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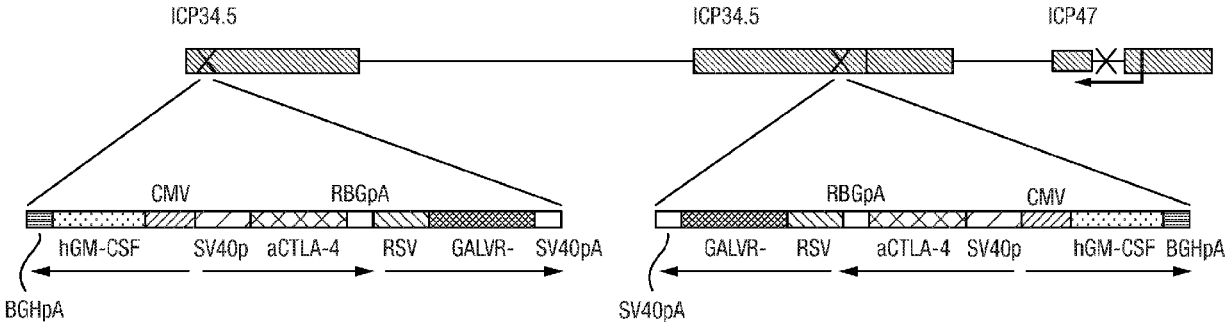

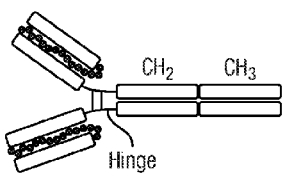

Fig. 3

(57) Abstract: The present invention relates to an oncolytic virus encoding a CTLA-4 inhibitor, such as an anti-CTLA-4 antibody, or an antigen binding fragment thereof.

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ALTERED VIRUS

Field of the Invention

The invention relates to an oncolytic immunotherapeutic agent and to the use of the oncolytic immunotherapeutic agent in treating cancer.

Background to the Invention

Viruses have a unique ability to enter cells at high efficiency. After entry into cells, viral genes are expressed and the virus replicates. This usually results in the death of the infected cell and the release of the antigenic components of the cell as the cell ruptures as it dies. As a result, virus mediated cell death tends to result in an immune response to these cellular components, including both those derived from the host cell and those encoded by or incorporated into the virus itself and enhanced due to the recognition by the host of so called damage associated molecular patterns (DAMPs) which aid in the activation of the immune response.

Viruses also engage with various mediators of the innate immune response as part of the host response to the recognition of a viral infection through e.g. toll-like receptors and cGAS/STING signalling and the recognition of pathogen associated molecular patterns (PAMPs) resulting in the activation of interferon responses and inflammation which are also immunogenic signals to the host. These immune responses may result in the immunogenic benefit to cancer patients such that immune responses to tumor antigens provide a systemic overall benefit resulting in the treatment of tumors which have not been infected with the virus, including micro-metastatic disease, and providing vaccination against relapse.

The combined direct ('oncolytic') effects of the virus, and immune responses against tumor antigens (including non-self 'neo-antigens', i.e. derived from the particular mutated genes in individual tumors) is termed 'oncolytic immunotherapy'.

Viruses may also be used as delivery vehicles ('vectors') to express heterologous genes inserted into the viral genome in infected cells. These properties make viruses useful for a variety of biotechnology and medical applications. For example, viruses expressing heterologous therapeutic genes may be used for gene therapy. In the context of oncolytic immunotherapy, delivered genes may include those encoding specific tumor

antigens, genes intended to induce immune responses or increase the immunogenicity of antigens released following virus replication and cell death, genes intended to shape the immune response which is generated, genes to increase the general immune activation status of the tumor, or genes to increase the direct oncolytic properties (i.e. cytotoxic effects) of the virus. Importantly, viruses have the ability to deliver encoded molecules which are intended to help to initiate, enhance or shape the systemic anti-tumor immune response directly and selectively to tumors, which may have benefits of e.g. reduced toxicity or of focusing beneficial effects on tumors (including those not infected by the virus) rather than off-target effects on normal (i.e. non-cancerous) tissues as compared to the systemic administration of these same molecules or systemic administration of other molecules targeting the same pathways.

It has been demonstrated that a number of viruses including, for example, herpes simplex virus (HSV) have utility in the oncolytic treatment of cancer. HSV for use in the oncolytic treatment of cancer must be disabled such that it is no longer pathogenic, but can still enter into and kill tumor cells. A number of disabling mutations to HSV, including disruption of the genes encoding ICP34.5, ICP6, and/or thymidine kinase, have been identified which do not prevent the virus from replicating in culture or in tumor tissue *in vivo*, but which prevent significant replication in normal tissue. HSVs in which only the ICP34.5 genes have been disrupted replicate in many tumor cell types *in vitro*, and replicate selectively in tumor tissue, but not in surrounding tissue, in mouse tumor models. Clinical trials of ICP34.5 deleted, or ICP34.5 and ICP6 deleted, HSV have also shown safety and selective replication in tumor tissue in humans.

As discussed above, an oncolytic virus, including HSV, may also be used to deliver a therapeutic gene in the treatment of cancer. An ICP34.5 deleted virus of this type additionally deleted for ICP47 and encoding a heterologous gene for GM-CSF has also been tested in clinical trials, including a phase 3 trial in melanoma in which safety and efficacy in man was shown. GM-CSF is a pro-inflammatory cytokine which has multiple functions including the stimulation of monocytes to exit the circulation and migrate into tissue where they proliferate and mature into macrophages and dendritic cells. GM-CSF is important for the proliferation and maturation of antigen presenting cells, the activity of which is needed for the activation of an anti-tumor immune response. The trial data demonstrated that tumor responses could be seen in injected tumors, and to a lesser extent

in uninjected tumors. Responses tended to be highly durable (months-years), and a survival benefit appeared to be achieved in responding patients. Each of these indicated engagement of the immune system in the treatment of cancer in addition to the direct oncolytic effect. However, this and other data with oncolytic viruses generally showed that not all tumors respond to treatment and not all patients achieve a survival advantage. Thus, improvements to the art of oncolytic therapy are clearly needed.

Recently it has been shown that oncolytic immunotherapy can result in additive or synergistic therapeutic effects in conjunction with immune co-inhibitory pathway blockade (i.e. inhibition or 'antagonism' of immune checkpoint pathways, also termed immune co-inhibitory pathways). Immune co-inhibitory pathway blockade is intended to block host immune inhibitory mechanisms which usually serve to prevent the occurrence of auto-immunity. However, in cancer patients these mechanisms can also serve to inhibit the induction of or block the potentially beneficial effects of any immune responses induced to tumors.

Systemic blockade of these pathways by agents targeting cytotoxic T lymphocyte-associated molecule -4 (CTLA-4), PD-1 or PD-L1 have shown efficacy in a number of tumor types, including melanoma and lung cancer. However, unsurprisingly, based on the mechanism of action, off target toxicity can occur due to the induction of auto-immunity. Even so, these agents are sufficiently tolerable to provide considerable clinical utility. Other immune co-inhibitory pathway and related targets for which agents (mainly antibodies) are in development include LAG-3, TIM-3, VISTA, CSF1R, IDO, CEACAM1, CD47. Optimal clinical activity of these agents, for example PD1, PDL1, LAG-3, TIM-3, VISTA, CSF1R, IDO, CD47, CEACAM1, may require systemic administration or presence in all tumors due to the mechanism of action, i.e. including targeting of the interface of immune effector cells with tumors or other immune inhibitory mechanisms in/of tumors. In some cases, more localised presence in e.g. just some tumors or in some lymph nodes may also be optimally effective, for example agents targeting CTLA-4.

An alternative approach to increasing the anti-tumor immune response in cancer patients is to target (activate) immune co-stimulatory pathways, i.e. in contrast to inhibiting immune co-inhibitory pathways. These pathways send activating signals into T cells and other immune cells, usually resulting from the interaction of the relevant ligands on antigen presenting cells (APCs) and the relevant receptors on the surface of T cells and other

immune cells. These signals, depending on the ligand/receptor, can result in the increased activation of T cells and/or APCs and/or NK cells and/or B cells, including particular sub-types, increased differentiation and proliferation of T cells and/or APCs and/or NK cells and/or B cells, including particular subtypes, or suppression of the activity of immune inhibitory T cells such as regulatory T cells. Activation of these pathways would therefore be expected to result in enhanced anti-tumor immune responses, but it might also be expected that systemic activation of these pathways, i.e. activation of immune responses generally rather than anti-tumor immune responses specifically or selectively, would result in considerable off target toxicity in non-tumor tissue, the degree of such off target toxicity depending on the particular immune co-stimulatory pathway being targeted. Nevertheless agents (mainly agonistic antibodies, or less frequently the soluble ligand to the receptor in question) targeting immune co-stimulatory pathways, including agents targeting GITR, 4-1-BB, OX40, CD40 or ICOS, and intended for systemic use (i.e. intravenous delivery) are in or have been proposed for clinical development.

For many of these approaches targeting immune co-inhibitory or co-inhibitory pathways to be successful, pre-existing immune responses to tumors are needed, i.e. so that a pre-existing immune response can be potentiated or a block to an anti-tumor immune response can be relieved. The presence of an inflamed tumor micro-environment, which is indicative of such an ongoing response, is also needed. Pre-existing immune responses to tumor neo-antigens appear to be particularly important for the activity of immune co-inhibitory pathway blockade and related drugs. Only some patients may have an ongoing immune response to tumor antigens including neoantigens and/or an inflamed tumor microenvironment, both of which are required for the optimal activity of these drugs. Therefore, oncolytic agents which can induce immune responses to tumor antigens, including neoantigens, and/or which can induce an inflamed tumor microenvironment are attractive for use in combination with immune co-inhibitory pathway blockade and immune potentiating drugs. This likely explains the promising combined anti-tumor effects of oncolytic agents and immune co-inhibitory pathway blockade in mice and humans that have so far been observed.

The above discussion demonstrates that there is still much scope for improving oncolytic agents and cancer therapies utilising oncolytic agents, anti-tumor immune responses and drugs which target immune co-inhibitory or co-stimulatory pathways.

Summary of the Invention

The present invention provides oncolytic viruses expressing an inhibitor of CTLA-4. The virus may further comprise other immunomodulatory agents. In particular the virus may comprise GM-CSF and/or at least one molecule targeting an immune co-stimulatory pathway. The CTLA-4 inhibitor acts to block a co-inhibitory pathway, i.e. interferes with the interaction between CTLA-4 and B7. GM-CSF aids in the induction of an inflammatory tumor micro-environment and stimulates the proliferation and maturation of antigen presenting cells, including dendritic cells, aiding the induction of an anti-tumor immune responses. These immune responses may be amplified through activation of an immune co-stimulatory pathway or pathways using an immune co-stimulatory pathway activating molecule or molecules also delivered by the oncolytic virus.

Oncolytic viruses replicate within tumors, causing lysis of tumor cells and release of tumor antigens, combined with local inflammation and activation of innate immune responses, all of which are beneficial for the activation of an anti-tumor immune response and for the activity of inhibitors of the CTLA-4/B7 interaction.

Delivery of molecules that inhibit the CTLA-4/B7 interaction directly into an immune response initiating-tumor, including where it would be expected to traffic to draining lymph nodes, focuses immune potentiation by the inhibitor on the tumor and therefore on tumor antigens present within it, reduces systemic toxicity and blocks regulatory T cell (Treg) activation that would otherwise inhibit T-cell activation at the site of immune response initiation. The use of an oncolytic virus to deliver molecules targeting CTLA-4, and optionally molecules targeting immune co-stimulatory pathways to tumors focuses the amplification of immune effects on anti-tumor immune responses, and reduces the amplification of immune responses to non-tumor antigens. Thus, immune cells in tumors and tumor draining lymph nodes are selectively affected by the molecules expressed by the virus rather than immune cells in general. This results in enhanced efficacy of immune cell stimulation, and can also result in reduced off target toxicity. It is also important for focusing the effects of combined systemic immune co-inhibitory pathway blockade and immune co-stimulatory pathway activation on tumors, i.e. such that the amplified immune responses from which co-inhibitory blocks are released are antitumor immune responses rather than responses to non-tumor antigens.

The invention utilizes the fact that, when delivered by an oncolytic virus, the site of action of CTLA-4 blockade and optionally co-stimulatory pathway activation and of GM-CSF expression is in the tumor and/or tumor draining lymph node, but the results of such activation (an amplified systemic anti-tumor-immune response) are systemic. This targets tumors generally, and not only tumors to which the oncolytic virus has delivered the immunomodulatory molecule or molecules. Oncolytic viruses of the invention therefore provide improved treatment of cancer through the generation of improved tumor focused immune responses. The oncolytic virus of the invention also offers improved anti-tumor immune stimulating effects such that the immune-mediated effects on tumors which are not destroyed by oncolysis, including micro-metastatic disease, are enhanced, resulting in more effective destruction of these tumors, and more effective long term anti-tumor vaccination to prevent future relapse and improve overall survival.

Anti-tumor efficacy is improved when an oncolytic virus of the invention is used as a single agent and also when the virus is used in combination with other anti-cancer modalities, including chemotherapy, treatment with targeted agents, radiation and, in preferred embodiments, immune checkpoint blockade drugs (i.e. antagonists of an immune co-inhibitory pathway, for example antibodies against PD1 or PD-L1) and/or agonists of an immune co-stimulatory pathway.

Accordingly, the present invention provides an oncolytic virus encoding a CTLA-4 inhibitor. The CTLA-4 inhibitor is preferably an anti-CTLA-4 antibody or antibody like molecule, or an antigen binding fragment thereof.

The virus may further comprise: (i) a GM-CSF-encoding gene; and/or (ii) an immune co-stimulatory pathway activating molecule or immune co-stimulatory pathway activating molecule-encoding gene. The virus may encode more than one immune co-stimulatory pathway activating molecule/gene.

The immune co-stimulatory pathway activating molecule is preferably GITRL, 4-1-BBL, OX40L, ICOSL or CD40L or a modified version of any thereof. Examples of modified versions include agonists of a co-stimulatory pathway that are secreted rather than being membrane bound, and/or agonists modified such that multimers of the protein are formed.

The virus may be a modified clinical isolate, such as a modified clinical isolate of a virus, wherein the clinical isolate kills two or more tumor cell lines more rapidly and/or at

a lower dose *in vitro* than one or more reference clinical isolates of the same species of virus.

The virus is preferably a herpes simplex virus (HSV), such as HSV1. The HSV typically does not express functional ICP34.5 and/or functional ICP47 and/or expresses the US11 gene as an immediate early gene.

The invention also provides:

- a pharmaceutical composition comprising a virus of the invention and a pharmaceutically acceptable carrier or diluent;
- the virus of the invention for use in a method of treating the human or animal body by therapy;
- the virus of the invention for use in a method of treating cancer, wherein the method optionally comprises administering a further anti-cancer agent;
- a product of manufacture comprising a virus of the invention in a sterile vial, ampoule or syringe;
- a method of treating cancer, which comprises administering a therapeutically effective amount of a virus or a pharmaceutical composition of the invention to a patient in need thereof, wherein the method optionally comprises administering a further anti-cancer agent;
- use of a virus of the invention in the manufacture of a medicament for use in a method of treating cancer, wherein the method optionally comprises administering a further anti-cancer agent.

The present invention also provides an oncolytic herpes simplex virus (HSV) encoding a CTLA-4 inhibitor, wherein:

(a) the virus further comprises a GM-CSF-encoding gene; and/or

(b) the virus further comprises an immune co-stimulatory pathway activating molecule-encoding gene encoding CD40 ligand (CD40L), ICOS ligand, GITR ligand, 4-1-BB ligand, OX40 ligand, TL1A, CD30 ligand, CD27 or flt3 ligand; and/or

(c) the virus further comprises an immune co-stimulatory pathway activating molecule-encoding gene, wherein the immune co-stimulatory pathway activating molecule is encoded by a codon optimized sequence so as to increase expression levels in target cells; and/or

(d) the virus further comprises a fusogenic protein-encoding gene; and/or

(e) the CTLA-4 inhibitor is encoded by a codon optimized sequence so as to increase expression levels in target cells; and/or

(f) the virus does not express functional ICP34.5, does not express functional ICP47, and expresses the US11 gene as an immediate early gene

Brief Description of the Figures

Figure 1 depicts the structures of the viruses used to construct exemplary viruses of the invention that comprise anti-mouse or anti-human CTLA-4 constructs that are codon optimized secreted scFv molecules linked to human or mouse IgG1 Fc regions. The scFvs contain light and heavy variable chains from 9D9 (the initial mouse antibody initially used to validate CTLA-4; WO2007/123737: mouse version) or from ipilimumab. (WO2014/066532; human version) linked by the 15-mer $[G_4S]_3$ (GGGGSGGGGSGGGGS). The viruses are modified versions of strain HSV1 RH018A (clinical strain 18). The ICP34.5 and ICP47 genes are inactivated in the viruses. The US11 gene is placed under the control of the ICP47 immediate early gene promoter by deletion of the ICP47 promoter. An expression cassette is inserted into the ICP34.5 gene loci. In virus 17, the expression cassette includes the human GM-CSF gene under the control of a CMV promoter and the GALV gene under the control of a RSV promoter. Virus 16 is the same as virus 17, except that human GM-CSF is included instead of mouse GM-CSF. Viruses 25 and 29 are the same as viruses 16 and 17, respectively, except that they each additionally comprise a GFP gene under the control of a MMLV promoter in the expression cassette. Viruses 27 and 31 are the same as viruses 25 and 29, respectively, except that the GFP gene is replaced with mouse anti-CTLA4 and human anti-CTLA4, respectively.

Figure 2 depicts the structures of the plasmids used to construct the exemplary viruses of the invention.

Figure 3 shows the structure of anti-mouse or human CTLA-4 constructs that are codon optimized secreted scFv molecules linked to human or mouse IgG1 Fc regions. The scFvs contain the linked ($[G_4S]_3$) light and heavy variable chains from 9D9 (the initial mouse antibody initially used to validate CTLA-4; US2011044953: mouse version) or

from ipilimumab (US20150283234; human version). The resulting structure of the CTLA-4 inhibitor is also shown.

Figure 4 is a western blot demonstrating that anti-mouse CTLA-4 is expressed from virus 27. The gel used was a reduced denatured PVDF membrane tris-glycine gel. Anti-CTLA-4 was detected using an alkaline phosphatase-tagged anti-mouse IgG1 antibody. Lane 1: spectra broad range ladder; lane 2 virus 27 neat supernatant; lane 3 virus 27 supernatant diluted 1 in 2; lane 4 virus 27 supernatant diluted 1 in 4; lane 5 virus 27 supernatant diluted 1 in 8; lane 6 virus 27 supernatant diluted 1 in 16; lane 7 virus 27 supernatant diluted 1 in 32; lane 8 negative control virus (neat supernatant). The expected size of anti-CTLA-4 (reduced) is 57kDa.

Figure 5 shows the superior tumor control and shrinkage in uninjected tumors of a virus expressing anti-mCTLA-4 (virus 27) compared to an otherwise identical virus that does not express CTLA-4 (virus 16). The dose of virus used was $5x10^4$ pfu (50ul of $1x10^6$ pfu/ml in each case), given three times over one week. This dose level of virus is subtherapeutic for uninjected tumors for virus 16, which allows the benefits of the delivery of the additional molecule encoded by virus 27 to clearly be seen.

Figure 6 shows the superior tumor control and shrinkage in both injected and uninjected tumors of a virus expressing anti-mCTLA-4 (virus 27) compared to an otherwise identical virus that does not express CTLA-4 (virus 16). The dose of the virus used was $5x10^4$ pfu over one week into the right tumor of a virus expressing anti-mCTLA-4 (virus 27) compared to an otherwise identical virus that does not express CTLA-4 (virus 16). Each line represents a different mouse.

Figure 7 shows the effect of combined treatment of bilateral mouse A20 tumors using anti-PD1 and virus 27 expressing mGM-CSF, GALVR and anti-mCTLA-4. The top panel shows the effect of anti-PD1 alone on both injected (right) and uninjected (left) tumors. The middle panel shows the effect of virus 27 alone on both injected (right) and uninjected (left) tumors. The bottom panel shows the superior tumor control and shrinkage achieved when anti-PD1 and virus 27 are both injected into the right tumor. The improved anti-tumor effect of the combined treatment is observed in both injected (right) and uninjected (left) tumors. Each line represents a different mouse.

Figure 8 shows the superior tumor control and shrinkage effects of virus 31 expressing hGM-CSF, GALVR and anti-human CTLA-4 compared to virus 17 expressing

only hGM-CSF and GALVR in mouse MC38 tumors in knock-in mice expressing human CTLA-4. The anti-tumor effects of virus 31 are observed when the virus is administered alone or in combination with anti-PD1. Superior tumor control and shrinkage in injected tumors is obtained with virus 31 which expresses anti-human CTLA-4 compared with an otherwise identical virus that does not express anti-human CTLA-4 (left panel). This effect is further enhanced when treatment with the virus is combined with anti-PD1 treatment. Superior tumor control and shrinkage is also observed in uninjected tumors (right panel) when treatment with either virus is combined with anti-PD1 treatment. This improvement is more marked for the virus 31 that expresses anti CTLA-4 than for virus 17 which does not. Each line represents a different mouse.

Brief Description of the Sequence Listing

SEQ ID NO: 1 is the light chain variable region amino acid sequence of the human CTLA-4 antibody used in the Examples.

SEQ ID NOs: 2 is the complete light chain amino acid sequence comprising the light chain variable region amino acid sequence of the human CTLA-4 antibody used in the Examples.

SEQ ID NO: 3 is the heavy chain variable region amino acid sequence of the human CTLA-4 antibody used in the Examples.

SEQ ID NO: 4 is the heavy chain CH1 amino acid sequence of the human CTLA-4 antibody used in the Examples.

SEQ ID NO: 5 is the heavy chain CH2/3 amino acid sequence of the human CTLA-4 antibody used in the Examples.

SEQ ID NO: 6 is the complete heavy chain amino acid sequence of the human CTLA-4 antibody used in the Examples.

SEQ ID NO: 7 is the amino acid sequence of the signal peptide present in the CTLA-4 antibodies of the Examples.

SEQ ID NO: 8 is the amino acid sequence of the linker present between the light chain variable region and the heavy chain variable region in the CTLA-4 antibodies of the Examples.

SEQ ID NO: 9 is the amino acid sequence of the human scFv CTLA-4 antibody of the Examples.

SEQ ID NO: 10 is the nucleotide sequence of the human scFv CTLA-4 antibody of the Examples.

SEQ ID NO: 11 is the light chain variable region amino acid sequence of the murine CTLA-4 antibody used in the Examples.

SEQ ID NO: 12 is the heavy chain variable region amino acid sequence of the murine CTLA-4 antibody used in the Examples.

SEQ ID NO: 13 is the complete heavy chain amino acid sequence of the murine CTLA-4 antibody used in the Examples.

SEQ ID NO: 14 is the amino acid sequence of the murine scFv CTLA-4 antibody of the Examples.

SEQ ID NO: 15 is the nucleotide sequence of the murine scFv CTLA-4 antibody of the Examples.

SEQ ID NO: 16 is the nucleotide sequence of the murine scFv CTLA-4 antibody of the Examples with inserted restriction sites for cloning purposes located at the N and C terminals, that is present in the exemplary virus. The restriction sites are the first six and last eight nucleotides of the sequence.

SEQ ID NO: 17 is the nucleotide sequence of the human scFv CTLA-4 antibody of the Examples with inserted restriction sites for cloning purposes located at the N and C terminals, that is present in the exemplary virus. The restriction sites are the first six and last eight nucleotides of the sequence.

SEQ ID NO: 18 is the nucleotide sequence of mouse GM-CSF.

SEQ ID NO: 19 is the nucleotide sequence of a codon optimized version of mouse GM-CSF.

SEQ ID NO: 20 is the nucleotide sequence of human GM-CSF.

SEQ ID NO: 21 is the nucleotide sequence of a codon optimized version of human GM-CSF.

SEQ ID NO: 22 is the amino acid sequence of mouse GM-CSF.

SEQ ID NO: 23 is the amino acid sequence of human GM-CSF.

SEQ ID NO: 24 is the nucleotide sequence of GALV-R-.

SEQ ID NO: 25 is the nucleotide sequence of a codon optimized version of GALV-R-.

SEQ ID NO: 26 is the amino acid sequence of GALV-R-.

SEQ ID NO: 27 is the nucleotide sequence of a codon optimized version of a human/mouse hybrid membrane bound version of CD40L.

SEQ ID NO: 28 is the amino acid sequence of a human/mouse hybrid membrane bound version of CD40L .

SEQ ID NO: 29 is the nucleotide sequence of a codon optimized version of a multimeric secreted version of human CD40L.

SEQ ID NO: 30 is the amino acid sequence of a multimeric secreted version of human CD40L.

SEQ ID NO: 31 is the nucleotide sequence of a codon optimized version of a multimeric secreted version of mouse CD40L.

SEQ ID NO: 32 is the amino acid sequence of a multimeric secreted version of mouse CD40L.

SEQ ID NO: 33 is the nucleotide sequence of wild-type human CD40L.

SEQ ID NO: 34 is the amino acid sequence of wild-type human CD40L.

SEQ ID NO: 35 is the nucleotide sequence of wild-type mouse CD40L.

SEQ ID NO: 36 is the amino acid sequence of wild-type mouse CD40L.

SEQ ID NO: 37 is the nucleotide sequence of the CMV promoter.

SEQ ID NO: 38 is the nucleotide sequence of the RSV promoter.

SEQ ID NO: 39 is the nucleotide sequence of BGH polyA.

SEQ ID NO: 40 is the nucleotide sequence of SV40 late polyA.

SEQ ID NO: 41 is the nucleotide sequence of rabbit beta-globulin polyA.

SEQ ID NO: 42 is the nucleotide sequence of GFP.

SEQ ID NO: 43 is the nucleotide sequence of retroviral LTR from MMLV.

SEQ ID NO: 44 is the nucleotide sequence of EF1a promoter.

SEQ ID NO: 45 is the nucleotide sequence of SV40 promoter.

SEQ ID NO: 46 is the nucleotide sequence of HGH polyA.

Detailed Description of the Invention

Oncolytic Virus

The virus of the invention is oncolytic. An oncolytic virus is a virus that infects and replicates in tumor cells, such that the tumor cells are killed. Therefore, the virus of the invention is replication competent. Preferably, the virus is selectively replication

competent in tumors. A virus is selectively replication competent in tumor tissue if it replicates more effectively in tumor tissue than in non-tumor tissue. The ability of a virus to replicate in different tissue types can be determined using standard techniques in the art.

The virus of the invention may be any virus which has these properties, including a herpes virus, pox virus, adenovirus, retrovirus, rhabdovirus, paramyxovirus or reovirus, or any species or strain within these larger groups. Viruses of the invention may be wild type (i.e. unaltered from the parental virus species), or with gene disruptions or gene additions. Which of these is the case will depend on the virus species to be used. Preferably the virus is a species of herpes virus, more preferably a strain of HSV, including strains of HSV1 and HSV2, and is most preferably a strain of HSV1. In particularly preferred embodiments the virus of the invention is based on a clinical isolate of the virus species to be used. The clinical isolate may have been selected on the basis of it having particular advantageous properties for the treatment of cancer.

The virus may be a modified clinical isolate, wherein the clinical isolate kills two or more tumor cell lines more rapidly and/or at a lower dose *in vitro* than one or more reference clinical isolate of the same species of virus. Typically, the clinical isolate will kill two or more tumor cell lines within 48 hours, preferably within 24 hours, of infection at multiplicities of infection (MOI) of less than or equal to 0.1. Preferably the clinical isolate will kill a broad range of tumor cell lines, such as 2, 3, 4, 5, 6, 7, 8, 9, 10 or, for example, all of the following human tumor cell lines: U87MG (glioma), HT29 (colorectal), LNCaP (prostate), MDA-MB-231 (breast), SK-MEL-28 (melanoma), Fadu (squamous cell carcinoma), MCF7 (breast), A549 (lung), MIAPACA-2 (pancreas), CAPAN-1(pancreas), HT1080 (fibrosarcoma).

In a preferred embodiment, the virus of the invention is a strain selected from:

strain RH018A having the accession number ECCAC 16121904;
strain RH004A having the accession number ECCAC 16121902;
strain RH031A having the accession number ECCAC 16121907;
strain RH040B having the accession number ECCAC 16121908;
strain RH015A having the accession number ECCAC 16121903;
strain RH021A having the accession number ECCAC 16121905;
strain RH023A having the accession number ECCAC 16121906; and
strain RH047A having the accession number ECCAC 16121909.

More preferably, the virus of the invention is a strain selected from:
strain RH018A having the accession number ECCAC 16121904;
strain RH004A having the accession number ECCAC 16121902;
strain RH031A having the accession number ECCAC 16121907;
strain RH040B having the accession number ECCAC 16121908; and
strain RH015A having the accession number ECCAC 16121903.

Most preferably, the virus of the invention is strain RH018A having the accession number EACC 16121904. Any one of the deposited strains may be modified as defined herein.

An HSV of the invention is capable of replicating selectively in tumors, such as human tumors. Typically, the HSV replicates efficiently in target tumors but does not replicate efficiently in non-tumor tissue. This HSV may comprise one or more mutations in one or more viral genes that inhibit replication in normal tissue but still allow replication in tumors. The mutation may, for example, be a mutation that prevents the expression of functional ICP34.5, ICP6 and/or thymidine kinase by the HSV.

In one preferred embodiment, the ICP34.5-encoding genes are mutated to confer selective oncolytic activity on the HSV. Mutations of the ICP34.5-encoding genes that prevent the expression of functional ICP34.5 are described in Chou *et al.* (1990) Science 250:1262-1266, Maclean *et al.* (1991) J. Gen. Virol. 72:631-639 and Liu *et al.* (2003) Gene Therapy 10:292-303, which are incorporated herein by reference. The ICP6-encoding gene and/or thymidine kinase-encoding gene may also be inactivated, as may other genes provided that such inactivation does not prevent the virus infecting or replicating in tumors.

The HSV may contain a further mutation or mutations which enhance replication of the HSV in tumors. The resulting enhancement of viral replication in tumors not only results in improved direct 'oncolytic' tumor cell killing by the virus, but also enhances the level of heterologous (i.e. a gene inserted into the virus, in the case of viruses of the invention genes encoding a CTLA-4 inhibitor, GM-CSF and/or an immune co-stimulatory pathway activating molecule(s)) gene expression and increases the amount of tumor antigen released as tumor cells die, both of which may also improve the immunogenic properties of the therapy for the treatment of cancer. For example, in a preferred embodiment of the invention, deletion of the ICP47-encoding gene in a manner that places

the US11 gene under the control of the immediate early promoter that normally controls expression of the ICP47 encoding gene leads to enhanced replication in tumors (see Liu *et al.*, 2003, which is incorporated herein by reference).

Other mutations that place the US11 coding sequence, which is an HSV late gene, under the control of a promoter that is not dependent on viral replication may also be introduced into a virus of the invention. Such mutations allow expression of US11 before HSV replication occurs and enhance viral replication in tumors. In particular, such mutations enhance replication of an HSV lacking functional ICP34.5-encoding genes.

Accordingly, in one embodiment the HSV of the invention comprises a US11 gene operably linked to a promoter, wherein the activity of the promoter is not dependent on viral replication. The promoter may be an immediate early (IE) promoter or a non-HSV promoter which is active in mammalian, preferably human, tumor cells. The promoter may, for example, be a eukaryotic promoter, such as a promoter derived from the genome of a mammal, preferably a human. The promoter may be a ubiquitous promoter (such as a promoter of β-actin or tubulin) or a cell-specific promoter, such as tumor-specific promoter. The promoter may be a viral promoter, such as the Moloney murine leukaemia virus long terminal repeat (MMLV LTR) promoter or the human or mouse cytomegalovirus (CMV) IE promoter. HSV immediate early (IE) promoters are well known in the art. The HSV IE promoter may be the promoter driving expression of ICP0, ICP4, ICP22, ICP27 or ICP47.

The genes referred to above, the functional inactivation of which provides the property of tumor selectivity to the virus, may be rendered functionally inactive by any suitable method, for example by deletion or substitution of all or part of the gene and/or control sequence of the gene or by insertion of one or more nucleic acids into or in place of the gene and/or the control sequence of the gene. For example, homologous recombination methods, which are standard in the art, may be used to generate the virus of the invention. Alternatively bacterial artificial chromosome (BAC)-based approaches may be used.

As used herein, the term "gene" is intended to mean the nucleotide sequence encoding a protein, i.e. the coding sequence of the gene. The various genes referred to above may be rendered non-functional by mutating the gene itself or the control sequences flanking the gene, for example the promoter sequence. Deletions may remove one or more portions of the gene, the entire gene or the entire gene and all or some of the control

sequences. For example, deletion of only one nucleotide within the gene may be made, resulting in a frame shift. However, a larger deletion may be made, for example at least about 25%, more preferably at least about 50% of the total coding and/or non-coding sequence. In one preferred embodiment, the gene being rendered functionally inactive is deleted. For example, the entire gene and optionally some of the flanking sequences may be removed from the virus. Where two or more copies of the gene are present in the viral genome both copies of the gene are rendered functionally inactive.

A gene may be inactivated by substituting other sequences, for example by substituting all or part of the endogenous gene with a heterologous gene and optionally a promoter sequence. Where no promoter sequence is substituted, the heterologous gene may be inserted such that it is controlled by the promoter of the gene being rendered non-functional. In an HSV of the invention it is preferred that the ICP34.5 encoding-genes are rendered non-functional by the insertion of a heterologous gene or genes and a promoter sequence or sequences operably linked thereto, and optionally other regulatory elements such as polyadenylation sequences, into each the ICP34.5-encoding gene loci.

A virus of the invention is used to express a CTLA-4 inhibitor, and optionally GM-CSF and/or an immune co-stimulatory pathway activating molecule, in tumors. This is typically achieved by inserting a heterologous gene encoding a CTLA-4 inhibitor, and optionally a heterologous gene encoding GM-CSF and/or a heterologous gene encoding the immune co-stimulatory pathway activating molecule, in the genome of a selectively replication competent virus wherein each gene is under the control of a promoter sequence. As replication of such a virus will occur selectively in tumor tissue, expression of the CTLA-4 inhibitor and, if present, expression of the GM-CSF and/or the immune co-stimulatory activating protein by the virus, is also enhanced in tumor tissue as compared to non-tumor tissue in the body. Enhanced expression occurs where expression is greater in tumors as compared to other tissues of the body. Proteins expressed by the oncolytic virus would also be expected to be present in oncolytic virus-infected tumor draining lymph nodes, including due to trafficking of expressed protein and of virus in and on antigen presenting cells from the tumor. Accordingly, the invention provides benefits of expression of the CTLA-4 inhibitor and any co-expressed GM-CSF and/or immune co-stimulatory pathway activating molecule selectively in tumors and tumor draining lymph nodes combined with the anti-tumor effect provided by oncolytic virus replication.

The virus of the invention comprises a CTLA-4 inhibitor. The CTLA-4 inhibitor is a molecule, typically a peptide or protein that binds to CTLA-4 and reduces or blocks signaling through CTLA-4. By reducing CTLA-4 signalling, the inhibitor reduces or removes the block of immune stimulatory pathways by CTLA-4.

The CTLA-4 inhibitor is preferably an antibody or an antigen binding fragment thereof.

The term "antibody" as referred to herein includes whole antibodies and any antigen binding fragment (*i.e.*, "antigen-binding portion") or single chains thereof. An antibody refers to a glycoprotein comprising at least two heavy (H) chains and two light (kappa)(L) chains inter-connected by disulfide bonds, or an antigen binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.,* effector cells) and the first component (Clq) of the classical complement system.

The antibody is typically a monoclonal antibody. The antibody may be a chimeric antibody. The antibody is preferably a humanised antibody and is more preferably a human antibody.

The term "antigen-binding fragment" of an antibody refers to one or more fragments of an antibody that retain the ability to specifically bind to CTLA-4. The antigen-binding fragment also retains the ability to inhibit CTLA-4 and hence to reduce or remove the CTLA-4 blockade of a stimulatory immune response. Examples of suitable fragments include a Fab fragment, a $F(ab')_2$ fragment, a Fab' fragment, a Fd fragment, a Fv fragment, a dAb fragment and an isolated complementarity determining region (CDR). Single chain antibodies such as scFv and heavy chain antibodies such as VHH and camel antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. In a preferred embodiment, the antibody is an scFv. Examples of suitable

scFv molecules are disclosed in, for example, WO2007/123737 and WO2014/066532, which are incorporated herein by reference.

The antibody encoding sequences typically encode an antibody or antibody fragment having a N-terminal signal sequence. The signal sequence may have the amino acid sequence shown in SEQ ID NO: 7. For example, this signal sequence is included in a scFv having the amino acid sequence shown in SEQ ID NO: 9 and encoded by the nucleotide sequence shown in SEQ ID NO: 10, and in a scFv having the amino acid sequence shown in SEQ ID NO: 14 and encoded by the nucleotide sequence shown in SEQ ID NO: 15.

In the antibody or antibody fragment, the light chain and heavy chain sequences may be joined by an amino acid linker. The linker typically comprises from about 15 to about 25 amino acids, such as about 18 or 20 amino acids. Any suitable linker may be used, such as linkers comprising glycine and serine residues, for example the amino acid sequence shown in SEQ ID NO: 8. For example, this linker is included in a scFv having the amino acid sequence shown in SEQ ID NO: 9 and encoded by the nucleotide sequence shown in SEQ ID NO: 10, and in a scFv having the amino acid sequence shown in SEQ ID NO: 14 and encoded by the nucleotide sequence shown in SEQ ID NO: 15. Both are preferred antibody fragments for use in the invention.

Other antibody fragments having similar structures are also preferred. Accordingly the virus of the invention may encode an antibody or fragment comprising, or consisting essentially of, a light chain variable region, a linker a heavy chain variable region, a heavy chain CH1 domain, a heavy chain CH2 domain and a heavy chain CH3 domain. The virus may further encode a signal sequence at the N-terminus of the antibody.

The antibodies or antibody fragments of the invention may preferably comprise an Fc region which is an IgG1, IgG2, IgG3 or IgG4 region, more preferably an IgG1 region. Preferably, the antibody is an scFv antibody in which the scFv is linked to IgG heavy chain CH2 and CH3 domains.

A preferred CTLA-4 antibody or fragment comprises the heavy chain variable region shown in SEQ ID NO:3 and/or the light chain variable region shown in SEQ ID NO: 1 or the heavy chain variable region shown in SEQ ID NO:11 and/or the light chain variable region shown in SEQ ID NO: 12. The antibody may comprise the heavy chain CH1 domain having the amino acid sequence shown in SEQ ID NO: 4 and/or the

CH2/CH3 domains shown in SEQ ID NO: 5. The antibody may comprise the light chain amino acid sequence shown in SEQ ID NO: 2. An antibody of the invention may alternatively comprise a variant of one of these heavy or light chain variable regions or CDR sequences. For example, a variant may be a substitution, deletion or addition variant of any of the above amino acid sequences.

A variant antibody may comprise 1, 2, 3, 4, 5, up to 10, up to 20, up to 30 or more amino acid substitutions and/or deletions from the specific sequences and fragments discussed above, whilst maintaining the activity of the antibodies described herein. "Deletion" variants may comprise the deletion of, for example, 1, 2, 3, 4 or 5 individual amino acids or of one or more small groups of amino acids such as 2, 3, 4 or 5 amino acids. "Substitution" variants preferably involve the replacement of one or more amino acids with the same number of amino acids and making conservative amino acid substitutions. For example, an amino acid may be substituted with an alternative amino acid having similar properties, for example, another basic amino acid, another acidic amino acid, another neutral amino acid, another charged amino acid, another hydrophilic amino acid, another hydrophobic amino acid, another polar amino acid, another aromatic amino acid or another aliphatic amino acid.

The virus of the invention comprises one or more polynucleotide sequence encoding the CTLA-4 inhibitor. The polynucleotide sequence is under the control of a suitable promoter. The virus may comprise a first polynucleotide sequence encoding an antibody heavy chain variable region and a second polynucleotide encoding an antibody light chain variable region. The first polynucleotide may encode a full length heavy chain and/or the second polynucleotide may encode a full length light chain. The first and second polynucleotide may be under the control of a single promoter, optionally with an IRES, or may be under the control of two separate promoters. The separates promoters may be the same or different.

The first polynucleotide may comprise, consist essentially of or consist of the heavy chain variable region encoding sequence shown in SEQ ID NO: 9 and/or the second polynucleotide may comprise, consist essentially of or consist of the heavy chain variable region encoding sequence shown in SEQ ID NO: 10. The first polynucleotide may comprise, consist essentially of or consist of the heavy chain variable region encoding sequence shown in SEQ ID NO: 19 and/or the second polynucleotide may comprise,

consist essentially of or consist of the heavy chain variable region encoding sequence shown in SEQ ID NO: 20.

A first and/or second polynucleotide sequences may be a variant of SEQ ID NO: 9, 10, 19 or 20. For example, a variant may be a substitution, deletion or addition variant of either of these nucleic acid sequences. A variant polynucleotide may comprise 1, 2, 3, 4, 5, up to 10, up to 20, up to 30, up to 40, up to 50, up to 75 or more nucleic acid substitutions and/or deletions from SEQ ID NO: 9, 10, 19 or 20.

Suitable variants may be at least 70% homologous to a polynucleotide of any one of nucleic acid sequences disclosed herein, preferably at least 80 or 90% and more preferably at least 95%, 97% or 99% homologous thereto. Preferably homology and identity at these levels is present at least with respect to the coding regions of the polynucleotides. Methods of measuring homology are well known in the art and it will be understood by those of skill in the art that in the present context, homology is calculated on the basis of nucleic acid identity. Such homology may exist over a region of at least 15, preferably at least 30, for instance at least 40, 60, 100, 200 or more contiguous nucleotides. Such homology may exist over the entire length of the unmodified polynucleotide sequence.

Methods of measuring polynucleotide homology or identity are known in the art. For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (e.g. used on its default settings) (Devereux *et al* (1984) Nucleic Acids Research 12, p387-395).

The PILEUP and BLAST algorithms can also be used to calculate homology or line up sequences (typically on their default settings), for example as described in Altschul S.F. (1993) J Mol Evol 36:290-300; Altschul, S, F *et al* (1990) J Mol Biol 215:403-10.

Software for performing BLAST analysis is publicly available through the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul *et al*, supra). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the

cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) Proc. Natl. Acad. Sci. USA 89:10915-10919) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g., Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5787. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

In one embodiment, a variant sequence may vary from the specific sequences given in the sequence listing by virtue of the redundancy in the genetic code. The DNA code has 4 primary nucleic acid residues (A, T, C and G) and uses these to "spell" three letter codons which represent the amino acids the proteins encoded in an organism's genes. The linear sequence of codons along the DNA molecule is translated into the linear sequence of amino acids in the protein(s) encoded by those genes. The code is highly degenerate, with 61 codons coding for the 20 natural amino acids and 3 codons representing "stop" signals. Thus, most amino acids are coded for by more than one codon - in fact several are coded for by four or more different codons. A variant polynucleotide of the invention may therefore encode the same polypeptide sequence as another polynucleotide of the invention, but may have a different nucleic acid sequence due to the use of different codons to encode the same amino acids. The codons may be optimized so as to increase expression levels of the encoded proteins in target cells as compared to if the unaltered sequence is used.

The virus of the invention preferably comprises GM-CSF. The sequence of the gene encoding GM-CSF may be codon optimized so as to increase expression levels of the respective proteins in target cells as compared to if the unaltered sequence is used.

The virus of the invention preferably comprises one or more immune co-stimulatory pathway activating molecules and/or one or more genes encoding an immune co-stimulatory pathway activating molecule. Immune co-stimulatory pathway activating molecules include proteins and nucleic acid molecules (e.g. aptamer sequences). Examples of immune co-stimulatory pathway activating molecules include CD40 ligand, GITR ligand, 4-1-BB ligand, OX40 ligand, ICOS ligand, flt3 ligand, TL1A, CD30 ligand, CD70 and single chain antibodies targeting the respective receptors for these molecules (CD40, GITR, 4-1-BB, OX40, ICOS, flt3, DR3, CD30, CD27).

Activators of immune co-stimulatory pathway include mutant or wild type, soluble, secreted and/or membrane bound ligands, and agonistic antibodies including single chain antibodies. Viruses of the invention preferably encode one or more of CD40L, ICOSL, 4-1-BBL, GITRL or OX40L.

Viruses of the invention may encode one or more immune co-stimulatory pathway activating molecules, preferably 1, 2, 3 or 4 immune co-stimulatory pathway activating molecules, more preferably 1 or 2 immune co-stimulatory pathway activating molecules.

The sequence of the gene encoding the immune co-stimulatory activating molecule may be codon optimized so as to increase expression levels of the respective protein(s) in target cells as compared to if the unaltered sequence is used.

The virus of the invention may comprise one or more further heterologous genes in addition to a CTLA-4 inhibitor, and GM-CSF and/or an immune co-stimulatory pathway activating molecule. In a preferred embodiment, the virus may further comprise a fusogenic protein such as GALVR-.

The fusogenic protein may be any heterologous protein capable of promoting fusion of a cell infected with the virus of the invention to another cell. A fusogenic protein, preferably a wild type or modified viral glycoprotein (i.e. modified to increase its fusogenic properties), is a protein which is capable in inducing the cell to cell fusion (syncitia formation) of cells in which it is expressed. Examples of fusogenic glycoproteins include VSV-G, syncitin-1 (from human endogenous retrovirus-W (HERV-W)) or syncitin-2 (from HERVFRDE1), paramyxovirus SV5-F, measles virus-H, measles virus-F,

RSV-F, the glycoprotein from a retrovirus or lentivirus, such as gibbon ape leukemia virus (GALV), murine leukemia virus (MLV), Mason-Pfizer monkey virus (MPMV) and equine infectious anemia virus (EIAV) with the R transmembrane peptide removed (R- versions). In a preferred embodiment the fusogenic protein is from GALV and has the R- peptide removed (GALV-R-).

The virus of the invention may optionally comprise multiple copies of the fusogenic protein-encoding gene, preferably 1 or 2 copies. The virus may comprise two or more different fusogenic proteins, including any of the fusogenic proteins listed above.

The fusogenic protein or proteins optionally expressed by a virus of the invention may be identical to a naturally occurring protein, or may be a modified protein.

The fusogenic protein-encoding gene (fusogenic gene) may have a naturally occurring nucleic acid sequence or a modified sequence. The sequence of the fusogenic gene may, for example, be modified to increase the fusogenic properties of the encoded protein, or to provide codon optimisation and therefore increase the efficiency of expression of the encoded protein.

The invention also provides a virus, such as a pox virus or a HSV, preferably HSV1, which expresses at least three heterologous genes, wherein each of the three heterologous genes is driven by a different promoter selected from the CMV promoter, the RSV promoter, the EF1a promoter, the SV40 promoter and a retroviral LTR promoter. The virus may, for example, express four heterologous genes, wherein each of the four heterologous genes is driven by a different promoter selected from the CMV promoter, the RSV promoter, the EF1a promoter, the SV40 promoter and a retroviral LTR promoter. The retroviral LTR is preferably from MMLV. The heterologous genes may be terminated by polyadenylation sequences. The polyadenylation sequences may be the same or different. Preferably each heterologous gene is terminated by a different polyadenylation sequence, which is preferably selected from the BGH, SV40, HGH and RBG polyadenylation sequences.The invention also provides a virus, such as a pox virus or a HSV, preferably HSV1, which expresses at least three heterologous genes, wherein each of the three heterologous genes is terminated by a different polyadenylation sequence selected from the BGH, SV40, HGH and RBG polyadenylation sequences. The virus may, for example, express four heterologous genes terminated by each of the BGH, SV40, HGH and RBG polyadenylation sequences, respectively.

The at least three heterologous genes may, for example, be selected from a CTLA-4 inhibitor, a gene encoding GM-CSF, a gene encoding an immune co-stimulatory pathway activating molecule and a fusogenic gene. Examples of the three heterologous genes are a CTLA-4 inhibitor, a gene encoding GM-CSF and a gene encoding an immune co-stimulatory pathway activating molecule; a CTLA-4 inhibitor, a gene encoding GM-CSF and a fusogenic gene; and a CTLA-4 inhibitor, a gene encoding an immune co-stimulatory pathway activating molecule and a fusogenic gene. The four heterologous genes may, for example, be a CTLA-4 inhibitor, a gene encoding GM-CSF, a gene encoding an immune co-stimulatory pathway activating molecule and a fusogenic gene. The three or four heterologous genes may comprise, for example, two ore more genes encoding immune co-stimulatory pathway activating molecules and/or two ore more fusogenic genes.

In one embodiment, the promoters controlling expression of the three heterologous genes are the CMV, RSV and MMLV promoters. For example, a preferred virus may comprise a GM-CSF gene under the control of a CMV promoter, a GALV gene under the control of a RSV promoter and a CTLA-4 inhibitor under the control of a MMLV promoter.

In one embodiment, the polyadenylation sequence terminating the at least three heterologous genes are SV40, BGH and RBG polyadenylation sequences. controlling expression of the three heterologous genes are the CMV, RSV and MMLV promoters. For example, a preferred virus may comprise a GM-CSF gene terminated by a BGH polyadenylation sequence, a GALV gene terminated by a SV40 polyadenylation sequence and a CTLA-4 inhibitor terminated by a RGB polyadenylation sequence.

Any combination of the various promoters and polyadenylation sequences may be used with any of the heterologous genes. For example, a preferred virus may comprise a GM-CSF gene under the control of a CMV promoter and terminated by a BGH polyadenylation sequence, a GALV gene under the control of a RSV promoter and terminated by a SV40 polyadenylation sequence, and a CTLA-4 inhibitor under the control of a MMLV promoter terminated by a RGB polyadenylation sequence.

Production of Virus

Viruses of the invention are constructed using methods well known in the art. For example plasmids (for smaller viruses and single and multiple genome component RNA

viruses) or BACs (for larger DNA viruses including herpes viruses) encoding the viral genome to be packaged, including the genes encoding the fusogenic and immune stimulating molecules under appropriate regulatory control, can be constructed by standard molecular biology techniques and transfected into permissive cells from which recombinant viruses can be recovered.

Alternatively, in a preferred embodiment plasmids containing DNA regions flanking the intended site of insertion can be constructed, and then co-transfected into permissive cells with viral genomic DNA such that homologous recombination between the target insertion site flanking regions in the plasmid and the same regions in the parental virus occur. Recombinant viruses can then be selected and purified through the loss or addition of a function inserted or deleted by the plasmid used for modification, e.g. insertion or deletion of a marker gene such as GFP or lacZ from the parental virus at the intended insertion site. In a most preferred embodiment the insertion site is the ICP34.5 locus of HSV, and therefore the plasmid used for manipulation contains HSV sequences flanking this insertion site, between which are an expression cassette encoding GM-CSF and the immune co-stimulatory pathway activating molecule. In this case, the parental virus may contain a cassette encoding GFP in place of ICP34.5 and recombinant virus plaques are selected through the loss of expression of GFP. In a most preferred embodiment the US11 gene of HSV is also expressed as an IE gene. This may be accomplished through deletion of the ICP47-encoding region, or by other means.

The CTLA-4 inhibitor, and optionally the GM-CSF encoding sequences and immune co-stimulatory pathway activating molecule encoding sequences and/or additional protein encoding sequence, such as a sequence encoding a fusogenic protein such as GALVR-, are inserted into the viral genome under appropriate regulatory control. This may be under the regulatory control of natural promoters of the virus species of the invention used, depending on the species and insertion site, or preferably under the control of heterologous promoters. Suitable heterologous promoters include mammalian promoters, such as the IEF2a promoter or the actin promoter. More preferred are strong viral promoters such as the CMV IE promoter, the RSV LTR, the MMLV LTR, other retroviral LTR promoters, or promoters derived from SV40. Preferably each exogenous gene (e.g. encoding the GM-CSF and immune co-stimulatory pathway activating molecule) will be under separate promoter control, but may also be expressed from a single

RNA transcript, for example through insertion of an internal ribosome entry sites (IRES) between protein coding sequences. RNA derived from each promoter is typically terminated using a polyadenylation sequence (e.g. mammalian sequences such as the bovine or human growth hormone (BGH) poly A sequence, synthetic polyadenylation sequences, the rabbit betaglobin polyadenylation sequence, or viral sequences such as the SV40 early or late polyadenylation sequence).

Each of the heterologous genes in the virus is typically under the control of a promoter. The promoters controlling expression of the heterologous genes may be the same or different. For example, the anti-CTLA-4, and one or more of the GM-CSF, fusogenic gene and immune co-stimulatory pathway activating molecule-encoding gene may each be under the control of the CMV promoter, the RSV promoter, the EF1a promoter, the SV40 promoter or a retroviral LTR promoter. Alternatively, for example, the anti-CTLA-4 may be under the control of a retroviral LTR promoter such as the MMLV promoter, the GM-CSF gene may be under the control of the CMV promoter and/or the fusogenic gene, such as GALVR- may be under the control of the RSV promoter.

Pharmaceutical Compositions

The invention provides a pharmaceutical composition comprising the virus and a pharmaceutically acceptable carrier or diluent. Suitable carriers and diluents include isotonic saline solutions, for example phosphate-buffered saline. The composition may further comprise other constituents such as sugars or proteins to improve properties such as stability of the product. Alternatively a lyophilized formulation may be used, which is reconstituted in a pharmaceutically acceptable carrier or diluent before use.

The choice of carrier, if required, is frequently a function of the route of delivery of the composition. Within this invention, compositions may be formulated for any suitable route and means of administration. Pharmaceutically acceptable carriers or diluents are those used in compositions suitable for intra-tumoral administration, intravenous/intraarterial administration, administration into the brain or administration into a body cavity (e.g. bladder, pleural cavity or by intraperitoneal administration). The composition may be administered in any suitable form, preferably as a liquid.

The present invention also provides a product of manufacture comprising a virus of the invention in a sterile vial, ampoule or syringe.

Medical Uses/Methods of Treatment

The invention provides the virus of the invention for use in the treatment of the human or animal body by therapy, particularly for use in a method of treating cancer. The cancer is typically in a mammal, preferably in a human. The virus kills infected tumour cells by lysis and by causing infected tumor cells to fuse with one another. The virus of the invention also elicits a systemic anti-tumor immune response, augmented through the expression of the CTLA-4 inhibitor, and optionally GM-CSF and the immune co-stimulatory pathway activating molecule, which also kills cancer cells.

The invention also provides a method of treating cancer, the method comprising administering a therapeutically effective amount of the virus of the invention to an individual in need thereof.

The invention additionally provides the use of the virus of the invention in the manufacture of a medicament for treating cancer.

The virus of the invention is particularly useful in treating any solid tumor including any adenocarcinoma, carcinoma, melanoma or sarcoma. For example, the virus of the invention is useful in treating head and neck, prostate, breast, ovarian, lung, liver, endometrial, bladder, gall bladder, pancreas, colon, kidney, stomach/gastric, esophageal, or cervical cancers, mesothelioma, melanoma or other skin cancer, lymphoma, glioma or other cancer of the nervous system, or sarcomas such as soft tissue sarcoma.

The virus of the invention may be used to treat malignant tumors, including tumors that have metastasised from the site of the original tumor. In this embodiment, the virus may be administered to the primary tumor or to one or more secondary tumors.

The virus of the invention may be administered in combination with other therapeutic agents, including chemotherapy, targeted therapy, immunotherapy (including immune checkpoint blockade, i.e. administration of one or more antagonist of an immune co-inhibitory pathway, and/or one or more agonist of an immune co-stimulatory pathway) and/or in combination with radiotherapy and/or in combination with any combination of these. The therapeutic agent is preferably an anti-cancer agent.

The virus of the invention may be administered in combination with a second virus, such as a second oncolytic virus.

For example, the therapeutic agent may comprise an immunogen (including a recombinant or naturally occurring antigen, including such an antigen or combination of antigens delivered as DNA or RNA in which it/they are encoded), to further stimulate an immune response, such as a cellular or humoral immune response, to tumor cells, particularly tumor neoantigens. The therapeutic agent may be an agent intended to increase or potentiate an immune response, such as a cytokine, an agent intended to inhibit an immune checkpoint pathway or stimulate an immune potentiating pathway or an agent which inhibits the activity of regulatory T cells (Tregs) or myeloid derived suppressor cells (MDSCs).

The therapeutic agent may be an agent known for use in an existing cancer therapeutic treatment. The therapeutic agent may be radiotherapy or a chemotherapeutic agent. The therapeutic agent may be selected from cyclophosmamide, alkylating-like agents such as cisplatin or melphalan, plant alkaloids and terpenoids such as vincristine or paclitaxel (Taxol), antimetabolites such as 5-fluorouracil, topoisomerase inhibitors type I or II such as camptothecin or doxorubicin, cytotoxic antibiotics such as actinomycin, anthracyclines such as epirubicin, glucocorticoids such as triamcinolone, inhibitors of protein, DNA and/or RNA synthesis such as methotrexate and dacarbaxine, histone deacetylase (HDAC) inhibitors, or any other chemotherapy agent.

The therapeutic agent may be one, or a combination of: immunotherapeutics or immunomodulators, such as TLR agonists; agents that down-regulate T-regulatory cells such as cyclophosphamide; or agents designed to block immune checkpoints or stimulate immune potentiating pathways, including but not limited to monoclonal antibodies, such as a CTLA-4 inhibitor, a PD-1 inhibitor, a PD-L1 inhibitor, a LAG-3 inhibitor, a TIM-3 inhibitor, a VISTA inhibitor, a CSF1R inhibitor, an IDO inhibitor, a CEACAM1 inhibitor, a GITR agonist, a 4-1-BB agonist, a KIR inhibitor, a SLAMF7 inhibitor, an OX40 agonist, a CD40 agonist, an ICOS agonist or a CD47 inhibitor. In a preferred embodiment, the therapeutic agent is a CTLA-4 inhibitor such as an anti-CTLA-4 antibody, a PD1 inhibitor, such as an anti-PD-1 antibody or a PD-L1 inhibitor such as an anti-PD-L1 antibody. Such inhibitors, agonists and antibodies can be generated and tested by standard methods known in the art.

Immunotherapeutic agents may also include bi-specific antibodies, cell based-therapies based on dendritic cells, NK cells or engineered T cells such CAR-T cells or T cells expressing engineered T cell receptors. Immunotherapeutic agents also include agents that target a specific genetic mutation which occurs in tumors, agents intended to induce immune responses to specific tumor antigens or combinations of tumor antigens, including neoantigens and/or agents intended to activate the STING/cGAS pathway, TLR or other innate immune response and/or inflammatory pathway, including intra-tumoral agents.

For example, a virus of the invention may be used: in combination with dacarbazine, a BRAF inhibitor and/or PD1 or PD-L1 blockade to treat melanoma; in combination with taxol, doxorubicin, vinorelbine, cyclophosphamide and/or gemcitabine to treat breast cancer; in combination with 5-fluorouracil and optionally leucovorin, irinoteacan and/or oxaliplatin to treat colorectal cancer; in combination with taxol, carboplatin, vinorelbine and/or gemcitabine, PD-1 or PD-L1 blockade to treat lung cancer; in combination with cisplatin and/or radiotherapy to treat head and neck cancer.

The therapeutic agent may be an inhibitor of the idoleamine 2,3-dioxygenase (IDO) pathway. Examples of IDO inhibitors include epacadostat (INCB024360), 1-methyl-tryptophan, indoximod (1-methyly-D-tryptophan), GDC-0919 or F001287.

The mechanism of action of IDO in suppressing anti-tumor immune responses may also suppress immune responses generated following oncolytic virus therapy. IDO expression is induced by toll like receptor (TLR) activation and interferon-γ both of which may result from oncolytic virus infection. One embodiment of the use of oncolytic virus therapy for cancer treatment includes combination of an oncolytic virus, including a virus expressing a CTLA-4 inhibitor, and optionally GM-CSF and/or an immune co-stimulatory pathway activating molecule or molecules and/or one or more additional protein encoding sequences, such as a sequence encoding a fusogenic protein such as GALVR-, with an inhibitor of the IDO pathway and optionally a further antagonist of an immune co-inhibitory pathway and/or one or more agonist of an immune co-stimulatory pathway, including those targeting PD-1 and/or PD-L1.

Where a therapeutic agent and/or radiotherapy is used in conjunction with a virus of the invention, administration of the virus and the therapeutic agent and/or radiotherapy may be contemporaneous or separated by time. The composition of the invention may be

administered before, together with or after the therapeutic agent or radiotherapy. The method of treating cancer may comprise multiple administrations of the virus of the invention and/or of the therapeutic agent and/or radiotherapy. In preferred embodiments, in the case of combination with immune checkpoint blockade or other immune potentiating agents, the virus of the invention is administered once or multiple times prior to the concurrent administration of the immune checkpoint blockade or other immune potentiating agent or agents thereafter, or concurrent with the administration of the immune checkpoint blockade or other immune potentiating agent or agents without prior administration of the virus of the invention.

The virus of the invention may be administered to a subject by any suitable route. Typically, a virus of the invention is administered by direct intra-tumoral injection. Intra-tumoral injection includes direct injection into superficial skin, subcutaneous or nodal tumors, and imaging guided (including CT, MRI or ultrasound) injection into deeper or harder to localize deposits including in visceral organs and elsewhere. The virus may be administered into a body cavity, for example into the pleural cavity, bladder or by intra-peritoneal administration. The virus may be injected into a blood vessel, preferably a blood vessel supplying a tumor.

Therapeutic agents which may be combined with a virus of the invention can be administered to a human or animal subject *in vivo* using a variety of known routes and techniques. For example, the composition may be provided as an injectable solution, suspension or emulsion and administered via parenteral, subcutaneous, oral, epidermal, intradermal, intramuscular, interarterial, intraperitoneal, intravenous injection using a conventional needle and syringe, or using a liquid jet injection system. The composition may be administered topically to skin or mucosal tissue, such as nasally, intratrachealy, intestinally, sublingually, rectally or vaginally, or provided as a finely divided spray suitable for respiratory or pulmonary administration. In preferred embodiments, the compositions are administered by intravenous infusion, orally, or directly into a tumor.

The virus and/or therapeutic agent may be administered to a subject in an amount that is compatible with the dosage composition that will be therapeutically effective. The administration of the virus of the invention is for a "therapeutic" purpose. As used herein, the term "therapeutic" or "treatment" includes any one or more of the following as its objective: the prevention of any metastasis or further metastasis occurring; the reduction or

elimination of symptoms; the reduction or complete elimination of a tumor or cancer, an increase in the time to progression of the patient's cancer; an increase in time to relapse following treatment; or an increase in survival time.

Therapeutic treatment may be given to Stage I, II, III, or IV cancers, preferably Stage II, III or IV, more preferably Stage III or IV, pre- or post-surgical intervention (i.e. following recurrence or incomplete removal of tumors following surgery), preferably before any surgical intervention (either for resection of primary or recurrent/metastatic disease), or following recurrence following surgery or following incomplete surgical removal of disease, i.e. while residual tumor remains.

Therapeutic treatment may be carried out following direct injection of the virus composition into target tissue which may be the tumor, into a body cavity, or a blood vessel. As a guide, the amount of virus administered is in the case of HSV in the range of from 10^4 to 10^{10} pfu, preferably from 10^5 to 10^9 pfu. In the case of HSV, an initial lower dose (e.g. 10^4 to 10^7 pfu) may be given to patients to seroconvert patients who are seronegative for HSV and boost immunity in those who are seropositive, followed by a higher dose then being given thereafter (e.g. 10^6 to 10^9 pfu). Typically up to 20ml of a pharmaceutical composition consisting essentially of the virus and a pharmaceutically acceptable suitable carrier or diluent may be used for direct injection into tumors, or up to 50ml for administration into a body cavity (which may be subject to further dilution into an appropriate diluent before administration) or into the bloodstream. However for some oncolytic therapy applications larger or smaller volumes may also be used, depending on the tumor and the administration route and site.

The routes of administration and dosages described are intended only as a guide since a skilled practitioner will be able to determine readily the optimum route of administration and dosage. The dosage may be determined according to various parameters, especially according to the location of the tumor, the size of the tumor, the age, weight and condition of the patient to be treated and the route of administration. Preferably the virus is administered by direct injection into the tumor or into a body cavity. The virus may also be administered by injection into a blood vessel. The optimum route of administration will depend on the location and size of the tumor. Multiple doses may be required to achieve an immunological or clinical effect, which, if required, will be typically administered between 2 days to 12 weeks apart, preferably 3-days to 3 weeks apart.

Repeat doses up to 5 years or more may be given, preferably for up to one month to two years dependent on the speed of response of the tumor type being treated and the response of a particular patient, and any combination therapy which may also be being given.

The following Examples illustrate the invention.

Example 1. Construction of a virus of the invention

The virus species used to exemplify the invention is HSV, specifically HSV1.

Diagrams of the plasmids used are shown in Figure 2. Diagrams of the viruses are shown in Figure 1. All viruses were constructed using HSV1 Strain RH018A. The plasmids used for virus construction were generated by a combination of gene synthesis and subcloning, conducted by Genscript Inc.

Viruses expressing anti-mouse CTLA4 together with mouse GM-CSF and GALV were constructed by co-transfection of Plasmid 77 with Virus 16 DNA, so as to insert GFP into Virus 16 by selection of plaques expressing GFP to give Virus 25. GFP was then knocked out of Virus 25 by co-transfection of Virus 25 DNA with Plasmid 119. This gave Virus 27.

Viruses expressing anti-human CTLA4 together with human GM-CSF and GALV were constructed by co-transfection of Plasmid 78 with Virus 17 DNA, so as to insert GFP into Virus 17 by selection of plaques expressing GFP to give Virus 29. GFP was then knocked out of Virus 29 by co-transfection of Virus 29 DNA with Plasmid 122. This gave Virus 31.

Viruses expressing anti-mouse CTLA-4 and co-stimulatory ligands together with mouse GM-CSF and GALV were constructed by co-transfection of a plasmid encoding GFP driven by an SV40 promoter between the mouse GM-CSF and anti-mouse CTLA-4 encoding sequences with Virus 27. GFP was then knocked out of the resulting virus with a plasmid enoding each of the individual mouse co-stimulatory ligands in place of GFP.

Viruses expressing anti-human CTLA-4 and co-stimulatory ligands together with human GM-CSF and GALV were constructed by co-transfection of a plasmid encoding GFP driven by an SV40 promoter between the human GM-CSF and anti-human CTLA-4 encoding sequences with Virus 31. GFP was then knocked out of the resulting virus with a plasmid encoding each of the individual human co-stimulatory ligands in place of GFP.

Figure 4 shows a western blot demonstrating expression of anti-mouse CTLA-4 from Virus 27.

Example 2. The effect of combined expression of GALV, GM-CSF and anti-CTLA4 from an oncolytic virus

The utility of the invention is demonstrated in the following way. A20 cells are administered into both flanks of Balb/c mice and the A20 tumors are allowed to grow to approximately 0.5cm in diameter.

The following treatments are then administered to groups of mice, into one flank of each mouse only (right tumor) 3 times per week for one week:

- 50µl of vehicle (1 group);
- 50µl of 10^6 pfu/ml of the HSV with only mouse GM-CSF and GALVR- inserted (Virus 16);
- 50µl of 10^6 pfu/ml of the HSV with GALVR-, mouse GM-CSF and the anti-mouse CTLA-4 antibody inserted (Virus 27);

Effects on tumor growth are then observed for up to one month. The dose of virus used was $5x10^4$ pfu (50ul of $1x10^6$ pfu/ml in each case), given three times over one week. This dose level of virus is subtherapeutic for uninjected tumors for virus 16, which allows the benefits of the delivery of the additional molecules encoded by virus 27 to clearly be seen. Figures 5 and 6 show the superior tumor control and shrinkage in uninjected tumors with the virus expressing anti-CTLA-4 compared to with virus 16, which does not express CTLA-4.

Example 3. The effect of combined expression of GALV, GM-CSF and anti-CTLA4 from an oncolytic virus with anti-PD-1

A20 cells are administered into both flanks of Balb/c mice and the A20 tumors are allowed to grow to approximately 0.5cm in diameter.

The following treatments are then administered to groups of mice (10 per group), into one flank of each mouse only 3 times per week for one week:

- 50µl of vehicle;
- Intraperitoneal anti-mouse PD1 (Bioxcell RMP-1-14 10mg/kg every three days);

- 50µl of 10^7 pfu/ml of the HSV with GALVR-, mouse GM-CSF and the anti-mouse CTLA-4 antibody inserted (Virus 27)
- 50µl of 10^7 pfu/ml, of the HSV with GALVR-, mouse GM-CSF and the anti-mouse CTLA-4 antibody inserted (Virus 27) together with intraperitoneal anti-mouse PD1 (10mg/kg every three days) (3 groups).

Effects on tumor growth are then observed for up to 80 days. Superior tumor control and shrinkage in both injected and un-injected tumors when treatment with the virus is combined treatment with anti-PD1. This data is shown in Figure 7.

Example 4. The effect of combined expression of GALV, GM-CSF and anti-humanCTLA4 from an oncolytic virus alone and in combination with anti-PD-1

MC38 cells are administered into both flanks of C57BL/6 mice engineered by gene editing to express human rather than mouse CTLA-4. This renders the mice susceptible to anti-human CTLA-4 antibodies such as ipilimumab. The MC38 tumors are allowed to grow to approximately 0.5cm in diameter.

The following treatments are then administered to groups of mice (10 per group), into one flank of each mouse only 3 times per week for two weeks:

- 50µl of vehicle;
- 50µl of 10^8 pfu/ml of Virus 17 (i.e. expressing hGM-CSF and GALV);
- 50µl of 10^8 pfu/mlof Virus 31 (i.e. expressing hGM-CSF, GALV and anti-human CTLA-4);
- 50µl of 10^8 pfu/ml of Virus 17 together with intraperitoneal anti-mouse PD1 (10mg/kg every three days);
- 50µl of 10^8 pfu/ml of Virus 31 together with intraperitoneal anti-mouse PD1 (10mg/kg every three days).

Effects on tumor growth are then observed for up to 35 days. Superior tumor control and shrinkage in injected tumors with the virus expressing anti-human CTLA-4 is seen, which is further enhanced with combined treatment with anti-PD1. Superior tumor control and shrinkage is observed in un-injected tumors when treatment with either virus is combined with anti PD1 treatment. The improvement is more marked for the virus that expresses anti CTLA4. This data is shown in Figure 8.

Example 5. The effect of combined expression of GALV, GM-CSF and anti-CTLA4 from an oncolytic virus with anti-PD-1

A20 cells are administered into both flanks of Balb/c mice and the A20 tumors are allowed to grow to approximately 0.5cm in diameter.

The following treatments are then administered to groups of mice (10 per group), into one flank of each mouse only 3 times per week for two weeks:

- 50µl of vehicle (1 group);
- Intraperitoneal anti-mouse PD1 (Bioxcell RMP-1-14 10mg/kg every three days);
- 50µl of 10^5 pfu/ml, 10^6 pfu/ml, or 10^7 pfu/ml of the HSV with only mouse GM-CSF and GALVR- inserted (3 groups);
- 50µl of 10^5 pfu/ml, 10^6 pfu/ml, or 10^7 pfu/ml of the HSV with GALVR-, mouse GM-CSF and the anti-mouse CTLA-4 antibody inserted together with intraperitoneal anti-mouse PD1 (10mg/kg every three days) (3 groups);
- 50µl of 10^5 pfu/ml, 10^6 pfu/ml, or 10^7 pfu/ml of the HSV with only mouse GM-CSF and GALVR- inserted together with intraperitoneal anti-mouse PD1 (10mg/kg every three days) (3 groups);
- 50µl of 10^5 pfu/ml, 10^6 pfu/ml, or 10^7 pfu/ml of the HSV with GALVR-, mouse GM-CSF and the anti-mouse CTLA-4 antibody inserted together with intraperitoneal anti-mouse PD1 (10mg/kg every three days) (3 groups).

Effects on tumor growth are then observed for up to one month. Superior tumor control and shrinkage in both injected and uninjected tumors with the virus expressing anti-CTLA-4 is seen, which is further enhanced with combined treatment with anti-PD1, as compared to the other groups is observed, including through an improved dose response curve.

Example 6. The effect of combined expression of GALV, GM-CSF and anti-human CTLA4 from an oncolytic virus alone and in combination with anti-PD-1

MC38 cells are administered into both flanks of C57BL/6 mice engineered by gene editing to express human rather than mouse CTLA-4. This renders the mice susceptible to anti-human CTLA-4 antibodies such as ipilimumab. The MC38 tumors are allowed to grow to approximately 0.5cm in diameter.

The following treatments are then administered to groups of mice (10 per group), into one flank of each mouse only 3 times per week for two weeks:

- 50µl of vehicle (1 group);
- Intraperitoneal anti-mouse PD1 (Bioxcell RMP-1-14 10mg/kg every three days);
- 50µl of 10^5 pfu/ml, 10^6 pfu/ml, or 10^7 pfu/ml of the HSV with only mouse GM-CSF and GALVR- inserted (3 groups);
- 50µl of 10^5 pfu/ml, 10^6 pfu/ml, or 10^7 pfu/ml of the HSV with GALVR-, mouse GM-CSF and the anti-mouse CTLA-4 antibody inserted together with intraperitoneal anti-mouse PD1 (10mg/kg every three days) (3 groups);
- 50µl of 10^5 pfu/ml, 10^6 pfu/ml, or 10^7 pfu/ml of the HSV with only mouse GM-CSF and GALVR- inserted together with intraperitoneal anti-mouse PD1 (10mg/kg every three days) (3 groups);
- 50µl of 10^5 pfu/ml, 10^6 pfu/ml, or 10^7 pfu/ml of the HSV with GALVR-, mouse GM-CSF and the anti-mouse CTLA-4 antibody inserted together with intraperitoneal anti-mouse PD1 (10mg/kg every three days) (3 groups).

Effects on tumor growth are then observed for up to one month. Superior tumor control and shrinkage in both injected and uninjected tumors with the virus expressing anti-CTLA-4 is seen, which is further enhanced with combined treatment with anti-PD1, as compared to the other groups is observed, including through an improved dose response curve.

Example 7. The effect of combined expression of GALV, GM-CSF, anti-CTLA4 and an immune co-stimulatory pathway activating molecule from an oncolytic virus

The experiment in Example 3 above is repeated but mice are dosed with the viruses additionally expressing the immune co-stimulatory pathway ligands as well as expressing GALV, mGM-CSF and anti-CTLA4.

More specifically, groups of mice receive:

(1) Vehicle;

(2) Intraperitoneal anti mouse PD1;

(3) HSV with mGM-CSF, GALVR- and anti-CTLA4 inserted as in Example 2;

(4) HSV with mGM-CSF, GALVR-, anti-CTLA4 and mouse CD40L inserted;

(5) HSV with mGM-CSF, GALVR-, anti-CTLA4 and mouse 4-1BBL inserted;
(6) HSV with mGM-CSF, GALVR-, anti-CTLA4 and mouse GITRL inserted;
(7) HSV with mGM-CSF, GALVR-, anti-CTLA4 and mouse OX40L inserted;
(8) HSV with mGM-CSF, GALVR-, anti-CTLA4 and mouse ICOSL inserted;
(9) HSV with mGM-CSF, GALVR- and anti-CTLA4 inserted as in Example 2, together with intraperitoneal anti-PD1;
(10) HSV with mGM-CSF, GALVR-, anti-CTLA4 and mouse CD40L inserted together with intraperitoneal anti-PD1;
(11) HSV with mGM-CSF, GALVR-, anti-CTLA4 and mouse 4-1BBL inserted together with intraperitoneal anti-PD1;
(12) HSV with mGM-CSF, GALVR-, anti-CTLA4 and mouse GITRL inserted together with intraperitoneal anti-PD1;
(13) HSV with mGM-CSF, GALVR-, anti-CTLA4 and mouse OX40L inserted together with intraperitoneal anti-PD1; or
(14) HSV with mGM-CSF, GALVR-, anti-CTLA4 and mouse ICOSL inserted together with intraperitoneal anti-PD1.

Superior tumor control is seen with the viruses expressing the immune co-stimulatory ligands.

Deposit Information

The following HSV1 strains were deposited at the ECACC, Culture Collections, Public Health England, Porton Down, Salisbury, SP4 0JG, United Kingdom on 19 December 2016 by Replimune Limited and were allocated the indicated accession numbers:

RH004A – Accession Number 16121902
RH015A – Accession Number 16121903
RH018A – Accession Number 16121904
RH021A – Accession Number 16121905
RH023A – Accession Number 16121906
RH031A – Accession Number 16121907

RH040B – Accession Number 16121908

RH047A – Accession Number 16121909.

CLAIMS

1. An oncolytic herpes simplex virus (HSV) encoding a CTLA-4 inhibitor, wherein:

(a) the virus further comprises a GM-CSF-encoding gene; and/or

(b) the virus further comprises an immune co-stimulatory pathway activating molecule-encoding gene encoding CD40 ligand (CD40L), ICOS ligand, GITR ligand, 4-1-BB ligand, OX40 ligand, TL1A, CD30 ligand, CD27 or flt3 ligand; and/or

(c) the virus further comprises an immune co-stimulatory pathway activating molecule-encoding gene, wherein the immune co-stimulatory pathway activating molecule is encoded by a codon optimized sequence so as to increase expression levels in target cells; and/or

(d) the virus further comprises a fusogenic protein-encoding gene; and/or

(e) the CTLA-4 inhibitor is encoded by a codon optimized sequence so as to increase expression levels in target cells; and/or

(f) the virus does not express functional ICP34.5, does not express functional ICP47, and expresses the US11 gene as an immediate early gene.

2. The virus of claim 1, wherein the CTLA-4 inhibitor is an anti-CTLA-4 antibody, or an antigen binding fragment thereof.

3. The virus of claim 2, wherein the fragment comprises a scFv molecule; the fragment is a scFv molecule linked to one or more IgG1 constant regions; or the antibody or fragment comprises a light chain variable region sequence linked to an IgG heavy chain.

4. The virus of claim 2 or 3, wherein the antibody or fragment:

(a) comprises the light chain variable region sequence shown in SEQ ID NO: 1 and the heavy chain variable region sequence shown in SEQ ID NO: 3;

(b) comprises the light chain variable region sequence shown in SEQ ID NO: 11 and the heavy chain variable region sequence shown in SEQ ID NO: 12;

(c) comprises the amino acid sequence of SEQ ID NO: 9;

(d) comprises the amino acid sequence of SEQ ID NO: 14;

(e) is encoded by the nucleotide sequence of SEQ ID NO: 10; or

(f) is encoded by the nucleotide sequence of SEQ ID NO: 15.

5. The virus of any one of claims 1 to 4, wherein the virus further comprises:

(a) a GM-CSF-encoding gene;

(b) an immune co-stimulatory pathway activating molecule or an immune co-stimulatory pathway activating molecule-encoding gene; and/or

(c) a fusogenic protein-encoding gene.

6. The virus of claim 5, wherein:

(a) the immune co-stimulatory pathway activating molecule-encoding gene encodes CD40 ligand (CD40L), ICOS ligand, GITR ligand, 4-1-BB ligand, OX40 ligand, TL1A, CD30 ligand, CD27 or flt3 ligand;

(b) the fusogenic protein is selected from the group consisting of vesicular stomatitis virus (VSV) G-protein, syncitin-1, syncitin-2, simian virus 5 (SV5) F-protein, measles virus (MV) H-protein, MV F-protein, respiratory syncytial virus (RSV) F-protein and a glycoprotein from gibbon ape leukemia virus (GALV), murine leukemia virus (MLV), Mason-Pfizer monkey virus (MPMV) or equine infectious anaemia virus (EIAV) from which the R peptide has been deleted; and/or

(c) the fusogenic protein is the glycoprotein from gibbon ape leukemia virus (GALV) and has the R transmembrane peptide mutated or removed (GALV-R-).

7. The virus of any one of claims 1 to 6, which encodes more than one immune co-stimulatory pathway activating molecule.

8. The virus of any one of claims 1 to 7, which is:

(i) a HSV1; or

(ii) a HSV1 which:

(a) does not express functional ICP34.5;

(b) does not express functional ICP47; and

(c) expresses the US11 gene as an immediate early gene.

9. The virus of any one of claims 1 to 8, which is a modified HSV1:
strain RH018A having the accession number ECACC 16121904;
strain RH004A having the accession number ECACC 16121902;
strain RH031A having the accession number ECACC 16121907;
strain RH040B having the accession number ECACC 16121908;
strain RH015A having the accession number ECACC 16121903;
strain RH021A having the accession number ECACC 16121905;
strain RH023A having the accession number ECACC 16121906; or
strain RH047A having the accession number ECACC 16121909.

10. The virus of claim 8 or 9, wherein:
(a) the CTLA-4 inhibitor is encoded by a gene inserted into the ICP34.5 encoding locus by insertion, partial deletion or complete deletion;
(b) the CTLA-4 inhibitor is encoded by a gene included in a cassette also including one or more immune stimulating gene(s) and/or an immune co-stimulatory pathway activating molecule encoding gene and/or a fusogenic protein encoding gene inserted into the ICP34.5 encoding locus by insertion, partial deletion or complete deletion; or
(c) the CTLA-4 inhibitor is encoded by a gene included in a cassette also including a GM-CSF encoding gene and/or an immune co-stimulatory pathway activating molecule encoding gene and/or a fusogenic protein encoding gene inserted into the ICP34.5 encoding locus by insertion, partial deletion or complete deletion.

11. The virus of any one of claims 1 to 10, wherein the virus comprises a codon optimized sequence encoding the CTLA-4 inhibitor, a codon optimized sequence encoding GM-CSF, a codon optimized sequence encoding an immune co-stimulatory pathway activating molecule and/or a codon optimized sequence encoding the fusogenic protein so as to increase expression levels in target cells.

12. The virus according to any one of claims 1 to 11, which expresses three heterologous genes, wherein each of the three heterologous genes is driven by a different

promoter selected from the CMV promoter, the RSV promoter, the SV40 promoter and a retroviral LTR promoter; and/or wherein each of the three heterologous genes is terminated by a different poly adenylation sequence selected from the BGH, SV40, HGH and RBG poly adenylation sequences.

13. The virus of any one of claims 1 to 11, which expresses four heterologous genes driven by each of the CMV promoter, the RSV promoter, the SV40 promoter and a retroviral LTR promoter, respectively; and/or terminated by each of the BGH, SV40, HGH and RBG poly adenylation sequences, respectively.

14. A pharmaceutical composition comprising a virus according to any one of claims 1 to 13 and a pharmaceutically acceptable carrier or diluent.

15. Use of the virus of any one of claims 1 to 13 in the manufacture of a medicament for treating cancer.

16. Use of the virus according to claim 15, wherein the cancer is a solid tumor; a tumor at its primary site and/or tumors that have metastasized from the site of the original tumor.

17. Use of the virus for use according to claim 15 or 16, wherein the medicament comprises:

(a) a further anti-cancer agent;

(b) a further anti-cancer agent selected from an agent targeting an immune co-inhibitory or immune co-stimulatory pathway, radiation and/or chemotherapy, an agent that targets a specific genetic mutation which occurs in tumors, an agent intended to induce an immune response to one or more tumor antigen(s) or neoantigen(s), a cellular product derived from T cells or NK cells, an agent intended to stimulate the STING, cGAS, TLR or other innate immune response and/or inflammatory pathway, a second virus, and combinations thereof;

(c) a further anti-cancer agent which is an agent targeting an immune co-inhibitory pathway which is a PD-1 inhibitor, a PD-L1 inhibitor, a LAG-3 inhibitor,

a TIM-3 inhibitor, a VISTA inhibitor, aCSF1R inhibitor, an IDO inhibitor, a KIR inhibitor, a SLAMF7 inhibitor, a CEACAM1 inhibitor or a CD47 inhibitor;

(d) a further anti-cancer agent which is an agent targeting an immune co-stimulatory pathway which is a GITR agonist, a 4-1-BB agonist, an OX40 agonist, a CD40 agonist or an ICOS agonist;

(e) a further anti-cancer agent which is a second oncolytic virus;

(f) a further anti-cancer agent which is an antibody; or

(g) an inhibitor of the indoleamine 2,3-dioxygenase (IDO) pathway and a further antagonist of an immune co-inhibitory pathway, or an agonist of an immune co-stimulatory pathway.

18. A method of treating cancer, which comprises administering a therapeutically effective amount of the virus of any one of claims 1 to 13 or a pharmaceutical composition according to claim 14 to a patient in need thereof.

19. The method according to claim 18, which further comprises:

(a) administering concurrently or separately to the virus a therapeutically effective amount of a further anti-cancer agent to the patient in need thereof;

(b) administering concurrently or separately to the virus a therapeutically effective amount of a further anti-cancer agent to the patient in need thereof , wherein the further anti-cancer agent is selected from the group consisting of an agent targeting an immune co-inhibitory or immune co-stimulatory pathway, radiation and/or chemotherapy, an agent that targets a specific genetic mutation which occurs in tumors, an agent intended to induce an immune response to one or more tumor antigen(s) or neoantigen(s), a cellular product derived from T cells or NK cells, an agent intended to stimulate the STING, cGAS, TLR or other innate immune response and/or inflammatory pathway, a second virus, and combinations thereof;

(c) administering concurrently or separately to the virus a therapeutically effective amount of a further anti-cancer agent to the patient in need thereof, wherein the further anti-cancer agent is an agent targeting an immune co-inhibitory pathway which is a PD-1 inhibitor, a PD-L1 inhibitor, a LAG-3 inhibitor, a TIM-3

inhibitor, a VISTA inhibitor, aCSF1R inhibitor, an IDO inhibitor, a KIR inhibitor, a SLAMF7 inhibitor, a CEACAM1 inhibitor or a CD47 inhibitor;

(d) administering concurrently or separately to the virus a therapeutically effective amount of a further anti-cancer agent to the patient in need thereof, wherein the further anti-cancer agent is an agent targeting an immune co-stimulatory pathway which is a GITR agonist, a 4-1-BB agonist, an OX40 agonist, a CD40 agonist or an ICOS agonist;

(e) administering concurrently or separately to the virus a therapeutically effective amount of a further anti-cancer agent to the patient in need thereof, wherein the further anti-cancer agent is a second oncolytic virus;

(f) administering concurrently or separately to the virus a therapeutically effective amount of a further anti-cancer agent to the patient in need thereof, wherein the further anti-cancer agent comprises an antibody; or

(g) administering an inhibitor of the indoleamine 2,3-dioxygenase (IDO) pathway and a further antagonist of an immune co-inhibitory pathway, or an agonist of an immune co-stimulatory pathway.

20. The method according to claim 18 or 19, wherein the cancer is a solid tumor, a tumor at its primary site and/or tumors that have metastasized from the site of the original tumor.

Fig. 1A

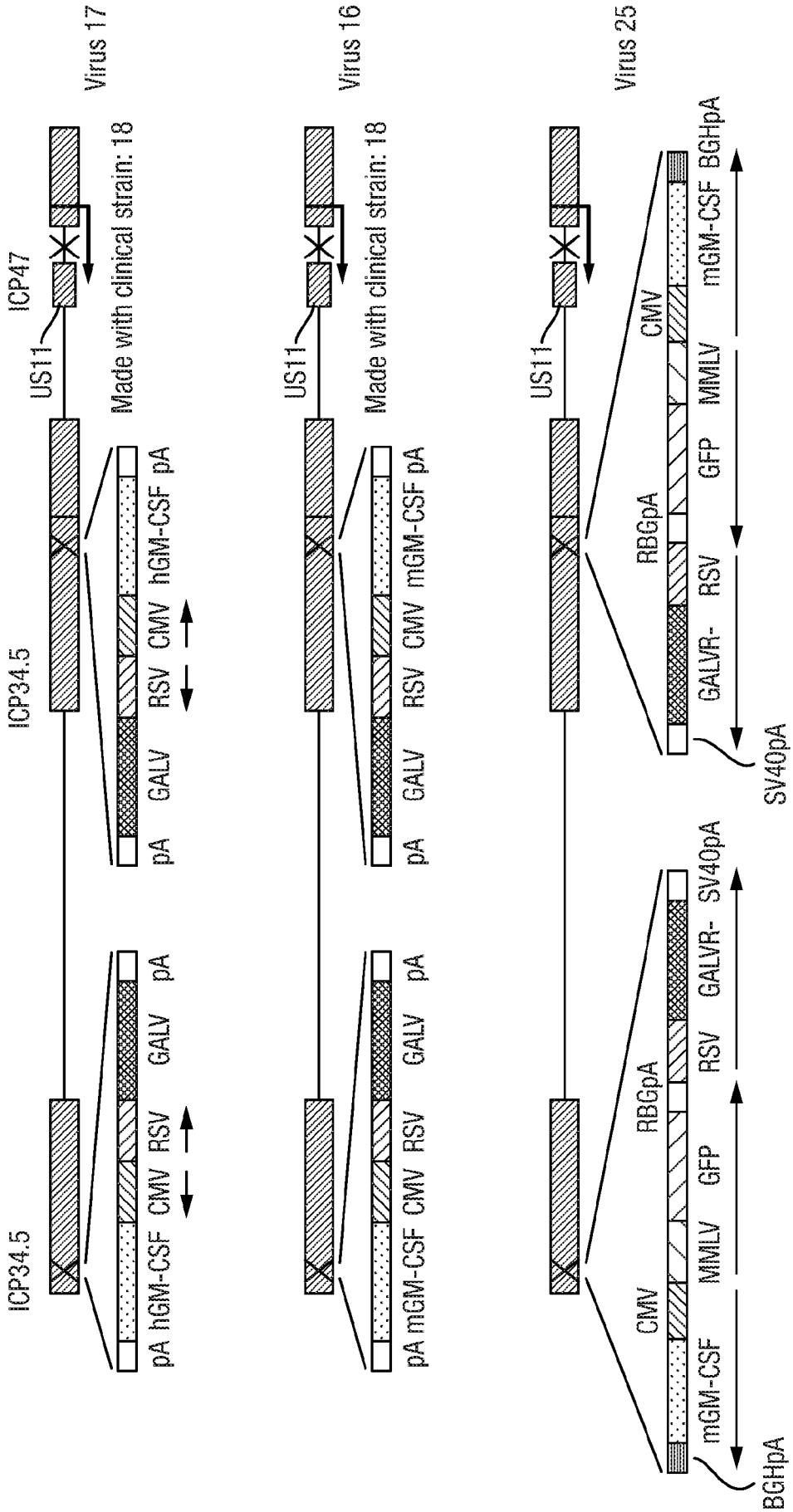

Virus 17
ICP47
US11
ICP34.5
Made with clinical strain: 18
pA hGM-CSF CMV RSV GALV pA
pA GALV RSV CMV hGM-CSF pA
Virus 16
US11
ICP34.5
Made with clinical strain: 18
pA mGM-CSF CMV RSV GALV pA
pA GALV RSV CMV mGM-CSF pA
Virus 25
US11
CMV
RBGpA
GFP
MMLV
mGM-CSF
BGHpA
GALVR-
RSV
SV40pA
CMV
RBGpA
mGM-CSF
MMLV
GFP
RSV
GALVR-
SV40pA
BGHpA

Fig. 1B

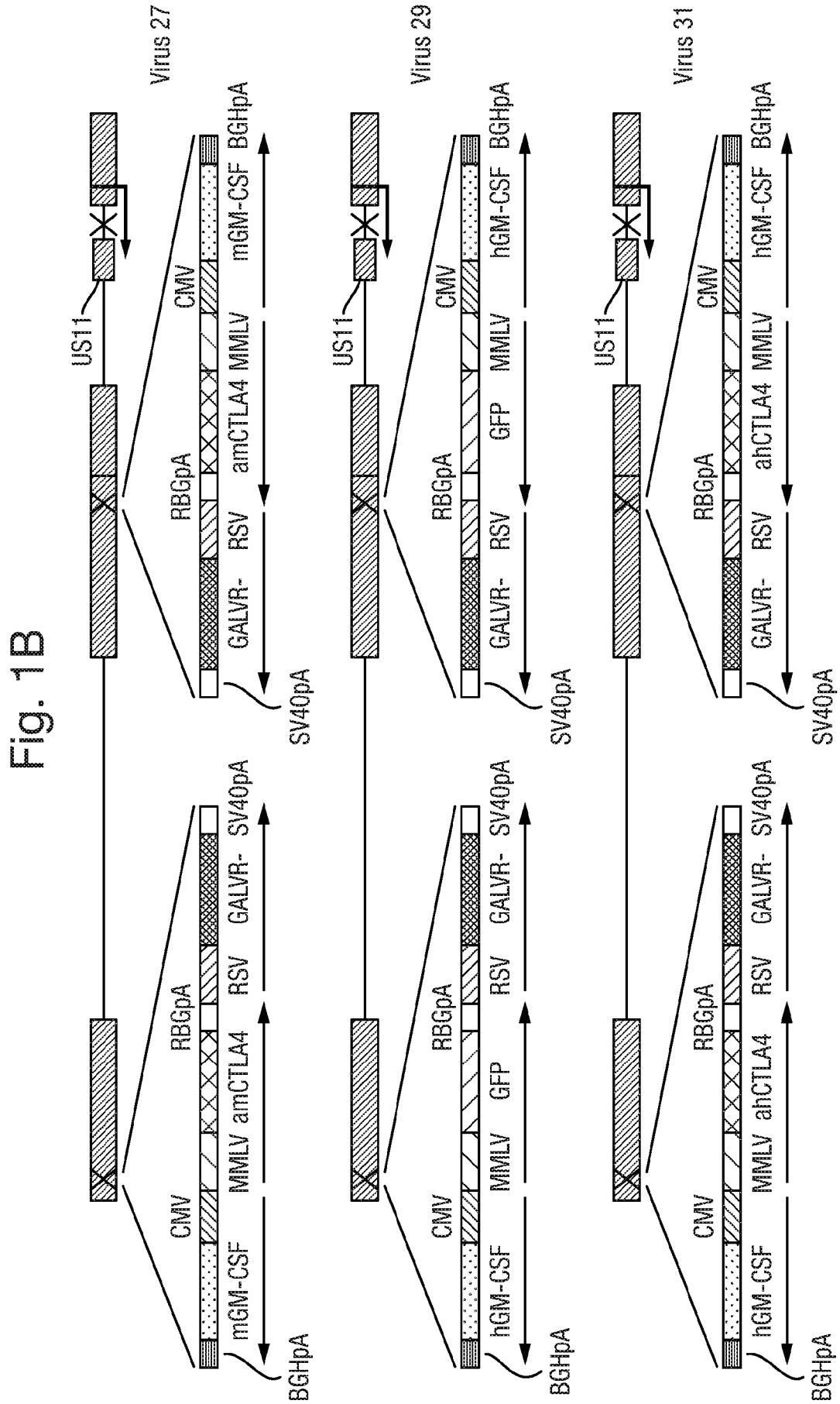

Virus 27
US11
CMV
mGM-CSF
MMLV
amCTLA4
RSV
GALVR-
SV40pA
RBGpA
CMV
RBGpA
amCTLA4
MMLV
mGM-CSF
BGHpA
SV40pA
BGHpA
Virus 29
US11
CMV
hGM-CSF
MMLV
GFP
RSV
GALVR-
SV40pA
RBGpA
CMV
RBGpA
GFP
MMLV
hGM-CSF
BGHpA
SV40pA
BGHpA
Virus 31
US11
CMV
hGM-CSF
MMLV
ahCTLA4
RSV
GALVR-
SV40pA
RBGpA
CMV
RBGpA
ahCTLA4
MMLV
hGM-CSF
BGHpA
SV40pA
BGHpA

Fig. 2A

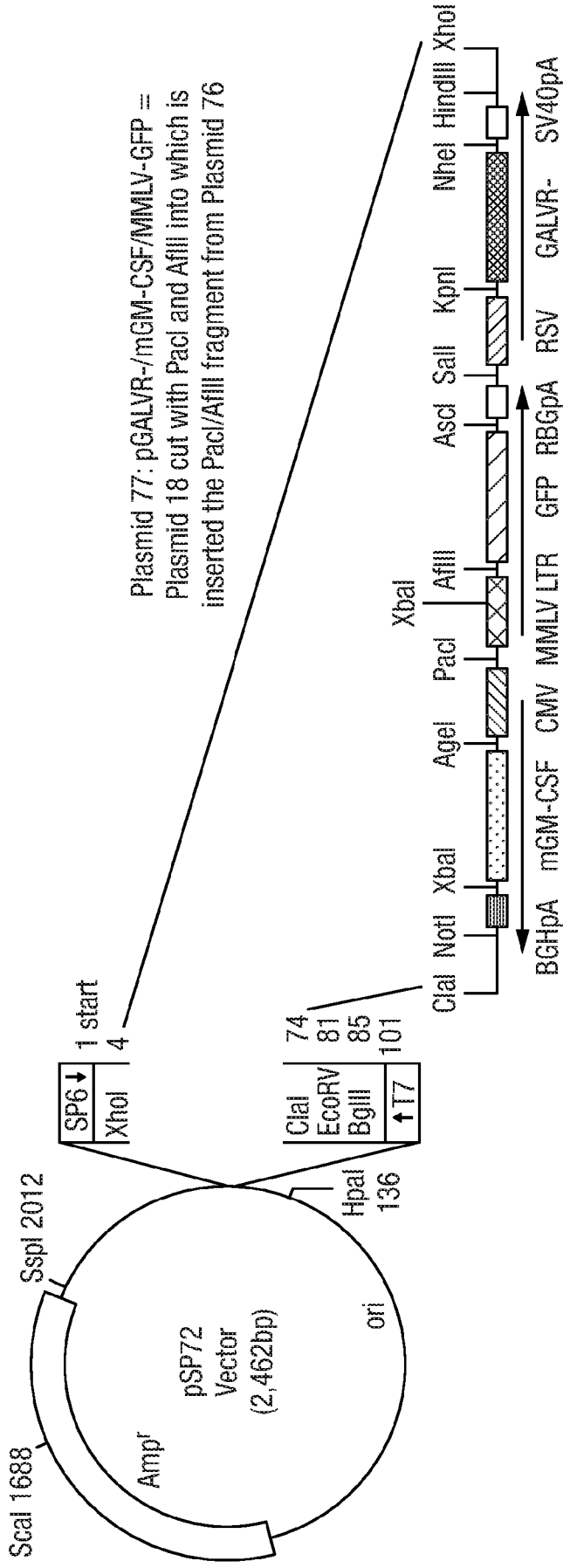

Plasmid 77: pGALVR-/mGM-CSF/MMLV-GFP =
Plasmid 18 cut with PacI and AflII into which is
inserted the PacI/AflII fragment from Plasmid 76
1 start
4
SP6
XhoI
74
81
85
101
ClaI
EcoRV
BglII
T7
HpaI
136
SspI 2012
ScaI 1688
Amp^r
pSP72
Vector
(2,462bp)
ori
ClaI NotI XbaI AgeI PacI AflII XbaI AscI SalI KpnI NheI HindIII XhoI
BGHpA mGM-CSF CMV MMLV LTR GFP RBGpA RSV GALVR- SV40pA

Fig. 2B

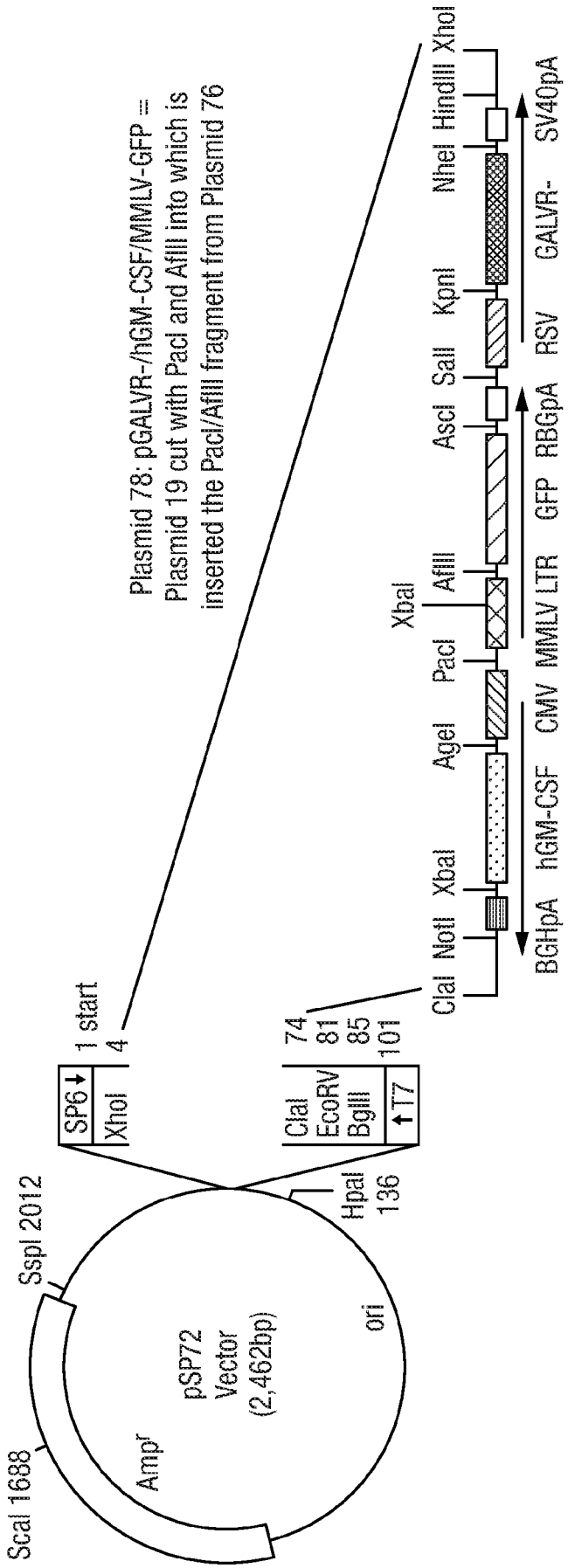

Plasmid 78: pGALVR-/hGM-CSF/MMLV-GFP =
Plasmid 19 cut with PacI and AflII into which is
inserted the PacI/AflII fragment from Plasmid 76
1 start
4
SP6
XhoI
74
81
85
101
ClaI
EcoRV
BglII
T7
HpaI
136
SspI 2012
ScaI 1688
Ampr
pSP72
Vector
(2,462bp)
ori
ClaI NotI XbaI AgeI PacI XbaI AflII AscI SalI KpnI NheI HindIII XhoI
BGHpA hGM-CSF CMV MMLV LTR GFP RBGpA RSV GALVR- SV40pA

Fig. 2C

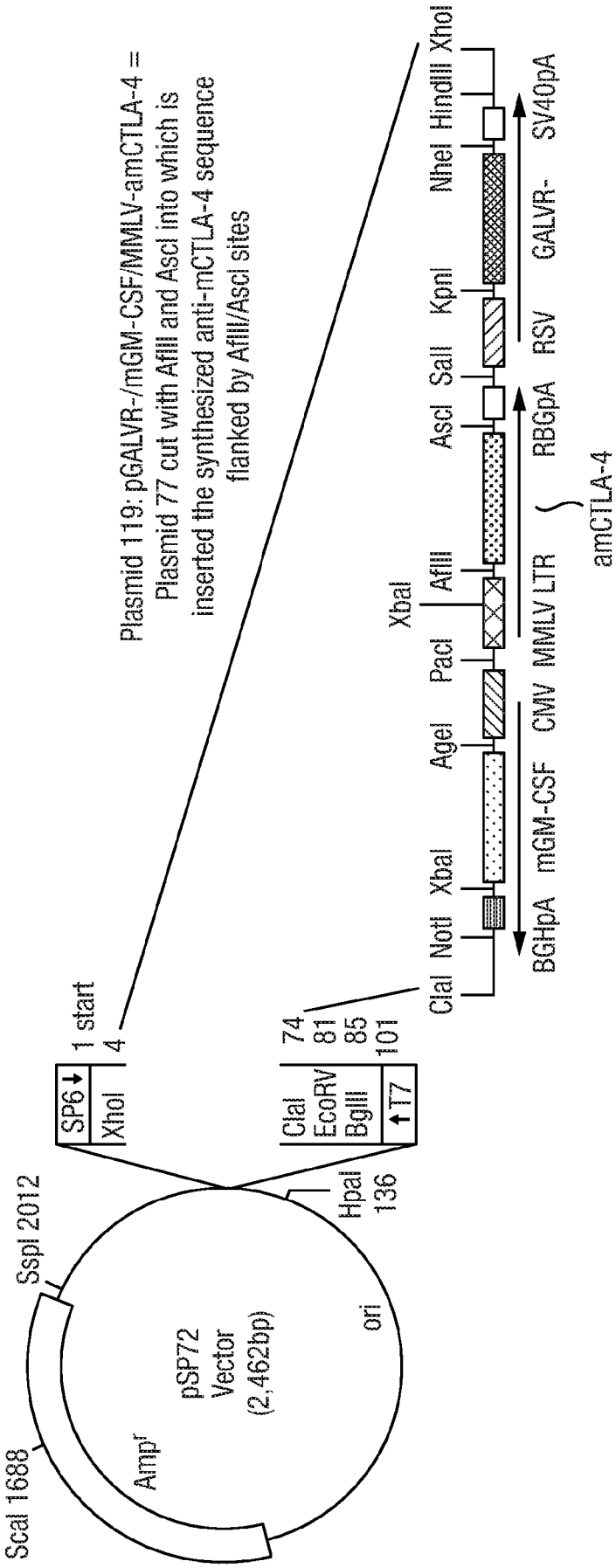

Plasmid 119: pGALVR-/mGM-CSF/MMLV-amCTLA-4 =
Plasmid 77 cut with AflIII and AscI into which is
inserted the synthesized anti-mCTLA-4 sequence
flanked by AflIII/AscI sites
1 start
4
SP6
XhoI
74
81
85
101
ClaI
EcoRV
BglII
T7
HpaI
136
SspI 2012
ScaI 1688
Ampr
pSP72
Vector
(2,462bp)
ori
ClaI NotI XbaI AgeI PacI XbaI AflIII AscI SalI KpnI NheI HindIII XhoI
BGHpA mGM-CSF CMV MMLV LTR amCTLA-4 RBGpA RSV GALVR- SV40pA

Fig. 2D

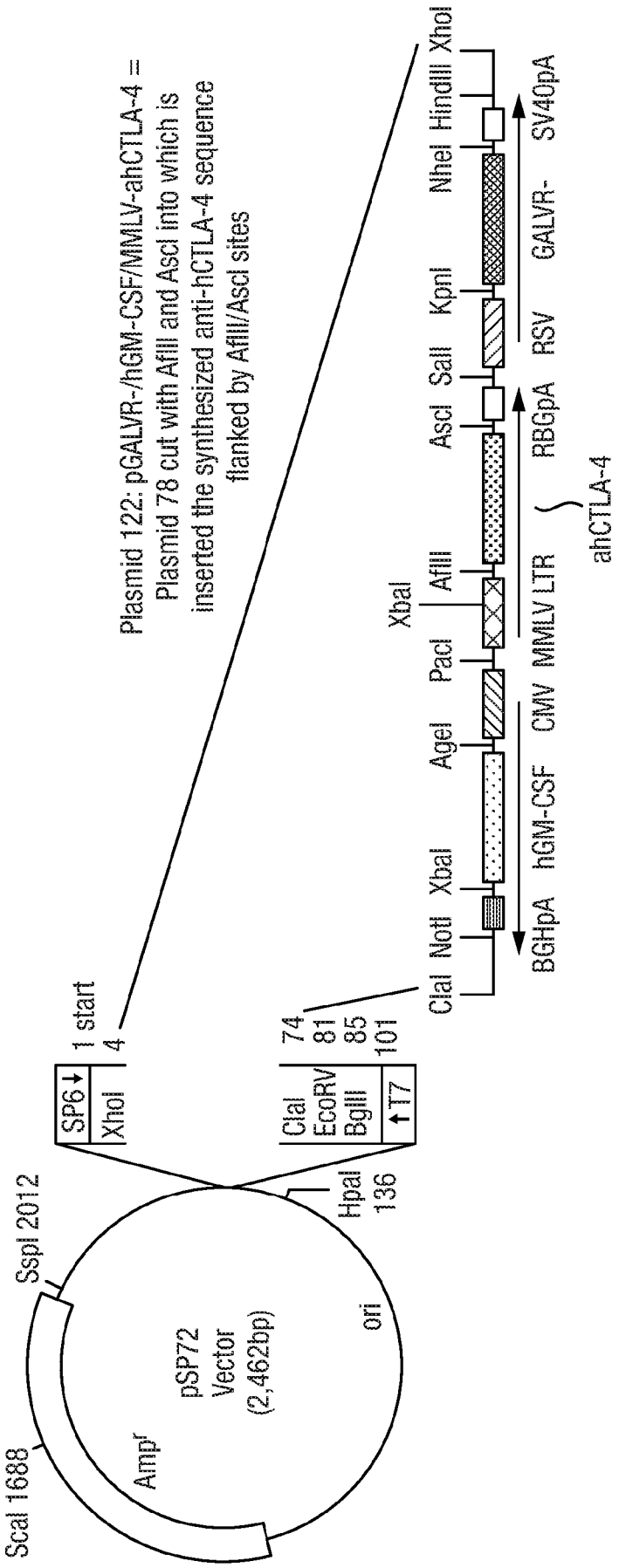

Plasmid 122: pGALVR-/hGM-CSF/MMLV-ahCTLA-4 =
Plasmid 78 cut with AflIII and AscI into which is
inserted the synthesized anti-hCTLA-4 sequence
flanked by AflIII/AscI sites
1 start
4
SP6
XhoI
74
81
85
101
ClaI
EcoRV
BglII
T7
HpaI
136
SspI 2012
ScaI 1688
Amp^r
pSP72
Vector
(2,462bp)
ori
ClaI
NotI
XbaI
hGM-CSF
AgeI
CMV
PacI
XbaI
AflIII
MMLV LTR
ahCTLA-4
AscI
SalI
RBGpA
KpnI
RSV
NheI
HindIII
GALVR-
XhoI
SV40pA
BGHpA

Fig. 3

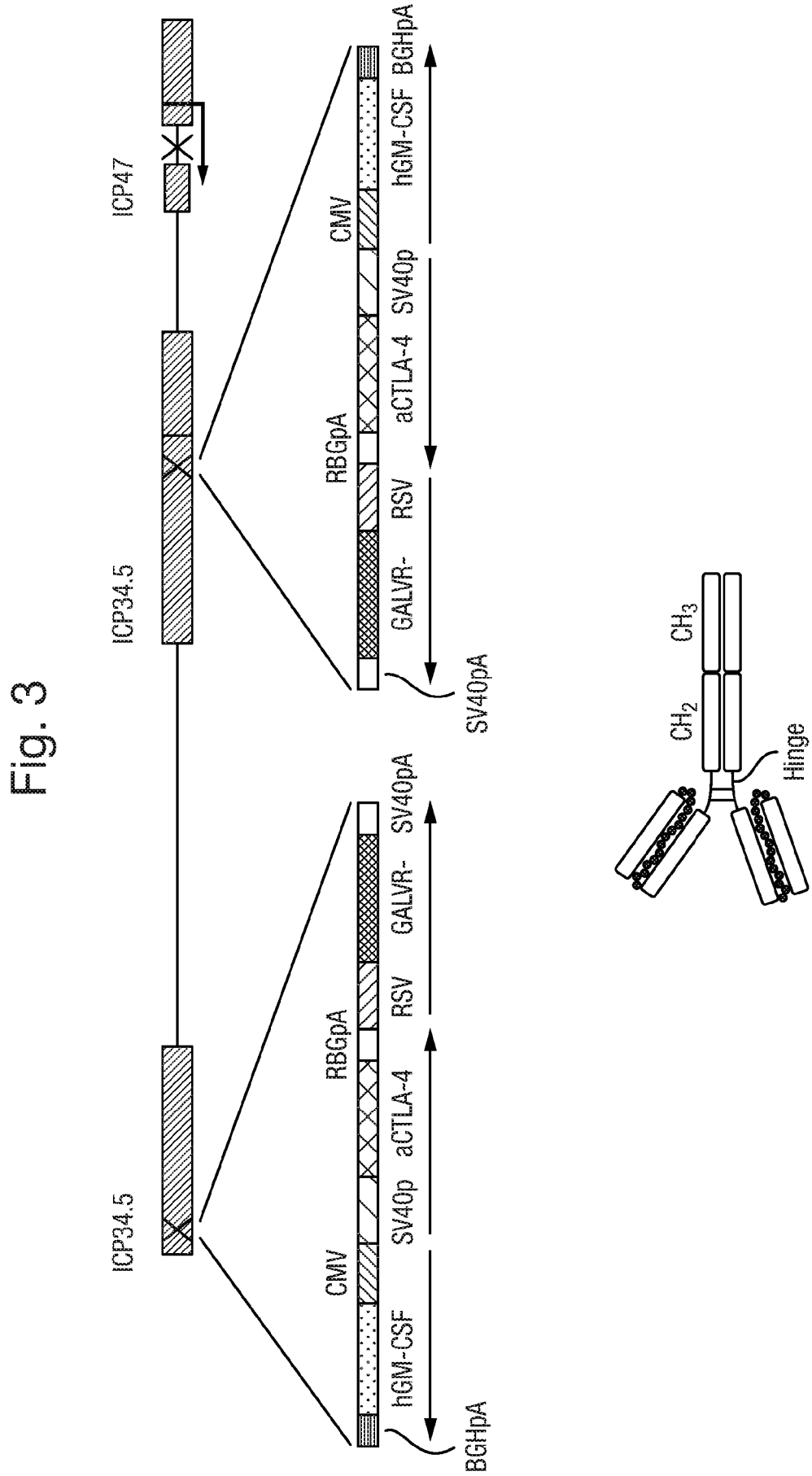

ICP47
ICP34.5
ICP34.5
CMV
hGM-CSF
BGHpA
SV40p
aCTLA-4
RBGpA
RSV
GALVR-
SV40pA
CMV
hGM-CSF
BGHpA
SV40p
aCTLA-4
RBGpA
RSV
GALVR-
SV40pA
CH3
CH2
Hinge

Fig. 4

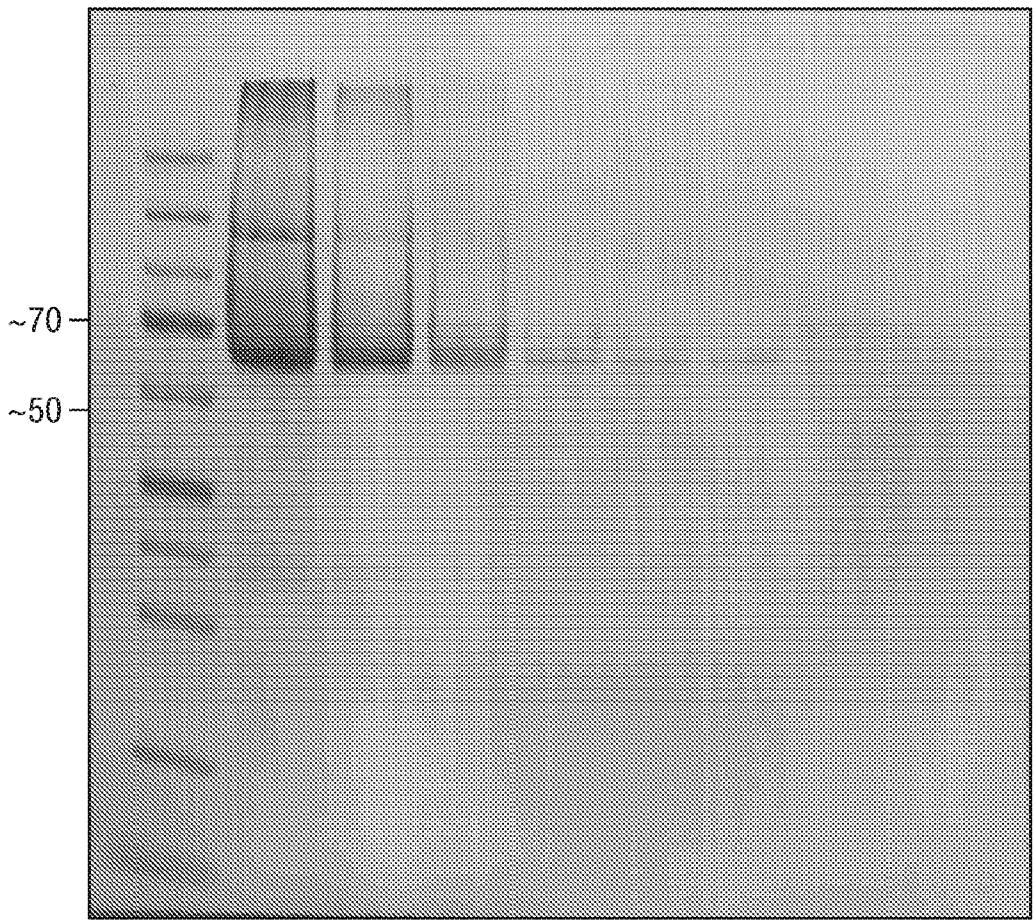
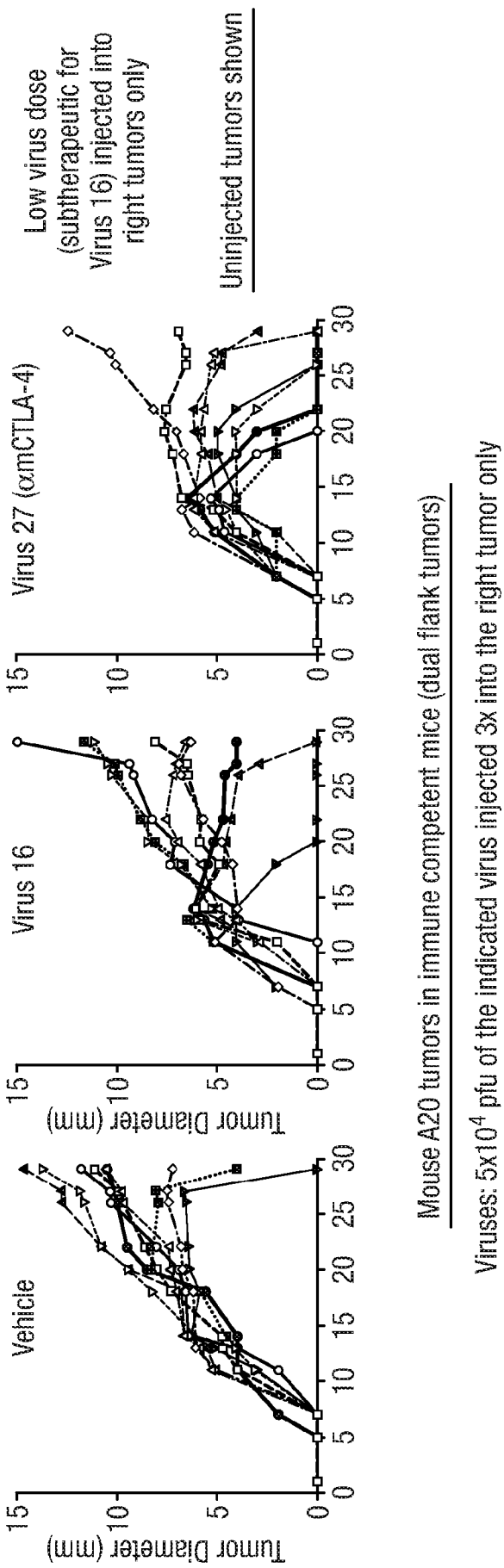


Fig. 5


Low virus dose (subtherapeutic for Virus 16) injected into right tumors only
Uninjected tumors shown
Virus 27 (αmCTLA-4)
Virus 16
Vehicle
Tumor Diameter (mm)
0
5
10
15
0
5
10
15
20
25
30
Mouse A20 tumors in immune competent mice (dual flank tumors)
Viruses: 5x10^4 pfu of the indicated virus injected 3x into the right tumor only

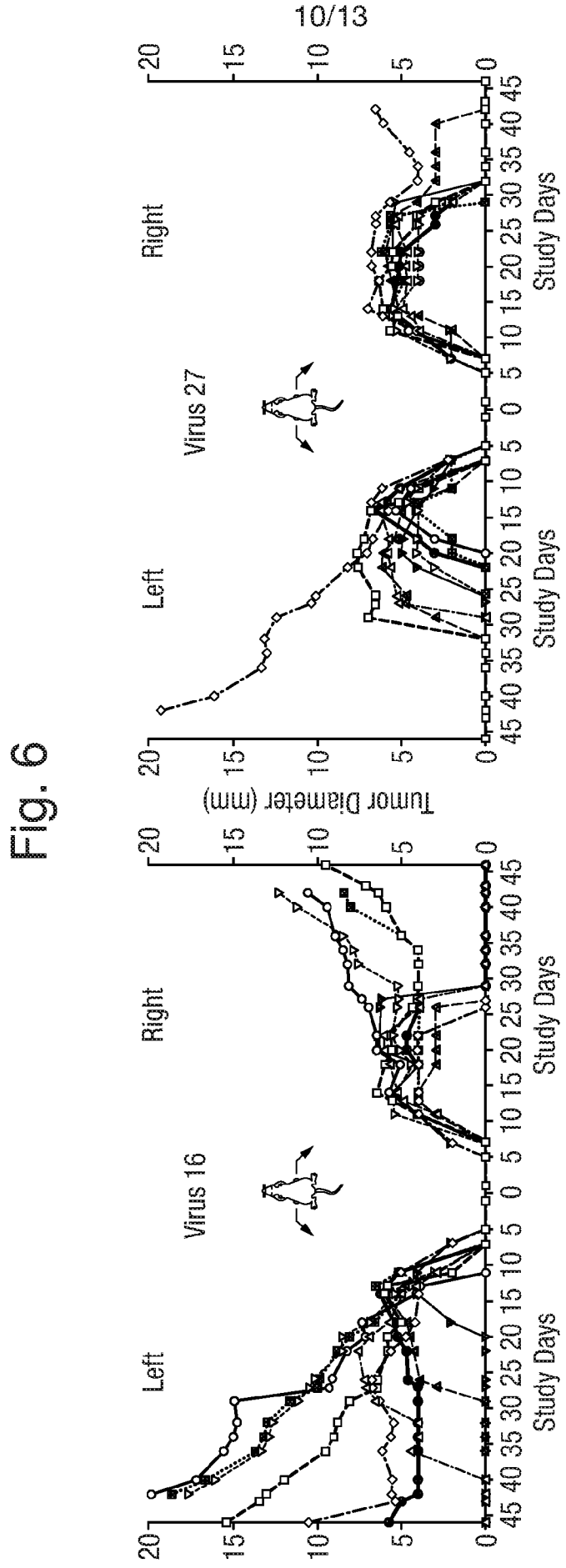

10/13
Left
Right
Virus 16
Virus 27
Tumor Diameter (mm)
Study Days
Mouse A20 bi-lateral tumors, immune competent mice
5x10^4 pfu of the indicated virus 3x over 1 wk into the right tumor

Fig. 6

Fig. 7

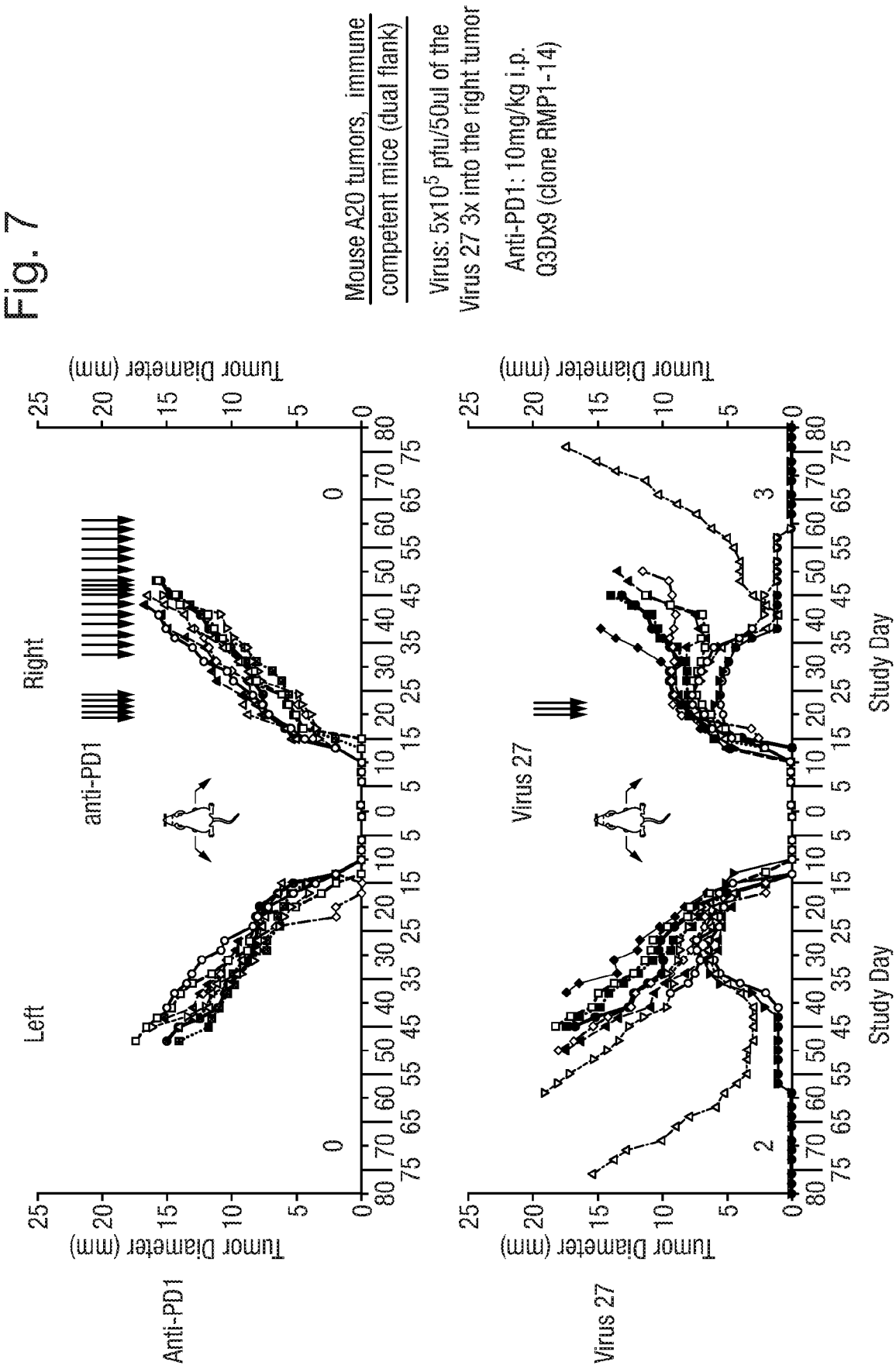

Mouse A20 tumors, immune competent mice (dual flank)
Virus: 5x10^5 pfu/50ul of the Virus 27 3x into the right tumor
Anti-PD1: 10mg/kg i.p. Q3Dx9 (clone RMP1-14)
Anti-PD1
Virus 27
Left
Right
anti-PD1
Virus 27
Tumor Diameter (mm)
Study Day
0
2
3

Fig. 7(Cont.)

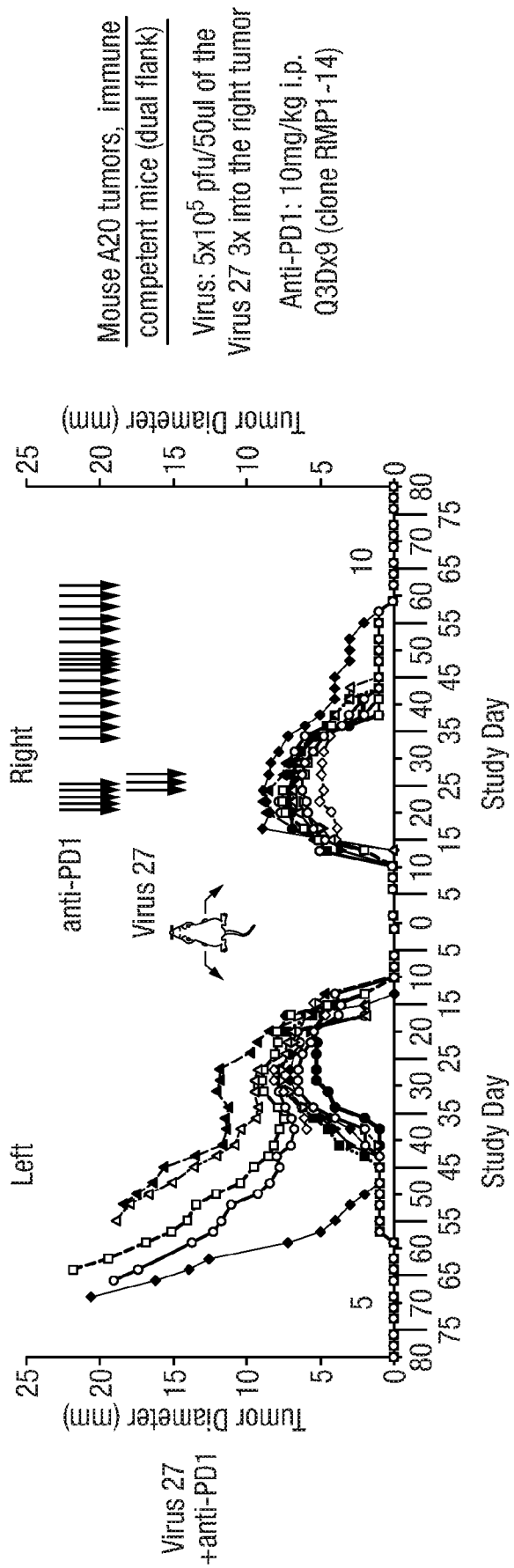
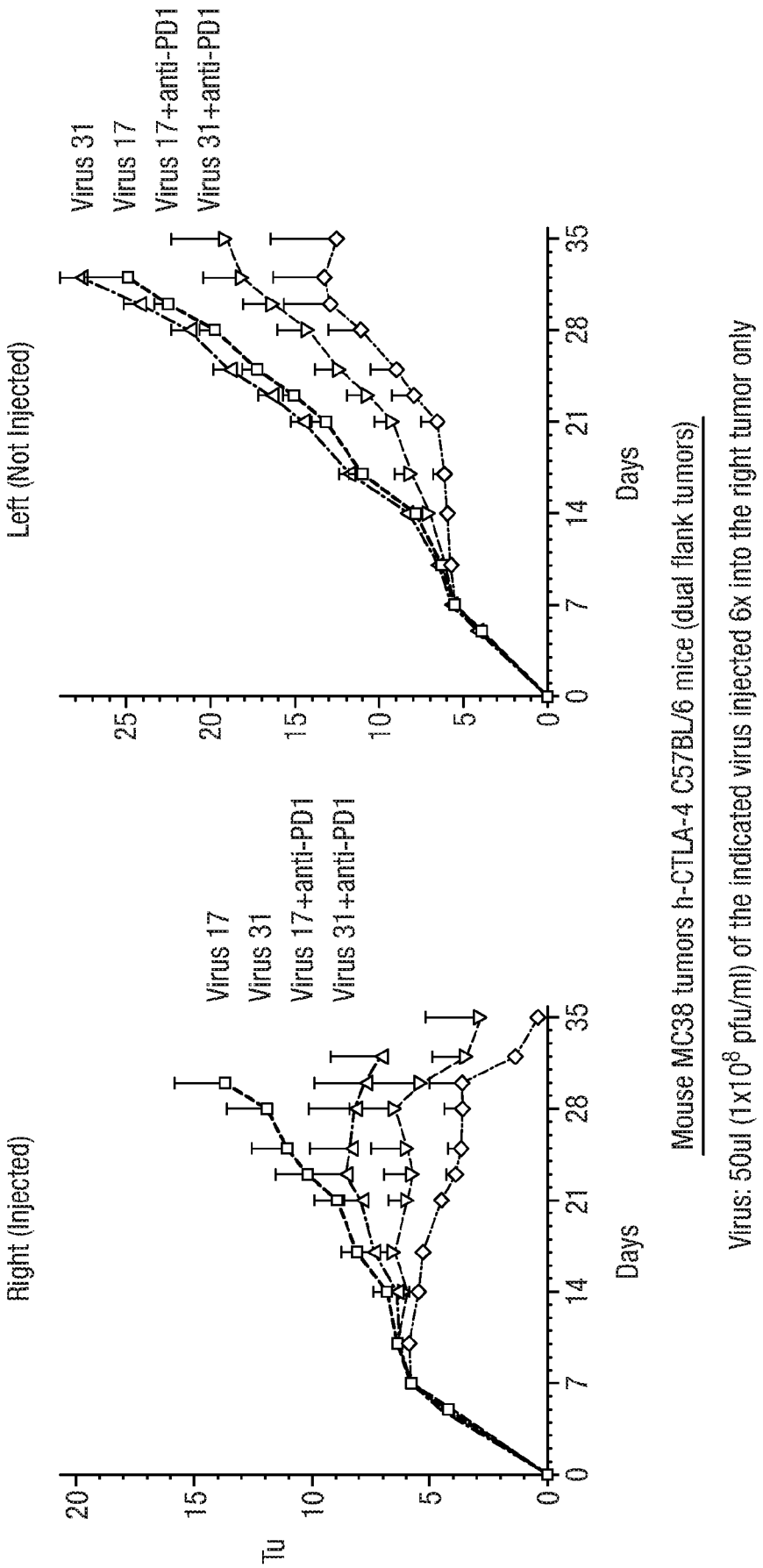

Mouse A20 tumors, immune competent mice (dual flank)
Virus: 5x10^5 pfu/50ul of the Virus 27 3x into the right tumor
Anti-PD1: 10mg/kg i.p. Q3Dx9 (clone RMP1-14)
Right
anti-PD1
Virus 27
Left
Virus 27 +anti-PD1
Tumor Diameter (mm)
Study Day

Fig. 8



Mouse MC38 tumors h-CTLA-4 C57BL/6 mice (dual flank tumors)

Virus: 50ul ($1x10^8$ pfu/ml) of the indicated virus injected 6x into the right tumor only

Anti-PD1: 10mg/kg i.p. Q3dx9

MC38 are very insensitive to HSV

SEQUENCE LISTING

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<120> ALTERED VIRUS

<130> N409771WO

<140>
<141> 2018-01-09

<150> GB 1700350.0
<151> 2017-01-09

<160> 46

<170> PatentIn version 3.5

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            20                  25                  30


Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35                  40                  45


Tyr Gly Ala Phe Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser Gly
    50                  55                  60


Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro
65                  70                  75                  80


Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro Trp
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Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
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Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Gly Ser Ser
            20                  25                  30


Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
        35                  40                  45


Ile Tyr Gly Ala Phe Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
    50                  55                  60


Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
65                  70                  75                  80


Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
                85                  90                  95


Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala
            100                 105                 110


Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
        115                 120                 125


Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
    130                 135                 140


Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
145                 150                 155                 160


Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
                165                 170                 175
```

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
180 185 190

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
195 200 205

Ser Phe Asn Arg Gly Glu Cys
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<213> Homo sapiens

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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Thr Phe Ile Ser Tyr Asp Gly Asn Asn Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Ile Tyr Tyr Cys
85 90 95

Ala Arg Thr Gly Trp Leu Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr
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Leu Val Thr Val Ser Ser
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Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
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Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
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Arg Val Glu Pro Lys Ser Cys
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Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
20 25 30

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
35 40 45

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Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    50                  55                  60

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
65                  70                  75                  80

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
                85                  90                  95

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
            100                 105                 110

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
        115                 120                 125

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
    130                 135                 140

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
145                 150                 155                 160

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                165                 170                 175

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            180                 185                 190

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        195                 200                 205

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    210                 215                 220

Pro Gly Lys
225
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<213> Homo sapiens

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            20                  25                  30


Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45


Thr Phe Ile Ser Tyr Asp Gly Asn Asn Lys Tyr Tyr Ala Asp Ser Val
    50                  55                  60


Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65                  70                  75                  80


Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Ile Tyr Tyr Cys
                85                  90                  95


Ala Arg Thr Gly Trp Leu Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr
            100                 105                 110


Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
        115                 120                 125


Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
    130                 135                 140


Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
145                 150                 155                 160


Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
                165                 170                 175


Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
            180                 185                 190
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Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
        195                 200                 205

Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr
    210                 215                 220

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
225                 230                 235                 240

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                245                 250                 255

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
            260                 265                 270

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
        275                 280                 285

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
    290                 295                 300

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
305                 310                 315                 320

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
                325                 330                 335

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
            340                 345                 350

Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
        355                 360                 365

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
    370                 375                 380

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
385                 390                 395                 400
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Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
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Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
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Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
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Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser
        35                  40                  45

Val Gly Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala
    50                  55                  60

Pro Arg Leu Leu Ile Tyr Gly Ala Phe Ser Arg Ala Thr Gly Ile Pro
65                  70                  75                  80

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
                85                  90                  95

Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr
            100                 105                 110

Gly Ser Ser Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
        115                 120                 125

Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
    130                 135                 140

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
145                 150                 155                 160

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
                165                 170                 175

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
            180                 185                 190

Thr Phe Ile Ser Tyr Asp Gly Asn Asn Lys Tyr Tyr Ala Asp Ser Val
        195                 200                 205

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
    210                 215                 220
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Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Ile Tyr Tyr Cys
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Ala Arg Thr Gly Trp Leu Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr
                245                 250                 255


Leu Val Thr Val Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro
            260                 265                 270


Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
        275                 280                 285


Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
    290                 295                 300


Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
305                 310                 315                 320


Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
                325                 330                 335


Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
            340                 345                 350


Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
        355                 360                 365


Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
    370                 375                 380


Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met
385                 390                 395                 400


Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
                405                 410                 415


Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
            420                 425                 430
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Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
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Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
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Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
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Lys Ser Leu Ser Leu Ser Pro Gly Lys
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ctgtcttgta gagccagcca gtctgtggga agctcctacc tggcttggta tcagcagaag 180

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gtccggcagg caccagggaa gggactggag tgggtgacct tcatcagcta tgacggcaac 600

aacaagtatt acgctgattc cgtgaaaggg aggtttacca ttagccgcga caactccaaa 660

aatacactgt acctgcagat gaacagcctg cgggccgagg atactgctat ctactattgc 720

gcaagaaccg ggtggctggg acccttcgac tattggggcc aggggactct ggtcaccgtg 780

tcctctgata agacacacac atgccctccc tgtcctgcac cagagctgct gggcgggcca 840

tccgtgttcc tgtttccacc caagcctaaa gacaccctga tgatcagccg gacacctgaa 900

gtcacttgcg tggtcgtgga cgtgagtcac gaggatccag aagtcaagtt taactggtac 960

gtggatggcg tcgaggtgca taatgccaag accaaacctc gcgaggaaca gtacaatagc 1020

acatatcgag tcgtgtccgt cctgactgtg ctgcatcagg attggctgaa cggcaaagag 1080

tataagtgca aagtgagcaa taaggcactg cctgccccaa tcgagaaaac aatttccaag 1140

gctaaaggcc agcccaggga acctcaggtg tacactctgc ctccaagtcg cgaggaaatg 1200

accaagaacc aggtgagcct gacctgtctg gtgaaagggt tctatccatc agacattgca 1260

gtggagtggg aaagcaatgg acagcccgaa aacaattaca agaccacacc ccctgtgctg 1320

gacagcgatg gctccttctt tctgtattct aagctgactg tggataaaag tcgctggcag 1380

caggggaacg tctttagctg ttccgtgatg catgaggctc tgcacaatca ttacacacag 1440

aagtctctga gtctgtcacc cggcaaatga 1470

<210> 11
<211> 127
<212> PRT
<213> Mus musculus

<400> 11

Ile Arg Arg Ala Asp Ile Val Met Thr Gln Thr Thr Leu Ser Leu Pro
1 5 10 15

Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser
20 25 30

Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys
35 40 45

Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe
50 55 60

Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
65 70 75 80

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr
85 90 95

Cys Phe Gln Gly Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys
100 105 110

Leu Glu Ile Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Gly Ser
115 120 125

<210> 12
<211> 134
<212> PRT
<213> Mus musculus

<400> 12

Glu Ala Lys Leu Gln Glu Ser Gly Pro Val Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Tyr Met Asn Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
35 40 45

Gly Val Ile Asn Pro Tyr Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Tyr Tyr Gly Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Ile Thr Val Ser Thr Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu
115 120 125

Ala Pro Arg Ser Ser Arg
130

<210> 13

<211> 356
<212> PRT
<213> Mus musculus

<400> 13

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Glu Ala Lys Leu Gln Glu Ser Gly Pro Val Leu Val Lys Pro Gly Ala
1               5                  10                  15


Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
            20                  25                  30


Tyr Met Asn Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
        35                  40                  45


Gly Val Ile Asn Pro Tyr Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
    50                  55                  60


Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80


Met Glu Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95


Ala Arg Tyr Tyr Gly Ser Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu
            100                 105                 110


Ile Thr Val Ser Thr Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu
        115                 120                 125


Ala Pro Arg Ser Ser Arg Gly Cys Lys Pro Cys Ile Cys Thr Val Pro
    130                 135                 140


Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu
145                 150                 155                 160


Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Ile Ser
                165                 170                 175


Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu
            180                 185                 190
```

```
Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr
        195                 200                 205

Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn
    210                 215                 220

Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro
225                 230                 235                 240

Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln
                245                 250                 255

Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val
            260                 265                 270

Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val
        275                 280                 285

Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln
    290                 295                 300

Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn
305                 310                 315                 320

Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val
                325                 330                 335

Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu Ser His
            340                 345                 350

Ser Pro Gly Lys
        355
```

<210> 14
<211> 519
<212> PRT
<213> Mus musculus

<400> 14

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Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1               5                  10                  15

Gly Ser Thr Gly Asp Ile Arg Arg Ala Asp Ile Val Met Thr Gln Thr
            20                  25                  30

Thr Leu Ser Leu Pro Val Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys
        35                  40                  45

Arg Ser Ser Gln Ser Ile Val His Ser Asn Gly Asn Thr Tyr Leu Glu
    50                  55                  60

Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys
65                  70                  75                  80

Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly
                85                  90                  95

Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp
            100                 105                 110

Leu Gly Val Tyr Tyr Cys Phe Gln Gly Ser His Val Pro Tyr Thr Phe
        115                 120                 125

Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Asp Ala Ala Pro Thr
    130                 135                 140

Val Ser Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
145                 150                 155                 160

Gly Gly Ser Glu Ala Lys Leu Gln Glu Ser Gly Pro Val Leu Val Lys
                165                 170                 175

Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
            180                 185                 190

Thr Asp Tyr Tyr Met Asn Trp Val Lys Gln Ser His Gly Lys Ser Leu
        195                 200                 205
```

```
Glu Trp Ile Gly Val Ile Asn Pro Tyr Asn Gly Asp Thr Ser Tyr Asn
    210                 215                 220

Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser
225                 230                 235                 240

Thr Ala Tyr Met Glu Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val
                245                 250                 255

Tyr Tyr Cys Ala Arg Tyr Tyr Gly Ser Trp Phe Ala Tyr Trp Gly Gln
            260                 265                 270

Gly Thr Leu Ile Thr Val Ser Thr Ala Lys Thr Thr Pro Pro Ser Val
        275                 280                 285

Tyr Pro Leu Ala Pro Arg Ser Ser Arg Gly Cys Lys Pro Cys Ile Cys
    290                 295                 300

Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys
305                 310                 315                 320

Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val
                325                 330                 335

Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp
            340                 345                 350

Asp Val Glu Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe
        355                 360                 365

Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp
    370                 375                 380

Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe
385                 390                 395                 400

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys
                405                 410                 415
```

```
Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys
            420                 425                 430

Asp Lys Val Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp
        435                 440                 445

Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys
    450                 455                 460

Asn Thr Gln Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser
465                 470                 475                 480

Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr
                485                 490                 495

Cys Ser Val Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser
            500                 505                 510

Leu Ser His Ser Pro Gly Lys
        515
```

<210> 15
<211> 1560
<212> DNA
<213> Mus musculus

<400> 15

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gacattcgac gggctgacat tgtgatgacc cagaccacac tgagcctgcc cgtgtccctg      120

ggcgaccagg ccagcatctc ctgccggagc tcccagtcta tcgtgcacag caacggaaac      180

acatacctgg agtggtatct gcagaagcct ggccagtccc caaagctgct gatctacaag      240

gtgtccaaca ggttcagcgg cgtgcctgac cgcttttctg gaagcggctc cggaacagat      300

ttcaccctga agatcagcag ggtggaggct gaggacctgg gcgtgtacta ctgcttccag      360

ggatcccacg tgccttacac ctttggcgga ggcacaaagc tggagatcaa gagagccgat      420

gctgctccaa ccgtgtctgg aagcggaggc gggggttctg gaggcggtgg gagcggtggc      480

ggagggtctg aggctaagct gcaggagagc ggccccgtgc tggtgaagcc tggagccagc      540
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gtgaagatgt cctgtaaggc ttctggatac accttcacag actactacat gaactgggtg 600

aagcagagcc acggcaagtc cctggagtgg atcggagtga tcaaccctta caacggcgac 660

acctcttaca accagaagtt taagggcaag gccaccctga cagtggataa gtctagctcc 720

accgcttaca tggagctgaa cagcctgaca tccgaggatt ctgccgtgta ctactgtgct 780

aggtactacg gaagctggtt cgcctactgg ggccagggaa cactgatcac cgtgtccaca 840

gccaagacca cacccctag cgtgtacccc ctggctccta ggtctagcag aggctgcaag 900

ccatgcatct gtaccgtgcc cgaggtgagc agcgtgttca tctttccacc caagcccaag 960

gacgtgctga ccatcacact gaccctaag gtgacatgcg tggtggtgga tatcagcaag 1020

gacgatccag aggtgcagtt ctcctggttt gtggacgatg tggaggtgca caccgcccag 1080

acacagccaa gggaggagca gttcaactcc acctttagat ccgtgtctga gctgcccatc 1140

atgcaccagg actggctgaa cggaaaggag ttcaagtgcc gggtgaactc cgccgctttt 1200

cctgctccaa tcgagaagac catctctaag acaaagggcc gcccaaaggc tccacaggtg 1260

tacaccatcc ctccacccaa ggagcagatg gctaaggata aggtgagcct gacctgtatg 1320

atcacagact tctttcccga ggatatcaca gtggagtggc agtggaacgg acagcctgcc 1380

gagaactaca agaacaccca gccaatcatg gacacagatg gctcttactt cgtgtacagc 1440

aagctgaacg tgcagaagtc taactgggag gctggcaaca ccttcacctg cagcgtgctg 1500

cacgaaggtc tccataatca ccacaccgaa aagagcctca gtcacagccc tgggaaatga 1560

<210> 16
<211> 1574
<212> DNA
<213> Mus musculus

<400> 16
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actggggaca ttcgacgggc tgacattgtg atgacccaga ccacactgag cctgcccgtg 120

tccctgggcg accaggccag catctcctgc cggagctccc agtctatcgt gcacagcaac 180

ggaaacacat acctggagtg gtatctgcag aagcctggcc agtccccaaa gctgctgatc 240

tacaaggtgt ccaacaggtt cagcggcgtg cctgaccgct tttctggaag cggctccgga 300

acagatttca ccctgaagat cagcagggtg gaggctgagg acctgggcgt gtactactgc 360

ttccagggat cccacgtgcc ttacaccttt ggcggaggca caaagctgga gatcaagaga 420

gccgatgctg ctccaaccgt gtctggaagc ggaggcgggg gttctggagg cggtgggagc 480

ggtggcggag ggtctgaggc taagctgcag gagagcggcc ccgtgctggt gaagcctgga 540

gccagcgtga agatgtcctg taaggcttct ggatacacct tcacagacta ctacatgaac 600

tgggtgaagc agagccacgg caagtccctg gagtggatcg gagtgatcaa cccttacaac 660

ggcgacacct cttacaacca gaagtttaag ggcaaggcca ccctgacagt ggataagtct 720

agctccaccg cttacatgga gctgaacagc ctgacatccg aggattctgc cgtgtactac 780

tgtgctaggt actacggaag ctggttcgcc tactggggcc agggaacact gatcaccgtg 840

tccacagcca agaccacacc ccctagcgtg tacccctgg ctcctaggtc tagcagaggc 900

tgcaagccat gcatctgtac cgtgcccgag gtgagcagcg tgttcatctt tccacccaag 960

cccaaggacg tgctgaccat cacactgacc cctaaggtga catgcgtggt ggtggatatc 1020

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gcccagacac agccaaggga ggagcagttc aactccacct ttagatccgt gtctgagctg 1140

cccatcatgc accaggactg gctgaacgga aaggagttca agtgccgggt gaactccgcc 1200

gcttttcctg ctccaatcga gaagaccatc tctaagacaa agggccgccc aaaggctcca 1260

caggtgtaca ccatccctcc acccaaggag cagatggcta aggataaggt gagcctgacc 1320

tgtatgatca cagacttctt tcccgaggat atcacagtgg agtggcagtg gaacggacag 1380

cctgccgaga actacaagaa cacccagcca atcatggaca cagatggctc ttacttcgtg 1440

tacagcaagc tgaacgtgca gaagtctaac tgggaggctg gcaacacctt cacctgcagc 1500

gtgctgcacg aaggtctcca taatcaccac accgaaaaga gcctcagtca cagccctggg 1560

aaatgaggcg cgcc 1574

<210> 17
<211> 1484
<212> DNA
<213> Homo sapiens

<400> 17
cttaagatgg aaactgacac cctgctgctg tgggtcctgc tgctgtgggt gcctggatcc 60

accggcgata tcgtgctgac ccagtctcct ggcacactga gtctgtcacc aggggagcga 120

gcaacactgt cttgtagagc cagccagtct gtgggaagct cctacctggc ttggtatcag 180

cagaagccag gccaggcacc caggctgctg atctacggag ccttcagccg ggccactggc 240

attccagaca ggttctctgg aagtggctca gggaccgact tcaccctgac catcagccga 300

ctggagcccg aagacttcgc cgtgtactat tgccagcagt acggctctag tccttggact 360

tttggacagg gcaccaaagt ggagatcaag cgcggcgggg gaggctctgg gggaggcggg 420

agtggaggcg ggggatcaca ggtccagctg gtggaaagcg gcgggggagt ggtccagcca 480

ggccggagcc tgcggctgag ctgcgccgct tcaggattca cattttcaag ctataccatg 540

cactgggtcc ggcaggcacc agggaaggga ctggagtggg tgaccttcat cagctatgac 600

ggcaacaaca agtattacgc tgattccgtg aaagggaggt ttaccattag ccgcgacaac 660

tccaaaaata cactgtacct gcagatgaac agcctgcggg ccgaggatac tgctatctac 720

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cctgaagtca cttgcgtggt cgtggacgtg agtcacgagg atccagaagt caagtttaac 960

tggtacgtgg atggcgtcga ggtgcataat gccaagacca aacctcgcga ggaacagtac 1020

aatagcacat atcgagtcgt gtccgtcctg actgtgctgc atcaggattg gctgaacggc 1080

aaagagtata agtgcaaagt gagcaataag gcactgcctg ccccaatcga gaaaacaatt 1140

tccaaggcta aaggccagcc cagggaacct caggtgtaca ctctgcctcc aagtcgcgag 1200

gaaatgacca agaaccaggt gagcctgacc tgtctggtga aagggttcta tccatcagac 1260

attgcagtgg agtgggaaag caatggacag cccgaaaaca attacaagac cacacccccc 1320

gtgctggaca gcgatggctc cttctttctg tattctaagc tgactgtgga taaaagtcgc 1380

tggcagcagg ggaacgtctt tagctgttcc gtgatgcatg aggctctgca caatcattac 1440

acacagaagt ctctgagtct gtcacccggc aaatgaggcg cgcc 1484

<210> 18
<211> 426

<212> DNA
<213> Mus musculus

<400> 18
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cgctcaccca tcactgtcac ccggccttgg aagcatgtag aggccatcaa agaagccctg 120

aacctcctgg atgacatgcc tgtcacattg aatgaagagg tagaagtcgt ctctaacgag 180

ttctccttca agaagctaac atgtgtgcag acccgcctga agatattcga gcagggtcta 240

cggggcaatt tcaccaaact caagggcgcc ttgaacatga cagccagcta ctaccagaca 300

tactgccccc caactccgga aacggactgt gaaacacaag ttaccaccta tgcggatttc 360

atagacagcc ttaaaacctt tctgactgat atccccttg aatgcaaaaa accagtccaa 420

aaatga 426

<210> 19
<211> 426
<212> DNA
<213> Mus musculus

<400> 19
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cgctcaccta tcactgtcac cagaccctgg aagcacgtgg aggccatcaa ggaggctctg 120

aacctgctgg acgatatgcc agtgaccctg aacgaggagg tggaggtggt gagcaacgag 180

ttctccttta agaagctgac ctgcgtgcag acaaggctga agatcttcga gcagggcctg 240

agaggaaact ttaccaagct gaagggcgcc ctgaacatga ccgcttctta ctaccagaca 300

tactgccccc ctacccccga gacagactgt gagacacagg tgaccacata cgccgacttc 360

attgatagcc tgaaaacatt cctgaccgac attccatttg agtgtaagaa gcccgtccag 420

aagtaa 426

<210> 20
<211> 435
<212> DNA
<213> Homo sapiens

<400> 20
atgtggctgc agagcctgct gctcttgggc actgtggcct gcagcatctc tgcacccgcc 60

cgctcgccca gccccagcac gcagccctgg gagcatgtga atgccatcca ggaggcccgg 120

cgtctcctga acctgagtag agacactgct gctgagatga atgaaacagt agaagtcatc 180

tcagaaatgt ttgacctcca ggagccgacc tgcctacaga cccgcctgga gctgtacaag 240

cagggcctgc ggggcagcct caccaagctc aagggcccct tgaccatgat ggccagccac 300

tacaagcagc actgccctcc aaccccggaa acttcctgtg caacccagat tatcaccttt 360

gaaagtttca aagagaacct gaaggacttt ctgcttgtca tccctttga ctgctgggag 420

ccagtccagg agtga 435

<210> 21
<211> 435
<212> DNA
<213> Homo sapiens

<400> 21
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aggtcaccaa gtccatctac tcagccttgg gagcacgtga acgcaatcca ggaggcacgg 120

cggctgctga acctgagccg ggacaccgcc gccgagatga acgagacagt ggaagtgatc 180

agcgagatgt tcgatctgca ggagcccacc tgcctgcaga caaggctgga gctgtacaag 240

cagggcctgc gcggctctct gaccaagctg aagggcccac tgacaatgat ggccagccac 300

tataagcagc actgcccccc taccccgag acaagctgtg ccacccagat catcacattc 360

gagtccttta aggagaacct gaaggatttt ctgctggtca ttccatttga ttgttgggag 420

cccgtccagg agtaa 435

<210> 22
<211> 141
<212> PRT
<213> Mus musculus

<400> 22

Met Trp Leu Gln Asn Leu Leu Phe Leu Gly Ile Val Val Tyr Ser Leu
1 5 10 15

Ser Ala Pro Thr Arg Ser Pro Ile Thr Val Thr Arg Pro Trp Lys His
20 25 30

```
Val Glu Ala Ile Lys Glu Ala Leu Asn Leu Leu Asp Asp Met Pro Val
        35                  40                  45


Thr Leu Asn Glu Glu Val Glu Val Val Ser Asn Glu Phe Ser Phe Lys
    50                  55                  60


Lys Leu Thr Cys Val Gln Thr Arg Leu Lys Ile Phe Glu Gln Gly Leu
65                  70                  75                  80


Arg Gly Asn Phe Thr Lys Leu Lys Gly Ala Leu Asn Met Thr Ala Ser
                85                  90                  95


Tyr Tyr Gln Thr Tyr Cys Pro Pro Thr Pro Glu Thr Asp Cys Glu Thr
            100                 105                 110


Gln Val Thr Thr Tyr Ala Asp Phe Ile Asp Ser Leu Lys Thr Phe Leu
        115                 120                 125


Thr Asp Ile Pro Phe Glu Cys Lys Lys Pro Val Gln Lys
    130                 135                 140


<210>  23
<211>  144
<212>  PRT
<213>  Homo sapiens

<400>  23

Met Trp Leu Gln Ser Leu Leu Leu Leu Gly Thr Val Ala Cys Ser Ile
1               5                   10                  15


Ser Ala Pro Ala Arg Ser Pro Ser Pro Ser Thr Gln Pro Trp Glu His
            20                  25                  30


Val Asn Ala Ile Gln Glu Ala Arg Arg Leu Leu Asn Leu Ser Arg Asp
        35                  40                  45


Thr Ala Ala Glu Met Asn Glu Thr Val Glu Val Ile Ser Glu Met Phe
    50                  55                  60
```

Asp Leu Gln Glu Pro Thr Cys Leu Gln Thr Arg Leu Glu Leu Tyr Lys
65 70 75 80

Gln Gly Leu Arg Gly Ser Leu Thr Lys Leu Lys Gly Pro Leu Thr Met
85 90 95

Met Ala Ser His Tyr Lys Gln His Cys Pro Pro Thr Pro Glu Thr Ser
100 105 110

Cys Ala Thr Gln Ile Ile Thr Phe Glu Ser Phe Lys Glu Asn Leu Lys
115 120 125

Asp Phe Leu Leu Val Ile Pro Phe Asp Cys Trp Glu Pro Val Gln Glu
130 135 140

<210> 24
<211> 2010
<212> DNA
<213> Gibbon leukemia virus

<400> 24
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atgagtcctg ggagctggaa aagactgatc atcctcttaa gctgcgtatt cggcggcggc 120

gggacgagtc tgcaaaataa gaacccccac cagcccatga ccctcacttg gcaggtactg 180

tcccaaactg gagacgttgt ctgggataca aaggcagtcc agccccttg gacttggtgg 240

cccacactta aacctgatgt atgtgccttg gcggctagtc ttgagtcctg ggatatcccg 300

ggaaccgatg tctcgtcctc taaacgagtc agacctccgg actcagacta tactgccgct 360

tataagcaaa tcacctgggg agccataggg tgcagctacc ctcgggctag gactagaatg 420

gcaagctcta ccttctacgt atgtccccgg gatggccgga ccctttcaga agctagaagg 480

tgcgggggggc tagaatccct atactgtaaa gaatgggatt gtgagaccac ggggaccggt 540

tattggctat ctaaatcctc aaaagacctc ataactgtaa aatgggacca aaatagcgaa 600

tggactcaaa aatttcaaca gtgtcaccag accggctggt gtaaccccct taaaatagat 660

ttcacagaca aaggaaaatt atccaaggac tggataacgg gaaaaacctg gggattaaga 720

ttctatgtgt ctggacatcc aggcgtacag ttcaccattc gcttaaaaat caccaacatg 780

ccagctgtgg cagtaggtcc tgacctcgtc cttgtggaac aaggacctcc tagaacgtcc 840

ctcgctctcc cacctcctct tcccccaagg gaagcgccac cgccatctct ccccgactct 900

aactccacag ccctggcgac tagtgcacaa actcccacgg tgagaaaaac aattgttacc 960

ctaaacactc cgcctcccac cacaggcgac agacttttg atcttgtgca gggggccttc 1020

ctaaccttaa atgctaccaa cccaggggcc actgagtctt gctggctttg tttggccatg 1080

ggcccccctt attatgaagc aatagcctca tcaggagagg tcgcctactc caccgacctt 1140

gaccggtgcc gctgggggac ccaaggaaag ctcaccctca ctgaggtctc aggacacggg 1200

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actggcctca ccccttgcct ctccacctca gtttttaatc agactagaga tttctgtatc 1380

caggtccagc tgattcctcg catctattac tatcctgaag aagttttgtt acaggcctat 1440

gacaattctc accccaggac taaaagagag gctgtctcac ttaccctagc tgttttactg 1500

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gacctccagc aaggcctgac aagcctccag atcgccatag atgctgacct ccgggccctc 1620

caagactcag tcagcaagtt agaggactca ctgacttccc tgtccgaggt agtgctccaa 1680

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aaagaaaaac tggataaaag acagttagag cgccagaaaa gccaaaactg gtatgaagga 1860

tggttcaata actccccttg gttcactacc ctgctatcaa ccatcgctgg gcccctatta 1920

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aatgatagga taagtgcagt taaaatttaa 2010

<210> 25
<211> 2013
<212> DNA
<213> Gibbon leukemia virus

<400> 25
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gcctacaagc agatcacctg gggcgccatc ggctgtagct atcctcgggc ccgcacaagg 420

atggccagct ccacctttta cgtgtgccca cgcgacggaa ggaccctgtc tgaggcaagg 480

agatgtggcg gcctggagag cctgtattgc aaggagtggg attgtgagac cacaggcaca 540

ggctactggc tgtctaagtc tagcaaggac ctgatcaccg tgaagtggga tcagaacagc 600

gagtggacac agaagttcca gcagtgccac cagaccggct ggtgtaatcc cctgaagatc 660

gactttacag ataagggcaa gctgtccaag gactggatca ccggcaagac atggggcctg 720

agattctacg tgtctggcca ccctggcgtg cagtttacaa tccggctgaa gatcaccaac 780

atgccagcag tggcagtggg accagacctg gtgctggtgg agcagggacc tccacgcacc 840

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ctgggactgg gaatcacagc aggaatcggc acaggcagca ccgccctgat caagggacca 1560

atcgacctgc agcagggact gacctccctg cagatcgcca tcgacgccga tctgagagcc 1620

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cagaacagga ggggcctgga cctgctgttc ctgaaggagg gaggactgtg cgccgccctg 1740

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ggctggttca acaattcccc ctggtttacc acactgctgt ctaccatcgc aggacctctg 1920

ttattactgc tgctgctgct gatcctgggc ccatgtatca tcaacaagct ggtgcagttt 1980

atcaacgacc gaatctccgc agtgaaaatc taa 2013

<210> 26
<211> 669
<212> PRT
<213> Gibbon leukemia virus

<400> 26

Met Val Leu Leu Pro Gly Ser Met Leu Leu Thr Ser Asn Leu His His
1 5 10 15

Leu Arg His Gln Met Ser Pro Gly Ser Trp Lys Arg Leu Ile Ile Leu
20 25 30

Leu Ser Cys Val Phe Gly Gly Gly Gly Thr Ser Leu Gln Asn Lys Asn
35 40 45

Pro His Gln Pro Met Thr Leu Thr Trp Gln Val Leu Ser Gln Thr Gly
50 55 60

Asp Val Val Trp Asp Thr Lys Ala Val Gln Pro Pro Trp Thr Trp Trp
65 70 75 80

Pro Thr Leu Lys Pro Asp Val Cys Ala Leu Ala Ala Ser Leu Glu Ser
85 90 95

Trp Asp Ile Pro Gly Thr Asp Val Ser Ser Ser Lys Arg Val Arg Pro
100 105 110

Pro Asp Ser Asp Tyr Thr Ala Ala Tyr Lys Gln Ile Thr Trp Gly Ala
115 120 125

```
Ile Gly Cys Ser Tyr Pro Arg Ala Arg Thr Arg Met Ala Ser Ser Thr
    130                 135                 140

Phe Tyr Val Cys Pro Arg Asp Gly Arg Thr Leu Ser Glu Ala Arg Arg
145                 150                 155                 160

Cys Gly Gly Leu Glu Ser Leu Tyr Cys Lys Glu Trp Asp Cys Glu Thr
                165                 170                 175

Thr Gly Thr Gly Tyr Trp Leu Ser Lys Ser Ser Lys Asp Leu Ile Thr
            180                 185                 190

Val Lys Trp Asp Gln Asn Ser Glu Trp Thr Gln Lys Phe Gln Gln Cys
        195                 200                 205

His Gln Thr Gly Trp Cys Asn Pro Leu Lys Ile Asp Phe Thr Asp Lys
    210                 215                 220

Gly Lys Leu Ser Lys Asp Trp Ile Thr Gly Lys Thr Trp Gly Leu Arg
225                 230                 235                 240

Phe Tyr Val Ser Gly His Pro Gly Val Gln Phe Thr Ile Arg Leu Lys
                245                 250                 255

Ile Thr Asn Met Pro Ala Val Ala Val Gly Pro Asp Leu Val Leu Val
            260                 265                 270

Glu Gln Gly Pro Pro Arg Thr Ser Leu Ala Leu Pro Pro Pro Leu Pro
        275                 280                 285

Pro Arg Glu Ala Pro Pro Pro Ser Leu Pro Asp Ser Asn Ser Thr Ala
    290                 295                 300

Leu Ala Thr Ser Ala Gln Thr Pro Thr Val Arg Lys Thr Ile Val Thr
305                 310                 315                 320

Leu Asn Thr Pro Pro Pro Thr Thr Gly Asp Arg Leu Phe Asp Leu Val
                325                 330                 335
```

```
Gln Gly Ala Phe Leu Thr Leu Asn Ala Thr Asn Pro Gly Ala Thr Glu
            340                 345                 350

Ser Cys Trp Leu Cys Leu Ala Met Gly Pro Pro Tyr Tyr Glu Ala Ile
        355                 360                 365

Ala Ser Ser Gly Glu Val Ala Tyr Ser Thr Asp Leu Asp Arg Cys Arg
    370                 375                 380

Trp Gly Thr Gln Gly Lys Leu Thr Leu Thr Glu Val Ser Gly His Gly
385                 390                 395                 400

Leu Cys Ile Gly Lys Val Pro Phe Thr His Gln His Leu Cys Asn Gln
                405                 410                 415

Thr Leu Ser Ile Asn Ser Ser Gly Asp His Gln Tyr Leu Leu Pro Ser
            420                 425                 430

Asn His Ser Trp Trp Ala Cys Ser Thr Gly Leu Thr Pro Cys Leu Ser
        435                 440                 445

Thr Ser Val Phe Asn Gln Thr Arg Asp Phe Cys Ile Gln Val Gln Leu
    450                 455                 460

Ile Pro Arg Ile Tyr Tyr Tyr Pro Glu Glu Val Leu Leu Gln Ala Tyr
465                 470                 475                 480

Asp Asn Ser His Pro Arg Thr Lys Arg Glu Ala Val Ser Leu Thr Leu
                485                 490                 495

Ala Val Leu Leu Gly Leu Gly Ile Thr Ala Gly Ile Gly Thr Gly Ser
            500                 505                 510

Thr Ala Leu Ile Lys Gly Pro Ile Asp Leu Gln Gln Gly Leu Thr Ser
        515                 520                 525

Leu Gln Ile Ala Ile Asp Ala Asp Leu Arg Ala Leu Gln Asp Ser Val
    530                 535                 540
```

```
Ser Lys Leu Glu Asp Ser Leu Thr Ser Leu Ser Glu Val Val Leu Gln
545                 550                 555                 560


Asn Arg Arg Gly Leu Asp Leu Leu Phe Leu Lys Glu Gly Gly Leu Cys
                565                 570                 575


Ala Ala Leu Lys Glu Glu Cys Cys Phe Tyr Ile Asp His Ser Gly Ala
            580                 585                 590


Val Arg Asp Ser Met Lys Lys Leu Lys Glu Lys Leu Asp Lys Arg Gln
        595                 600                 605


Leu Glu Arg Gln Lys Ser Gln Asn Trp Tyr Glu Gly Trp Phe Asn Asn
    610                 615                 620


Ser Pro Trp Phe Thr Thr Leu Leu Ser Thr Ile Ala Gly Pro Leu Leu
625                 630                 635                 640


Leu Leu Leu Leu Leu Leu Ile Leu Gly Pro Cys Ile Ile Asn Lys Leu
                645                 650                 655


Val Gln Phe Ile Asn Asp Arg Ile Ser Ala Val Lys Ile
            660                 665


<210>  27
<211>  759
<212>  DNA
<213>  Artificial Sequence

<220>
<223>  Human-Mouse hybrid

<400>  27
atgatcgaga cctacaatca gacaagccca cggtccgccg caaccggact gcctatcagc       60

atgaagatct tcatgtacct gctgaccgtg tttctgatca cacagatgat cggctccgcc      120

ctgttcgccg tgtatctgca caggagactg gacaagatcg aggatgagcg caatctgcac      180

gaggacttcg tgtttatgaa gaccatccag cggtgcaaca caggcgagag gagcctgtct      240

ctgctgaatt gtgaggagat caagtcccag ttcgagggct ttgtgaagga tatcatgctg      300
```

aacaaggagg agacaaagaa ggacgaggat ccacagatcg cagcacacgt ggtgtccgag 360

gcaaactcta atgccgccag cgtgctgcag tgggccaaga agggctacta taccatgaag 420

tctaacctgg tgacactgga gaatggcaag cagctgaccg tgaagaggca gggcctgtac 480

tatatctatg cccaggtgac attctgctct aacagagagg caagctccca ggcacccttc 540

atcgtgggac tgtggctgaa gccctctagc ggcagcgaga ggatcctgct gaaggccgcc 600

aatacccact cctctagcca gctgtgcgag cagcagtcca tccacctggg aggcgtgttc 660

gagctgcagc ctggagccag cgtgttcgtg aacgtgacag acccatctca ggtgagccac 720

ggcaccggct tcacaagctt tggcctgctg aagctgtga 759

<210> 28
<211> 252
<212> PRT
<213> Artificial Sequence

<220>
<223> Human-Mouse hybrid

<400> 28

Met Ile Glu Thr Tyr Asn Gln Thr Ser Pro Arg Ser Ala Ala Thr Gly
1 5 10 15

Leu Pro Ile Ser Met Lys Ile Phe Met Tyr Leu Leu Thr Val Phe Leu
20 25 30

Ile Thr Gln Met Ile Gly Ser Ala Leu Phe Ala Val Tyr Leu His Arg
35 40 45

Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp Phe Val
50 55 60

Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser Leu Ser
65 70 75 80

Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe Val Lys
85 90 95

Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Asp Glu Asp Pro Gln

```
            100                 105                 110

Ile Ala Ala His Val Val Ser Glu Ala Asn Ser Asn Ala Ala Ser Val
        115                 120                 125

Leu Gln Trp Ala Lys Lys Gly Tyr Tyr Thr Met Lys Ser Asn Leu Val
    130                 135                 140

Thr Leu Glu Asn Gly Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr
145                 150                 155                 160

Tyr Ile Tyr Ala Gln Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser
                165                 170                 175

Gln Ala Pro Phe Ile Val Gly Leu Trp Leu Lys Pro Ser Ser Gly Ser
            180                 185                 190

Glu Arg Ile Leu Leu Lys Ala Ala Asn Thr His Ser Ser Ser Gln Leu
        195                 200                 205

Cys Glu Gln Gln Ser Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro
    210                 215                 220

Gly Ala Ser Val Phe Val Asn Val Thr Asp Pro Ser Gln Val Ser His
225                 230                 235                 240

Gly Thr Gly Phe Thr Ser Phe Gly Leu Leu Lys Leu
                245                 250
```

<210> 29
<211> 1416
<212> DNA
<213> Homo sapiens

<400> 29
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gagatgaaga gcctgtccca gagatctgtg cctaacacct gcacactggt catgtgcagc 120

cccaccgaga atggactgcc tggaagggac ggaagggatg gaagggaggg ccctcggggc 180

gagaagggcg acccaggact gcctggacca atgggactga gcggactgca gggaccaaca 240

ggacctgtgg gaccaaaggg agagaacgga tccgccggag agccaggccc taagggcgag 300

aggggcctgt ctggcccccc tggcctgcca ggcatcccag gccccgccgg caaggagggc 360

ccatccggca agcagggcaa tatcggcccc cagggcaagc ctggcccaaa gggcgaggca 420

ggaccaaagg gagaagtggg agcacctggc atgcagggat ccaccggagc aaagggatct 480

acaggaccaa agggcgagcg cggcgcccca ggcgtgcagg gcgcccccgg caatgcagga 540

gcagcaggac cagcaggacc tgcaggccca cagggcgccc ctggctctag gggcccaccc 600

ggcctgaagg gcgacagggg agtgcctggc gataggggca tcaagggaga gagcggactg 660

ccagattccg ccgccctgag gcagcagatg gaggccctga agggcaagct gcagaggctg 720

gaggtggcct tctcccacta ccagaaggcc gccctgtttc cagacggcca caggagactg 780

gacaagatcg aggatgagcg caacctgcac gaggatttcg tgtttatgaa gaccatccag 840

agatgcaaca caggcgagcg gtctctgagc ctgctgaatt gtgaggagat caagtctcag 900

ttcgagggct ttgtgaagga catcatgctg aacaaggagg agaccaagaa ggagaatagc 960

ttcgagatgc agaagggcga tcagaatccc cagatcgcag cacacgtgat cagcgaggca 1020

agctccaaga ccacatccgt gctgcagtgg gccgagaagg gctactatac catgtccaac 1080

aatctggtga cactggagaa cggcaagcag ctgaccgtga agagacaggg cctgtactat 1140

atctatgccc aggtgacatt ctgctctaat cgggaggcct ctagccaggc ccctttatc 1200

gcctctctgt gcctgaagag cccaggcaga ttcgagcgga tcctgctgag ggccgccaac 1260

acccactcct ctgccaagcc atgcggacag cagagcatcc acctgggagg cgtgttcgag 1320

ctgcagccag gagcctccgt gtttgtgaat gtgacagacc catcccaggt gtctcacgga 1380

accggcttca catcctttgg cctgctgaag ctgtga 1416

<210> 30
<211> 471
<212> PRT
<213> Homo sapiens

<400> 30

Met Leu Pro Phe Leu Ser Met Leu Val Leu Leu Val Gln Pro Leu Gly
1 5 10 15

```
Asn Leu Gly Ala Glu Met Lys Ser Leu Ser Gln Arg Ser Val Pro Asn
            20                  25                  30

Thr Cys Thr Leu Val Met Cys Ser Pro Thr Glu Asn Gly Leu Pro Gly
        35                  40                  45

Arg Asp Gly Arg Asp Gly Arg Glu Gly Pro Arg Gly Glu Lys Gly Asp
    50                  55                  60

Pro Gly Leu Pro Gly Pro Met Gly Leu Ser Gly Leu Gln Gly Pro Thr
65                  70                  75                  80

Gly Pro Val Gly Pro Lys Gly Glu Asn Gly Ser Ala Gly Glu Pro Gly
                85                  90                  95

Pro Lys Gly Glu Arg Gly Leu Ser Gly Pro Pro Gly Leu Pro Gly Ile
            100                 105                 110

Pro Gly Pro Ala Gly Lys Glu Gly Pro Ser Gly Lys Gln Gly Asn Ile
        115                 120                 125

Gly Pro Gln Gly Lys Pro Gly Pro Lys Gly Glu Ala Gly Pro Lys Gly
    130                 135                 140

Glu Val Gly Ala Pro Gly Met Gln Gly Ser Thr Gly Ala Lys Gly Ser
145                 150                 155                 160

Thr Gly Pro Lys Gly Glu Arg Gly Ala Pro Gly Val Gln Gly Ala Pro
                165                 170                 175

Gly Asn Ala Gly Ala Ala Gly Pro Ala Gly Pro Ala Gly Pro Gln Gly
            180                 185                 190

Ala Pro Gly Ser Arg Gly Pro Pro Gly Leu Lys Gly Asp Arg Gly Val
        195                 200                 205

Pro Gly Asp Arg Gly Ile Lys Gly Glu Ser Gly Leu Pro Asp Ser Ala
    210                 215                 220
```

```
Ala Leu Arg Gln Gln Met Glu Ala Leu Lys Gly Lys Leu Gln Arg Leu
225                 230                 235                 240


Glu Val Ala Phe Ser His Tyr Gln Lys Ala Ala Leu Phe Pro Asp Gly
                245                 250                 255


His Arg Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp
            260                 265                 270


Phe Val Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser
        275                 280                 285


Leu Ser Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe
    290                 295                 300


Val Lys Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser
305                 310                 315                 320


Phe Glu Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val
                325                 330                 335


Ile Ser Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu
            340                 345                 350


Lys Gly Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly
        355                 360                 365


Lys Gln Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln
    370                 375                 380


Val Thr Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile
385                 390                 395                 400


Ala Ser Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu
                405                 410                 415


Arg Ala Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser
            420                 425                 430
```

Ile His Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe
435 440 445

Val Asn Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr
450 455 460

Ser Phe Gly Leu Leu Lys Leu
465 470

<210> 31
<211> 1412
<212> DNA
<213> Mus musculus

<400> 31
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ccaaccgaga atggactgcc aggaagggac ggaagagatg gaagggaggg accaagggga 180

gagaagggcg accctggact gcctggacca atgggactgt ccggactgca gggaccaaca 240

ggccctgtgg gaccaaaggg agagaatgga agcgccggag agccaggacc taagggagag 300

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ggaccaaagg gagaagtggg agcacccggc atgcagggca gcaccggagc aaagggatcc 480

accggcccta agggagagag aggagcacct ggagtgcagg gcgccccagg caatgcagga 540

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ggcctgaagg gcgacagggg agtgccaggc gataggggca tcaagggaga gtccggactg 660

ccagactctg ccgccctgag gcagcagatg gaggccctga agggcaagct gcagaggctg 720

gaggtggcct tctcccacta ccagaaggcc gccctgtttc cagacggaca caggagactg 780

gataaggtgg aggaggaggt gaacctgcac gaggatttcg tgttcatcaa gaagctgaag 840

aggtgcaaca agggcgaggg cagcctgtcc ctgctgaatt gtgaggagat gcggcgccag 900

ttcgaggacc tggtgaagga tatcaccctg aacaaggagg agaagaagga gaattctttt 960

gagatgcaga ggggcgacga ggatcctcag atcgcagcac acgtggtgtc cgaggcaaac 1020

tctaatgccg ccagcgtgct gcagtgggcc aagaagggct actataccat gaagtctaac 1080

ctggtcatgc tggagaatgg caagcagctg acagtgaaga gagagggcct gtactacgtg 1140

tacacccagg tgacattctg cagcaacaga gagcccagct cccagcggcc ttttatcgtg 1200

ggcctgtggc tgaagccctc tatcggaagc gagaggatcc tgctgaaggc agccaatacc 1260

cactctagct cccagctgtg cgagcagcag tccgtgcacc tgggaggcgt gttcgagctg 1320

caggcaggag caagcgtgtt cgtgaacgga cagaggccag ccaggtcatc cacagagtgg 1380

gcttctctag ctttggcctg ctgaagctgt ga 1412

<210> 32
<211> 470
<212> PRT
<213> Mus musculus

<400> 32

Met Leu Pro Phe Leu Ser Met Leu Val Leu Leu Val Gln Pro Leu Gly
1 5 10 15

Asn Leu Gly Ala Glu Met Lys Ser Leu Ser Gln Arg Ser Val Pro Asn
20 25 30

Thr Cys Thr Leu Val Met Cys Ser Pro Thr Glu Asn Gly Leu Pro Gly
35 40 45

Arg Asp Gly Arg Asp Gly Arg Glu Gly Pro Arg Gly Glu Lys Gly Asp
50 55 60

Pro Gly Leu Pro Gly Pro Met Gly Leu Ser Gly Leu Gln Gly Pro Thr
65 70 75 80

Gly Pro Val Gly Pro Lys Gly Glu Asn Gly Ser Ala Gly Glu Pro Gly
85 90 95

Pro Lys Gly Glu Arg Gly Leu Ser Gly Pro Pro Gly Leu Pro Gly Ile
100 105 110

Pro Gly Pro Ala Gly Lys Glu Gly Pro Ser Gly Lys Gln Gly Asn Ile

```
        115                 120                 125

Gly Pro Gln Gly Lys Pro Gly Pro Lys Gly Glu Ala Gly Pro Lys Gly
    130                 135                 140

Glu Val Gly Ala Pro Gly Met Gln Gly Ser Thr Gly Ala Lys Gly Ser
145                 150                 155                 160

Thr Gly Pro Lys Gly Glu Arg Gly Ala Pro Gly Val Gln Gly Ala Pro
                165                 170                 175

Gly Asn Ala Gly Ala Ala Gly Pro Ala Gly Pro Ala Gly Pro Gln Gly
            180                 185                 190

Ala Pro Gly Ser Arg Gly Pro Pro Gly Leu Lys Gly Asp Arg Gly Val
        195                 200                 205

Pro Gly Asp Arg Gly Ile Lys Gly Glu Ser Gly Leu Pro Asp Ser Ala
    210                 215                 220

Ala Leu Arg Gln Gln Met Glu Ala Leu Lys Gly Lys Leu Gln Arg Leu
225                 230                 235                 240

Glu Val Ala Phe Ser His Tyr Gln Lys Ala Ala Leu Phe Pro Asp Gly
                245                 250                 255

His Arg Arg Leu Asp Lys Val Glu Glu Glu Val Asn Leu His Glu Asp
            260                 265                 270

Phe Val Phe Ile Lys Lys Leu Lys Arg Cys Asn Lys Gly Glu Gly Ser
        275                 280                 285

Leu Ser Leu Leu Asn Cys Glu Glu Met Arg Arg Gln Phe Glu Asp Leu
    290                 295                 300

Val Lys Asp Ile Thr Leu Asn Lys Glu Glu Lys Lys Glu Asn Ser Phe
305                 310                 315                 320

Glu Met Gln Arg Gly Asp Glu Asp Pro Gln Ile Ala Ala His Val Val
```

325 330 335

Ser Glu Ala Asn Ser Asn Ala Ala Ser Val Leu Gln Trp Ala Lys Lys
340 345 350

Gly Tyr Tyr Thr Met Lys Ser Asn Leu Val Met Leu Glu Asn Gly Lys
355 360 365

Gln Leu Thr Val Lys Arg Glu Gly Leu Tyr Tyr Val Tyr Thr Gln Val
370 375 380

Thr Phe Cys Ser Asn Arg Glu Pro Ser Ser Gln Arg Pro Phe Ile Val
385 390 395 400

Gly Leu Trp Leu Lys Pro Ser Ile Gly Ser Glu Arg Ile Leu Leu Lys
405 410 415

Ala Ala Asn Thr His Ser Ser Ser Gln Leu Cys Glu Gln Gln Ser Val
420 425 430

His Leu Gly Gly Val Phe Glu Leu Gln Ala Gly Ala Ser Val Phe Val
435 440 445

Asn Val Thr Glu Ala Ser Gln Val Ile His Arg Val Gly Phe Ser Ser
450 455 460

Phe Gly Leu Leu Lys Leu
465 470

<210> 33
<211> 786
<212> DNA
<213> Homo sapiens

<400> 33
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atgaaaattt ttatgtattt acttactgtt tttcttatca cccagatgat tgggtcagca 120

ctttttgctg tgtatcttca tagaaggttg gacaagatag aagatgaaag gaatcttcat 180

gaagattttg tattcatgaa aacgatacag agatgcaaca caggagaaag atccttatcc 240

ttactgaact gtgaggagat taaaagccag tttgaaggct ttgtgaagga tataatgtta 300

aacaaagagg agacgaagaa agaaaacagc tttgaaatgc aaaaaggtga tcagaatcct 360

caaattgcgg cacatgtcat aagtgaggcc agcagtaaaa caacatctgt gttacagtgg 420

gctgaaaaag gatactacac catgagcaac aacttggtaa ccctggaaaa tgggaaacag 480

ctgaccgtta aaagacaagg actctattat atctatgccc aagtcacctt ctgttccaat 540

cgggaagctt cgagtcaagc tccatttata gccagcctct gcctaaagtc ccccggtaga 600

ttcgagagaa tcttactcag agctgcaaat acccacagtt ccgccaaacc ttgcgggcaa 660

caatccattc acttgggagg agtatttgaa ttgcaaccag gtgcttcggt gtttgtcaat 720

gtgactgatc caagccaagt gagccatggc actggcttca cgtcctttgg cttactcaaa 780

ctctga 786

<210> 34
<211> 261
<212> PRT
<213> Homo sapiens

<400> 34

Met Ile Glu Thr Tyr Asn Gln Thr Ser Pro Arg Ser Ala Ala Thr Gly
1 5 10 15

Leu Pro Ile Ser Met Lys Ile Phe Met Tyr Leu Leu Thr Val Phe Leu
20 25 30

Ile Thr Gln Met Ile Gly Ser Ala Leu Phe Ala Val Tyr Leu His Arg
35 40 45

Arg Leu Asp Lys Ile Glu Asp Glu Arg Asn Leu His Glu Asp Phe Val
50 55 60

Phe Met Lys Thr Ile Gln Arg Cys Asn Thr Gly Glu Arg Ser Leu Ser
65 70 75 80

Leu Leu Asn Cys Glu Glu Ile Lys Ser Gln Phe Glu Gly Phe Val Lys
85 90 95

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Asp Ile Met Leu Asn Lys Glu Glu Thr Lys Lys Glu Asn Ser Phe Glu
            100                 105                 110

Met Gln Lys Gly Asp Gln Asn Pro Gln Ile Ala Ala His Val Ile Ser
        115                 120                 125

Glu Ala Ser Ser Lys Thr Thr Ser Val Leu Gln Trp Ala Glu Lys Gly
    130                 135                 140

Tyr Tyr Thr Met Ser Asn Asn Leu Val Thr Leu Glu Asn Gly Lys Gln
145                 150                 155                 160

Leu Thr Val Lys Arg Gln Gly Leu Tyr Tyr Ile Tyr Ala Gln Val Thr
                165                 170                 175

Phe Cys Ser Asn Arg Glu Ala Ser Ser Gln Ala Pro Phe Ile Ala Ser
            180                 185                 190

Leu Cys Leu Lys Ser Pro Gly Arg Phe Glu Arg Ile Leu Leu Arg Ala
        195                 200                 205

Ala Asn Thr His Ser Ser Ala Lys Pro Cys Gly Gln Gln Ser Ile His
    210                 215                 220

Leu Gly Gly Val Phe Glu Leu Gln Pro Gly Ala Ser Val Phe Val Asn
225                 230                 235                 240

Val Thr Asp Pro Ser Gln Val Ser His Gly Thr Gly Phe Thr Ser Phe
                245                 250                 255

Gly Leu Leu Lys Leu
            260
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<210> 35
<211> 783
<212> DNA
<213> Mus musculus

<400> 35
atgatagaaa catacagcca accttccccc agatccgtgg caactggact tccagcgagc 60

atgaagattt ttatgtattt acttactgtt ttccttatca cccaaatgat tggatctgtg 120

ctttttgctg tgtatcttca tagaagattg gataaggtcg aagaggaagt aaaccttcat 180

gaagattttg tattcataaa aaagctaaag agatgcaaca aaggagaagg atctttatcc 240

ttgctgaact gtgaggagat gagaaggcaa tttgaagacc ttgtcaagga tataacgtta 300

aacaaagaag agaaaaaaga aaacagcttt gaaatgcaaa gaggtgatga ggatcctcaa 360

attgcagcac acgttgtaag cgaagccaac agtaatgcag catccgttct acagtgggcc 420

aagaaaggat attataccat gaaaagcaac ttggtaatgc ttgaaaatgg gaaacagctg 480

acggttaaaa gagaaggact ctattatgtc tacactcaag tcaccttctg ctctaatcgg 540

gagccttcga gtcaacgccc attcatcgtc ggcctctggc tgaagcccag cagtggatct 600

gagagaatct tactcaaggc ggcaaatacc cacagttcct cccagctttg cgagcagcag 660

tctgttcact tgggcggagt gtttgaatta caagctggtg cttctgtgtt tgtcaacgtg 720

actgaagcaa gccaagtgat ccacagagtt ggcttctcat cttttggctt actcaaactc 780

tga 783

<210> 36
<211> 260
<212> PRT
<213> Mus musculus

<400> 36

Met Ile Glu Thr Tyr Ser Gln Pro Ser Pro Arg Ser Val Ala Thr Gly
1 5 10 15

Leu Pro Ala Ser Met Lys Ile Phe Met Tyr Leu Leu Thr Val Phe Leu
20 25 30

Ile Thr Gln Met Ile Gly Ser Val Leu Phe Ala Val Tyr Leu His Arg
35 40 45

Arg Leu Asp Lys Val Glu Glu Glu Val Asn Leu His Glu Asp Phe Val
50 55 60

Phe Ile Lys Lys Leu Lys Arg Cys Asn Lys Gly Glu Gly Ser Leu Ser

65 70 75 80

Leu Leu Asn Cys Glu Glu Met Arg Arg Gln Phe Glu Asp Leu Val Lys
85 90 95

Asp Ile Thr Leu Asn Lys Glu Glu Lys Lys Glu Asn Ser Phe Glu Met
100 105 110

Gln Arg Gly Asp Glu Asp Pro Gln Ile Ala Ala His Val Val Ser Glu
115 120 125

Ala Asn Ser Asn Ala Ala Ser Val Leu Gln Trp Ala Lys Lys Gly Tyr
130 135 140

Tyr Thr Met Lys Ser Asn Leu Val Met Leu Glu Asn Gly Lys Gln Leu
145 150 155 160

Thr Val Lys Arg Glu Gly Leu Tyr Tyr Val Tyr Thr Gln Val Thr Phe
165 170 175

Cys Ser Asn Arg Glu Pro Ser Ser Gln Arg Pro Phe Ile Val Gly Leu
180 185 190

Trp Leu Lys Pro Ser Ser Gly Ser Glu Arg Ile Leu Leu Lys Ala Ala
195 200 205

Asn Thr His Ser Ser Ser Gln Leu Cys Glu Gln Gln Ser Val His Leu
210 215 220

Gly Gly Val Phe Glu Leu Gln Ala Gly Ala Ser Val Phe Val Asn Val
225 230 235 240

Thr Glu Ala Ser Gln Val Ile His Arg Val Gly Phe Ser Ser Phe Gly
245 250 255

Leu Leu Lys Leu
260

<210> 37

<211> 632
<212> DNA
<213> Artificial Sequence

<220>
<223> CMV promoter

<400> 37
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gcccatatat ggagttccgc gttacataac ttacggtaaa tggcccgcct ggctgaccgc 120

ccaacgaccc ccgcccattg acgtcaataa tgacgtatgt tcccatagta acgccaatag 180

ggactttcca ttgacgtcaa tgggtggagt atttacggta aactgcccac ttggcagtac 240

atcaagtgta tcatatgcca agtacgcccc ctattgacgt caatgacggt aaatggcccg 300

cctggcatta tgcccagtac atgaccttat gggactttcc tacttggcag tacatctacg 360

tattagtcat cgctattacc atggtgatgc ggttttggca gtacatcaat gggcgtggat 420

agcggtttga ctcacgggga tttccaagtc tccaccccat tgacgtcaat gggagtttgt 480

tttggcacca aaatcaacgg gactttccaa aatgtcgtaa caactccgcc ccattgacgc 540

aaatgggcgg taggcgtgta cggtgggagg tctatataag cagagctctc tggctaacta 600

gagaacccac tgcttactgg cttatcgaaa tt 632

<210> 38
<211> 394
<212> DNA
<213> Artificial Sequence

<220>
<223> RSV promoter

<400> 38
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gcttcggttg tacgcggtta ggagtcccct caggatatag tagtttcgct tttgcatagg 120

gaggggggaaa tgtagtctta tgcaatacac ttgtagtctt gcaacatggt aacgatgagt 180

tagcaacatg ccttacaagg agagaaaaag caccgtgcat gccgattggt ggaagtaagg 240

tggtacgatc gtgccttatt aggaaggcaa cagacaggtc tgacatggat tggacgaacc 300

actgaattcc gcattgcaga gataattgta tttaagtgcc tagctcgata caataaacgc 360

catttgacca ttcaccacat tggtgtgcac ctcc 394

<210> 39
<211> 188
<212> DNA
<213> Artificial Sequence

<220>
<223> BGH polyA

<400> 39
ctgtgccttc tagttgccag ccatctgttg tttgcccctc ccccgtgcct tccttgaccc 60

tggaaggtgc cactcccact gtcctttcct aataaaatga ggaaattgca tcgcattgtc 120

tgagtaggtg tcattctatt ctgggggggtg gggtggggca ggacagcaag ggggaggatt 180

gggaagac 188

<210> 40
<211> 249
<212> DNA
<213> Artificial Sequence

<220>
<223> SV40 late polyA

<400> 40
gacatgataa gatacattga tgagtttgga caaaccacaa ctagaatgca gtgaaaaaaa 60

tgctttattt gtgaaatttg tgatgctatt gctttatttg tgaaatttgt gatgctattg 120

ctttatttgt aaccattata agctgcaata aacaagttaa caacaacaat tgcattcatt 180

ttatgtttca ggttcagggg gaggtgtggg aggtttttta aagcaagtaa aacctctaca 240

aatgtggta 249

<210> 41
<211> 99
<212> DNA
<213> Artificial Sequence

<220>
<223> Rabbit beta-globulin polyA

<400> 41
gacctctggc taataaagga aatttatttt cattgcaata gtgtgttgga atttttttgtg 60

tctctcactc ggaaggacat atgggagggc aaatcattt 99

<210> 42
<211> 723
<212> DNA
<213> Artificial Sequence

<220>
<223> GFP

<400> 42
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gacggcgacg taaacggcca caagttcagc gtgtccggcg agggcgaggg cgatgccacc 120

tacggcaagc tgaccctgaa gttcatctgc accaccggca agctgcccgt gccctggccc 180

accctcgtga ccaccctgac ctacggcgtg cagtgcttca gccgctaccc cgaccacatg 240

aagcagcacg acttcttcaa gtccgccatg cccgaaggct acgtccagga gcgcaccatc 300

ttcttcaagg acgacggcaa ctacaagacc cgcgccgagg tgaagttcga gggcgacacc 360

ctggtgaacc gcatcgagct gaagggcatc gacttcaagg aggacggcaa catcctgggg 420

cacaagctgg agtacaacta caacagccac aacgtctata tcatggccga caagcagaag 480

aacggcatca aggtgaactt caagatccgc cacaacatcg aggacggcag cgtgcagctc 540

gccgaccact accagcagaa cacccccatc ggcgacggcc ccgtgctgct gcccgacaac 600

cactacctga gcacccagtc cgccctgagc aaagacccca acgagaagcg cgatcacatg 660

gtcctgctgg agttcgtgac cgccgccggg atcactctcg gcatggacga gctgtacaag 720

taa 723

<210> 43
<211> 454
<212> DNA
<213> Artificial Sequence

<220>
<223> MoMuLV LTR

<400> 43
ttaattaagt aacgccattt tgcaaggcat ggaaaaatac ataactgaga atagagaagt 60

tcagatcaag gtcaggaaca gatggaacag ctgaatatgg gccaaacagg atatctgtgg 120

taagcagttc ctgccccggc tcagggccaa gaacagatgg aacagctgaa tatgggccaa 180

acaggatatc tgtggtaagc agttcctgcc ccggctcagg gccaagaaca gatggtcccc 240

agatgcggtc cagccctcag cagtttctag agaaccatca gatgtttcca gggtgcccca 300

aggacctgaa atgaccctgt gccttatttg aactaaccaa tcagttcgct tctcgcttct 360

gttcgcgcgc ttctgctccc cgagctcaat aaaagagccc acaacccctc actcggggcg 420

ccagtcctcc gattgactga gtcgcccgct taag 454

<210> 44
<211> 1349
<212> DNA
<213> Artificial Sequence

<220>
<223> EF1alpha promoter

<400> 44
ttaattaaga gtaattcata caaaaggact cgcccctgcc ttggggaatc ccagggaccg 60

tcgttaaact cccactaacg tagaacccag agatcgctgc gttcccgccc cctcacccgc 120

ccgctctcgt catcactgag gtggagaaga gcatgcgtga ggctccggtg cccgtcagtg 180

ggcagagcgc acatcgccca cagtccccga gaagttgggg ggaggggtcg gcaattgaac 240

cggtgcctag agaaggtggc gcggggtaaa ctgggaaagt gatgtcgtgt actggctccg 300

ccttttccc gagggtgggg gagaaccgta tataagtgca gtagtcgccg tgaacgttct 360

ttttcgcaac gggtttgccg ccagaacaca ggtaagtgcc gtgtgtggtt cccgcgggcc 420

tggcctcttt acgggttatg gcccttgcgt gccttgaatt acttccacgc ccctggctgc 480

agtacgtgat tcttgatccc gagcttcggg ttggaagtgg gtgggagagt tcgaggcctt 540

gcggttaagg agcccttcg cctcgtgctt gagttgaggc ctggcttggg cgctggggcc 600

gccgcgtgcg aatctggtgg caccttcgcg cctgtctcgc tgctttcgat aagtctctag 660

ccatttaaaa tttttgatga cctgctgcga cgctttttt ctggcaagat agtcttgtaa 720

atgcgggcca agatctgcac actggtattt cggtttttgg ggccgcgggc ggcgacgggg 780

cccgtgcgtc ccagcgcaca tgttcggcga ggcggggcct gcgagcgcgg ccaccgagaa 840

tcggacgggg gtagtctcaa gctggccggc ctgctctggt gcctggcctc gcgccgccgt 900

gtatcgcccc gccctgggcg gcaaggctgg cccggtcggc accagttgcg tgagcggaaa 960

gatggccgct tcccggccct gctgcaggga gctcaaaatg gaggacgcgg cgctcgggag 1020

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catgtgactc cacggagtac cgggcgccgt ccaggcacct cgattagttc tcgagctttt 1140

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agtgggtgga gactgaagtt aggccagctt ggcacttgat gtaattctcc ttggaatttg 1260

ccctttttga gtttggatct tggttcattc tcaagcctca gacagtggtt caaagttttt 1320

ttcttccatt tcaggtgtcg tgacttaag 1349

<210> 45
<211> 345
<212> DNA
<213> Artificial Sequence

<220>
<223> SV40 enhancer promoter

<400> 45
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tatgcaaagc atgcatctca attagtcagc aaccaggtgt ggaaagtccc caggctcccc 120

agcaggcaga agtatgcaaa gcatgcatct caattagtca gcaaccatag tcccgccccc 180

aactccgccc atcccgcccc taactccgcc cagttccgcc cattctccgc cccatggctg 240

actaattttt tttatttatg cagaggccga ggccgcctcg gcctctgagc tattccagaa 300

gtagtgagga ggctttttg gaggcctagg cttttgcaaa aagct 345

<210> 46
<211> 481
<212> DNA
<213> Artificial Sequence

<220>
<223> HGH polyA

<400> 46
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ccagtgccca ccagccttgt cctaataaaa ttaagttgca tcattttgtc tgactaggtg 120

tccttctata atattatggg gtggaggggg gtggtatgga gcaaggggca agttgggaag 180

acaacctgta gggcctgcgg ggtctattgg gaaccaagct ggagtgcagt ggcacaatct 240

tggctcactg caatctccgc ctcctgggtt caagcgattc tcctgcctca gcctcccgag 300

ttgttgggat tccaggcatg catgaccagg ctcagctaat ttttgttttt ttggtagaga 360

cggggtttca ccatattggc caggctggtc tccaactcct aatctcaggt gatctaccca 420

ccttggcctc ccaaattgct gggattacag gcgtgaacca ctgctccctt ccctgtcctt 480

t 481