with MC38-CEA cells (2×10^5 , i.d. in the back flank). Mice were treated on days 1 and 15 with MVA-BN-CV301 (1×10^7 Inf.U., s.c. above the tail base). Mice were treated with anti-LAG-3 on days 1 and 15 (200 μ g i.p.). The results are shown in Figure 18. ** $p < 0.01$, *** $p < 0.001$, Repeated measures Two-way ANOVA with Tukey's Multiple Comparisons post-test.

Example 20

Induction of an anti-tumor response in mice treated with MVA-BN-CV301 and anti-PD-1 and anti-LAG-3

[0211] Female C57BL/6 mice (6-8 weeks old, ~20 g, Simonsen Laboratories, Gilroy CA) were implanted on day 1 with MC38-CEA cells (2×10^5 , i.d. in the back flank). Mice were treated

on days 1 and 15 with MVA-BN-CV301 (1E7 Inf.U., s.c. above the tail base). Mice were treated with anti-PD1 and anti-LAG-3 on days 1 and 15 (200 µg i.p. each). ** p<0.01, **** p<0.0001, Repeated measures Two-way ANOVA with Tukey's Multiple Comparisons post-test. Shown in Figure 19, the combination treatment of MVA-BN CV301 and anti-PD1 and anti-LAG3 resulted in a decrease in tumor volume as compared to anti-PD1 and anti-LAG3 alone or MVA-BN-CV301 alone.

Example 21

Induction of an anti-tumor response in mice treated with PROSTVAC and anti-PD-1

[0212] Male BALB/c mice (6-8 weeks old, ~20 g, Simonsen Laboratories, Gilroy CA) were implanted on day 1 with E6 cells (RM11-PSA, 1.5×10^5 , i.d. in the back flank). Mice were treated on day 1 with PROSTVAC-V (2E7 Inf. U., s.c. at the tail base), and on days 8 and 15 with PROSTVAC-F (1E8 Inf. U., s.c. at the tail base). Mice were treated on days 1 and 15 with anti-PD-1 (200 µg i.p.). The results of the treatment are shown in Figure 20.

Example 22

Induction of an anti-tumor response in mice treated with PROSTVAC and anti-LAG-3

[0213] Male BALB/c mice (6-8 weeks old, ~20 g, Simonsen Laboratories, Gilroy CA) were implanted on day 1 with E6 cells (RM11-PSA, 1.5×10^5 , i.d. in the back flank). Mice were treated on day 1 with PROSTVAC-V (2E7 Inf. U., s.c. at the tail base), and on days 8 and 15 with PROSTVAC-F (1E8 Inf. U., s.c. at the tail base). Mice were treated on days 1 and 15 with anti-LAG-3 (200 µg i.p.). The results of the treatment are shown in Figure 21.

Example 23

Induction of an anti-tumor response in mice treated with PROSTVAC and anti-PD-1 and anti-LAG-3

[0214] Male BALB/c mice (6-8 weeks old, ~20 g, Simonsen Laboratories, Gilroy CA) were implanted on day 1 with E6 cells (RM11-PSA, 1.5×10^5 , i.d. in the back flank). Mice were treated on day 1 with PROSTVAC-V (2E7 Inf. U., s.c. at the tail base), and on days 8 and 15 with PROSTVAC-F (1E8 Inf. U., s.c. at the tail base). Mice were treated with on days 1 and 15 with anti-PD-1 and anti-LAG-3 (200 μ g each, i.p.). Shown in Figure 22, the combination treatment of PROSTVAC with anti-PD-1 and anti-LAG-3 resulted in a decrease in tumor volume as compared to anti-PD-1 and anti-LAG-3 alone or PROSTVAC alone.

Example 24

Induction of an anti-tumor response in mice treated with PANVAC (CV301-V/F) and anti-PD-1

[0215] Female C57/BL6 mice transgenic for human CEA ((Tg(CEA)18/B6j, received from Jack Shively from the City of Hope National Medical Center, see also Clarke et al. Cancer Research, 58:1469ff., 1998) were implanted on day 1 with MC38-CEA cells (3.0×10^5 , i.d. in the back flank). Mice were treated on day 4 with CV301-Vaccinia (CV301-V) (also known as PANVAC-V) (2E7 Inf. U., s.c. at the tail base), and on days 11 and 18 with CV301-Fowlpox (CV-301-F) (also known as PANVAC-F)(1E8 Inf. U., s.c. at the tail base). CV301-V/F treatments were admixed with Fowlpox GM-CSF (1E7 Inf. U.) on days 4, 11, and 18. Mice were treated with on days 4, 11, and 18 with anti-PD-1 (200 μ g i.p.). ** $p < 0.01$, **** $p < 0.0001$, Repeated measures Two-way ANOVA with Tukey's Multiple Comparisons post-test. Shown in Figure 23, the combination treatment of CV301-V/F with anti-PD-1 delayed tumor growth compared to control mice.

[0216] It will be apparent that the precise details of the methods or compositions described herein may be varied or modified without departing from the spirit of the described

invention. We claim all such modifications and variations that fall within the scope and spirit of the claims below.

CLAIMS

We claim:

1. A therapy for the treatment of a human cancer patient, comprising: (a) a recombinant orthopoxvirus comprising a nucleic acid encoding a polypeptide of at least one tumor antigen; and (b) at least one of an anti-PD-1 antagonist, an anti-LAG-3 antagonist, or an anti-ICOS agonist; wherein (a) and (b) are to be administered as a combination treatment.
2. The therapy of claim 1, wherein the recombinant orthopoxvirus is selected from a vaccinia virus, a modified vaccinia Ankara (MVA) virus, or MVA-BN.
3. The therapy of claims 1-2, further comprising: (a) a recombinant avipoxvirus comprising a nucleic acid encoding a polypeptide of at least one tumor antigen; and (b) at least one of an anti-PD-1 antagonist, an anti-LAG-3 antagonist, or an anti-ICOS agonist; wherein (a) and (b) are to be administered as a combination treatment.
4. The therapy of claims 1-3, wherein the recombinant avipoxvirus and antagonist or agonist combination is to be administered after the recombinant orthopoxvirus and antagonist or agonist combination of claim 3.
5. The therapy of claim 3-4, wherein the avipoxvirus is a fowlpoxvirus.
6. The therapy of claim 1-2, further comprising: (a) two or more recombinant orthopoxviruses comprising a nucleic acid encoding a polypeptide of at least one tumor antigen; and (b) at least one of an anti-PD-1 antagonist, an anti-LAG-3 antagonist, or an anti-ICOS agonist; wherein (a) and (b) are to be administered as a combination treatment.

7. The combination therapy of claims 1-6, wherein the anti-PD-1 antagonist, anti-LAG-3 antagonist, or anti-ICOS agonist comprises an antibody
8. The combination therapy of claims 1-7, wherein the at least one tumor antigen is selected from a CEA, MUC-1, PAP, PSA, and a HER-2 antigen.
9. The combination therapy of claims 1-8, wherein the cancer is breast cancer, lung cancer, colorectal cancer, gastric cancer, pancreatic cancer, prostate cancer, bladder cancer, or ovarian cancer.
10. The combination therapy of claims 1-7, wherein the at least one tumor antigen is a PAP antigen.
11. The combination therapy of claims 1-7, wherein the at least one tumor antigen is a PSA antigen.
12. The combination therapy of claims 10-11, wherein the cancer is prostate cancer.
13. The combination therapy of claims 1-7, wherein the at least one tumor antigen is selected from a MUC-1 and a CEA antigen.
14. The combination therapy of claim 13, wherein the cancer is selected from breast cancer, colorectal cancer, lung cancer, gastric cancer, pancreatic cancer, bladder cancer, and ovarian cancer.
15. The combination therapy of claims 1-7, wherein the at least one tumor antigen is a HER-2 antigen.
16. The combination therapy of claims 15, wherein the cancer is breast cancer.
17. A method for treating a human cancer patient comprising:
 - (a) administering to the patient a recombinant orthopoxvirus comprising a nucleic acid encoding a polypeptide of at least one tumor antigen; and

- (b) administering to the patient at least one of an anti-PD-1 antagonist, an anti-LAG-3 antagonist, or an anti-ICOS agonist.

18. The method of claim 17, further comprising:

- (a) administering to the patient a recombinant avipoxvirus comprising a nucleic acid encoding a polypeptide of at least one tumor antigen; and
- (b) at least one of an anti-PD-1 antagonist, an anti-LAG-3 antagonist, or an anti-ICOS agonist.

19. The method of claim 18, wherein the avipox virus is a fowlpox virus.

20. The method of claim 17, further comprising administering to the patient one or more subsequent recombinant orthopoxviruses comprising a nucleic acid encoding a polypeptide of at least one tumor antigen and at least one of an anti-PD-1 antagonist, an anti-LAG-3 antagonist, or an anti-ICOS agonist.

21. The method of claims 17 and 20, wherein the orthopoxvirus is a vaccinia virus.

22. The method of claim 21, wherein the vaccinia virus is selected from a modified Vaccinia Ankara (MVA) virus and MVA-BN.

23. The method of claims 17-22, comprising administering to the patient an anti-PD-1 antagonist antibody.

24. The method of claims 17-22, comprising administering to the patient an anti-LAG-3 antagonist antibody.

25. The method of claims 17-22, comprising administering to the patient an anti-ICOS agonist antibody.

26. The method of claims 17-22, comprising administering to the patient an anti-PD-1 antagonist antibody and an anti-LAG-3 antagonist antibody

27. The method of claims 17-26, wherein the at least one tumor antigen is a CEA, MUC-1, PAP, PSA, or HER-2 antigen.
28. The method of claims 17-27, wherein the cancer treatment is directed against prostate cancer, breast cancer, lung cancer, gastric cancer, pancreatic cancer, bladder cancer, or ovarian cancer.
29. The method of claims 17-26, wherein the at least one tumor antigen is a CEA or a MUC-1 antigen.
30. The method of claim 29, wherein the cancer treatment is directed against breast cancer, colorectal cancer, lung cancer, gastric cancer, pancreatic cancer, bladder cancer, or ovarian cancer.
31. The method of claims 17-26, wherein the at least one tumor antigen is a PAP antigen.
32. The method of claims 17-26, wherein the at least one tumor antigen is a PSA antigen.
33. The method of claims 31-32, wherein the cancer treatment is directed against prostate cancer.
34. The method of claims 17-26, wherein the at least one tumor antigen is a HER-2 antigen.
35. The method of claim 34, wherein the cancer treatment is directed against breast cancer.
36. The method of claims 17-18 and 23-35, wherein the recombinant orthopoxvirus and the recombinant avipoxvirus in combination with the at least one of an anti-PD-1 antagonist; an anti-LAG-3 antagonist, or an anti-ICOS agonist are administered as part of a heterologous prime-boost regimen;

wherein the heterologous prime-boost regimen comprises a first prime dose of the recombinant orthopoxvirus in combination with the antagonist or agonist and one or more subsequent boost doses of the recombinant avipoxvirus in combination with the antagonist or agonist.

37. The method of claim 36, wherein the heterologous prime-boost regimen is PROSTVAC or CV301.
38. The method of claims 17-35, wherein the recombinant orthopoxviruses in combination with the at least one of anti-PD-1 antagonist; an anti-LAG-3 antibody, or an anti-ICOS agonist are administered as part of a homologous prime-boost regimen; wherein the homologous prime-boost regimen comprises a first prime dose of the recombinant orthopoxvirus in combination with the antagonist or agonist and one or more subsequent boost doses of a same recombinant orthopoxvirus in combination with the antagonist or agonist.
39. A combination therapy for the treatment of a human cancer patient, the combination comprising:
 - (a) a recombinant orthopoxvirus or a recombinant avipoxvirus, each comprising a nucleic acid encoding a polypeptide of at least one tumor antigen; and
 - (b) an anti-PD-1 antagonist, an anti-LAG-3 antagonist, or an anti-ICOS agonist.
40. The combination therapy of claim 40, wherein the recombinant orthopoxvirus is selected from a vaccinia virus, a modified vaccinia Ankara (MVA) virus, or MVA-BN.

41. The combination therapy of claim 39, wherein the recombinant avipoxvirus is a fowlpox virus.
42. The combination therapy of claim 39-41, wherein (b) includes an anti-PD-1 antagonist antibody.
43. The combination therapy of claim 39-41, wherein (b) includes an anti-LAG-3 antagonist antibody
44. The combination therapy of claim 39-41, wherein (b) includes an anti-PD-1 antagonist antibody and an anti-LAG-3 antagonist antibody.
45. The combination therapy of claims 39-44, wherein the at least one tumor antigen is selected from a CEA, MUC-1, PAP, PSA, and a HER-2 antigen.
46. The combination therapy of claims 39-45, further comprising one or more subsequent recombinant orthopoxviruses or avipoxviruses each comprising a nucleic acid encoding a polypeptide of at least one tumor antigen combined with at least one of an anti-PD-1 antagonist antibody, an anti-LAG-3 antagonist antibody, or an anti-ICOS agonist antibody.
47. A kit for treating cancer in a human cancer patient, the kit comprising: (a) a recombinant orthopoxvirus comprising a nucleic encoding a polypeptide of at least one tumor antigen; and (b) at least one of an anti-PD-1 antagonist antibody, an anti-LAG-3 antagonist antibody, or an anti-ICOS agonist antibody.
48. The kit of claim 47, further comprising a recombinant avipoxvirus comprising a nucleic encoding a polypeptide of at least one tumor antigen.
49. The kit of claims 47-48, wherein the at least one tumor antigen is selected from a CEA, MUC-1, PAP, PSA, and a HER-2 antigen.

50. A medicament or composition for use in the treatment of a human cancer patient, comprising: a first recombinant orthopoxvirus comprising a nucleic acid encoding a polypeptide of at least one tumor antigen; and (b) at least one of an anti-PD-1 antagonist, an anti-LAG-3 antagonist, or an anti-ICOS agonist; wherein (a) and (b) are to be administered as a combination.
51. The medicament or composition of claim 50, further comprising: (a) a recombinant avipoxvirus comprising a nucleic acid encoding a polypeptide of at least one tumor antigen; and (b) at least one of an anti-PD-1 antagonist, an anti-LAG-3 antagonist, or an anti-ICOS agonist; wherein (a) and (b) are to be administered as a combination.
52. Use of a composition in the preparation of a pharmaceutical composition or medicament for the treatment of a human cancer patient, the composition comprising: a) a recombinant orthopoxvirus comprising a nucleic acid encoding a polypeptide of at least one tumor antigen; and (b) an anti-PD-1 antagonist, an anti-LAG-3 antagonist, or an anti-ICOS agonist.
53. The use of claim 52, wherein the composition further comprises : (a) a recombinant avipoxvirus comprising a nucleic acid encoding a polypeptide of at least one tumor antigen; and (b) at least one of an anti-PD-1 antagonist, an anti-LAG-3 antagonist, or an anti-ICOS agonist; wherein (a) and (b) are to be administered as a combination.
54. The medicament or composition of claims 50-51 or the use of the claims 52-53, wherein the recombinant orthopoxvirus is selected from a vaccinia virus, a modified vaccinia Ankara (MVA), and MVA-BN.
55. The medicament or composition of claim 51 or the use of claim 53, wherein the recombinant avipoxvirus is a fowlpox virus.

56. The medicament or composition of claims 50-55, wherein the at least one tumor antigen is selected from a CEA, MUC-1, PAP, PSA, and a HER-2 antigen.

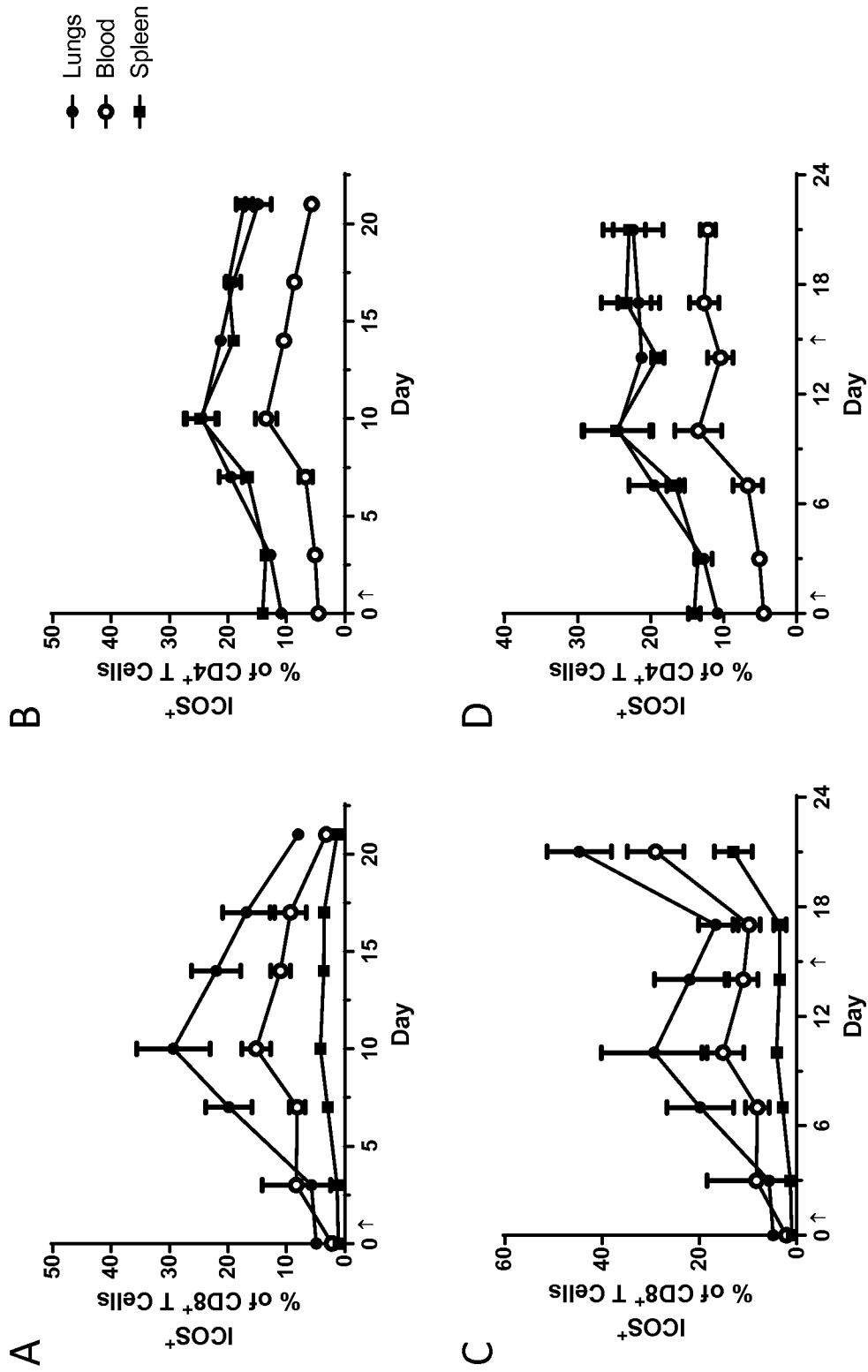


Figure 1

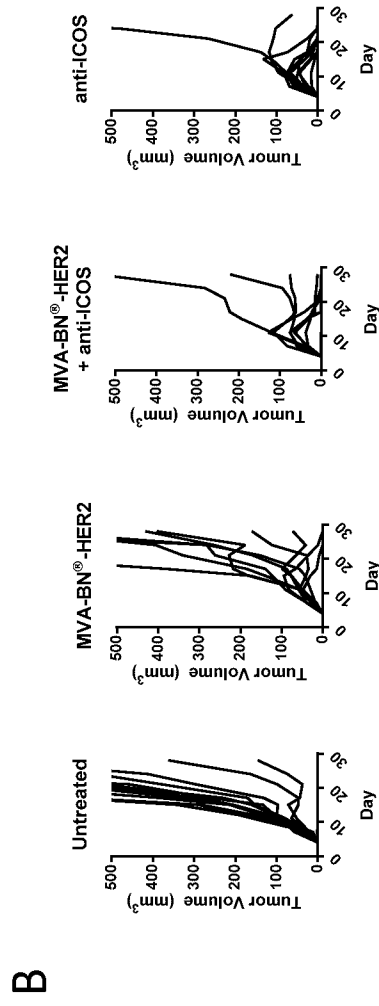
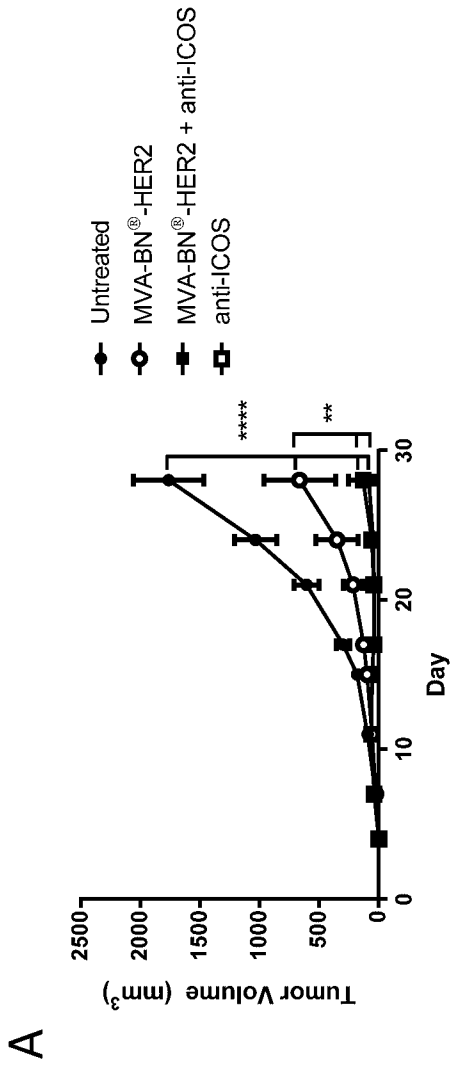


Figure 2

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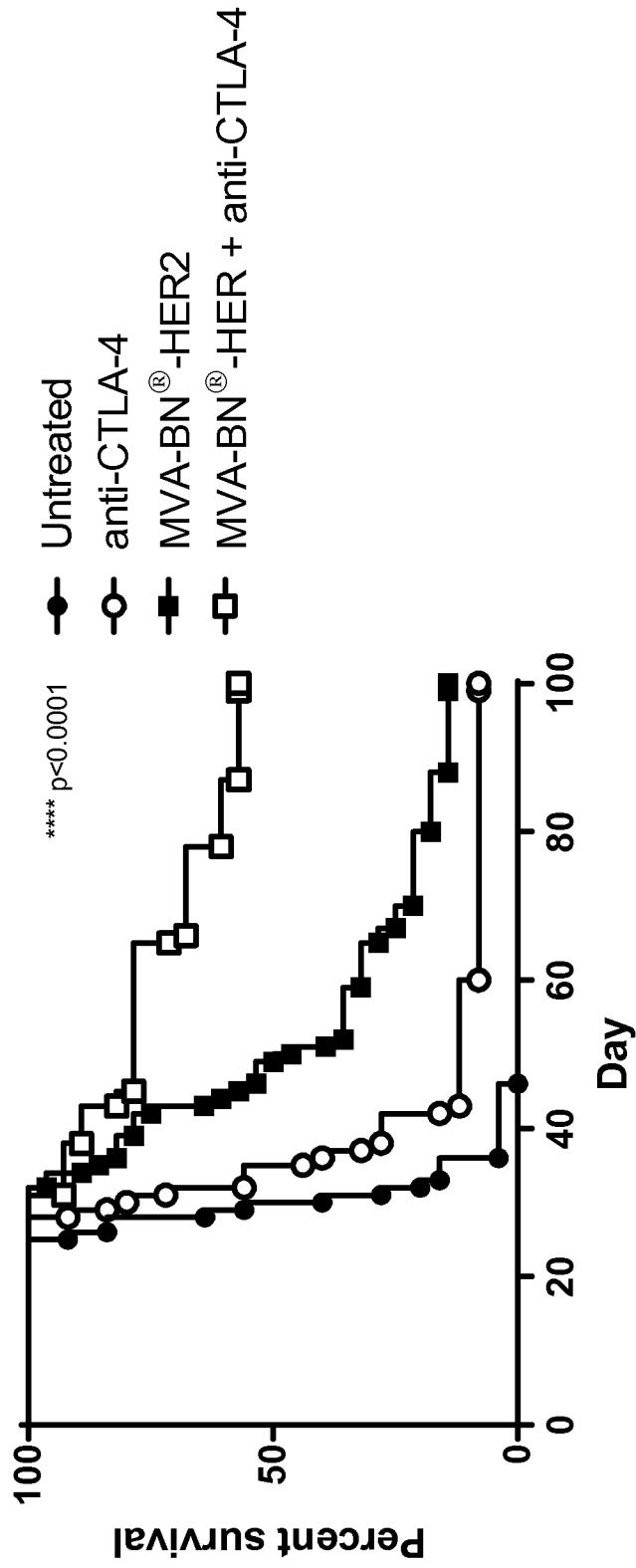


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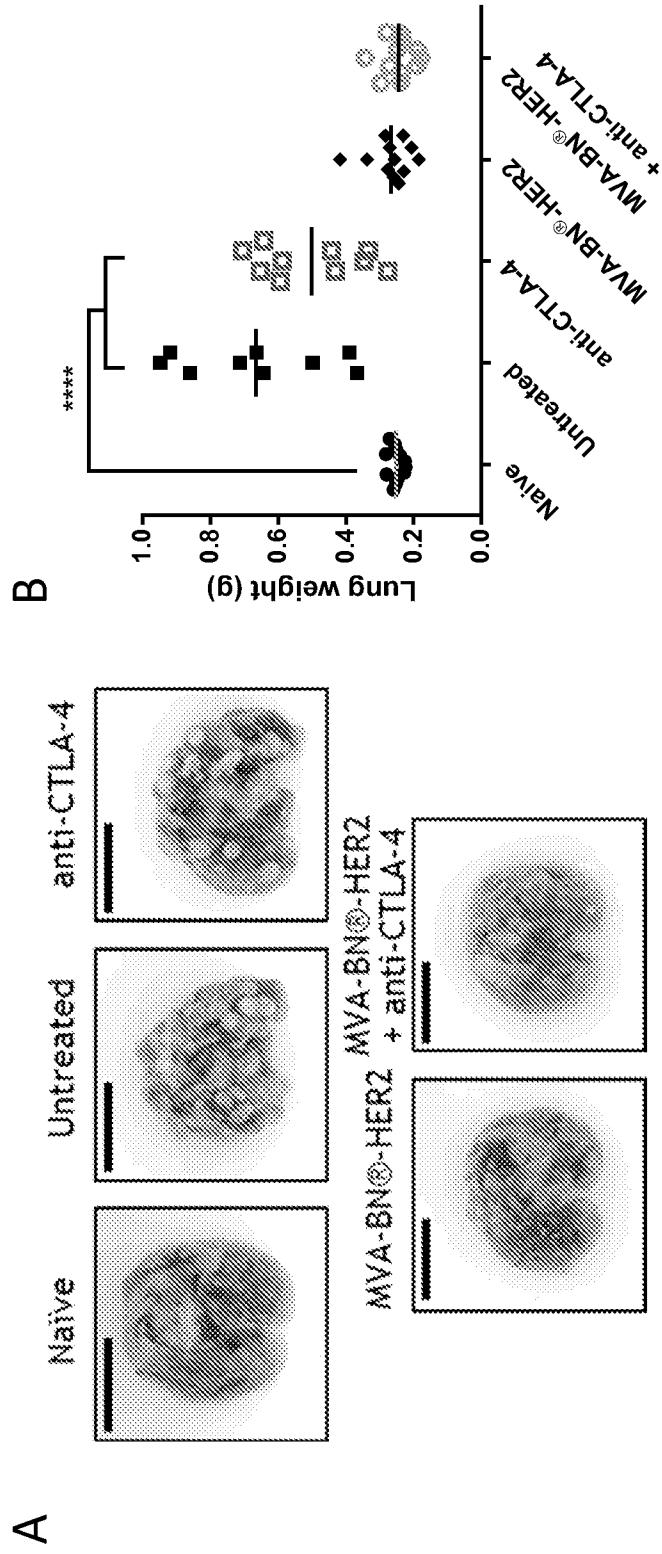


Figure 4

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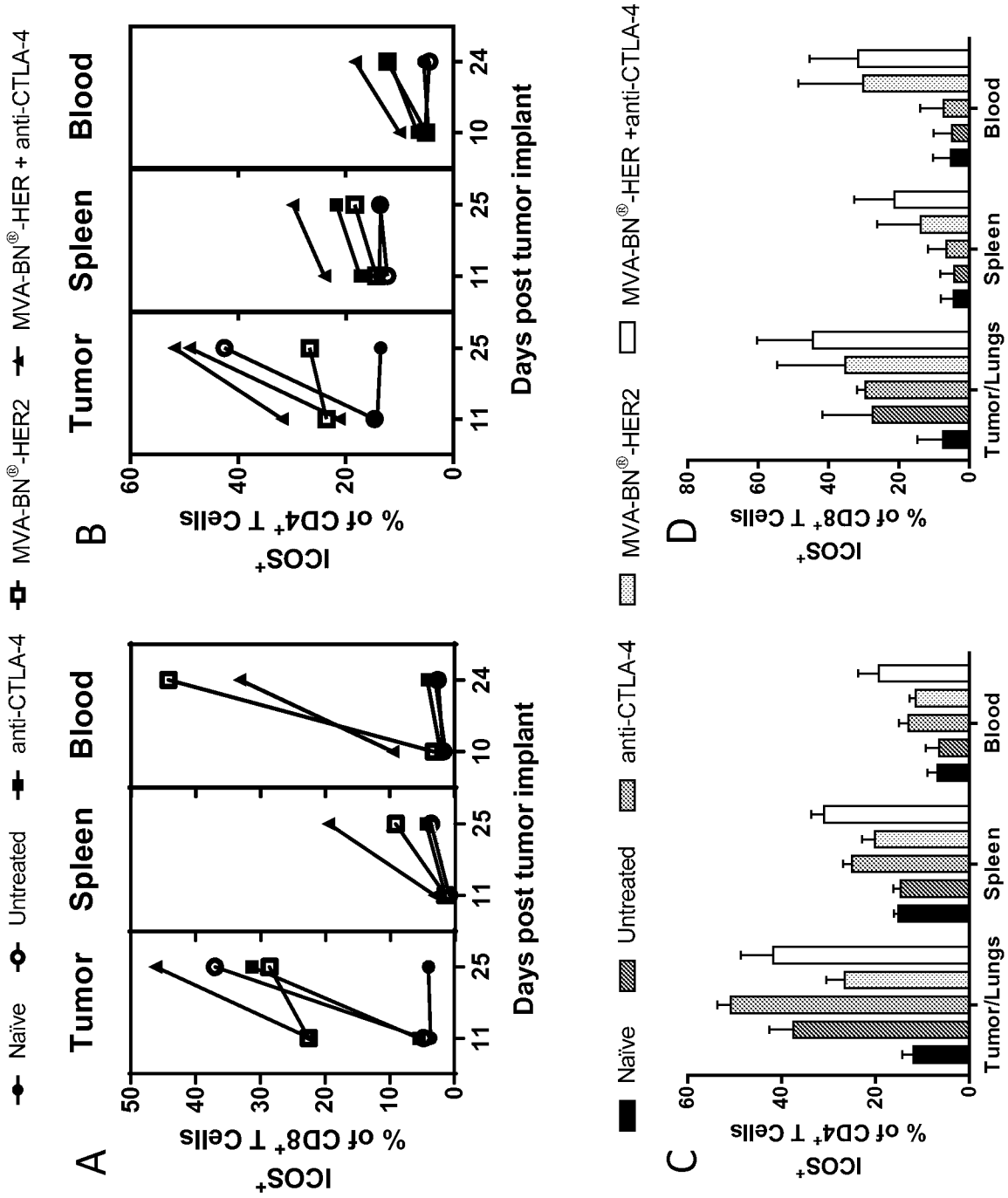


Figure 5

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Naive
 Untreated
 anti-CTLA-4
 MVA-BN[®]-HER2
 MVA-BN[®]-HER +anti-CTLA-4

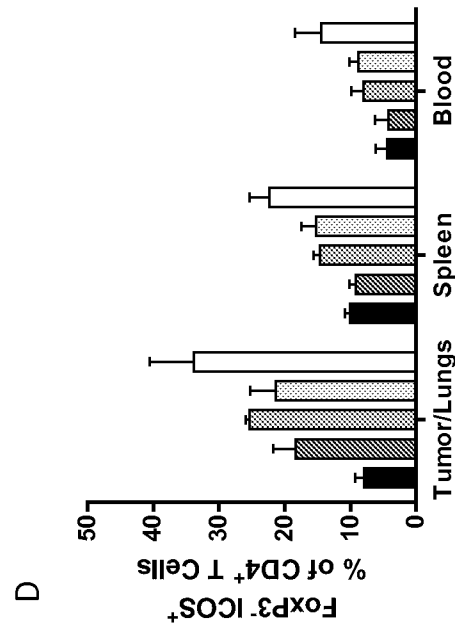
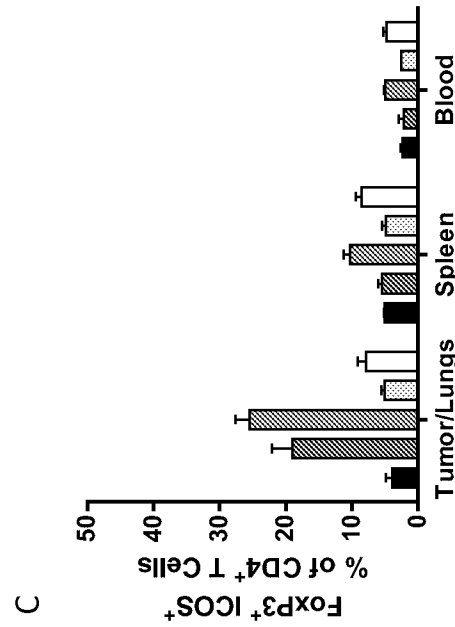
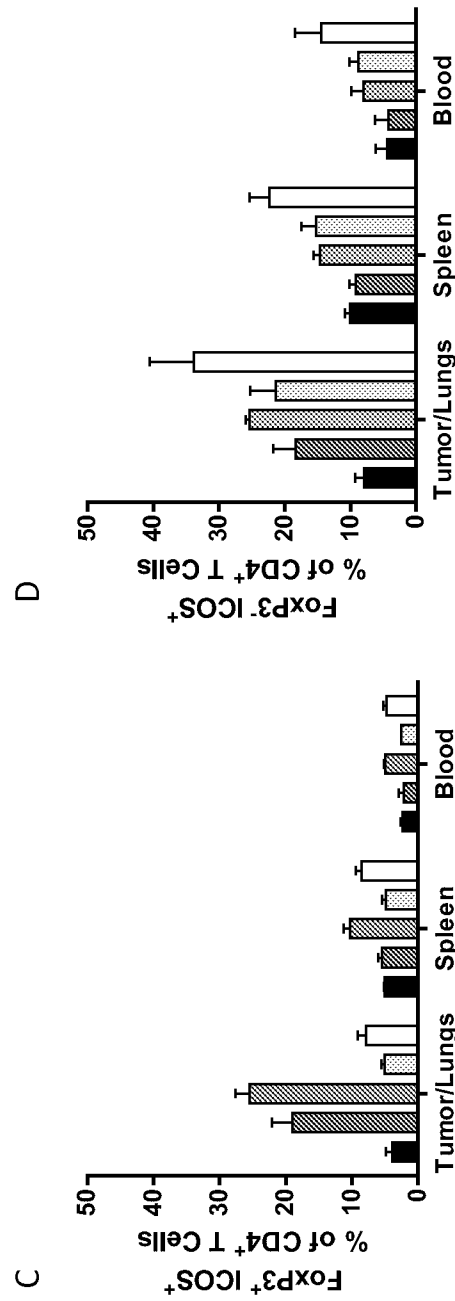
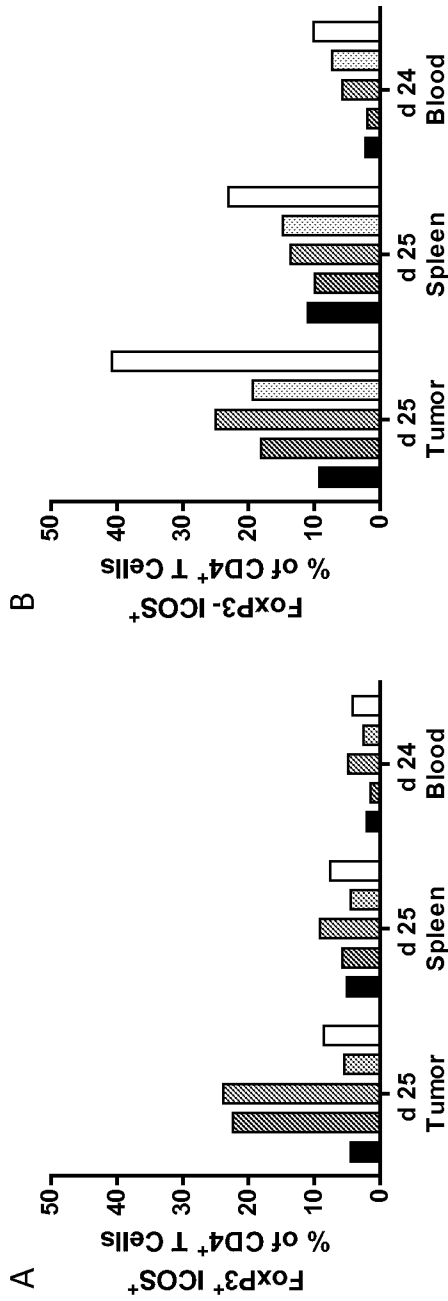


Figure 6

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Naive
 Untreated
 anti-CTLA-4
 MVA-BN[®]-HER2
 MVA-BN[®]-HER +anti-CTLA-4

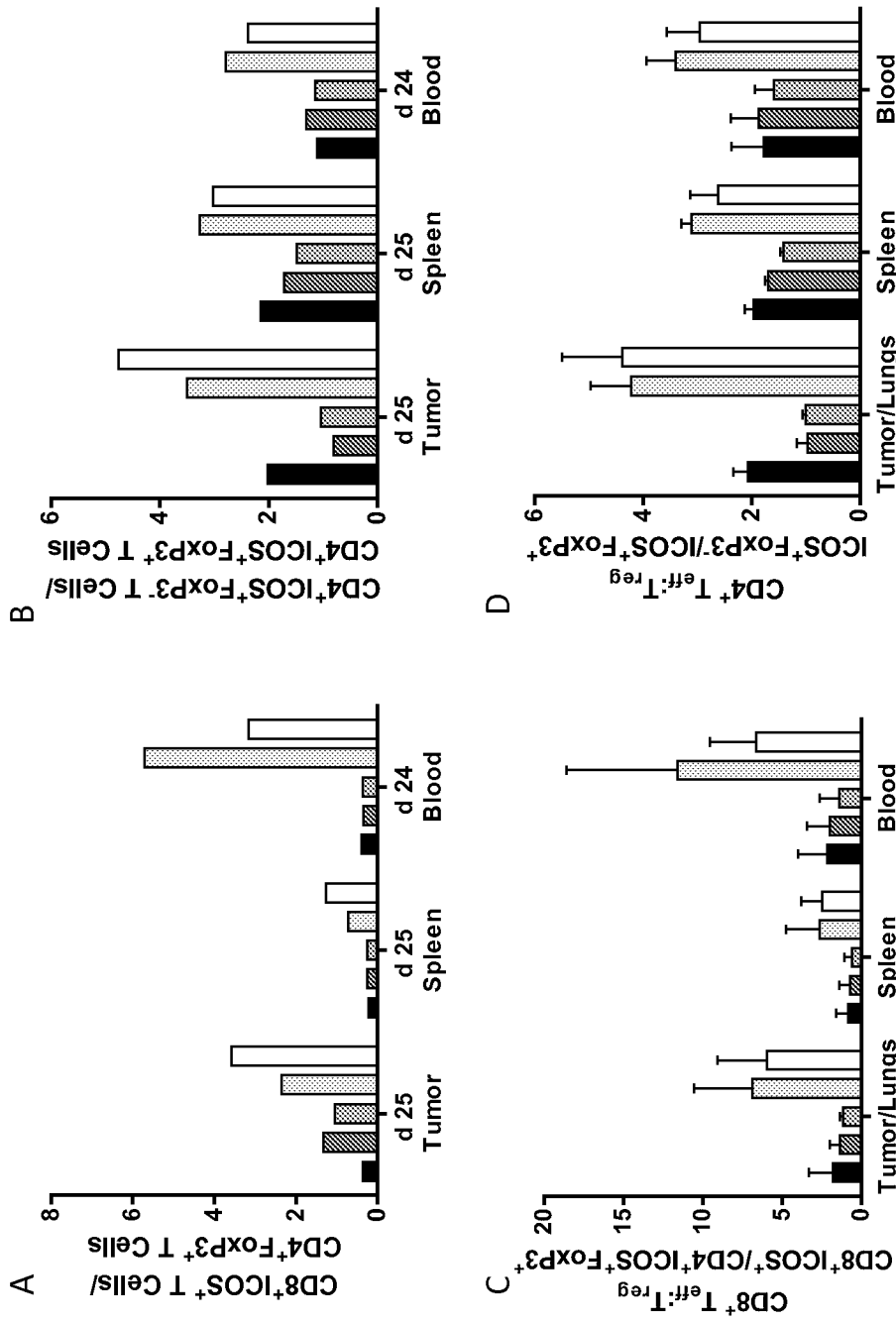


Figure 7

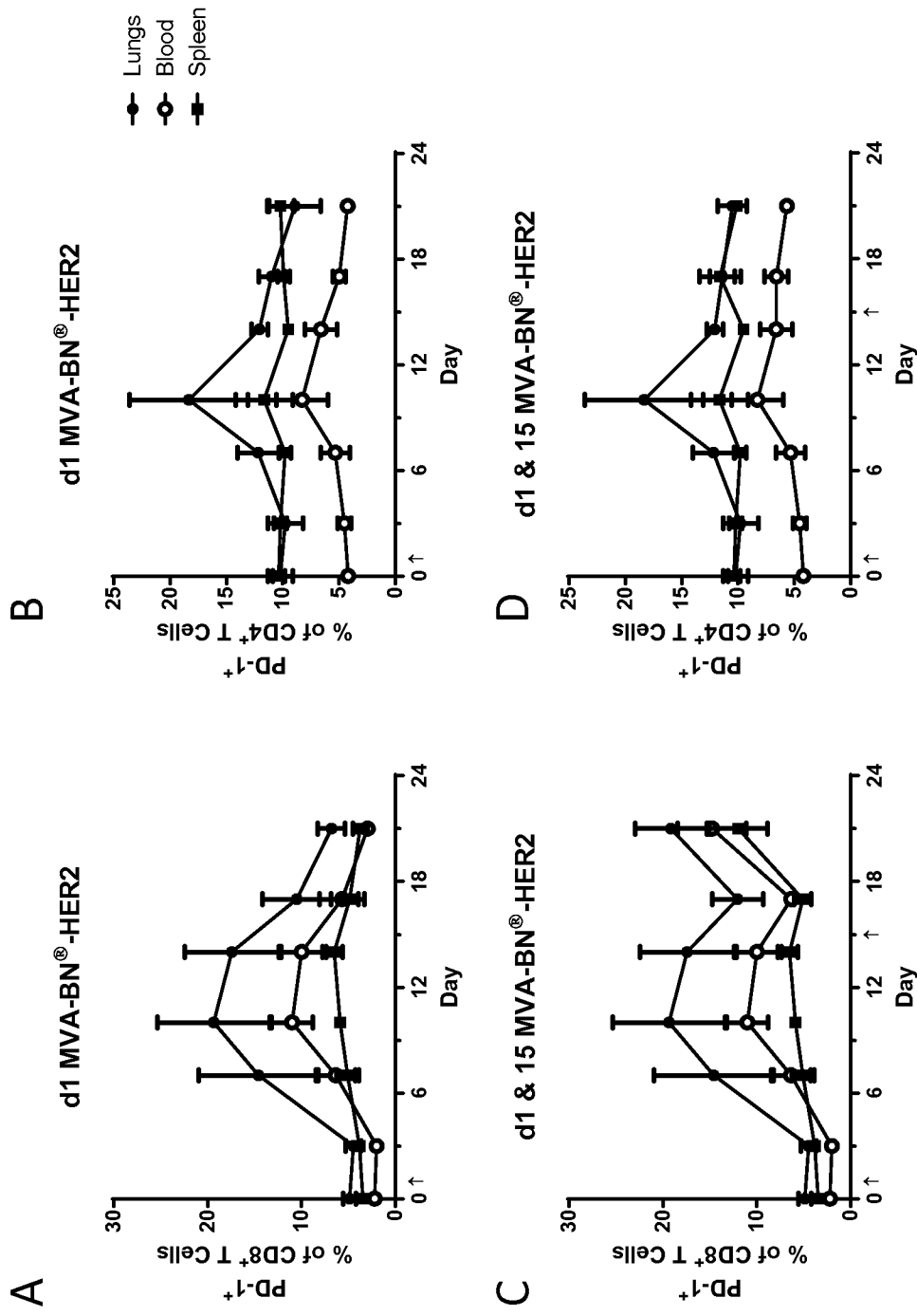


Figure 8

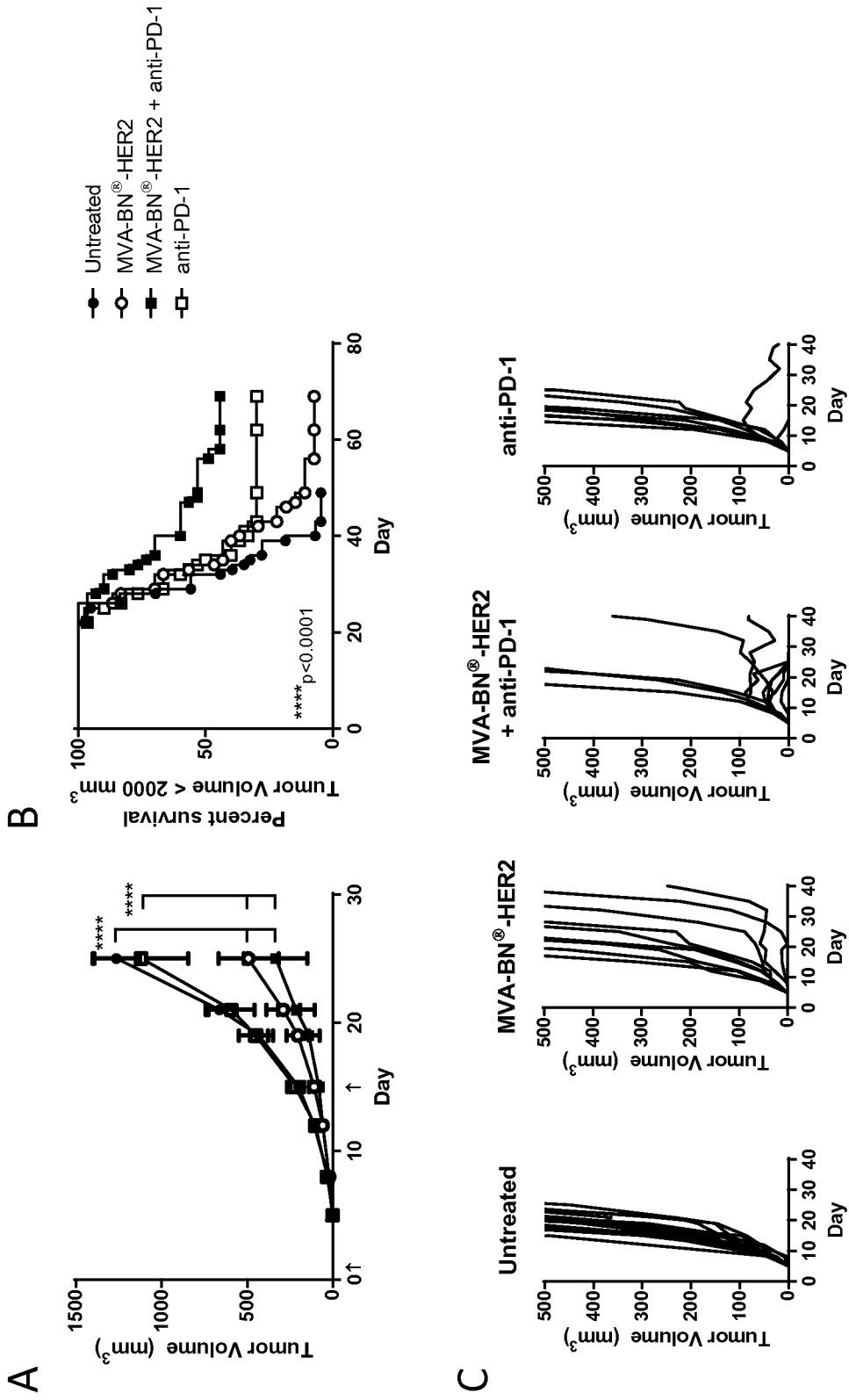


Figure 9

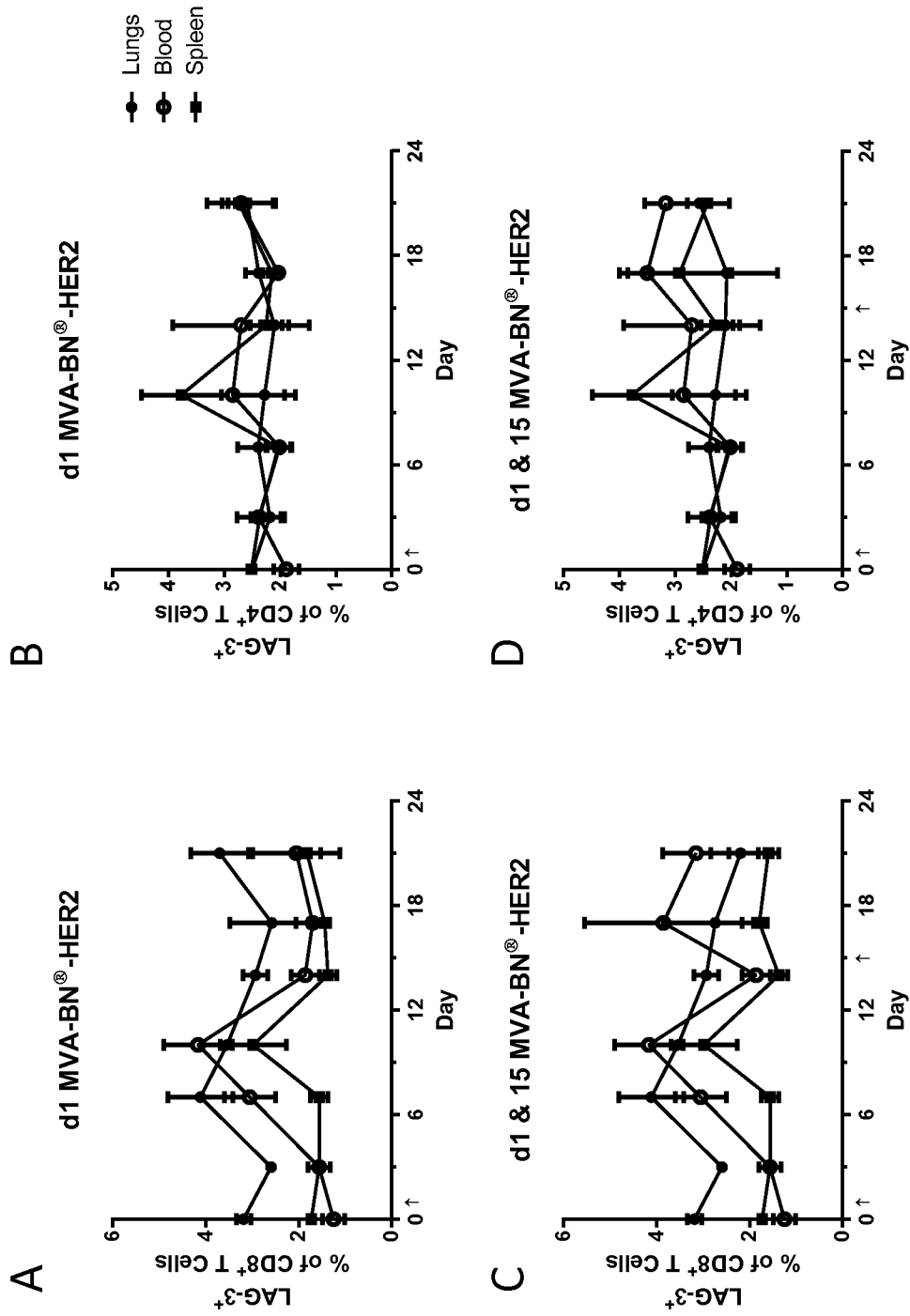


Figure 10

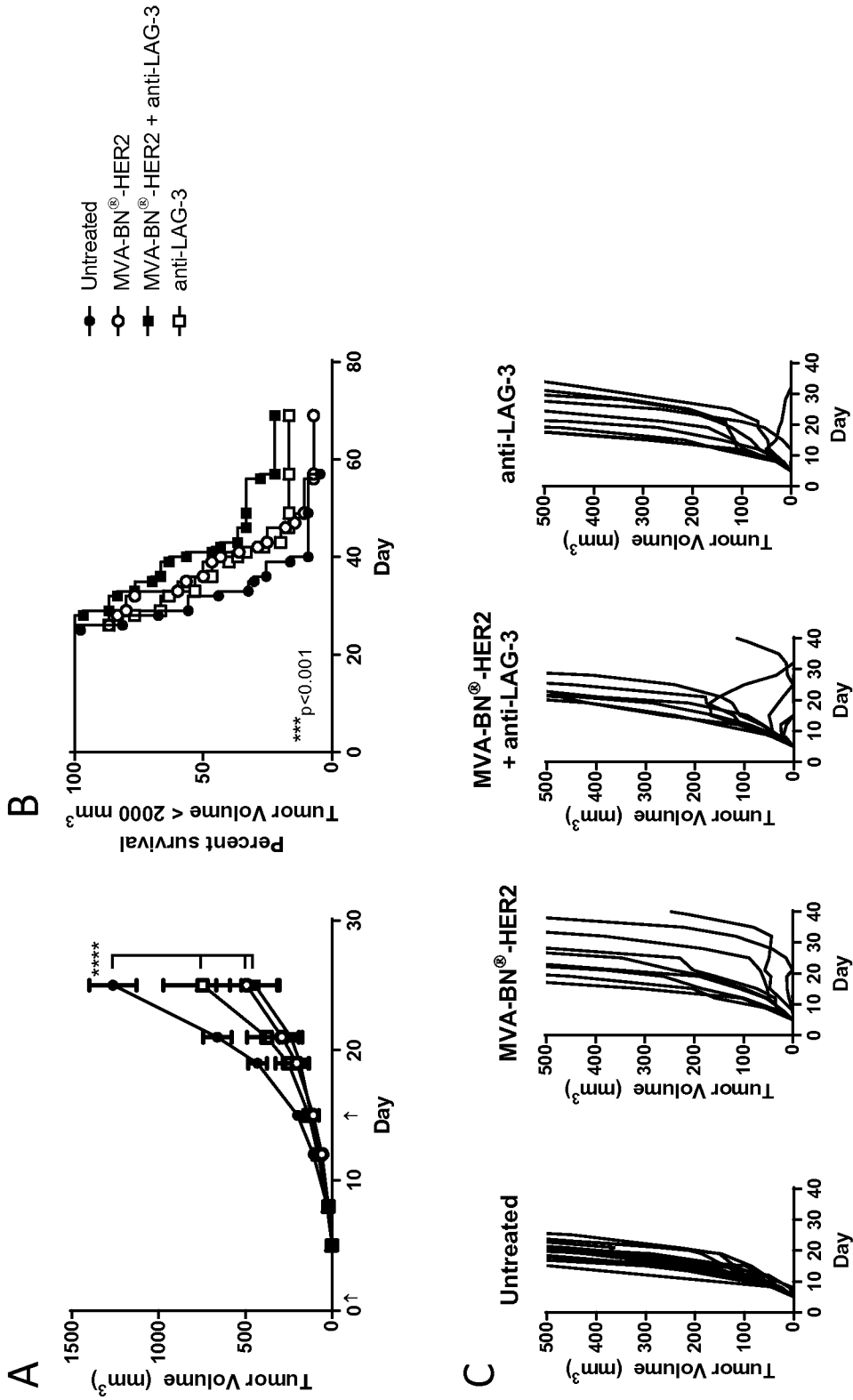


Figure 11

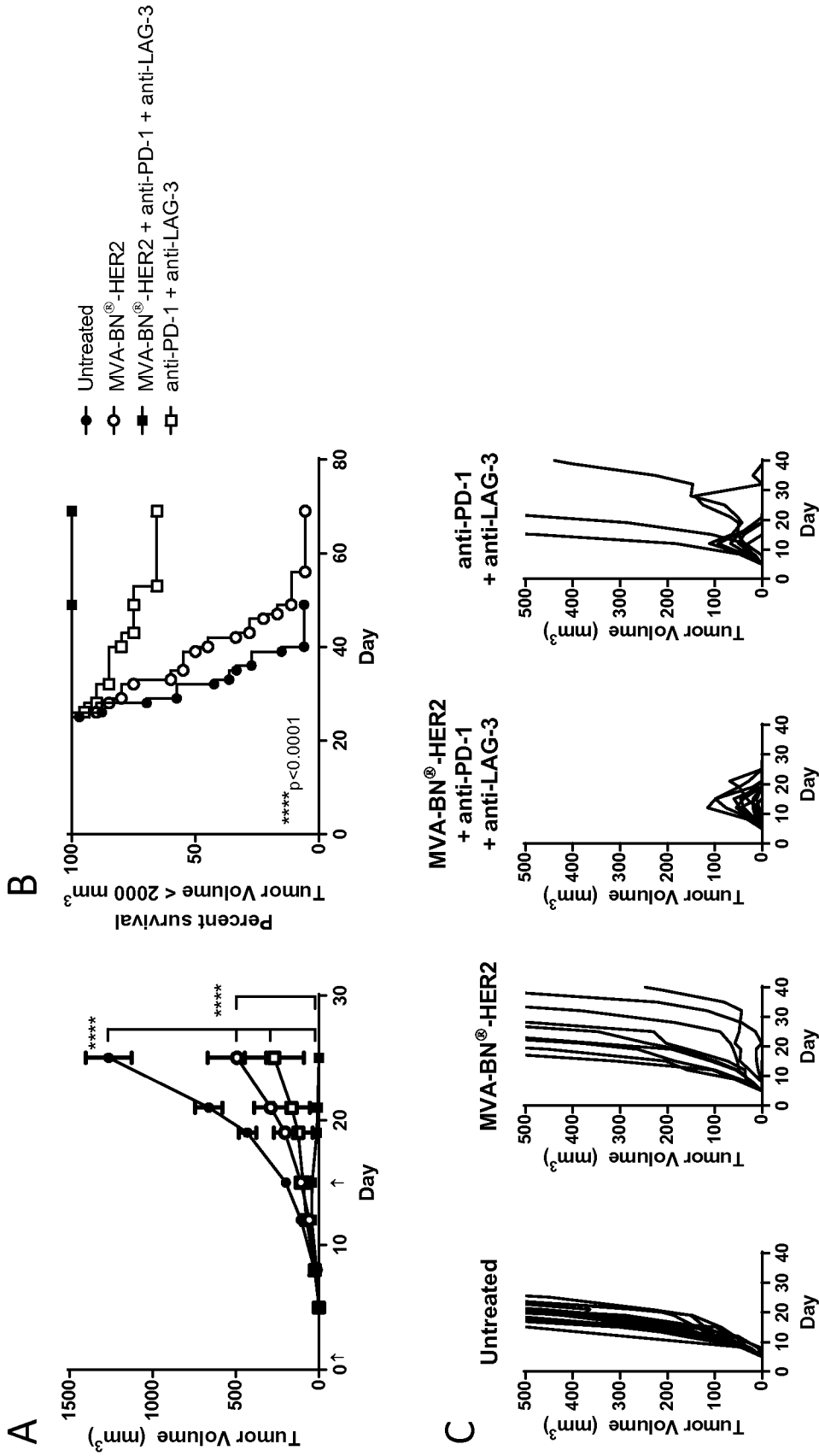


Figure 12

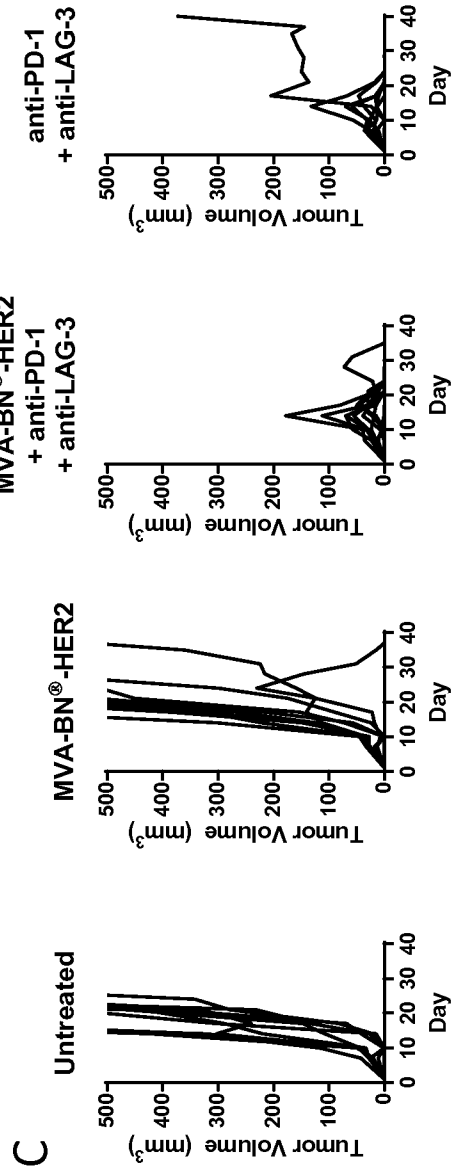
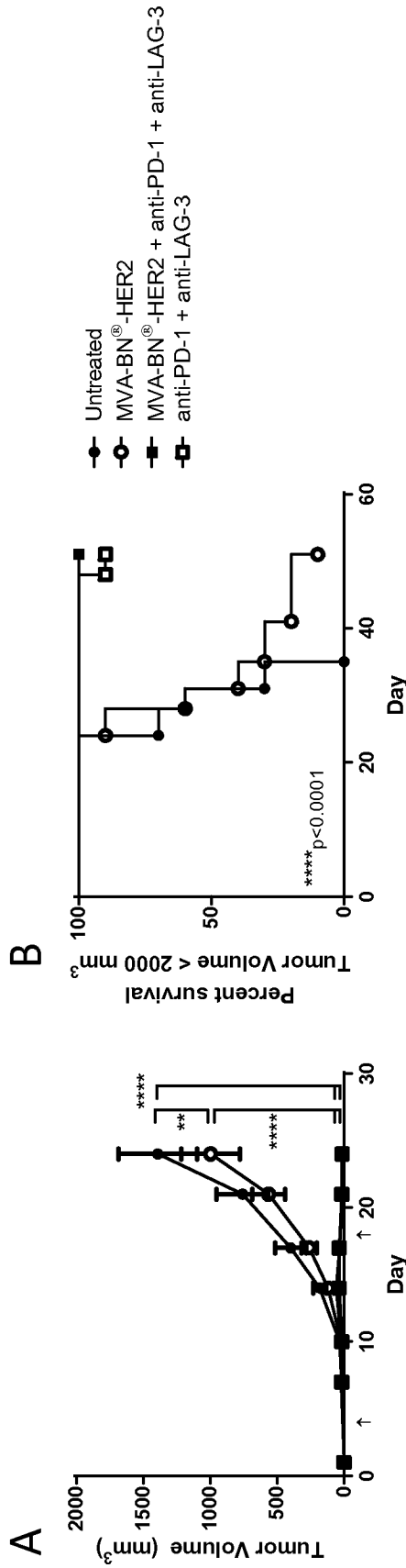


Figure 13

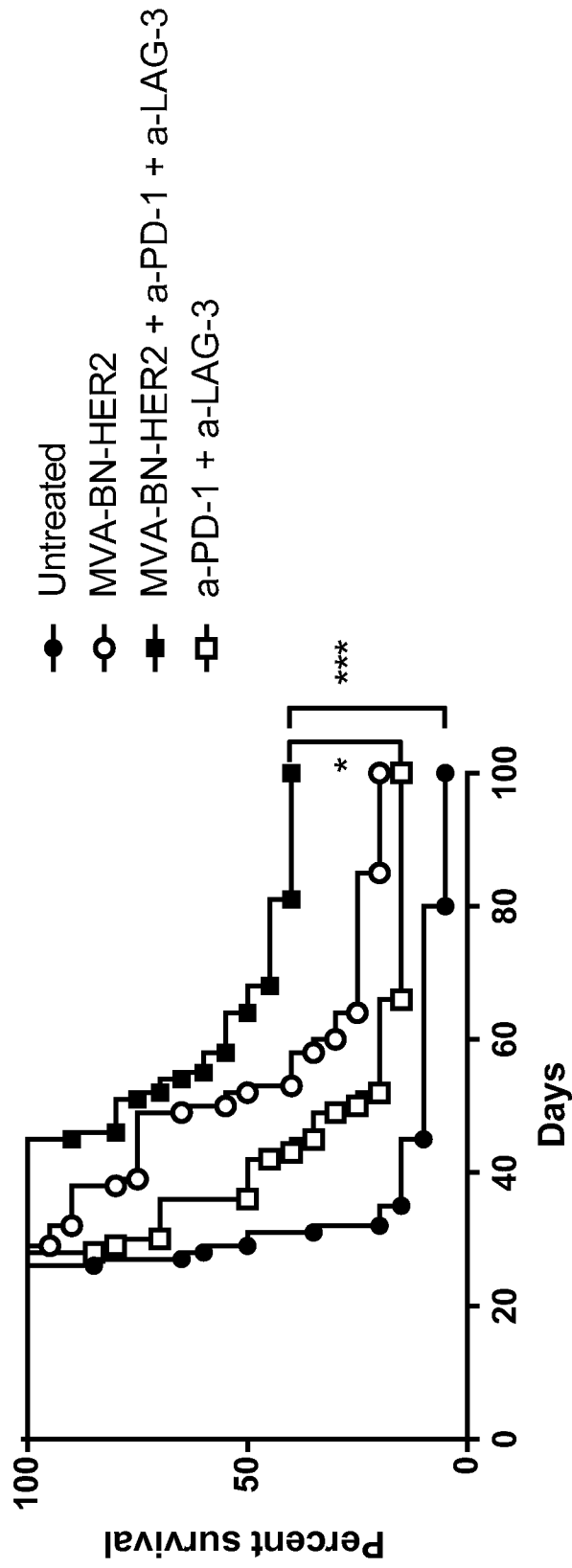


Figure 14

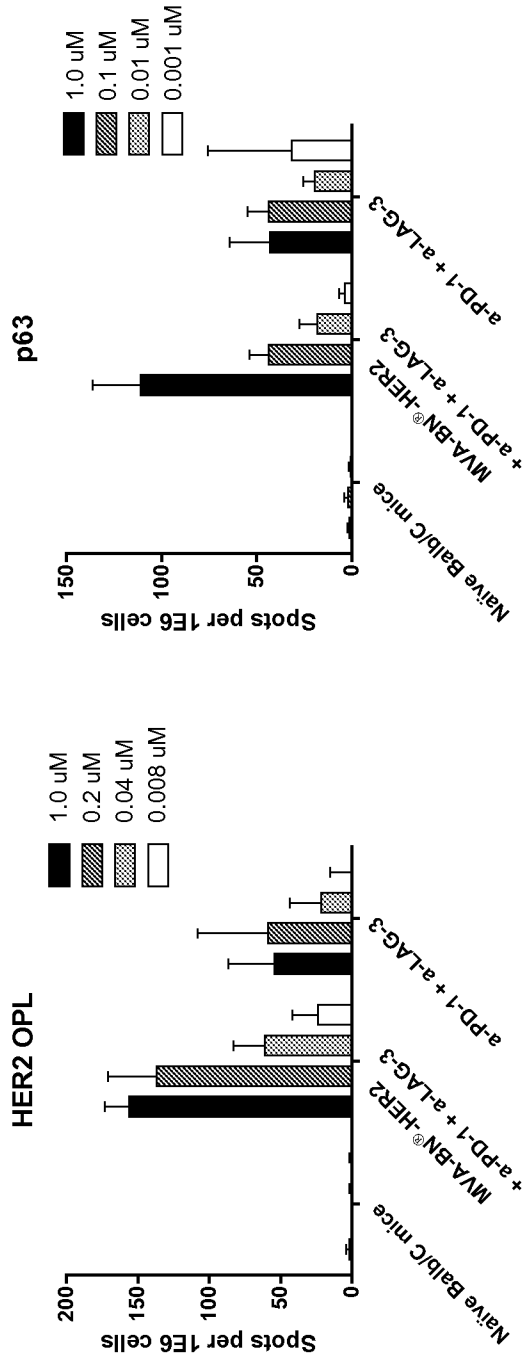


Figure 15

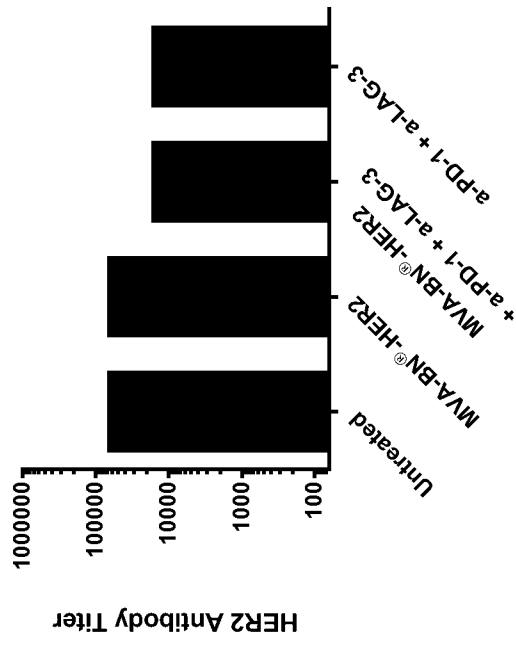


Figure 16

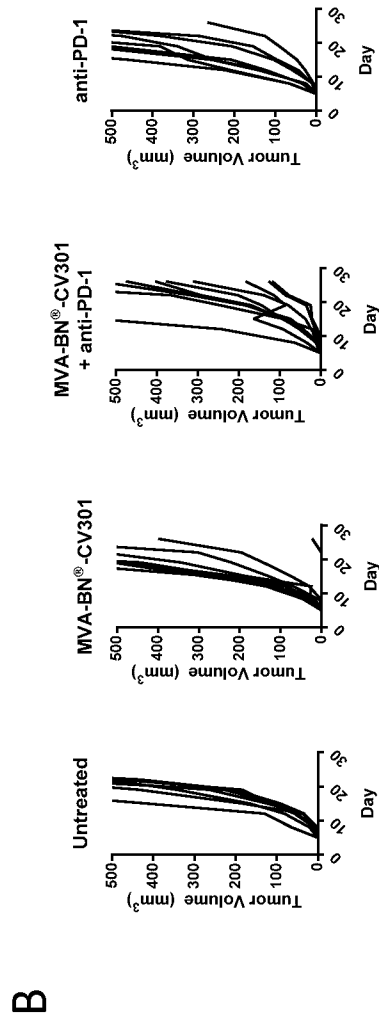
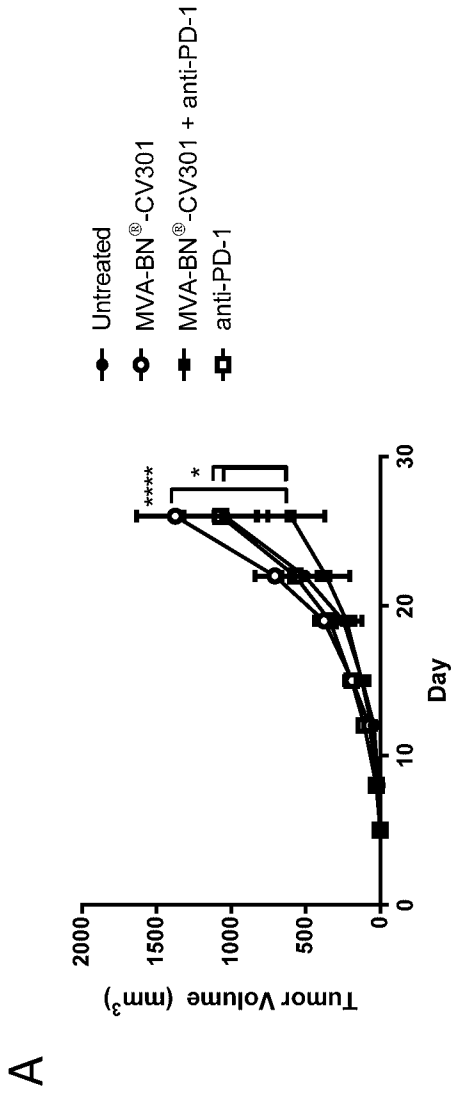


Figure 17

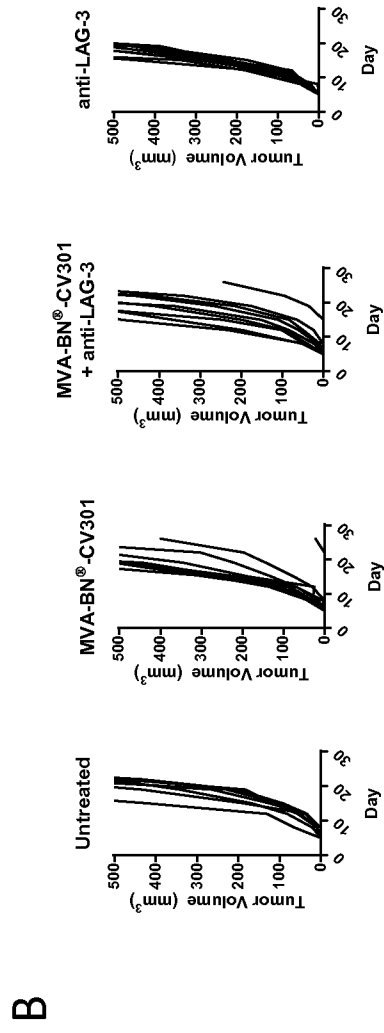
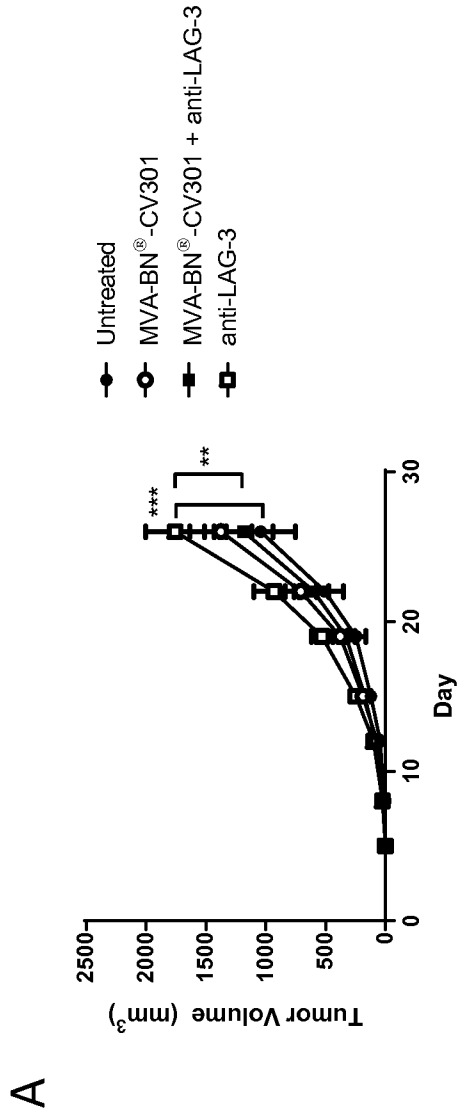


Figure 18

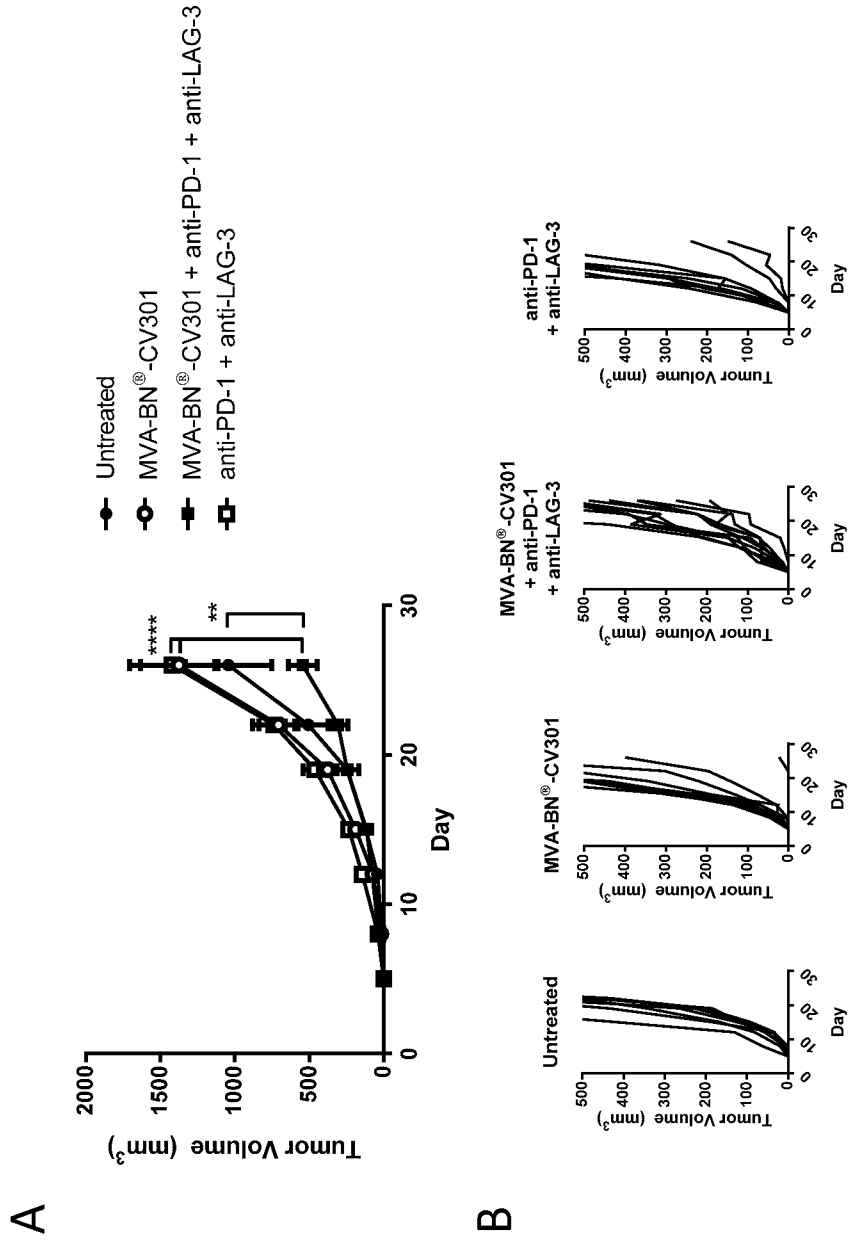


Figure 19

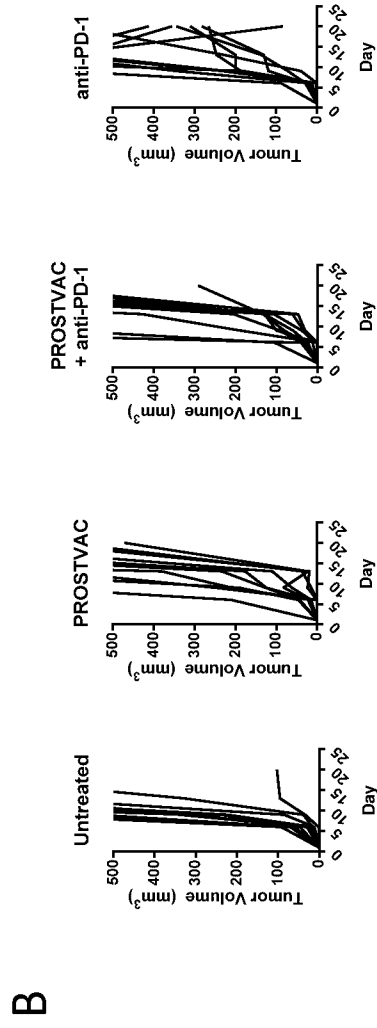
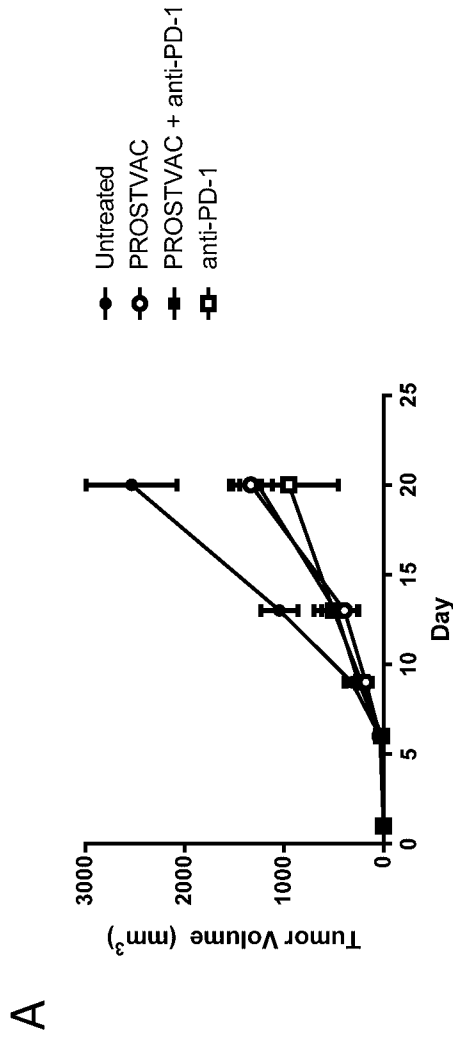


Figure 20

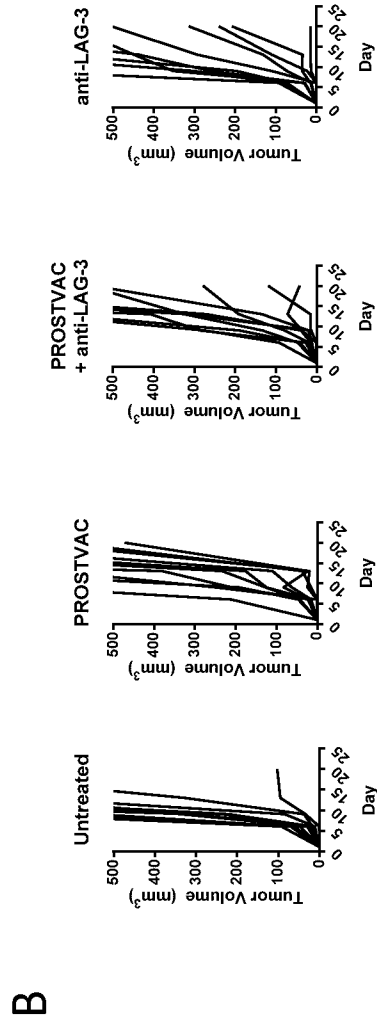
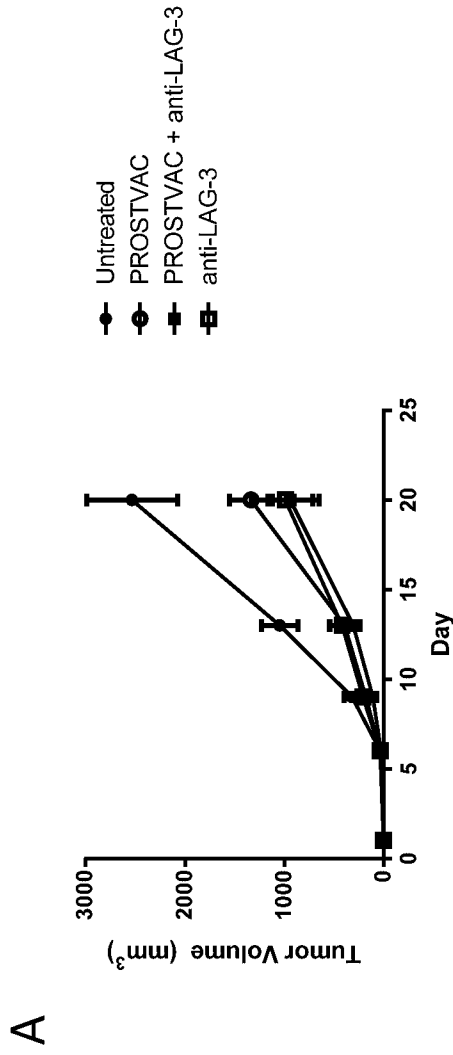


Figure 21

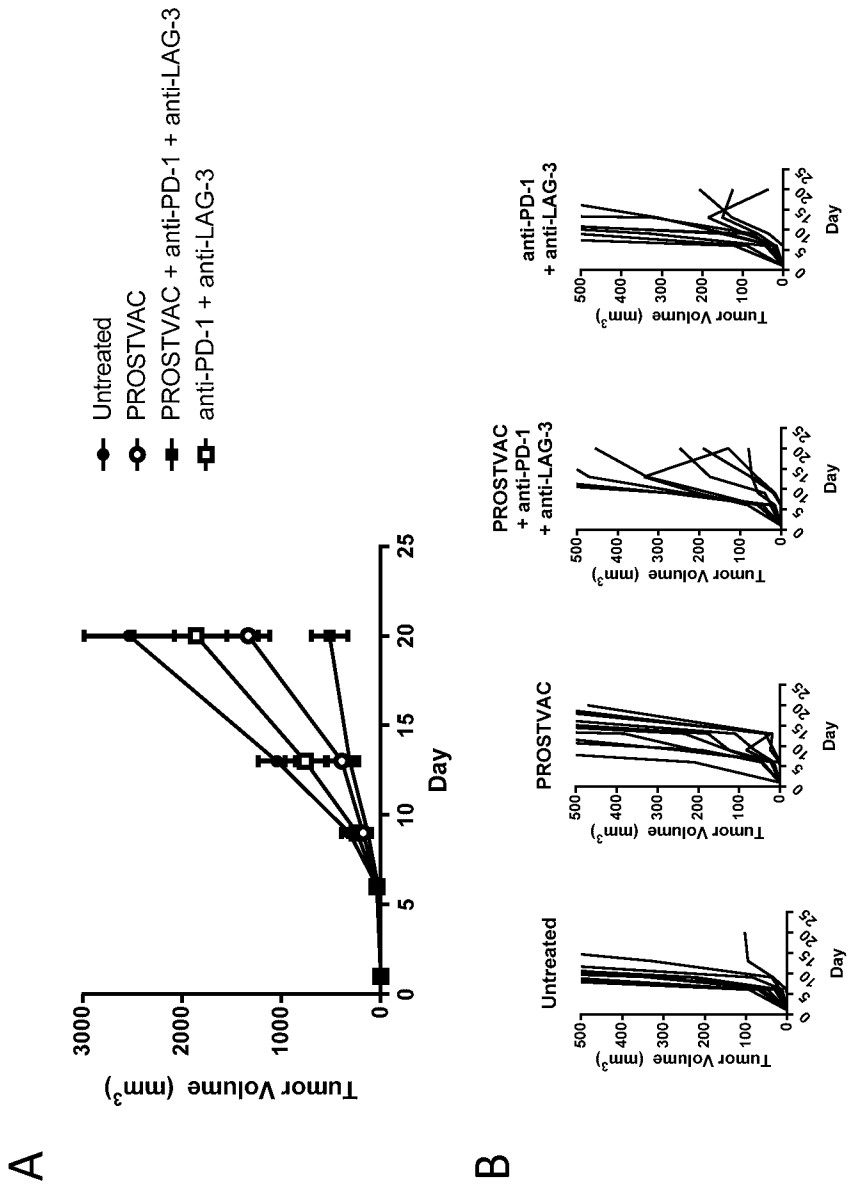


Figure 22

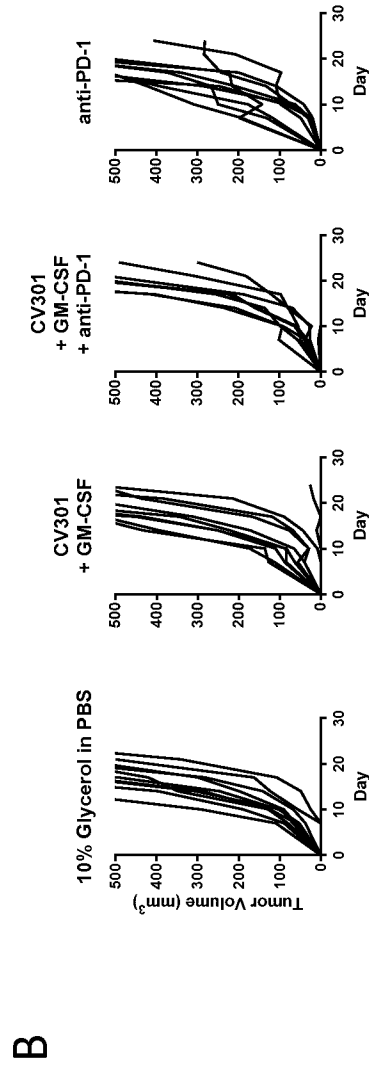
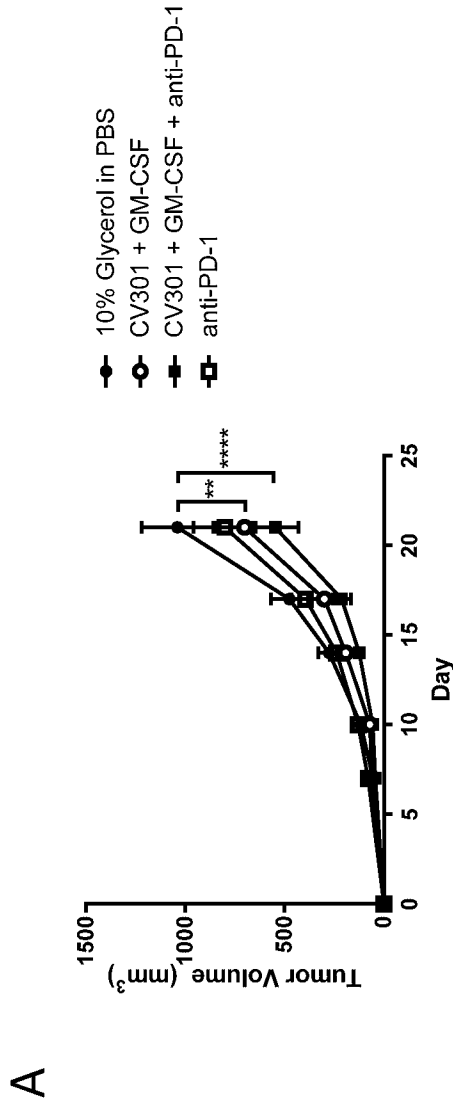


Figure 23

INTERNATIONAL SEARCH REPORT

International application No PCT/US2014/063516
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A. CLASSIFICATION OF SUBJECT MATTER INV. A61K39/00 ADD.				
According to International Patent Classification (IPC) or to both national classification and IPC				
B. FIELDS SEARCHED				
Minimum documentation searched (classification system followed by classification symbols) A61K C12N				
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched				
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) EPO-Internal, WPI Data, BIOSIS				
C. DOCUMENTS CONSIDERED TO BE RELEVANT				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
Y	RAVI A. MADAN ET AL.: "Ipilimumab and a poxviral vaccine targeting prostate-specific antigen in metastatic castration-resistant prostate cancer: a phase 1 dose-escalation trial", LANCET ONCOLOGY, vol. 13, no. 5, 10 February 2012 (2012-02-10), pages 501-508, XP002735511, page 501, right-hand column, paragraph 2 - page 502, left-hand column, paragraph 3 page 502, right-hand column, paragraph 3 page 506, left-hand column, paragraph 2 page 507, left-hand column, paragraph 4 - right-hand column, paragraph 1 ----- -/--	1-56		
<table style="width: 100%; border: none;"> <tr> <td style="width: 50%; border: none;"><input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C.</td> <td style="width: 50%; border: none;"><input checked="" type="checkbox"/> See patent family annex.</td> </tr> </table>			<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C.	<input checked="" type="checkbox"/> See patent family annex.
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C.	<input checked="" type="checkbox"/> See patent family annex.			
* Special categories of cited documents :				
"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family			
Date of the actual completion of the international search	Date of mailing of the international search report			
5 February 2015	19/02/2015			
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Montero Lopez, B			

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2014/063516

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MADAN RAVI A ET AL: "Combination of vaccine and immune checkpoint inhibitor is safe with encouraging clinical activity", ONCOIMMUNOLOGY, vol. 1, no. 7, October 2012 (2012-10), pages 1167-1168, XP002735512, the whole document -----	1-56
Y	WO 2011/041613 A2 (SLOAN KETTERING INST CANCER [US]; ALLISON JAMES [US]; SHARMA PADMANEE) 7 April 2011 (2011-04-07) cited in the application page 5, paragraph 0017 - paragraph 0021 page 7, paragraph 0027; example 1 -----	1-56
Y	S.-R. WOO ET AL: "Immune Inhibitory Molecules LAG-3 and PD-1 Synergistically Regulate T-cell Function to Promote Tumoral Immune Escape", CANCER RESEARCH, vol. 72, no. 4, 20 December 2011 (2011-12-20), pages 917-927, XP055151722, ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-11-1620 cited in the application page 918, paragraph 4 - page 919, paragraph 1 page 921, paragraph 3 page 924, paragraph 3 - page 925, paragraph 1 page 925, last paragraph -----	1-56
A	WO 98/04727 A1 (THERION BIOLOG CORP [US]; US GOV HEALTH & HUMAN SERV [US]) 5 February 1998 (1998-02-05) page 5, line 13 - line 28 page 6, paragraph 23 - page 8, line 2 page 11, line 5 - line 26; examples -----	1-56
A	STEFANIE J MANDL ET AL: "Immunotherapy with MVA-BN-HER2 induces HER-2-specific Th1 immunity and alters the intratumoral balance of effector and regulatory T cells", CANCER IMMUNOLOGY, IMMUNOTHERAPY, SPRINGER, BERLIN, DE, vol. 61, no. 1, 7 August 2011 (2011-08-07), pages 19-29, XP019995547, ISSN: 1432-0851, DOI: 10.1007/S00262-011-1077-4 cited in the application the whole document -----	1-56
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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2014/063516

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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