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(54) Title: TREATMENT OF CANCER BY INFUSION OF ONCOLYTIC HERPES SIMPLEX VIRUS TO THE BLOOD

		Th1 response						Pro-inflammatory		Th2 response				
Patient	No. doses	IL-2	IL-12	IFN-γ	IP-10	MIG	TNF-α	IL-6	IL-1α	IL-4	IL-10	IFN-α	VEGF	IL-21
01	1	↓	-	++	+++	+++	++	+	-	-	+	-	↓	-
02	1	↓	↓	-	↓	-	+/↓	↓	+	-	+	-	+++	+
03	1	↓	++	++	+++	+++	+	+	-	-	-/↓	-	+++	↓
04	2	↓	-	+++++	+++	++++	+++	+	-	-	+	-	↓	-
05	2	↓	-	-	-	-	↓	↓	-	-	-/↓	-	++	+
06*	2													
07	4	++	+++	+++++	++++	+++	+	+	-	-	++	-	-	↓
08	4	++	++	+++++	+++	+++	+	+++	-	↓	+++	+	↓	+
09	4	++	++	++	+++	+++	tba	+	tba	+++	+	tba	+++	+

Figure 1

(57) Abstract: An oncolytic herpes simplex virus is disclosed for use in a method of treating cancer in a human subject, the method comprising administering to the human subject at least one dose of oncolytic herpes simplex virus by infusion to the blood, wherein the oncolytic herpes simplex virus reaches cells of the cancer in which it replicates.

WO 2017/013421 A1

Treatment of Cancer by Infusion of Oncolytic Herpes Simplex Virus to the Blood

Field of the Invention

The present invention relates to methods of treatment of human cancers involving administration of an oncolytic herpes simplex virus to a human subject by infusion of the virus to the blood.

Background to the Invention

Oncolytic virotherapy concerns the use of lytic viruses which selectively infect and kill cancer cells. Some oncolytic viruses are promising therapies as they display exquisite selection for replication in cancer cells and their self-limiting propagation within tumors results in fewer toxic side effects. Several oncolytic viruses have shown great promise in the clinic (Bell, J., Oncolytic Viruses: An Approved Product on the Horizon? Mol Ther. 2010; 18(2): 233–234; Russell et al., (Oncolytic Virotherapy. Nature Biotechnology Vol. 30 No.7 July 2012).

Talimogene laherparepvec

After a long period of development, and question marks over the validity of the approach, oncolytic virotherapy has significantly moved forward with the first approval by the US Food and Drug Administration ("FDA") and European Medicines Agency ("EMA") of an oncolytic virus therapy.

The oncolytic virus talimogene laherparepvec (IMLYGIC®) was approved by FDA on 27th October 2015 for the "local treatment of unresectable cutaneous, subcutaneous, and nodal lesions in patients with melanoma recurrent after initial surgery" and by EMA on 16th December 2015 for the "treatment of of adults with unresectable melanoma that is regionally or distantly metastatic (Stage IIIB, IIIC and IVM1a) with no bone, brain, lung or other visceral disease".

Talimogene laherparepvec is an attenuated herpes simplex virus type-1 (HSV-1) derived by functional deletion of two genes (ICP34.5 and ICP47) and insertion of coding sequence for human granulocyte macrophage colony-stimulating factor (GM-CSF) and is produced in Vero cells by recombinant DNA technology.

The safety and efficacy of Imlygic® monotherapy compared with subcutaneously administered GMCSF were evaluated in a phase 3, multinational, open-label, and

randomised clinical study of patients with stage IIIB, IIIC, and IV melanoma that was not considered to be surgically resectable. Previous systemic treatment for melanoma was allowed but not required. Patients with active brain metastases, bone metastases, extensive visceral disease, primary ocular or mucosal melanoma, evidence of immunosuppression, or receiving treatment with a systemic anti-herpetic agent were excluded from the study.

The results of the study are summarised in the EMA's Summary of Product Characteristics, which states "*In an analysis to evaluate systemic activity of Imlygic, 27 of 79 patients (34.2%) had a ≥ 50% overall decrease in non-visceral lesions that were not injected with Imlygic, and 8 of 71 patients (11.3%) had a ≥ 50% overall decrease in visceral lesions that were not injected with Imlygic.*"

Exploratory subgroup analyses for durable response rate (DRR) and overall survival by stage of disease were also carried out. While the pivotal study was not powered to evaluate efficacy in these individual subgroups, patients with no visceral disease derived greater benefit from Imlygic® treatment than those with more advanced disease. The secondary endpoint on overall survival just missed statistical significance.

The labels approved by both FDA and EMA indicate that talimogene laherparepvec is approved for injection into cutaneous, subcutaneous, and/or nodal lesions.

The FDA approval sets out the dosing regime for talimogene laherparepvec, as follows: "*Your healthcare professional will inject Imlygic directly into your tumour(s) with a needle and syringe. Your second injection will be given 3 weeks after the first injection. After that, you will receive injections every 2 weeks for as long as you have the tumour(s). Your healthcare professional will decide which tumour(s) to inject and may not inject every tumour. Your existing tumour(s) may increase in size and new tumour(s) could appear while you are being treated with Imlygic. You can expect to be treated with Imlygic for at least 6 months or longer.*" The EMA approval contains a similar statement.

The dosing schedule for Imlygic® involves an initial injection of a dose of 10^6 pfu/ml into the largest lesion and other lesions are prioritised by lesion size for injection until the maximum injection volume of 4 ml is reached. In the second and subsequent treatments the dose of virus is 10^8 pfu/ml, new lesions are injected first and other lesions are prioritised by lesion size for injection until the maximum injection volume of 4 ml is

reached. In subsequent visits new lesions developing since previous treatments are injected first and other lesions are prioritised by lesion size for injection until the maximum injection volume of 4 ml is reached.

The volume of Imlygic® to be injected into each lesion is dependent on the size of the lesion and is determined according to a table. The total injection volume for each treatment session is a maximum of 4 ml.

Patients may experience increase in size of existing lesion(s) or the appearance of a new lesion prior to achieving a response. As long as there are injectable lesion(s) remaining, Imlygic® should be continued for at least 6 months unless the physician considers that the patient is not benefitting from Imlygic® treatment or that other treatment is required.

Imlygic® treatment may be reinitiated if new lesions appear following a complete response and the physician considers that the patient will benefit from treatment.

The dosing regime involves numerous injections in different locations, which is generally disadvantageous for the patient. Injection site reactions were very common in the Phase 3 study with 27.2% of patients reporting such incidents and included: injection site pain, erythema, haemorrhage, swelling, inflammation, secretion and discharge. In particular, cellulitis and systemic bacterial infection were reported.

During preclinical testing in immunocompetent mice, rats, and dogs, talimogene laherparepvec was administered by subcutaneous, intravenous or intratumoral injection. Talimogene laherparepvec was injected into various xenograft tumours at doses up to 2 x 10^8 PFU/kg in immunodeficient mice (nude and SCID). Lethal systemic viral infection was observed in up to 20% of nude mice (primarily deficient in T lymphocyte function) and 100% of SCID mice (devoid of both T and B lymphocytes). Across the studies, fatal disseminated viral infection was observed in 14% of nude mice following treatment with talimogene laherparepvec at doses that are 10 to 100-fold higher than those that result in 100% lethality with wild-type HSV-1. Imlygic has not been studied in immunocompromised human patients but the animal data indicates that patients who are severely immunocompromised may be at an increased risk of disseminated herpetic infection and the FDA approval indicates that they should not be treated with Imlygic®.

In summary, the pre-clinical and clinical studies of talimogene laherparepvec indicate that it has some measurable clinical benefit in a sub-group of melanoma patients with local disease but no significant benefit in patients with visceral lesions or advanced metastatic disease. It has also failed to statistically establish an overall survival benefit in any group of patients and shows limited evidence of a systemic effect, particularly in late stage and visceral disease. Administration to human patients is exclusively by injection with notable patient reactions and the pre-clinical data indicates an unsuitability for systemic administration.

Experience with HSV1716

In many animal models, oncolytic viruses (including oncolytic herpes simplex viruses) work spectacularly well but repeating the results in human trials has proven challenging. The difficulties in translating biologic agents with complex (and not fully understood) structures and complex mechanisms of action will be apparent and common general knowledge to those skilled in the art. For example, when it comes to developing treatments for human patients that involve directed stimulation of parts of the human immune system studies of the effect of oncolytic virus on the immune response in mice (and other non-human animals) must be treated with caution owing to acknowledged differences in immune response between mouse models and humans (Mestas and Hughes., J Immunol 2004; 172:2731-2738).

HSV1716 has been in clinical development for a number of years. Clinical studies have investigated safety and mechanism of action of direct intra-tumoral administration for the treatment of localised brain tumors, melanoma, head and neck cancer. These studies have demonstrated some proof of safety and mechanism of action in tumors in which the agent was directly administered by injection but no evidence of systemic benefit in uninjected lesions (as seen in animal models) has been observed.

Local intra-tumoral injection is relatively straight-forward in certain types of tumor such as some head and neck cancers and melanomas, which accounts for the preponderance of clinical investigations with oncolytic viruses in those indications. In other indications the treatment of local disease requires considerable resource and skill to place the agent into active regions of tumor (discussed below). Brain tumors, deep-seated visceral tumors and multiple active metastases pose considerable barriers to successful intratumoral administration and attempts to treat such tumors are often accompanied by significant safety and technical issues, cost and resource requirements.

As reported in the Imlygic® data, the proposed use of intra-tumoral injection of an augmented oncolytic HSV expressing a cytokine (GM-CSF) to create a systemic therapy had limited success in advanced or visceral disease – even in melanoma where multiple intra-tumoral injections are relatively straight forward to carry out.

The optimal dose and route of administration, particularly for advanced, metastatic or visceral disease, remains a problem in the art of oncolytic viruses.

Mace et al (Potential for efficacy of the oncolytic herpes simplex virus 1716 in patients with oral squamous cell carcinoma. Head Neck, 2008 Aug; 30(8):1045-51) utilized direct intratumoral injection of herpes simplex virus HSV1716 in a Phase I study designed to investigate safety and mechanism of action of HSV716 when injected up to 14 days prior to planned resection of the tumor. On page 1050 Mace et al state: "*In conclusion, intratumoral injection of HSV1716 at a dose of 10^5 pfu or $5x10^5$ pfu into oral SCC is safe and well tolerated but with little biological activity. As has been seen with other oncolytic viruses, the effective application is not as straightforward as laboratory studies might have indicated. The principal problem areas involve optimizing the dose, delivery and distribution of HSV1716 into a dense heterogeneous SCC tumor cell matrix. Increasing our knowledge of the interactions between HSV1716, the HNSCC tumor cell, and the immune system will help to optimize antitumor efficacy*".

As stated by Mace et al, the question remains – how to optimise methods of treatment of cancer using oncolytic virus, particularly oncolytic herpes simplex viruses. These questions and challenges are also discussed by Russell et al (Oncolytic Virotherapy. Nature Biotechnology Vol. 30 No.7 July 2012) and in Seymour and Fisher (British Journal of Cancer (2016) 114, 357-361).

Systemic administration of herpes simplex virus has been seen as problematic outside of model systems in the laboratory. The main barriers to overcome being considered as (i) non-specificity of HSV-1 binding and associated losses to non-target tissues; (ii) the presence of circulating immune cells considered likely to neutralise the HSV; and (iii) the inability to detect HSV in the circulation immediately following IV infusion. These problems are discussed in Russell et al., (Oncolytic virotherapy. Nature Biotechnology Vol. 30 No.7 July 2012) which discusses the challenges presented by administration of virus to the blood in terms of neutralization of the virus by serum factors, sequestration of

virus by the mononuclear phagocytic system and lack of extravasation. Seymour and Fisher (British Journal of Cancer (2016) 114, 357-361) also discuss the challenges of translating the promise of oncolytic viral therapy to success via systemic delivery.

Ongoing clinical studies conducted by the Applicant have for the first time investigated regional and systemic delivery of HSV1716 in human patients. These studies in mesothelioma and in advanced pediatric solid tumors have provided new data and insight which have led to the invention described herein.

Clinical trial NCT00931931 is the first clinical study designed to investigate intravenous infusion of an oncolytic herpes simplex virus. This is a Phase 1 trial investigating the treatment of non-central nervous system solid tumors.

The study was originally established to deliver HSV1716 by direct injection into deep-seated tumors guided by imaging in an interventional radiology suite. Patients eligible for the study had disease refractory to all established treatments and presented with large tumors and/or extensive metastatic disease. Despite the skill of the interventional radiologists, intra-tumoral injection had limited application to patients with such large tumor burden or advanced metastatic disease. An amendment to the study was sought in order to investigate, for the first time, whether systemic delivery of HSV1716 was feasible, safe and had any biological effect in this patient group.

Head and Neck Cancer

Squamous cell carcinoma of the head and neck (hereafter "head and neck cancer" or "HNSCC") is the most common malignancy (90%) of the upper aero-digestive tract. Head and neck cancer is the sixth most common malignancy worldwide and results in approx. 350,000 deaths per year. The incidence of HNSCC has increased over the past 10 years due to increasing prevalence of of human papillomavirus (HPV). There are >45,000 estimated new US cases reported each year (American Cancer Society, 2015) and >375,000 new cases worldwide (Globocan, 2012).

Intratumoral injection of the herpes simplex virus talimogene laherparepvec (IMLYGIC®) was also investigated in a Phase III study in head and neck cancer in combination with cisplatin and radiation (Clinicaltrials.gov identifier NCT01161498) but the study was terminated prior to completion and its results have not been reported. The agent was

subsequently approved for the treatment of melanoma by the FDA based on a protocol of direct intratumoral injection.

Intratumoral injection of herpes simplex viruses in clinical studies for the treatment of head and neck cancer has therefore led to disappointing results. The development of an appropriate strategy for the use of oncolytic herpes simplex virus in order to provide an effective treatment for Stage III or Stage IV head and neck cancer is an outstanding problem in the field.

Immune Response

Cancer cells normally acquire antigenicity that can be recognised by the adaptive immune system as non-self and lead to generation of an immune response involving proliferation of antigen-specific lymphocytes. Tumors are now established to co-opt certain immune checkpoint pathways as a mechanism of immune resistance against T-cells specific for tumor antigens (Drew M. Pardoll., The blockade of immune checkpoints in cancer immunotherapy. Nature Reviews Cancer Vol. 12 April 2012 252-264).

Tumor infiltration with M2-phenotype macrophages and myeloid derived suppressor cells (MDSC) promotes tumor progression whereas infiltration of memory cytotoxic T cells and T helper 1 (Th1) T lymphocytes are often associated with a good clinical outcome and good response to immunotherapy. Therefore, one aim of immunotherapies is to modify the context of immune, inflammatory and angiogenic elements to favour a strong Th1 cytotoxic microenvironment (Giraldo et al., The immune contexture of primary and metastatic human tumors. Current Opinion in Immunology 2014, 27:8-15).

Haabeth et al (Inflammation driven by tumor-specific Th1 cells protects against B-cell cancer. Nature Communications. 2:240 15 Mar 2011 [DOI:10.1038/ncomms1239]) also report that successful cancer immunosurveillance mediated by tumor-specific $CD4^+$ T cells is consistently associated with elevated levels of both proinflammatory (IL-1α, IL-1β and IL-6) and Th1 associated (interferon-γ (IFN-γ), IL-2 and IL-12) cytokines. They describe cancer eradication as a collaboration between tumor specific Th1 cells and tumor infiltrating, antigen presenting macrophages in which Th1 cells induce secretion of IL-1β and IL-6 by macrophages and Th1 associated IFN-γ renders macrophages directly cytotoxic to cancer cells and causes them to secrete the angiostatic chemokines CXCL9/MIG (monokine induced by IFN-γ) and CXCL10/IP-10 (IFN-γ inducible protein 10), and conclude that inflammation driven by tumor specific Th1 cells may prevent rather

than promote cancer. Haabeth et al also report a panel of nine cytokines consistently associated with successful cancer immunosurveillance, including proinflammatory (IL-1α, IL-1β and IL-6) and Th1 associated (IL-2, IL-3, IL-12, IFN-γ, CXCL9 and CXCL10) cytokines.

Whilst the pattern of cytokine production that characterises the T helper type 1 (Th1) and type 2 (Th2) responses is reasonably well understood, it is much less clear how the direction of differentiation towards Th1 or Th2 is decided.

Herpes simplex virus type 1 has evolved several strategies to evade the production and function of interferons (IFNs) and cytokines generated by the innate immune system (which plays a role in activation of the adaptive immune response). HSV counteracts the production of IFN, diminishes IFN-signalling, and blocks the actions of PKR and activation of the 2'-5' A system through several viral products, including ICP0, ICP27, ICP34.5 and vhs (Melchjorsen et al., Activation and Evasion of Innate Antiviral Immunity by Herpes Simplex Virus. *Viruses* 2009, *1*, 737-759; doi:10.3390/v1030737; L. Aurelian., Herpes Simplex Virus Type 2 Vaccines: New Ground for Optimism? Clinical and Diagnostic Laboratory Immunology, May 2004, p. 437–445).

Young J Kim (Subverting the adaptive immune resistance mechanism to improve clinical responses to immune checkpoint blockade therapy. OncoImmunology 3:12 e954868; December 2014) describes a mechanistic and clinical rationale for combining IFN-γ Th1 inducing cancer vaccines with the blockade of the immune checkpoint protein Programmed cell death 1 (PD-1, also called CD279), and indicates that strategies to increase tumor-infiltrating cytotoxic T lymphocyte anticancer activities with immune checkpoint inhibitors may convert anti-PD-1 blockade non-responders to responders, thereby circumventing immune evasion.

Sagiv-Barfi et al reported that ibrutinib, an inhibitor of ITK, an essential enzyme in Th2 T cells can shift the balance between Th1 and Th2 T cells towards Th1 and thereby enhance anti-tumor responses. They describe the combination of anti-PD-L1 antibody and ibrutinib to suppress tumor growth in mouse models of lymphoma that are intrinsically insensitive to ibrutinib, as well as in models of breast and colon cancer (Sagiv-Barfi et al., Therapeutic antitumor immunity by checkpoint blockade is enhanced by ibrutinib, an inhibitor of both BTK and ITK. PNAS E966-972 Published online February 17, 2015).

Accordingly, there are advantages for treatment of tumors associated with promoting a Th1 response in the tumor microenvironment, and further ways of establishing such a treatment-favourable environment are required.

WO2014/036412 describes the treatment of stage IIIb to stage IV melanoma by a method comprising administering to a patient with stages IIIb to IV melanoma an effective amount of an immune checkpoint inhibitor and a herpes simplex virus, wherein the herpes simplex virus lacks functional ICP34.5 genes, lacks a functional ICP47 gene and comprises a gene encoding human GM-CSF.

Summary of the Invention

The inventors have shown that following infusion of oncolytic herpes simplex virus to the blood of a human subject having a cancer the virus is undetectable in the blood after about 24 hours, which is consistent with proposed mechanisms of viral neutralization and sequestration discussed above. However, after about 72 hours in some subjects viral DNA is detectable again in the blood. This observation and accompanying tumor imaging observations indicate that the virus has reached cancer cells in the subject, has infected those cells and replicated. Lysis of cancer cells is consistent with release of viral particles and fragments, including viral DNA, into the blood which may then be detected.

These remarkable observations provide the first indication that cancer cells can be treated in human subjects at a location distant to the site of administration by a mechanism that involves the virus reaching those cancer cells, e.g. in which the virus infects, replicates in and lyses the cancer cells, rather than relying on induction of an immune response to treat cancer cells not directly injected with virus.
These findings open the door to treatment of cancers which are not suitable for direct injection with virus, such as cancers occurring in the internal organs, cancers occurring at multiple sites, and metastatic cancers.

The treatment of cancer in human subjects may therefore be effected by infusion of a therapeutically effective amount of oncolytic herpes simplex virus to the subject's blood. Methods of treatment may further involve taking of blood or tumor tissue samples to determine the presence of herpes simplex virus and provide an indicator that virus has reached cells of the cancer.

In one aspect of the present invention an oncolytic herpes simplex virus for use in a method of treating cancer in a human subject is provided, the method comprising administering to the human subject at least one dose of oncolytic herpes simplex virus by infusion to the blood, wherein the oncolytic herpes simplex virus reaches cells of the cancer in which it replicates.

In another aspect of the present invention the use of an oncolytic herpes simplex virus in the manufacture of a medicament for use in a method of treating cancer in a human subject is provided, the method comprising administering to the human subject at least one dose of oncolytic herpes simplex virus by infusion to the blood, wherein the oncolytic herpes simplex virus reaches cells of the cancer in which it replicates.

In another aspect of the present invention a method of treating cancer in a human subject in need of treatment is provided, the method comprising administering to the human subject at least one dose of oncolytic herpes simplex virus by infusion to the blood, wherein the oncolytic herpes simplex virus reaches cells of the cancer in which it replicates.

In some embodiments, upon infusion oncolytic herpes simplex is absorbed by cells or is neutralised, but is able to reach cancer cells where it infects, replicates and lyses cancer cells, lysis of cancer cells releasing viral DNA which is detectable in the blood. In some embodiments, oncolytic herpes simplex virus DNA is not detectable in a sample of the subject's blood within about 24 hours of infusion, but is detectable in a sample of the subject's blood taken after one of about 72 hours, about 96 hours, about 120 hours, about 144 hours, about 168 hours, about 2 weeks, about 3 weeks or about 4 weeks. In some embodiments, the method comprises determining whether oncolytic herpes simplex virus DNA is present in a sample of the subject's blood. The method may comprise taking a blood sample from the subject, and determining whether herpes simplex virus DNA is present in the blood sample.

In some embodiments, the method comprises determining whether oncolytic herpes simplex virus is present in a sample of the subject's tumor tissue. The method may comprise taking a sample of tumor tissue from the subject and determining whether oncolytic herpes simplex virus is present in a sample of the subject's tumor tissue.

The oncolytic herpes simplex virus may be a mutant of HSV-1 strain 17 or is HSV1716. The cancer may be a solid tumor, a recurrent or metastatic solid tumor, or a non-CNS solid tumor.

The dose of oncolytic herpes simplex virus administered may be at least $1x10^6$ iu.

In some embodiments, a dose of oncolytic herpes simplex virus is administered over a period of 3 hours or less.

In some embodiments, the administered oncolytic herpes simplex virus is formulated as about 0.5ml to about 5ml of a suspension of virus in about 200ml to about 300ml of lactated Ringer's solution.

In some embodiments, the method comprises administering to the human subject at least one treatment cycle of oncolytic herpes simplex virus, wherein a treatment cycle comprises, or consists, of at least two doses of oncolytic herpes simplex virus, each dose administered by infusion to the blood wherein the second and subsequent doses are each administered within about 17 days of the preceding dose, each dose of oncolytic herpes simplex virus being in the range about $1x10^6$ iu to about $1x10^8$ iu. In some embodiments, one dose of oncolytic herpes simplex virus is administered per week. In some other embodiments, two doses of oncolytic herpes simplex virus are administered per week. Each dose of oncolytic herpes simplex virus may be in the range about 1 x 10^7 iu to about 1 x 10^8 iu. The treatment cycle may further comprise, or consist of, administration of a therapeutically effective amount of an immune checkpoint inhibitor which may be selected from the group consisting of an inhibitor of PD-1, PD-L1, CTLA4, TIM-3 or LAG-3. In some embodiments, the subject may receive two or more treatment cycles.
In some embodiments, the method comprises determining the presence of a Th1 response in the subject. In some embodiments, the level of IL-2 and/or IL-12 and/or IFN-γ in the subject's blood may be upregulated for more than 7 days after one or more treatment cycles.

In another aspect of the present invention an oncolytic herpes simplex virus for use in a method of treating cancer in a human subject is provided, the method comprising administering to the human subject at least one treatment cycle of oncolytic herpes simplex virus, wherein a treatment cycle comprises, or consists, of at least two doses of

oncolytic herpes simplex virus, each dose administered by infusion to the blood wherein the second and subsequent doses are each administered within about 17 days of the preceding dose, each dose of oncolytic herpes simplex virus being in the range about $1x10^6$ iu to about $1x10^8$ iu.

In another aspect of the present invention the use of an oncolytic herpes simplex in the manufacture of a medicament for us in a method of treating cancer in a human subject is provided, the method comprising administering to the human subject at least one treatment cycle of oncolytic herpes simplex virus, wherein a treatment cycle comprises, or consists, of at least two doses of oncolytic herpes simplex virus, each dose administered by infusion to the blood wherein the second and subsequent doses are each administered within about 17 days of the preceding dose, each dose of oncolytic herpes simplex virus being in the range about $1x10^6$ iu to about $1x10^8$ iu.

In another aspect of the present invention a method of treating cancer in a human subject in need of treatment is provided, the method comprising administering to the human subject at least one treatment cycle of oncolytic herpes simplex virus, wherein a treatment cycle comprises, or consists, of at least two doses of oncolytic herpes simplex virus, each dose administered by infusion to the blood wherein the second and subsequent doses are each administered within about 17 days of the preceding dose, each dose of oncolytic herpes simplex virus being in the range about $1x10^6$ iu to about $1x10^8$ iu.

A treatment cycle may comprise 2 doses, more than 2 doses, up to 4 doses, 4 doses, up to 6 doses, 6 doses, up to 8 doses, or 8 doses, up to 10 doses, or 10 doses, of oncolytic herpes simplex virus.

The second and subsequent doses may be administered within about 14 (e.g. 14±1, 14±2, 14±3, or 14±4) or about 7 (e.g. 7±1 or 7±2) days of the preceding dose. In some embodiments one dose of oncolytic herpes simplex virus may be administered per week. Administration of each weekly dose of oncolytic herpes simplex virus may be separated by 7±1 or 7±2 days. In some embodiments two doses of oncolytic herpes simplex virus may be administered per week. Administration of each twice weekly dose of oncolytic herpes simplex virus may be separated by 4±1 or 4±2 days.

In some embodiments each dose of oncolytic herpes simplex virus may be in the range about 1 x 10^7 iu to about 1 x 10^8 iu.

The subject may receive administration of one or a plurality (preferably a plurality) of doses of herpes simplex virus. Each dose of herpes simplex virus is preferably in the range 1x10^6 iu to 1x10^8 iu, and may be 2x10^6 iu or greater than 2x10^6 iu. Doses may be in a range selected from the group consisting of: 2x10^6 to 9x10^6 iu, 2x10^6 to 5x10^6 iu, 5x10^6 to 9x10^6 iu, 2x10^6 to 1x10^7 iu, 2x10^6 to 5x10^7 iu, 2x10^6 to 1x10^8 iu, 2x10^6 to 5x10^8 iu, 2x10^6 to 1x10^9 iu, 5x10^6 to 1x10^7 iu, 5x10^6 to 5x10^7 iu, 5x10^6 to 1x10^8 iu, 5x10^6 to 5x10^8 iu, 5x10^6 to 1x10^9 iu, 5x10^6 to 5x10^9 iu, 1x10^7 to 9x10^7 iu, 1x10^7 to 5x10^7 iu, 1x10^8 to 9x10^8 iu, 1x10^8 to 5x10^8 iu. In some embodiments suitable doses may be in the range 2x10^6 to 9x10^6 iu, 1x10^7 to 9 x10^7 iu, or 1x10^8 to 9 x10^8 iu. In some embodiments suitable doses may be about 1x10^7 iu or about 1x10^8 iu. Dosage figures may optionally be +/- half a log value.

In some embodiments the treatment cycle further comprises, or consists, of administration of a therapeutically effective amount of an immune checkpoint inhibitor.

In some embodiments the treatment cycle of oncolytic herpes simplex virus, comprises, or consists, of:

(i) four doses of oncolytic herpes simplex virus over a period of about four weeks, one dose administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu; or

(ii) four doses of oncolytic herpes simplex virus over a period of about two weeks, two doses administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu.

As such, in one aspect of the present invention an oncolytic herpes simplex virus for use in a method of treating cancer in a human subject is provided, the method comprising administering to the human subject at least one treatment cycle of oncolytic herpes simplex virus, wherein a treatment cycle comprises, or consists, of:

(i) four doses of oncolytic herpes simplex virus over a period of about four weeks, one dose administered per week by infusion to the blood, each dose of

oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu; or

(ii) four doses of oncolytic herpes simplex virus over a period of about two weeks, two doses administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu.

In another aspect of the present invention the use of an oncolytic herpes simplex in the manufacture of a medicament for us in a method of treating cancer in a human subject is provided, the method comprising administering to the human subject at least one treatment cycle of oncolytic herpes simplex virus, wherein a treatment cycle comprises, or consists, of:

(i) four doses of oncolytic herpes simplex virus over a period of about four weeks, one dose administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu; or

(ii) four doses of oncolytic herpes simplex virus over a period of about two weeks, two doses administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu.

In another aspect of the present invention a method of treating cancer in a human subject in need of treatment is provided, the method comprising administering to the human subject at least one treatment cycle of oncolytic herpes simplex virus, wherein a treatment cycle comprises, or consists, of:

(i) four doses of oncolytic herpes simplex virus over a period of about four weeks, one dose administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu; or

(ii) four doses of oncolytic herpes simplex virus over a period of about two weeks, two doses administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu.

In another aspect of the present invention an oncolytic herpes simplex virus for use in a method of treating cancer in a human subject is provided, the method comprising

administering to the human subject an oncolytic herpes simplex virus and a therapeutically effective amount of an immune checkpoint inhibitor, wherein the method comprises at least one treatment cycle of oncolytic herpes simplex virus comprising, or consisting, of:

(i) four doses of oncolytic herpes simplex virus over a period of about four weeks, one dose administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu; or

(ii) four doses of oncolytic herpes simplex virus over a period of about two weeks, two doses administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu.

In another aspect of the present invention the use of an oncolytic herpes simplex in the manufacture of a medicament for us in a method of treating cancer in a human subject is provided, the method comprising administering to the human subject an oncolytic herpes simplex virus and a therapeutically effective amount of an immune checkpoint inhibitor, wherein the method comprises at least one treatment cycle of oncolytic herpes simplex virus comprising, or consisting, of:

(i) four doses of oncolytic herpes simplex virus over a period of about four weeks, one dose administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu; or

(ii) four doses of oncolytic herpes simplex virus over a period of about two weeks, two doses administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu.

In another aspect of the present invention a method of treating cancer in a human subject in need of treatment is provided, the method comprising administering to the human subject an oncolytic herpes simplex virus and a therapeutically effective amount of an immune checkpoint inhibitor, wherein the method comprises at least one treatment cycle of oncolytic herpes simplex virus comprising, or consisting, of:

(i) four doses of oncolytic herpes simplex virus over a period of about four weeks, one dose administered per week by infusion to the blood, each dose of

oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu; or

(ii) four doses of oncolytic herpes simplex virus over a period of about two weeks, two doses administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu.

In some embodiments administration of each weekly dose of oncolytic herpes simplex virus in is separated by 7±1 or 7±2 days. In some embodiments administration of each twice weekly dose of oncolytic herpes simplex virus is separated by 4±1 or 4±2 days.

In some embodiments the method comprises administering an immune checkpoint inhibitor according to at least one treatment cycle of immune checkpoint inhibitor, wherein the periods of time of the oncolytic herpes simplex treatment cycle and immune checkpoint inhibitor treatment cycle overlap. In some embodiments the method comprises at least one treatment cycle of immune checkpoint inhibitor comprising, or consisting, of 1 or 2 doses of immune checkpoint inhibitor administered within a period of about 3 or 4 weeks. In some embodiments the method comprises at least one treatment cycle of immune checkpoint inhibitor comprising, or consisting, of a period of about 3 weeks in which 1 dose of immune checkpoint inhibitor is administered. In some embodiments the method comprises at least one treatment cycle of immune checkpoint inhibitor comprising, or consisting, of 1 dose of immune checkpoint inhibitor administered about every 3 weeks. In some embodiments the method comprises at least two treatment cycles of oncolytic herpes simplex virus according to (i) and at least two treatment cycles of immune checkpoint inhibitor each comprising, or consisting, of a period of about 3 weeks in which 1 dose of immune checkpoint inhibitor is administered, wherein the periods of time of the first treatment cycle of oncolytic herpes simplex virus and first treatment cycle of immune checkpoint inhibitor overlap. In some embodiments the method comprises at least two treatment cycles of oncolytic herpes simplex virus according to (ii) and a treatment cycle of immune checkpoint inhibitor comprising, or consisting, of a period of about 3 weeks in which 1 dose of immune checkpoint inhibitor is administered, wherein the periods of time of the treatment cycles of oncolytic herpes simplex virus and treatment cycle of immune checkpoint inhibitor overlap.

The immune checkpoint inhibitor may be an inhibitor of at least one of PD-1, PD-L1, CTLA4, TIM-3 or LAG-3. The immune checkpoint inhibitor may be selected from an anti-

PD-1 antibody, anti-PD-L1 antibody, anti-CTLA4 antibody, anti-TIM-3 antibody or anti-LAG-3 antibody. The immune checkpoint inhibitor may be selected from the group consisting of pembrolizumab, nivolumab or ipilumab.

In another aspect of the present invention an oncolytic herpes simplex virus for use in a method of treating cancer in a human subject is provided, the method comprising administering:

a herpes simplex virus by intravenous infusion at a dose of greater than $2x10^6$ iu at day 1 of week 1 followed by a dose of greater than $2x10^6$ iu at day 1 of week 2, and every week thereafter until surgery is scheduled, toxicity or disease progression, a predetermined maximum number of doses is reached, a fixed number of doses is reached, complete response, disease progression, or intolerance of the treatment, wherein the herpes simplex virus lacks functional ICP34.5 genes; and

an immune checkpoint inhibitor by intravenous infusion every 3 weeks for at least 3 infusions beginning with or after the second or third dose of the herpes simplex virus.

In another aspect of the present invention the use of an oncolytic herpes simplex in the manufacture of a medicament for us in a method of treating cancer in a human subject is provided, the method comprising administering:

a herpes simplex virus by intravenous infusion at a dose of greater than $2x10^6$ iu at day 1 of week 1 followed by a dose of greater than $2x10^6$ iu at day 1 of week 2, and every week thereafter until surgery is scheduled, toxicity or disease progression, a predetermined maximum number of doses is reached, a fixed number of doses is reached, complete response, disease progression, or intolerance of the treatment, wherein the herpes simplex virus lacks functional ICP34.5 genes; and

an immune checkpoint inhibitor by intravenous infusion every 3 weeks for at least 3 infusions beginning with or after the second or third dose of the herpes simplex virus.

In another aspect of the present invention a method for the treatment of cancer is provided, the method comprising administering:

a herpes simplex virus by intravenous infusion at a dose of greater than $2x10^6$ iu at day 1 of week 1 followed by a dose of greater than $2x10^6$ iu at day 1 of week 2, and every week thereafter until surgery is scheduled, toxicity or disease progression, a predetermined maximum number of doses is reached, a fixed number of doses is reached, complete response, disease progression, or intolerance of the treatment, wherein the herpes simplex virus lacks functional ICP34.5 genes; and

an immune checkpoint inhibitor by intravenous infusion every 3 weeks for at least 3 infusions beginning with or after the second or third dose of the herpes simplex virus.

In some embodiments a dose of oncolytic herpes simplex virus is administered over a period of 3 hours or less.

In some embodiments the administered oncolytic herpes simplex virus is formulated as about 0.5ml to about 5ml of a suspension of virus in about 200ml to about 300ml of lactated Ringer's solution. In some embodiments the administered oncolytic herpes simplex virus is formulated as about 1.0ml of a suspension of virus in about 250ml of lactated Ringer's solution.

The subject may receive two or more treatment cycles of oncolytic herpes simplex virus, which may be consecutive or each treatment cycle may be separated by a break from treatment. A break from treatment may be about 1, 2, 3, or 4 weeks.

The first 1, 2 or 3 treatment cycles of oncolytic herpes simplex virus may comprise administration of a dose of oncolytic herpes simplex virus that is lower than the dose administered in later treatment cycles.

In some embodiments the method may further comprise determining the presence of a Th1 response in the subject. The method may comprise measuring the level of IFNγ, IL-2 and/or IL-12 in a sample obtained from a subject. The level of IL-2 and/or IL-12 and/or IFN-γ in the subject's blood may be upregulated for more than 7 days after one or more treatment cycles.

In some embodiments all copies of the ICP34.5 gene in the genome of the herpes simplex virus are modified such that the ICP34.5 gene is incapable of expressing a functional ICP34.5 gene product. As such the herpes simplex virus may be an ICP34.5 null mutant.

In some embodiments one or both of the ICP34.5 genes in the genome of the herpes simplex virus are modified such that the ICP34.5 gene is incapable of expressing a functional ICP34.5 gene product.

In some embodiments the herpes simplex virus is a mutant of HSV-1 strain 17. In preferred embodiments the herpes simplex virus is HSV1716 (ECACC Accession No. V92012803). HSV1716 is also called SEPREHVIR®. In some embodiments the herpes simplex virus is a mutant of HSV-1 strain 17 mutant 1716.

The cancer to be treated may be a solid tumor, a recurrent or metastatic tumor, a recurrent or metastatic solid tumor, a non-CNS tumor, or a non-CNS solid tumor. In some embodiments the cancer is not a melanoma.

In some embodiments human subject may be a child.

In some embodiments the oncolytic herpes simplex virus does not encode (or is not further modified to contain nucleic acid encoding) a cytokine or chemokine, an interleukin, an interferon, a tumor necrosis factor, a colony stimulating factor, an immune modulator, a member of the CC family, a member of the CXC family or a member of the CXC family. In some embodiments the oncolytic herpes simplex virus does not express GMCSF. In some embodiments the oncolytic herpes simplex virus encodes a functional ICP47 and/or ICP6 gene.

In another aspect of the present invention a kit comprising a herpes simplex virus lacking functional ICP34.5 genes, and a package insert or label with directions to treat cancer by using a combination of the herpes simplex virus and an immune checkpoint inhibitor is provided.

The directions may comprise instructions to administer to a human subject with cancer at least one treatment cycle of oncolytic herpes simplex virus, wherein a treatment cycle comprises, or consists, of at least two doses of oncolytic herpes simplex virus, each dose administered by infusion to the blood wherein the second and subsequent doses are each administered within about 17 days of the preceding dose, each dose of oncolytic herpes simplex virus being in the range about $1x10^6$ iu to about $1x10^8$ iu.

The directions may comprise instructions to administer to a human subject with cancer at least one treatment cycle of oncolytic herpes simplex virus, wherein a treatment cycle comprises, or consists, of:

(i) four doses of oncolytic herpes simplex virus over a period of about four weeks, one dose administered per week by infusion to the blood, each dose of

oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu; or

(ii) four doses of oncolytic herpes simplex virus over a period of about two weeks, two doses administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu.

The directions may comprise instructions to administer to a human subject with cancer an oncolytic herpes simplex virus and a therapeutically effective amount of an immune checkpoint inhibitor, wherein the instructions for administration of the oncolytic herpes simplex virus comprise instructions to administer at least one treatment cycle of oncolytic herpes simplex virus comprising, or consisting, of:

(i) four doses of oncolytic herpes simplex virus over a period of about four weeks, one dose administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu; or

(ii) four doses of oncolytic herpes simplex virus over a period of about two weeks, two doses administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu.

The directions may comprise instructions to administer to a patient with cancer a herpes simplex virus administered by intravenous infusion at a dose of greater than $2x10^6$ iu at day 1 of week 1 followed by a dose of greater than $2x10^6$ iu at day 1 of week 2, and every week thereafter until surgery is scheduled, toxicity or disease progression, a predetermined maximum number of doses is reached, a fixed number of doses is reached, complete response, disease progression, or intolerance of the treatment; and an immune checkpoint inhibitor administered intravenously every 3 weeks for at least 3 infusions beginning with or after the second or third dose of the herpes simplex virus.

A method of manufacturing the kit is also provided.

In another aspect of the present invention a method of promoting a combination treatment comprising a herpes simplex virus lacking functional ICP34.5 genes and an immune checkpoint inhibitor, for the treatment of a human patient with cancer, is provided.

In some embodiments the promotion is by a package insert, wherein the package insert provides instructions to receive cancer treatment with a herpes simplex virus in combination with an immune checkpoint inhibitor. In some embodiments the promotion is by a package insert accompanying a formulation comprising the herpes simplex virus. In some embodiments the promotion is by written communication to a physician or health care provider. In some embodiments the promotion is by oral communication to a physician or health care provider. In some embodiments the promotion is followed by the treatment of the patient with the herpes simplex virus.

In another aspect of the present invention a method of instructing a human patient with cancer by providing instructions to receive a combination treatment with a herpes simplex virus lacking functional ICP34.5 genes and an immune checkpoint inhibitor to extend survival of the patient, is provided.

Doses of herpes simplex virus are preferably administered by intravenous infusion, which may take place over a period of several hours, e.g. about 30 minutes to about 3 or about 4 hours. For example, Infusion of a dose of herpes simplex virus may take place over about 1 hour, about 2 hours or about 3 hours.

A subject will commonly receive a plurality of doses of herpes simplex virus as part of a course of treatment, preferably 3 or more doses. The doses may be administered in accordance with a dosing regime. For example, each dose of herpes simplex virus may be administered within 1 to 7, 1 to 14, or 1 to 21 days of the preceding dose.

Doses of herpes simplex virus may be administered at regular intervals, e.g. every 7 days, every 14 days, every 21 days, or every 28 days (+/- 1, 2 or 3 days). The number of doses of herpes simplex virus administered in a course of treatment may be any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 doses or more, preferably at least 3 or at least 4 doses, or up to 8 doses.

A dosing regime for a herpes simplex virus may be designed to continue dosing until: surgery is scheduled, toxicity or disease progression, a predetermined maximum number of doses is reached, e.g. 3, 4, 5, 6, 7, 8, 9 or 10 doses, or a fixed number of doses is reached, e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 doses, complete response, disease progression, or intolerance of the treatment.

The immune checkpoint inhibitor may be administered intravenously. The subject may receive a single intravenous administration of a combined preparation of herpes simplex virus and immune checkpoint inhibitor, or may receive separate intravenous administrations of herpes simplex virus and immune checkpoint inhibitor. Herpes simplex virus and immune checkpoint inhibitor may be administered on the same day, e.g. during the same hospital visit, or on separate days.

A subject may receive a plurality of doses of immune checkpoint inhibitor during the course of treatment. The doses may be administered in accordance with a dosing regime. For example, each dose of immune checkpoint inhibitor may be administered within 1 to 21 or 1 to 28 days of the preceding dose. Doses of immune checkpoint inhibitor may be administered at regular intervals, e.g. every 7 days, every 14 days, every 21 days, or every 28 days (+/- 1, 2 or 3 days).

Suitable doses of immune checkpoint inhibitor will vary depending on the immune checkpoint inhibitor selected and the prescribing information of the medical practitioner. By way of example, suitable doses may be one of 100mg, 200mg, 300mg or 400mg, or 1mg/kg, 2mg/kg, 3mg/kg or 4mg/kg.

Doses of immune checkpoint inhibitor may be administered by intravenous infusion, which may take place over a period of up to several hours, e.g. about 30 minutes to about 3 or about 4 hours. For example, Infusion of a dose of immune checkpoint inhibitor may take place over about 1 hour, about 2 hours or about 3 hours.

By way of example, pembrolizumab may be administered by intravenous infusion once every 3 weeks, optionally at a dose of about 200mg. In another example, pembrolizumab may be administered by intravenous infusion once every week, optionally at a dose of about 200mg, and optionally in combination with chemotherapy, e.g. in the form of platinum and/or 5-fluoruracil.

A combined dosing regime for a herpes simplex virus and immune checkpoint inhibitor may be designed to continue dosing until: toxicity or disease progression, a predetermined maximum number of doses of one or both agents is reached, e.g. 3, 4, 5, 6, 7, 8, 9 or 10 doses, a fixed number of doses of one or both agents is reached, e.g. 3, 4, 5, 6, 7, 8, 9, or 10 doses, complete response, disease progression, or intolerance of the treatment.

In some embodiments herpes simplex virus may be administered more regularly than the immune checkpoint inhibitor. Administration of herpes simplex virus may optionally precede, i.e. commence before, administration of the immune checkpoint inhibitor. For example, herpes simplex virus may be administered once weekly, whereas the immune checkpoint inhibitor may be administered once every 2, 3 or 4 weeks, optionally commencing in the first, second or third week of treatment. The dosing schedule may be designed such that administration of herpes simplex virus is on the same day as administration of the immune checkpoint inhibitor.

The immune checkpoint inhibitor may be an inhibitor of at least one of PD-1, PD-L1, CTLA4, TIM-3 or LAG-3. The immune checkpoint inhibitor may be an anti-PD-1 antibody, anti-PD-L1 antibody, anti-CTLA4 antibody, anti-TIM-3 antibody or anti-LAG-3 antibody. The immune checkpoint inhibitor may be one of pembrolizumab, nivolumab or ipilimumab.

In one aspect of the present invention a pharmaceutical composition comprising a herpes simplex virus and an immune checkpoint inhibitor is provided. The herpes simplex virus may be a mutant of HSV-1 strain 17. In preferred embodiments the herpes simplex virus is HSV1716.

In one aspect of the present invention a kit is provided, the kit comprising a predetermined amount of herpes simplex virus and a predetermined amount of an immune checkpoint inhibitor. The herpes simplex virus may be a mutant of HSV-1 strain 17. In preferred embodiments the herpes simplex virus is HSV1716. The kit may be provided together with instructions for the administration of the herpes simplex virus, and immune checkpoint inhibitor sequentially or simultaneously in order to provide a treatment for cancer.

In one aspect of the present invention products are provided containing therapeutically effective amounts of:

(i) HSV1716, and

(ii) an immune checkpoint inhibitor

for simultaneous or sequential use in a method of medical treatment, preferably treatment of cancer. The products may be pharmaceutically acceptable formulations and may optionally be formulated as a combined preparation for coadministration.

Optionally, in some embodiments the herpes simplex virus does not express GMCSF. Optionally, in some embodiments the herpes simplex virus encodes a functional ICP47 gene. Optionally, in some embodiments the herpes simplex virus is not a herpes simplex virus that lacks functional ICP34.5 genes and lacks a functional ICP47 gene and comprises a gene encoding human GM-CSF.

Optionally, in some embodiments the cancer is not a melanoma. Optionally, in some embodiments the cancer is not a primary melanoma. Optionally, in some embodiments the cancer is not a metastatic (secondary) melanoma. Optionally, in some embodiments the cancer is not stage IIIb to stage IV melanoma. Optionally, in some embodiments the cancer is not a head and neck cancer.

Description

Without being bound to any particular theory of invention, the inventors have identified that herpes simplex virus oncolytic immunotherapy works through a multi-modal approach.

Systemic Administration

Contrary to the disclosure in Mace et al (*supra*) and the experience with IMLYGIC®, the inventors have realised that simply increasing the dosage of herpes simplex virus by intra-tumoral injection is unlikely to generate a satisfactory response for a number of reasons:

(i) ever higher doses of oncolytic treatment risk triggering an uncontrollable cytokine response raising safety concerns;

(ii) under high multiplicity of infection, the tumor cells at the site of injection are unlikely to support virus replication with evidence that the cells may adopt an apoptotic pathway and commence shut down to prevent viral spread thus undermining the basis of oncolytic therapy;

(iii) tumor heterogeneity limits the spread of an oncolytic virus from the point of injection;

(iv) injection into accessible tumors – which may even be amenable to surgical treatment – is unlikely to be effective for disseminated or invasive disease, e.g. as commonly found in Stage III and IV head and neck cancer..

The barriers to effective systemic administration of HSV in human subjects are common general knowledge in the art (e.g. as discussed in Russell et al., (Oncolytic virotherapy. Nature Biotechnology Vol. 30 No.7 July 2012) and Seymour and Fisher (British Journal of Cancer (2016) 114, 357-361).

Despite the teaching in the art, the inventors pursued the systemic administration of herpes simplex virus, i.e. to the blood, as a therapeutic strategy in a Phase I trial.

Surprisingly, even at the relatively low doses used in the Phase I trial, evidence of virus targeting and replication in the subjects has emerged and is reported below.

The inventors have therefore identified systemic administration of herpes simplex virus, i.e. to the blood, as a therapeutic strategy. Optionally the systemic administration of herpes simplex virus may be coupled with monitoring the subject to determine whether the virus has successfully reached the tumor and replicated at the site of the tumor. Such monitoring is possible by taking a sample of blood or tumor tissue and analysing the sample for the presence of herpes simplex virus, e.g. HSV DNA or envelope proteins or HSV antigens. Based on the determination the subject may be selected to receive further treatment with the herpes simplex virus, optionally in combination with treatment with an immune checkpoint inhibitor.

In the context of cancer indicated for surgery, optionally head and neck cancer, herpes simplex virus may be administered to the blood prior to surgery and removal of tumor tissue during surgery permits analysis to determine whether the herpes simplex virus has been able to reach and replicate in the tumor tissue, and also provides an option to establish whether a localised immune response has been induced in the tumor tissue.

Immune response

The inventors have identified that loco-regional administration of oncolytic herpes simplex virus to patients having cancer induces a strong Th1 immune response. The response appears to mature and become sustained with time and be stronger in patients receiving more than one dose of virus.

Oncolytic herpes simplex virus transfected into tumor cells will replicate in and selectively lyse the tumor cells bringing about tumor cell necrosis and the liberation of tumor antigens, which trigger a Th1 immune response.

Infiltration of memory cytotoxic T cells and T helper 1 (Th1) T lymphocytes are often associated with a good clinical outcome and good response to immunotherapy. Therefore, one aim of immunotherapies is to modify the context of immune, inflammatory and angiogenic elements to favour a strong Th1 cytotoxic microenvironment (Giraldo et al., The immune contexture of primary and metastatic human tumors. Current Opinion in Immunology 2014, 27:8-15).

Patients treated with oncolytic herpes simplex virus are therefore well-suited to treatment with an immune checkpoint inhibitor so as to provide either an enhanced treatment effect and/or to convert patients having no or sub-optimal response to immune checkpoint inhibitor therapy to become responders to such therapy.

Accordingly, patients may optionally be treated with an oncolytic herpes simplex virus in order to stimulate and establish a Th1 immune response thereby priming the subject for treatment with an immune checkpoint inhibitor.

Without wishing to be bound by theory of the invention, treatment with an oncolytic herpes simplex virus may optionally be used to establish a Th1 immune response in the subject. Having established a Th1 response, treatment with an immune checkpoint inhibitor may increase the magnitude of tumor specific T cell responses as compared to treatment with an immune checkpoint inhibitor alone. In particular, the response of a subject to treatment with an immune checkpoint inhibitor may be improved by use of an oncolytic herpes simplex virus to establish a Th1 response. Subjects who are non-responders or poor responders to immune checkpoint inhibitor treatment may be converted to responders.

As regards "response" to treatment, the mechanism of action of herpes simplex virus therapy alone, immune checkpoint therapy alone, and together in combination may result in the appearance of disease progression on imaging using conventional RECIST criteria. Response in clinical studies therefore needs to account for the potential for "pseudo-progression" with these agents and to recognise that an immunological response may take longer to manifest itself as compared to chemotherapeutic agents. Imaging guidelines have been modified to account for these factors and immune related response criteria ("irRC") should be used to evaluate imaging response.

In general, the combination of treatments may enhance the systemic T-cell activation and the anti-tumor response to tumor antigens following the lytic replication of oncolytic herpes simplex virus in the cells of the cancer. This may improve the rate of overall tumor response and duration of response. Overall, these effects may provide an extension in overall survival, particularly when compared to treatment using an immune checkpoint inhibitor alone.

T helper (Th) cells play an important role in the adaptive immune system, regulating the activity of other immune cells by releasing certain cytokines. They are essential in the activation and proliferation of cytotoxic T cells and in optimising the activity of macrophages. Mature Th cells express CD4 (i.e. are $CD4^+$ T cells). Proliferating Th cells differentiate from a Th0 state into effector T cells of two main subtypes: Type 1 (Th1) and Type 2 (Th2) (Kidd P. Altern Med Rev. 2003;8(3):223-46; Sallusto et al., Trends in Immunology. Volume 19, Issue 12, p568-574, 1 December 1998).

Differentiation towards Th1 is primarily triggered by IL-12 and IL-2. The primary effector cytokine of the Th1 response is IFN-γ, although cytokines secreted by Th1 cells include Tumor Necrosis Factor (TNF-α), IFN-γ and interleukins (IL) 2, 12, and 18. IFN-γ promotes production of IL-12 from dendritic cells and macrophages which further promotes IFN-γ production in Th cells by a positive feedback mechanism. IFN-γ production also inhibits production of IL-4, and thereby inhibits the Th2 response. Th1 immunity is mainly effected through macrophages, $CD8^+$ T cells, IgG B cells and IFN-γ $CD4^+$ T cells. Th1 cytokines tend to produce pro-inflammatory cytokines such as IL-6.

Differentiation towards Th2 is primarily triggered by IL-4 and the effector cytokines of the Th2 response include IL-4, IL-5, IL-9, IL-10 and IL-13. Th2 immunity is mainly effected through eosinophils, basophils and mast cells, as well as B cells and IL-4 / IL-5 $CD4^+$ T cells. IL-10 acts to suppress Th1 cell differentiation by inhibiting IL-2 and IFN-γ in Th cells and IL-12 in dendritic cells and macrophages.

Data presented herein from a trial in human patients shows that single dose non-intratumoral administration of oncolytic herpes simplex virus can be sufficient to induce an IFNγ response (e.g. Figures 1 and 22) but the response is not robustly translated into upregulation of IL-2 and IL-12 (Figures 5, 9 and 23). IFNγ is pleiotropic (Trincheri and Perussia., Immune interferon: a pleiotropic lymphokine with multiple effects. Immunology Today, Volume 6 , Issue 4 , 131 – 136), and upregulation of IFNγ alone, without induction

of threshold levels of IL-2 and/or IL-12, is not a reliable indicator of induction of a robust or sustained Th1 response.

Administration of multiple doses of oncolytic herpes simplex virus is shown to further upregulate IFNγ and lead to upregulation of IL-2 and IL-12 (Figure 23) and expansion of T cells associated with a Th1 response (Figure 49). IL-2 and IL-12 upregulation leads to expansion of a Th1 cell population by a positive feedback mechanism (Busse et al., Competing feedback loops shape IL-2 signaling between helper and regulatory T lymphocytes in cellular microenvironments. PNAS 2010 107 (7) 3058-3063; Vignali and Kuchroo., IL-12 family cytokines: immunological playmakers. Nature Immunology 13, 722-728 (2012)) which is modulated by upregulated IL-10 (Taga and Tosato., IL-10 inhibits human T cell proliferation and IL-2 production. J Immunol. 1992 feb 15;148(4):1143-8).

The data obtained (Figures 23 and 49) show that multiple dose administration of oncolytic herpes simplex virus leads to upregulated IFNγ, IL-2, IL-12, IL-10 in human subjects, and an appropriate T cell population, clearly indicating establishment of a sustained Th1 response.

The inventors have identified that administration of oncolytic herpes simplex virus to human patients having cancer induces a strong Th1 immune response. The response appears to mature and become sustained with time and be stronger in patients receiving more than one dose of virus. Oncolytic herpes simplex virus transfected into tumor cells will replicate in and selectively lyse the tumor cells bringing about tumor cell necrosis and the liberation of tumor antigens, which trigger a Th1 immune response. Indeed, the inventors have noted that oncolytic herpes simplex virus remodels the tumor microenvironment away from immunosuppression by directly interacting with tumor infiltrating immune cells.

Human patients treated with oncolytic herpes simplex virus are therefore well-suited to treatment with an immune checkpoint inhibitor so as to provide either an enhanced treatment effect and/or to convert patients having no or sub-optimal response to immune checkpoint inhibitor therapy to become responders to such therapy.

Accordingly, human patients may be treated with an oncolytic herpes simplex virus in order to stimulate and establish a Th1 immune response thereby priming the subject for treatment with an immune checkpoint inhibitor.

Without wishing to be bound by theory of the invention, treatment with an oncolytic herpes simplex virus may be used to establish a Th1 immune response in the subject. Having established a Th1 response treatment with an immune checkpoint inhibitor may increase the magnitude of tumor specific T cell responses as compared to treatment with an immune checkpoint inhibitor alone. In particular, the response of a subject to treatment with an immune checkpoint inhibitor may be improved by use of an oncolytic herpes simplex virus to establish a Th1 response. Subjects who are non-responders or poor responders to immune checkpoint inhibitor treatment may be converted to responders.

In general, the combination of treatments may enhance the systemic T-cell activation and the anti-tumor response to tumor antigens following the lytic replication of oncolytic herpes simplex virus in the cells of the cancer. This may lead to enhanced destruction of tumors to which the virus has been administered, e.g. by direct injection, but may also enhance the destruction of tumors to which the virus has not been administered and/or are distant to the site of administration, e.g. secondary / metastatic tumors. This may improve the rate of overall tumor response and duration of response. Overall, these effects may provide an extension in overall survival, particularly when compared to treatment using an immune checkpoint inhibitor alone.

The inventors have noted that mice that receive combination therapy with oncolytic herpes simplex virus and an immune checkpoint inhibitor showed more T cell recruitment to the tumor, and displayed higher immune inflammatory responses and a less immunosuppressive microenvironment, as measured by increased proportions of CD4+ and CD8+ T cells relative to CD4+/CD25+/Fox3P+ Treg cells and immunosuppressive cytokines. The combination therapy did not result in more NK, NKT or B cell recruitment or affect *in vivo* virus activity but induced more inflammatory responses with less immune regulatory/suppressive responses.

The inventors have identified that induction of a Th1 response through administration of an effective amount of oncolytic herpes simplex virus is effective to trigger an anti-tumor Th1 response in the subject. They have also identified that the anti-tumor Th1 response

has a memory effect, and may be used to vaccinate the subject against relapse of the cancer at both primary and distal sites

Herpes Simplex Virus

The herpes simplex virus (HSV) genome comprises two covalently linked segments, designated long (L) and short (S). Each segment contains a unique sequence flanked by a pair of inverted terminal repeat sequences. The long repeat (RL or R_L) and the short repeat (RS or R_S) are distinct.

The HSV ICP34.5 (also called γ34.5) gene, which has been extensively studied, has been sequenced in HSV-1 strains F and syn17+ and in HSV-2 strain HG52. One copy of the ICP34.5 gene is located within each of the RL repeat regions. Mutants inactivating one or both copies of the ICP34.5 gene are known to lack neurovirulence, i.e. be avirulent/ non-neurovirulent (non-neurovirulence is defined by the ability to introduce a high titre of virus (approx 10^6 plaque forming units (pfu)) to an animal or patient without causing a lethal encephalitis such that the LD_{50} in animals, e.g. mice, or human patients is in the approximate range of $\geq 10^6$ pfu), and be oncolytic.

An oncolytic virus is a virus that will lyse cancer cells (oncolysis), preferably in a preferential or selective manner. Viruses that selectively replicate in dividing cells over non-dividing cells are often oncolytic. Oncolytic viruses are well known in the art and are reviewed in Molecular Therapy Vol.18 No.2 Feb 2010 pg 233-234.

Preferred herpes simplex virus are replication-competent, being replication-competent at least in the target tumor/cancer cells.

HSV that may be used in the present invention include HSV in which one or both of the γ34.5 (also called ICP34.5) genes are modified (e.g. by mutation which may be a deletion, insertion, addition or substitution) such that the respective gene is incapable of expressing, e.g. encoding, a functional ICP34.5 protein. Preferably, in HSV according to the invention both copies of the γ34.5 gene are modified such that the modified HSV is not capable of expressing, e.g. producing, a functional ICP34.5 protein.

In some embodiments the herpes simplex virus may be an ICP34.5 null mutant where all copies of the ICP34.5 gene present in the herpes simplex virus genome (two copies are normally present) are disrupted such that the herpes simplex virus is incapable of

producing a functional ICP34.5 gene product. In other embodiments the herpes simplex virus may lack at least one expressible ICP34.5 gene. In some embodiments the herpes simplex virus may lack only one expressible ICP34.5 gene. In other embodiments the herpes simplex virus may lack both expressible ICP34.5 genes. In still other embodiments each ICP34.5 gene present in the herpes simplex virus may not be expressible. Lack of an expressible ICP34.5 gene means, for example, that expression of the ICP34.5 gene does not result in a functional ICP34.5 gene product.

Herpes simplex virus may be derived from any HSV including any laboratory strain or clinical isolate (non-laboratory strain) of HSV. In some preferred embodiments the HSV is a mutant of HSV-1 or HSV-2. Alternatively the HSV may be an intertypic recombinant of HSV-1 and HSV-2. The mutant may be of one of laboratory strains HSV-1 strain 17, HSV-1 strain F or HSV-2 strain HG52. The mutant may be of the non-laboratory strain JS-1. Preferably the mutant is a mutant of HSV-1 strain 17. The herpes simplex virus may be one of HSV-1 strain 17 mutant 1716, HSV-1 strain F mutant R3616, HSV-1 strain F mutant G207, HSV-1 mutant NV1020, or a further mutant thereof in which the HSV genome contains additional mutations and/or one or more heterologous nucleotide sequences. Additional mutations may include disabling mutations, which may affect the virulence of the virus or its ability to replicate. For example, mutations may be made in any one or more of ICP6, ICP0, ICP4, ICP27. Preferably, a mutation in one of these genes (optionally in both copies of the gene where appropriate) leads to an inability (or reduction of the ability) of the HSV to express the corresponding functional polypeptide. By way of example, the additional mutation of the HSV genome may be accomplished by addition, deletion, insertion or substitution of nucleotides. In some embodiments the HSV genome does not have a mutation in ICP6, or in ICP0, ICP4, ICP27.

A number of oncolytic herpes simplex viruses are known in the art. Examples include HSV1716, R3616 (e.g. see Chou & Roizman, Proc. Natl. Acad. Sci. Vol.89, pp.3266-3270, April 1992), G207 (Toda et al, Human Gene Therapy 9:2177-2185, October 10, 1995), NV1020 (Geevarghese et al, Human Gene Therapy 2010 Sep; 21(9):1119-28), RE6 (Thompson et al, Virology 131, 171-179 (1983)), and Oncovex™ (Simpson et al, Cancer Res 2006; 66:(9) 4835-4842 May 1, 2006; Liu et al, Gene Therapy (2003): 10, 292-303), dlsptk, hrR3,R4009, MGH-1, MGH-2, G47Δ, Myb34.5, DF3γ34.5, HF10, NV1042, RAMBO, rQNestin34.5, R5111, R-LM113, CEAICP4, CEAγ34.5, DF3γ34.5, KeM34.5 (Manservigi et al, The Open Virology Journal 2010; 4:123-156), rRp450, M032 (Campadelli-Fiume et al, Rev Med. Virol 2011; 21:213-226), Baco1 (Fu et al, Int. J.

Cancer 2011; 129(6):1503-10) and M032 and C134 (Cassady et al, The Open Virology Journal 2010; 4:103-108).

In some preferred embodiments the herpes simplex virus is HSV-1 strain 17 mutant 1716 (HSV1716). HSV 1716 is an oncolytic, non-neurovirulent HSV and is described in EP 0571410, WO 92/13943, Brown et al (Journal of General Virology (1994), 75, 2367-2377) and MacLean et al (Journal of General Virology (1991), 72, 631-639). HSV 1716 has been deposited on 28 January 1992 at the European Collection of Animal Cell Cultures, Vaccine Research and Production Laboratories, Public Health Laboratory Services, Porton Down, Salisbury, Wiltshire, SP4 0JG, United Kingdom under accession number V92012803 in accordance with the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure (herein referred to as the 'Budapest Treaty').

In some embodiments the herpes simplex virus is a mutant of HSV-1 strain 17 modified such that both ICP34.5 genes do not express a functional gene product, e.g. by mutation (e.g. insertion, deletion, addition, substitution) of the ICP34.5 gene, but otherwise resembling or substantially resembling the genome of the wild type parent virus HSV-1 strain 17+. That is, the virus may be a variant of HSV1716, having a genome mutated so as to inactivate both copies of the ICP34.5 gene of HSV-1 strain 17+ but not otherwise altered to insert or delete/modify other protein coding sequences.

In some embodiments the genome of an Herpes Simplex Virus according to the present invention may be further modified to contain nucleic acid encoding at least one copy of a polypeptide that is heterologous to the virus (i.e. is not normally found in wild type virus) such that the polypeptide can be expressed from the nucleic acid. As such, the virus may also be an expression vector from which the polypeptide may be expressed. Examples of such viruses are described in WO2005/049846 and WO2005/049845.

In order to effect expression of the polypeptide, nucleic acid encoding the polypeptide is preferably operably linked to a regulatory sequence, e.g. a promoter, capable of effecting transcription of the nucleic acid encoding the polypeptide. A regulatory sequence (e.g. promoter) that is operably linked to a nucleotide sequence may be located adjacent to that sequence or in close proximity such that the regulatory sequence can effect and/or control expression of a product of the nucleotide sequence. The encoded product of the nucleotide sequence may therefore be expressible from that regulatory sequence.

In some preferred embodiments, the Herpes Simplex Virus is not modified to contain nucleic acid encoding at least one copy of a polypeptide (or other nucleic acid encoded product) that is heterologous to the virus. That is the virus is not an expression vector from which a heterologous polypeptide or other nucleic acid encoded product may be expressed. Such HSV are not suitable for, or useful in, gene therapy methods and the method of medical treatment for which they are employed may optionally be one that does not involve gene therapy.

In some embodiments the genome of an oncolytic Herpes Simplex Virus according to the present invention does not encode (or is not further modified to contain nucleic acid encoding) a cytokine or chemokine, e.g. a mammalian or human cytokine or chemokine. For example, the genome of an oncolytic Herpes Simplex Virus according to the present invention does not encode an interleukin, e.g. IL-2 and/or IL-12, an interferon, e.g. IFN-γ, a tumor necrosis factor, a colony stimulating factor (e.g. GM-CSF, G-CSF), an immune modulator, a member of the CC family, e.g. CCL5, a member of the CXC family or a member of the CXC family.

In some embodiments the herpes simplex virus has an intact ICP0 gene, capable of expressing functional ICP0. In some embodiments the herpes simplex virus has an intact ICP27 gene, capable of expressing functional ICP27. In some embodiments the herpes simplex virus has an intact vhs gene, capable of expressing functional vhs.

In some embodiments the herpes simplex virus has an intact ICP47 gene, capable of expressing functional ICP47. In some embodiments the oncolytic herpes simplex virus has an intact ICP6 gene, capable of expressing functional ICP6.

Optionally, in some embodiments the herpes simplex virus does not encode or express (granulocyte macrophage colony stimulating factor) GMCSF.

Optionally, in some embodiments the herpes simplex virus is not a herpes simplex virus that lacks functional ICP34.5 genes and lacks a functional ICP47 gene and comprises a gene encoding human GM-CSF.

In some optional embodiments the herpes simplex virus is not Talimogene laherparepvec, HSV-1 [strain JS1] ICP34.5-/ICP47-/hGM-CSF also known as OncoVEX

GM-CSF (Lui et al., Gene Therapy, 10:292-303, 2003; US Patent No. 7,223,593 and US Patent No. 7,537,924)]. In talimogene laherparepvec, the HSV-1 viral genes encoding ICP34.5 are functionally deleted, the ICP47 is functionally deleted, the coding sequence for human GM-CSF is inserted into the viral genome such that it replaces nearly all of the ICP34.5 gene and the HSV thymidine kinase (TK) gene remains intact.

In some optional embodiments the herpes simplex virus is not a herpes simplex virus that lacks only one of the two functional copies of the $\gamma_1$34.5 gene. For example, in some optional embodiments the herpes simplex virus is not NV1020 or a variant thereof.

Herpes simplex viruses may be formulated as medicaments and pharmaceutical compositions for clinical use and in such formulations may be combined with a pharmaceutically acceptable carrier, diluent or adjuvant. The composition is preferably formulated for intravenous or intra-arterial routes of administration which may include infusion or injection. Suitable formulations may comprise the virus in a sterile or isotonic medium. Medicaments and pharmaceutical compositions may be formulated in fluid (including gel) or solid (e.g. tablet) form. Fluid formulations may be formulated for administration by injection or via catheter to a selected region of the human or animal body.

Administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the disease being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 20th Edition, 2000, pub. Lippincott, Williams & Wilkins.

Targeting therapies may be used to deliver the virus to certain types of cell, e.g. by the use of targeting systems such as antibody or cell specific ligands. Targeting may be desirable for a variety of reasons; for example if the virus is unacceptably toxic in high dose, or if it would otherwise require too high a dosage, or if it would not otherwise be able to enter the target cells.

HSV capable of targeting cells and tissues are described in (PCT/GB2003/000603; WO 03/068809), hereby incorporated in its entirety by reference.

An HSV may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. Such other treatments may include chemotherapy (including either systemic treatment with a chemotherapeutic agent or targeted therapy using small molecule or biological molecule (e.g. antibody) based agents that target key pathways in tumor development, maintenance or progression) or radiotherapy provided to the subject as a standard of care for treatment of the cancer.

In addition to direct action of oncolytic herpes simplex virus (oHSV) on tumors, there is growing evidence that the host immune response plays an important role in establishing the efficacy of the anti-tumor response through innate immune effectors, adaptive antiviral immune responses and adaptive antitumor immune responses (e.g. *see* Prestwich et al., Oncolytic viruses: a novel form of immunotherapy. Expert Rev Anticancer Ther. Oct 2008; 8(10): 1581-1588).

Several studies have shown that oHSV is capable of inducing an anti-tumor immune response. This can manifest as tumor growth reduction in lesions treated with oHSV and in untreated lesions in the same animal, efficacy of oHSV requiring an intact immune response, induction of anti-tumor cytokine response, reversal of tumor immune dysfunction and facilitation of tumor antigen presentation. Induction of an anti-tumor immune response can reduce establishment of metastases, or contribute to their elimination, and protect from re-occurrence of tumor.

For example, in Benencia et al., ((2008) Herpes virus oncolytic therapy reverses tumor immune dysfunction and facilitates tumor antigen presentation. Cancer Biol.Ther. 7, 1194-1205) growth reduction in treated and untreated lesions was reported. In Miller and Fraser ((2003) Requirement of an integrated immune response for successful neuroattenuated HSV-1 therapy in an intracranial metastatic melanoma model. Mol. Ther. 7(6):741-747) efficacy of HSV176 required an intact immune response which was mediated by a tumor-specific proliferative T cell response.

Administration of herpes simplex virus

Administration of herpes simplex virus is preferably for a period of time sufficient to allow virus to reach the site of the tumor tissue, and preferably to begin to replicate in the tumor tissue. It may also be for a period of time sufficient to induce or elicit a Th1 response in the subject.

This may involve administration at regular intervals, e.g. weekly or fortnightly, of doses of herpes simplex virus sufficient to allow accumulation of virus at the tumor site and/or to induce a sustained Th1 response over a period of time. For example, doses may be given at regular, defined, intervals over a period of one of at least 1, 2, 3, 4, 5, 6, 7, 8, weeks or 1, 2, 3, 4, 5 or 6 months.

As such, multiple doses of herpes simplex virus may be administered. For example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more doses of herpes simplex virus may be administered to a subject as part of a course of treatment. In some embodiments one of at least 1, 2, 3, or 4 doses of herpes simplex virus are administered to the subject, preferably at regular intervals (e.g. weekly). In some embodiments this may prime the subject for treatment with an immune checkpoint inhibitor.

Doses of herpes simplex virus may be separated by a predetermined time interval, which may be selected to be one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or 31 days, or 1, 2, 3, 4, 5, or 6 months. By way of example, doses may be given once every 7, 14, 21 or 28 days (plus or minus 3, 2, or 1 days). The dose of herpes simplex virus given at each dosing point may be the same, but this is not essential. For example, it may be appropriate to give a higher priming dose at the first, second and/or third dosing points.

Administration of oncolytic herpes simplex virus may be of one or more treatment cycles, e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more treatment cycles. A subject receiving multiple treatment cycles may be given subsequent treatment cycles consecutively, without a break from treatment, or may separate all or selected treatment cycles by a break from treatment, e.g. a break of 1, 2, 3, 4, 5, 6, 7, 8 or 9 days or about 1, 2, 3, or 4 weeks.

In some embodiments a treatment cycle may comprise, or consist of, 4 doses of oncolytic herpes simplex virus, one dose per week over a period of 4 weeks. In some embodiments a treatment cycle may comprise, or consist of, 8 doses of oncolytic herpes simplex virus, one dose per week over a period of 8 weeks. Weekly doses may be

separated by 7 ±1 or 7±2 days. For example, weekly doses may be given on days 1, 8, 15 and 22.

In some embodiments a treatment cycle may comprise, or consist of, 4 doses of oncolytic herpes simplex virus, two doses per week over a period of 2 weeks. In some embodiments a treatment cycle may comprise, or consist of, 8 doses of oncolytic herpes simplex virus, two doses per week over a period of 4 weeks. Twice weekly doses may be separated by 4 ±1 or 4±2 days. For example, weekly doses may be given on days 1, 5, 8, 13 or 1, 5, 8, 12.

Subjects may receive the same dosage at each administration within a given treatment cycle, e.g. a dosage of 1×10^7 iu or 1×10^8 iu, or between $1x10^6$ and $1x10^8$ iu or between 1×10^7 iu and 1×10^8 iu. In some embodiments the first 1, 2 or 3 treatment cycles may comprise administration of a lower dosage amount at each administration, e.g. 1×10^7 iu, and later treatment cycles may comprise administration of a higher dosage amount at each administration, e.g.1×10^8 iu.

Administration of oncolytic herpes simplex virus may be by infusion to the blood (intravenous or intra-arterial) and subjects will preferably attend clinic on the scheduled administration days for administration of oncolytic herpes simplex virus. Administration of oncolytic herpes simplex virus may continue until development of severe toxicity or withdrawal of consent.

A tumour biopsy may be taken in the period commencing 14 days before the first dose of oncolytic herpes simplex virus (Day 1). A tumour biopsy or surgical resection sample may be taken after completion of a cycle of treatment, e.g. within 14 days of the last dose of oncolytic herpes simplex virus in a given treatment cycle. Samples obtained from tumour biopsy or surgical resection may be used to determine the presence and/or maintenance of a Th1 response.

Blood or serum samples may be taken at the stage of initial subject assessment (before treatment with oncolytic herpes simplex virus), and during a or each treatment cycle, e.g. on days 1, 8, 15, 22, for weekly administration, days 1, 5, 8, 13, or days 1, 5, 8, 12 for twice weekly administration. Blood or serum samples may be used to determine the presence and/or maintenance of a Th1 response.

Where a Th1 response has been induced the subject may continue with dosing of oncolytic herpes simplex virus on the same dosing schedule in order to maintain the

response. Alternatively, dosing frequency may be reduced and the subject may receive either no further doses of oncolytic herpes simplex virus, e.g. in cases where the Th1 response is self-sustaining following induction, or the status of the Th1 response in the subject may be monitored and the subject may be given booster administrations as and when considered appropriate, e.g. by a medical practitioner, in order to maintain the sustained Th1 response.

Suitable dosage amounts of herpes simplex virus may be in the range 10^6 to 10^9 iu or $2x10^6$ to 10^9 iu. Doses of herpes simplex virus in this range may be particularly required for systemic, e.g. intravenous, administration where the viral dose is diluted by administration to the blood. Each dose of herpes simplex virus is preferably of greater than $2x10^6$ iu. Each dose of virus may be in a range selected from the group consisting of: $2x10^6$ to $9x10^6$ iu, $2x10^6$ to $5x10^6$ iu, $5x10^6$ to $9x10^6$ iu, $2x10^6$ to $1x10^7$ iu, $2x10^6$ to $5x10^7$ iu, $2x10^6$ to $1x10^8$ iu, $2x10^6$ to $5x10^8$ iu, $2x10^6$ to $1x10^9$ iu, $5x10^6$ to $1x10^7$ iu, $5x10^6$ to $5x10^7$ iu, $5x10^6$ to $1x10^8$ iu, $5x10^6$ to $5x10^8$ iu, $5x10^6$ to $1x10^9$ iu, $5x10^6$ to $5x10^9$ iu, $1x10^7$ to $9x10^7$ iu, $1x10^7$ to $5x10^7$ iu, $1x10^8$ to $9x10^8$ iu, $1x10^8$ to $5x10^8$ iu. In some embodiments suitable doses may be in the range $2x10^6$ to $9x10^6$ iu, $1x10^7$ to $9 x10^7$ iu, or $1x10^8$ to $9 x10^8$ iu. In some embodiments suitable doses may be about $1x10^7$ iu or $1x10^8$ iu. Dosage figures may optionally be +/- half a log value.

The term 'infectious units' is used to refer to virus concentrations derived using the TCID50 method and 'plaque forming units (pfus)' to refer to plaque-based assay results. As 1iu will form a single plaque in a titration assay, 1 iu is equivalent to 1 pfu.

In general, administration is preferably in a "effective amount", this optionally being sufficient to induce a Th1 response in the individual and/or for the virus to have an independent treatment effect on the cancer. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the disease being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 20th Edition, 2000, pub. Lippincott, Williams & Wilkins.

In some embodiments herpes simplex virus is administered in the form of monotherapy, i.e. not part of a combination treatment, although subjects may also receive, or continue to receive, standard of care chemotherapy or radiation therapy. In other embodiments herpes simplex virus may be administered as part of a programme of treatment in which an immune checkpoint inhibitor is also administered, the two agents providing a combination therapy treatment.

Administration of herpes simplex virus may be carried out for a period of time prior to treatment with an immune checkpoint inhibitor in which period the subject receives herpes simplex virus but does not receive an immune checkpoint inhibitor. Treatment is preferably for a period of time suitable, or sufficient, to allow virus to reach the site of the tumor tissue, and preferably to begin to replicate in the tumor tissue so as to be detectable in a tumor tissue sample, e.g. biopsy, and/or to induce or elicit a Th1 immune response in the subject. This may be referred to as "pre-treatment" with herpes simplex virus.

In some preferred embodiments pre-treatment may involve a period of time in which the subject is administered herpes simplex virus but is not administered an immune checkpoint inhibitor (called "herpes simplex virus monotherapy" herein). The period of herpes simplex virus monotherapy may be sufficient to allow virus to reach the site of the tumor tissue and/or to induce or elicit a Th1 response in the subject. During a period of herpes simplex virus monotherapy the subject may also receive treatment in the form of simultaneous, sequential or separate administration of other chemotherapy or radiation therapy, e.g. which may be part of the standard of care for the cancer being treated, but in that time period the patient will not receive a therapeutically effective dose of an immune checkpoint inhibitor.

As such, methods according to the present invention may comprise administration of an herpes simplex virus for a period of time in which the subject receives herpes simplex virus but does not receive an immune checkpoint inhibitor. The period of time may be suitable, or sufficient, to allow virus to reach the site of the tumor tissue and/or to induce a Th1 response in the subject.

Following the pre-treatment a determination may be made as to whether virus has reached the site of the tumor tissue and/or whether a Th1 response has been induced or elicited in the subject. The determination may involve analysis of a tumor tissue sample

obtained during surgery and following herpes simplex virus monotherapy. A selection of subject(s) suitable for treatment with an immune checkpoint inhibitor may then be made.

A subject may then begin treatment with an immune checkpoint inhibitor. That is, the method may then further comprises the administration of an immune checkpoint inhibitor to the subject.

Accordingly, at a selected time point the period of pre-treatment may end and the subject may then be administered an immune checkpoint inhibitor. Optionally the subject will continue to be administered herpes simplex virus simultaneously, sequentially or separately such that the subject receives co-therapy with immune checkpoint inhibitor and herpes simplex virus. The subject may also receive, or continue to receive, treatment in the form of simultaneous, sequential or separate administration of other chemotherapy or radiation therapy, e.g. which may be part of the standard of care for the cancer being treated.

For example, pre-treatment may occur for one of at least 1, 2, 3, 4, or 5 weeks in which the subject receives herpes simplex virus but does not receive an immune checkpoint inhibitor. Preferably the period of time is sufficient to allow virus to reach the site of the tumor tissue and/or to induce or elicit a Th1 response in the subject. By way of example, a subject may receive herpes simplex virus monotherapy in the form of weekly doses of herpes simplex virus for one of at least 1, 2, 3, 4, 5, 6, 7, 8, weeks or 1, 2, 3, 4, 5 or 6 months.

In other embodiments a subject may receive herpes simplex virus monotherapy as described above and may discontinue treatment with herpes simplex virus and begin receiving treatment with an immune checkpoint inhibitor. In such embodiments there may be no day on which a subject is receiving co-therapy, i.e. no day on which an ongoing scheduled programme of treatment with herpes simplex virus and immune checkpoint inhibitor overlaps.

In other embodiments there may a substantial overlap of treatment with herpes simplex virus. In one arrangement, co-therapy with herpes simplex virus and immune checkpoint inhibitor may commence at the start of treatment, or during a period in which the subject is receiving an herpes simplex virus, e.g. in order to induce or elicit a Th1 response, preferably a sustained Th1 response. In other arrangements a short period of herpes

simplex virus monotherapy may be provided, not necessarily suitable or sufficient to induce or elicit a Th1 response, after which the subject begins to also receive treatment with an immune checkpoint inhibitor, i.e. co-therapy. During co-therapy the herpes simplex virus and immune checkpoint inhibitor may be administered on the same day or on different days.

Co-therapy may comprises simultaneous or sequential administration of herpes simplex virus and immune checkpoint inhibitor.

Simultaneous administration refers to administration of the herpes simplex virus and immune checkpoint inhibitor together, for example as a pharmaceutical composition containing both agents, or immediately after each other and optionally via the same route of administration, e.g. to the same artery, vein or other blood vessel.

Sequential administration refers to administration of one of the herpes simplex virus or immune checkpoint inhibitor followed after a given time interval by separate administration of the other agent. It is not required that the two agents are administered by the same route, although this is the case in some embodiments. The time interval may be any time interval.

Whilst simultaneous or sequential administration may be intended such that both the herpes simplex virus and immune checkpoint inhibitor are delivered to the same tumor tissue to effect treatment it is not essential for both agents to be present in the tumor tissue in active form at the same time.

However, in some embodiments of sequential administration the time interval is selected such that the herpes simplex virus and immune checkpoint inhibitor are expected to be present in the tumor tissue in active form at the same time, thereby allowing for a combined, additive or synergistic effect of the two agents in treating the tumor. In such embodiments the time interval selected may be any one of 5 minutes or less, 10 minutes or less, 15 minutes or less, 20 minutes or less, 25 minutes or less, 30 minutes or less, 45 minutes or less, 60 minutes or less, 90 minutes or less, 120 minutes or less, 180 minutes or less, 240 minutes or less, 300 minutes or less, 360 minutes or less, or 720 minutes or less, or 1 day or less, or 2 days or less.

In some embodiments, a subject will receive oncolytic herpes simplex virus before treatment with the immune checkpoint inhibitor that is intended to take advantage of the Th1 response which the oncolytic herpes simplex virus may induce or elicit when providing a treatment effect.

Where co-therapy with an herpes virus occurs it may continue for as long as desired or prescribed. In some embodiments, treatment with herpes simplex virus may be discontinued in favour of continued treatment with the immune checkpoint inhibitor.

Doses of immune checkpoint inhibitor may also be separated by a predetermined time interval, which may be selected to be one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or 31 days, or 1, 2, 3, 4, 5, or 6 months. Administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the disease being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 20th Edition, 2000, pub. Lippincott, Williams & Wilkins.

During co-therapy the subject may also receive, or continue to receive, treatment in the form of simultaneous, sequential or separate administration of other chemotherapy or radiation therapy, e.g. which may be part of the standard of care for the cancer being treated.

A Th1 response is preferably a sustained Th1 response. By "sustained Th1 response" we mean that the level of one or more relevant cytokines and/or relevant T cell population is upregulated for more than 7 days, and preferably for one of at least 2, 3, 4, 5, 6, 7, or 8 weeks, or for one of at least 1, 2, 3, 4, 5, or 6 months.

Immune Checkpoint Proteins and Inhibitors

The term "immune checkpoint inhibitor" refers to molecules that totally or partially reduce, inhibit, interfere with or modulate one or more immune checkpoint proteins. An inhibitor

may inhibit or block the interaction of an immune checkpoint protein with one of its ligands or receptors.

Immune checkpoint proteins negatively regulate T-cell activation or function. Numerous immune checkpoint proteins are known, such as CTLA-4 (Cytotoxic T-Lymphocyte-Associated protein 4) and its ligands CD80 and CD86; and PD-1 (Programmed Death 1) with its ligands PD-L1 and PD-L2 (Pardoll, Nature Reviews Cancer 12: 252-264, 2012), TIM-3 (T-cell Immunoglobulin domain and Mucin domain 3), LAG-3 (Lymphocyte Activation Gene-3), BTLA (CD272 or B and T Lymphocyte Attenuator), KIR (Killer-cell Immunoglobulin-like Receptor), VISTA (V-domain immunoglobulin suppressor of T-cell activation), and A2aR (Adenosine A2A receptor),. These proteins are responsible for down-regulating T-cell responses. Immune checkpoint proteins regulate and maintain self-tolerance and the duration and amplitude of physiological immune responses. Immune checkpoint inhibitors include antibodies and small molecule inhibitors.

Cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) is an immune checkpoint protein that down-regulates pathways of T-cell activation (Fong et al., Cancer Res. 69(2):609-615, 2009; Weber Cancer Immunol. Immunother, 58:823-830, 2009). CTLA-4 is a negative regulator of T-cell activation. Blockade of CTLA-4 has been shown to augment T-cell activation and proliferation. Inhibitors of CTLA-4 include anti-CTLA-4 antibodies. Anti-CTLA-4 antibodies bind to CTLA-4 and block the interaction of CTLA-4 with its ligands CD80/CD86 expressed on antigen presenting cells and thereby blocking the negative down regulation of the immune responses elicited by the interaction of these molecules. Examples of anti-CTLA-4 antibodies are described in US Patent Nos: 5,811,097; 5,811,097; 5,855,887; 6,051,227; 6,207,157; 6,682,736; 6,984,720; and 7,605,238.

Anti-CTLA-4 antibodies include tremelimumab, (ticilimumab, CP-675,206), ipilimumab (also known as IODI, MDX-DOIO; marketed under the name Yervoy™ and) a fully human monoclonal IgG antibody that binds to CTLA-4 approved for the treatment of unresectable or metastatic melanoma.

Another immune checkpoint protein is programmed cell death 1 (PD-1). PD-1, also called CD279, is a type I membrane protein encoded in humans by the *PDCD1* gene. It has two ligands, PD-L1 and PD-L2.The PD-1 pathway is a key immune-inhibitory mediator of T-cell exhaustion. Blockade of this pathway can lead to T-cell activation, expansion, and

enhanced effector functions. As such, PD-1 negatively regulates T cell responses. PD-1 has been identified as a marker of exhausted T cells in chronic disease states, and blockade of PD-1:PD-1L interactions has been shown to partially restore T cell function. (Sakuishi et al., *JEM* Vol. 207, September 27, 2010, pp2187-2194). PD-1 limits the activity of T cells in peripheral tissues at the time of an inflammatory response to infection and to limit autoimmunity. PD-1 blockade *in vitro* enhances T-cell proliferation and cytokine production in response to a challenge by specific antigen targets or by allogeneic cells in mixed lymphocyte reactions. A strong correlation between PD-1 expression and response was shown with blockade of PD-1 (Pardoll, Nature Reviews Cancer, 12: 252-264, 2012). PD-1 blockade can be accomplished by a variety of mechanisms including antibodies that bind PD-1 or its ligand, PD-L1, or soluble PD-1 decoy receptors (e.g. sPD-1, *see* Pan et al., Oncology Letters 5: 90-96, 2013).. Examples of PD-1 and PD-L1 blockers are described in US Patent Nos. 7,488,802; 7,943,743; 8,008,449; 8,168,757; 8,217,149, and PCT Published Patent Application No.s: W003042402, W02008156712, W02010089411, W02010036959, W02011066342, W02011159877, W02011082400, and W02011161699.

PD-1 blockers include anti-PD-1 and anti-PD-L1 antibodies and proteinaceous binding agents. Nivolumab (BMS-936558) is an anti–PD-1 antibody that was approved for the treatment of melanoma in Japan in July 2014. It is a fully human IgG4 antibody that binds to and blocks the activation of PD-1 by its ligands PD-L1 and PD-L2. Other anti-PD-1 antibodies include pembrolizumab (lambrolizumab; MK-3475 or SCH 900475), a humanized monoclonal IgG4 antibody against PD-1; CT-011 a humanized antibody that binds PD-1. AMP-224 is a fusion protein of B7-DC; an antibody Fc portion; BMS-936559 (MDX-1105-01) for PD-L1 (B7-HI) blockade. Other anti-PD-1 antibodies are described in WO 2010/077634, WO 2006/121168, WO2008/156712 and WO2012/135408. AUNP-12 (Aurigene) is a branched 29 amino acid peptide antagonist of the interaction of PD-1 with PD-L1 or PD-L2 and has been shown to inhibit tumor growth and metastasis in preclinical models of cancer.

T cell immunoglobulin mucin 3 (TIM-3) is an immune regulator identified as being upregulated on exhausted $CD8^+$ T cells (Sakuishi et al., *JEM* Vol. 207, September 27, 2010, pp2187-2194 and Fourcade et al., 2010, J. Exp. Med. 207:2175-86). TIM-3 was originally identified as being selectively expressed on IFN-γ–secreting Th1 and Tc1 cells. Interaction of TIM-3 with its ligand, galectin-9, triggers cell death in $TIM\text{-}3^+$ T cells. Anti-

TIM-3 antibodies are described in Ngiow et al (Cancer Res. 2011 May 15;71(10):3540-51),and in US8,552,156

Other immune-checkpoint inhibitors include lymphocyte activation gene-3 (LAG-3) inhibitors, such as IMP321, a soluble Ig fusion protein (Brignone et al., 2007, J. Immunol.179:4202-4211). Other immune-checkpoint inhibitors include B7 inhibitors, such as B7-H3 and B7-H4 inhibitors. In particular, the anti-B7-H3 antibody MGA271 (Loo et al., 2012, 5 Clin. Cancer Res. July 15 (18) 3834).

Reference to an "antibody" includes a fragment or derivative thereof, or a synthetic antibody or synthetic antibody fragment. Antibodies may be provided in isolated form or may be formulated as a medicament or pharmaceutical composition, e.g. combined with a pharmaceutically acceptable adjuvant, carrier, diluent or excipient.

In view of today's techniques in relation to monoclonal antibody technology, antibodies can be prepared to most antigens. The antigen-binding portion may be a part of an antibody (for example a Fab fragment) or a synthetic antibody fragment (for example a single chain Fv fragment [ScFv]). Suitable monoclonal antibodies to selected antigens may be prepared by known techniques, for example those disclosed in "Monoclonal Antibodies: A manual of techniques ", H Zola (CRC Press, 1988) and in "Monoclonal Hybridoma Antibodies: Techniques and Applications ", J G R Hurrell (CRC Press, 1982). Chimaeric antibodies are discussed by Neuberger et al (1988, 8th International Biotechnology Symposium Part 2, 792-799).

Monoclonal antibodies (mAbs) are useful in the methods of the invention and are a homogenous population of antibodies specifically targeting a single epitope on an antigen.

Polyclonal antibodies may also be useful in the methods of the invention. Monospecific polyclonal antibodies are preferred. Suitable polyclonal antibodies can be prepared using methods well known in the art.

Fragments of antibodies, such as Fab and Fab_2 fragments may also be provided as can genetically engineered antibodies and antibody fragments. The variable heavy (V_H) and variable light (V_L) domains of the antibody are involved in antigen recognition, a fact first recognised by early protease digestion experiments. Further confirmation was found by

"humanisation" of rodent antibodies. Variable domains of rodent origin may be fused to constant domains of human origin such that the resultant antibody retains the antigenic specificity of the rodent parented antibody (Morrison et al (1984) Proc. Natl. Acad. Sd. USA 81, 6851-6855).

That antigenic specificity is conferred by variable domains and is independent of the constant domains is known from experiments involving the bacterial expression of antibody fragments, all containing one or more variable domains. These molecules include Fab-like molecules (Better et al (1988) Science 240, 1041); Fv molecules (Skerra et al (1988) Science 240, 1038); single-chain Fv (ScFv) molecules where the V_H and V_L partner domains are linked via a flexible oligopeptide (Bird et al (1988) Science 242, 423; Huston et al (1988) Proc. Natl. Acad. Sd. USA 85, 5879) and single domain antibodies (dAbs) comprising isolated V domains (Ward et al (1989) Nature 341, 544). A general review of the techniques involved in the synthesis of antibody fragments which retain their specific binding sites is to be found in Winter & Milstein (1991) Nature 349, 293- 299.

By "ScFv molecules" we mean molecules wherein the V_H and V_L partner domains are covalently linked, e.g. by a flexible oligopeptide.

Fab, Fv, ScFv and dAb antibody fragments can all be expressed in and secreted from E. coli, thus allowing the facile production of large amounts of the said fragments.

Whole antibodies, and $F(ab')_2$ fragments are "bivalent". By "bivalent" we mean that the said antibodies and $F(ab')_2$ fragments have two antigen combining sites. In contrast, Fab, Fv, ScFv and dAb fragments are monovalent, having only one antigen combining site. Synthetic antibodies which bind to an immune checkpoint protein may also be made using phage display technology as is well known in the art.

Administration of immune checkpoint inhibitor

Administration of immune checkpoint inhibitor may involve administration at regular intervals, e.g. weekly, fortnightly, or once every three or four weeks. For example, doses may be given at regular, defined, intervals over a period of one of at least 1, 2, 3, 4, 5, 6, 7, 8, weeks or 1, 2, 3, 4, 5 or 6 months.

As such, multiple doses of immune checkpoint inhibitor may be administered. For example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more doses of immune checkpoint inhibitor may be

administered to a subject as part of a course of treatment. In some preferred embodiments 1 or 2 doses, optionally 3 or more doses, of immune checkpoint inhibitor are administered to the subject, preferably at regular intervals (e.g. weekly, fortnightly, or once every three or four weeks). Each dose is preferably administered within a single day, e.g. over a period of 1, 2, 3, 4, 5, or 6 hours, and optionally at the same time as a dose of oncolytic herpes simplex virus.

Doses of immune checkpoint inhibitor may be separated by a predetermined time interval, which may be selected to be one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or 31 days, or 1, 2, 3, 4, 5, or 6 months. By way of example, doses may be given once every 7, 14, 21 or 28 days (plus or minus 3, 2, or 1 days). The dose of immune checkpoint inhibitor given at each dosing point may be the same, but this is not essential. For example, it may be appropriate to give a higher priming dose at the first, second and/or third dosing points.

Administration of immune checkpoint inhibitor may be of one or more treatment cycles, e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more treatment cycles. A subject receiving multiple treatment cycles may be given subsequent treatment cycles consecutively, without a break from treatment, or may separate all or selected treatment cycles by a break from treatment, e.g. a break of 1, 2, 3, 4, 5, 6, 7, 8 or 9 days or about 1, 2, 3, or 4 weeks.

In some embodiments a treatment cycle may comprise, or consist of, 1 or 2 doses of immune checkpoint inhibitor administered per period of about 3 or about 4 weeks. In some embodiments a treatment cycle may comprise, or consist of, one dose of immune checkpoint inhibitor administered per three week period. Doses may be separated by 21±3, 21±2 or 21±1 days. For example, doses may be given on days 1, 22, 43 etc.

A treatment cycle of immune checkpoint inhibitor may be given in conjunction with a treatment cycle of oncolytic herpes simplex virus to provide a combined treatment. The treatment cycles are not required to commence on the same day, although they may. For example, a treatment cycle of oncolytic herpes simplex virus may commence on day 1 and a treatment cycle of immune checkpoint inhibitor may commence on day 8, or on any of days 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14. As such a treatment cycle of immune checkpoint inhibitor may commence after a treatment cycle of oncolytic herpes simplex virus (e.g. about a week after) or before a treatment cycle of oncolytic herpes simplex virus (e.g. about a week before). Preferably both treatment cycles will have a duration

that causes them to overlap, e.g. by at least one day or more preferably by about one or about two weeks.

Subjects may receive the same dosage of immune checkpoint inhibitor at each administration within a given treatment cycle, e.g. a dosage of 50mg, 100mg, 150mg, 200mg, 250mg, 300mg, 350mg or 400mg. In some embodiments the first 1, 2 or 3 treatment cycles may comprise administration of a lower dosage amount at each administration, e.g. 150mg, and later treatment cycles may comprise administration of a higher dosage amount at each administration, e.g. 200mg.

Administration of immune checkpoint inhibitor may be by infusion to the blood (intravenous or intra-arterial) and subjects will preferably attend clinic on the scheduled administration days for administration of immune checkpoint inhibitor. Administration of immune checkpoint inhibitor may continue until development of severe toxicity or withdrawal of consent.

In general, administration is preferably in a "effective amount", this being sufficient to induce a treatment effect in the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the disease being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 20th Edition, 2000, pub. Lippincott, Williams & Wilkins.

Two stage programme of treatment

In some aspects and embodiments of the present invention a subject may initially receive one dose, preferably several doses, of herpes simplex virus, preferably by intravenous administration, without receiving an immune checkpoint inhibitor. The subject's response to treatment with herpes simplex virus is monitored and based on the determined response the subject may be selected for treatment with an immune checkpoint inhibitor, preferably in addition to continued treatment with herpes simplex virus – the subject thereby progressing to a further stage of treatment in which they receive combined treatment with herpes simplex virus and an immune checkpoint inhibitor.

The response to initial treatment with herpes simplex virus may involve determining whether the herpes simplex virus has reached the cancer. This may involve detecting

herpes simplex virus, or fragments of herpes simplex virus such as HSV DNA, HSV proteins of HSV antigens, in a blood sample or in a tumor tissue sample.

Additionally, or alternatively, the response to initial treatment with herpes simplex virus may involve determining whether a Th1 response has been induced in the cancer. Detection of a Th1 response may be indicative of the herpes simplex virus successfully reaching the tumor and initiating an anti-cancer response, which may involve lysis of tumor cells by the herpes simplex virus and/or induction of a host anti-cancer immune response. This may involve detecting a Th1 response in a blood sample or in a tumor tissue sample.

In cases where a determination is made that the herpes simplex virus has reached the cancer or has induced a Th1 response, the subject may be selected for a further stage of treatment with an immune checkpoint inhibitor. In this stage, the subject preferably continues treatment with herpes simplex virus and treatment with immune checkpoint inhibitor is provided as an additional treatment. The subject thereby receiving combined treatment with herpes simplex virus and immune checkpoint inhibitor.

Subjects selected for the initial stage of treatment with herpes simplex virus may be indicated for surgical removal of tumor tissue (referred to herein as 'tumor resection'). For example, they may have a cancer considered, by a medical practitioner, operable to remove some or all of the tumor tissue. Treatment with herpes simplex virus may commence prior to tumor resection.

Tumor tissue removed during tumor resection may be analysed to determine whether herpes simplex virus has reached the tumor, e.g. by detecting *in vitro* the presence of HSV DNA in excised tumor tissue and/or to determine whether a Th1 response is present in the cancer, e.g. by detecting *in vitro* the presence of a Th1 response in excised tumor tissue.

A determination that herpes simplex virus has reached the cancer and/or that a Th1 response has been induced in the cancer may be indicative of the herpes simplex virus successfully reaching the tumor and initiating an anti-cancer response, which may involve lysis of tumor cells by the herpes simplex virus and/or induction of a host anti-cancer immune response. In such cases, the subject may be selected for a further stage of treatment with an immune checkpoint inhibitor. In this stage, the subject preferably

continues treatment with herpes simplex virus and treatment with immune checkpoint inhibitor is provided as an additional treatment. The subject thereby receiving combined treatment with herpes simplex virus and immune checkpoint inhibitor.

Accordingly, in one aspect of the present invention a herpes simplex virus or immune checkpoint inhibitor is provided for use in a method for the treatment of cancer, the method comprising:

(a) intravenous administration to a subject having cancer of one or more doses of a herpes simplex virus, wherein the herpes simplex virus lacks functional ICP34.5 genes;

(b) determining, preferably by *in vitro* analysis of a blood sample or of a tumor tissue sample, one or both of: (i) whether the herpes simplex virus has reached the tumor; and/or (ii) the presence of a Th1 response; and

(c) administering to a subject in which one or both of (i) herpes simplex virus has reached the tumor; and/or (ii) a Th1 response is present, a herpes simplex virus and an immune checkpoint inhibitor.

In another aspect of the present invention the use of a herpes simplex virus or an immune checkpoint inhibitor in the manufacture of a medicament is provided for use in a method for the treatment of cancer, the method comprising:

(a) intravenous administration to a subject having cancer of one or more doses of a herpes simplex virus, wherein the herpes simplex virus lacks functional ICP34.5 genes;

(b) determining, preferably by *in vitro* analysis of a blood sample or tumor tissue sample, one or both of: (i) whether the herpes simplex virus has reached the tumor; and/or (ii) the presence of a Th1 response; and

(c) administering to a subject in which one or both of (i) herpes simplex virus has reached the tumor; and/or (ii) a Th1 response is present, a herpes simplex virus and an immune checkpoint inhibitor.

In another aspect of the present invention a method for the treatment of cancer is provided, the method comprising:

(a) intravenous administration to a subject having cancer of one or more doses of a herpes simplex virus, wherein the herpes simplex virus lacks functional ICP34.5 genes;

(b) determining, preferably by *in vitro* analysis of a blood sample or tumor tissue sample, one or both of: (i) whether the herpes simplex virus has reached the tumor; and/or (ii) the presence of a Th1 response; and

(c) administering to a subject in which one or both of (i) herpes simplex virus has reached the tumor; and/or (ii) a Th1 response is present, a herpes simplex virus and an immune checkpoint inhibitor.

In (a) the subject may be a subject indicated for surgical removal of some or all of the tumor, and administration of the herpes simplex virus is prior to said surgery. In (b) the tumor tissue sample may be a sample of tumor tissue removed during surgery following intravenous administration of a herpes simplex virus according to (a).

In some embodiments methods according to the present invention comprise administering one or more doses of herpes simplex virus to the subject, determining the presence of a Th1 immune response in the subject, administering an immune checkpoint inhibitor to a subject in which a Th1 immune response is induced or elicited in combination with said herpes simplex virus.

In some embodiments methods according to the present invention comprise administering one or more doses of herpes simplex virus to the subject, determining whether herpes simplex virus has reached the tumor, administering an immune checkpoint inhibitor to a subject in which herpes simplex virus has reached the tumor in combination with said herpes simplex virus. In some embodiments determining whether herpes simplex virus has reached the tumor may comprise determining the presence of HSV DNA in tissue of the cancer, e.g. in a tumor tissue sample which may have been obtained during surgical removal of some or all of the tumor tissue.

In some embodiments the subject may receive administration of a plurality of doses of herpes simplex virus. The doses of herpes simplex virus may be administered in two stages, each stage may involve administration of one or a plurality of doses.

Where a subject has undergone surgery to remove some or all of the tumor tissue the timing of the next dose of herpes simplex virus may be varied to accommodate the condition of the subject and may, for example, be within 14 days of the day of surgery.

Where surgery is indicated, administration of herpes simplex virus may commence prior to the subject undergoing surgery for removal of tumor tissue, and preferably prior to receiving treatment with immune checkpoint inhibitor. The subject may not receive treatment with the immune checkpoint inhibitor, in particular as a combined treatment with herpes simplex virus, until after surgery for removal, and preferably analysis, of tumor tissue.

In a first stage, the subject does not receive treatment with an immune checkpoint inhibitor, although the subject may receive concomitant chemotherapy or radiation therapy, e.g. as part of standard of care treatment. Preferably, this stage takes place prior to determining, whether the herpes simplex virus has reached the tumor, and/or whether a Th1 response has been induced.

The subject response to treatment of herpes simplex virus in the first stage is thereby monitored to determine whether treatment with an immune checkpoint inhibitor as an additional agent will be helpful. Surgical removal of tumor tissue or the taking of a blood sample, followed by determination of the subject response to herpes simplex virus monotherapy may form a decision point in terms of selecting a subject for entry into a second stage of treatment.

Where a subject is selected for treatment with an immune checkpoint inhibitor in a second stage of treatment the subject will preferably continue to receive treatment with herpes simplex virus, preferably receiving the herpes simplex virus by intravenous administration. The dosing schedule and amount of herpes simplex virus may continue unchanged from the first to second stage, or may be changed as considered necessary by the responsible medical practitioner.

In another aspect of the present invention a method for selecting patients with cancer for treatment with an immune checkpoint inhibitor and a herpes simplex virus is provided, the method comprising:

intravenous administration of a herpes simplex virus to a subject having cancer, wherein the herpes simplex virus lacks functional ICP34.5 genes;

determining, preferably *in vitro* in a sample of blood or tumor tissue obtained from the subject, one or both of: (i) whether the herpes simplex virus has reached the tumor; and/or (ii) the presence of a Th1 response; and

selecting a subject in which one or both of (i) herpes simplex virus has reached the tumor; and/or (ii) a Th1 response is present, for treatment with an immune checkpoint inhibitor in combination with said herpes simplex virus.

In another aspect of the present invention a method for selecting patients with cancer for treatment with an immune checkpoint inhibitor and a herpes simplex virus is provided, the method comprising:

intravenous administration of a herpes simplex virus to a subject having cancer, the subject being indicated for surgical removal of some or all of the tumor and the intravenous administration of herpes simplex virus being prior to the surgery, wherein the herpes simplex virus lacks functional ICP34.5 genes;

determining, preferably *in vitro*, one or both of: (i) whether the herpes simplex virus has reached the tumor; and/or (ii) the presence of a Th1 response in tumor tissue removed during the surgery; and

selecting a subject in which one or both of (i) herpes simplex virus has reached the tumor; and/or (ii) a Th1 response is present in tumor tissue removed during the surgery, for treatment with an immune checkpoint inhibitor in combination with said herpes simplex virus.

In some embodiments determining whether herpes simplex virus has reached the tumor may comprise determining, *in vitro*, the presence of HSV DNA in a blood sample or tumor tissue sample, e.g. tumor tissue sample removed during surgery.

In some embodiments the subject has been treated with herpes simplex virus and is, or has been, selected for treatment with the immune checkpoint inhibitor as having a Th1 immune response.

In some embodiments methods according to the present invention may comprise administering one or more doses of the herpes simplex virus effective to induce a Th1 immune response.

Accordingly, in some embodiments, the method comprises administering one or more doses of a herpes simplex virus effective to induce a Th1 immune response in the

subject, and administering a therapeutically effective amount of an immune checkpoint inhibitor.

In some aspects of the present invention a method of treating cancer in a subject in need of treatment is provided, the method comprising administering one or more doses of a herpes simplex virus to a subject, and administering to a subject in which a Th1 immune response is induced or elicited a therapeutically effective amount of an immune checkpoint inhibitor.

In some embodiments the method may comprise the step of determining the presence of a Th1 immune response in the subject, optionally prior to administration of the immune checkpoint inhibitor.

In some embodiments the method may comprise the step of selecting a subject in which a Th1 immune response is induced or elicited by the herpes simplex virus for treatment with the immune checkpoint inhibitor, said selection preferably being made prior to administration of the immune checkpoint inhibitor.

In some embodiments the method comprises administering one or more doses of a herpes simplex virus to the subject, determining the presence of a Th1 immune response in the subject, administering to a subject in which a Th1 immune response is induced or elicited a therapeutically effective amount of an immune checkpoint inhibitor.

In some embodiments the method comprises administering one or more doses of a herpes simplex virus to the subject, selecting a subject in which a Th1 immune response is induced or elicited for treatment with an immune checkpoint inhibitor, and administering to a selected subject a therapeutically effective amount of an immune checkpoint inhibitor.

In some embodiments the method comprises selecting a subject in which a Th1 immune response is induced or elicited by the herpes simplex virus for treatment with the immune checkpoint inhibitor. In some embodiments administration of the herpes simplex virus is for a period of time sufficient to induce or elicit a Th1 response in the subject.

In some embodiments the method comprises selecting a subject in which herpes simplex virus administered in a first stage of treatment has reached the tumor for treatment with

the immune checkpoint inhibitor. In some embodiments administration of the herpes simplex virus is for a period of time sufficient to allow the herpes simplex virus to reach the tumor.

In some embodiments administration of the herpes simplex virus is for a period of time prior to treatment with an immune checkpoint inhibitor in which period the subject is administered herpes simplex virus but is not administered an immune checkpoint inhibitor.

Determining whether herpes simplex virus has reached the tumor

In some embodiments a determination is made as to whether herpes simplex virus administered to the subject has reached the site of a tumor. This is of particular relevance where the herpes simplex virus is administered by intravenous administration as the site of administration will be distal from the tumor and the herpes simplex virus must reach the tumor and preferably be able to replicate in the tumor.

The determination may be made by detecting intact herpes simplex virus, or components or fragments of herpes simplex virus (e.g. HSV DNA, HSV proteins or HSV antigens), in a sample obtained from the subject, preferably in either a blood sample or tumor tissue sample.

Intact herpes simplex virus may be detected by viral culture of a sample, e.g. shell vial culture (e.g. *see* Tse et al., J Clin Microbiol. 1989 Jan 27(1): 199-200).

HSV DNA may be detected by routine techniques such as qPCR or Real-Time PCR (e.g. *see* Kessler et al., J Clin Microbiol. 2000 Jul; 38(7):2638-2642; Pandori et al BMC Infectious Diseases 2006 6:104; Hong et al., BioMed Research International Vol 2014 (2014) Article ID 261947, 5 pages).

In some embodiments samples may be collected at selected intervals, e.g. before treatment with herpes simplex virus and at selected intervals after treatment has commenced, to monitor the detection of herpes simplex virus, components or fragments of herpes simplex virus.

For example, samples, e.g. blood samples, may be collected after treatment with herpes simplex virus has commenced at one of about 24 hours, about 48 hours, about 72 hours,

about 96 hours, about 120 hours, about 144 hours, about one week, about two weeks, about three weeks or about four weeks. The level of HSV DNA in the sample may be detected.

Such monitoring may show an inability to detect, or detection of only low levels of, herpes simplex virus, components or fragments thereof, immediately after commencement of treatment and/or during initial stages of treatment followed by ability to detect herpes simplex virus, components or fragments thereof as treatment progresses. This re-emergence of detection of herpes simplex virus, components or fragments thereof may indicate that the herpes simplex virus has reached the tumor, and optionally that it is replicating in the tumor.

Accordingly, in some embodiments the determination may be made after passage of a period of time from administration of herpes simplex virus sufficient for the virus or viral fragments or components to be detectable in a sample, e.g. a blood or tumor tissue sample.

Detecting a Th1 immune response

A Th1 immune response may be characterised by upregulation of one or more relevant cytokines selected from the group consisting of IFN-γ, IL-2, IL-12, IP-10, MIG, TNF-α, IL-18, IL-27, TNF-β. In some embodiments a Th1 response may be characterised by upregulation of IFN-γ, IL-2, or IL-12.

Not all subjects respond to administration of oncolytic HSV by induction of a Th1 response and subjects may therefore be monitored to determine their response to treatment with the oncolytic HSV. Subjects who respond may continue treatment with oncolytic HSV at a dose and/or dosage regime designed to maintain the sustained Th1 response. Subjects who do not respond to treatment by generation of a Th1 response may discontinue treatment with oncolytic HSV, or may transition to combination treatment with oncolytic HSV and one or more additional chemotherapeutic agents.

Accordingly, the presence of a Th1 response may be determined by measuring the level of a Th1 cytokine in a sample obtained from a subject.

In addition to the cytokines secreted by Th1 cells, the expression of specific cell surface proteins or receptors, including IL-12 R beta 2, IL-27 R alpha/WSX-1, IFN-gamma R2,

CCR5, and CXCR3, can be used to distinguish Th1 cells from other T cell subtypes. T cells associated with a Th1 response can also be partially characterised as $CD4^+$ or $CD8^+$. Accordingly, the presence of a Th1 response may also be determined or partially determined by measuring the expression of Th1 cell surface proteins or receptors by T cells in a sample obtained from a subject and/or by measuring the proportion of T cells that are $CD4^+$ or $CD8^+$ in a sample obtained from a subject.

In some preferred embodiments a Th1 response may be characterised by upregulation of IFN-γ. In some preferred embodiments a Th1 response may be characterised by upregulation of IL-2. In some preferred embodiments a Th1 response may be characterised by upregulation of IL-12. In some preferred embodiments a Th1 response may be characterised by upregulation of IFN-γ and determination of T cell $CD8^+$ status. In some preferred embodiments a Th1 response may be characterised by upregulation of IL-2 and determination of T cell $CD8^+$ status. In some preferred embodiments a Th1 response may be characterised by upregulation of IL-12 and determination of T cell $CD8^+$ status. In some preferred embodiments a Th1 response may be characterised by upregulation of IFN-γ and one or both of IL-2 and IL-12, and optionally determination of T cell $CD8^+$ status. In some preferred embodiments a Th1 response may be characterised by upregulation of IL-2 and one or both of IFN-γ and IL-12, and optionally determination of T cell $CD8^+$ status. In some preferred embodiments a Th1 response may be characterised by upregulation of IL-12 and one or both of IFN-γ and IL-2, and optionally determination of T cell $CD8^+$ status. In some preferred embodiments a Th1 response may be characterised by upregulation of IL-2 and/or IL-12.

The detection and characterisation of a Th1 immune response is a routine procedure for one of ordinary skill in the art. Numerous cytokine and cell detection assays are available including single and multiplexed ELISAs, reverse-transcription-PCR, Taqman real-time PCR, immunohistochemistry and flow cytometry (e.g. *see* Whiteside TL. Cytokine assays. Biotechniques 2002;Suppl:4 – 8, 10, 12 – 5; Pala P, Hussell T, Openshaw PJ. Flow cytometric measurement of intracellular cytokines. J Immunol Methods 2000;243:107 – 24; Jason J, Larned J. Single-cell cytokine profiles in normal humans: comparison of flow cytometric reagents and stimulation protocols. J Immunol Methods 1997;207:13 – 22; Farrell AM et al. A rapid flow cytometric assay to detect CD4+ and CD8+ T-helper (Th) 0, Th1 and Th2 cells in whole blood and its application to study cytokine levels in atopic dermatitis before and after cyclosporin therapy. Br J Dermatol. 2001 Jan;144(1):24-33).

The induction of a Th1 response and maintenance of the response over time may therefore be monitored at desired intervals by analysing the level of cytokine expression in a sample taken from a subject. Such monitoring may be conducted, daily, weekly, fortnightly, monthly or yearly as desired and/or consider appropriate by a medical practitioner.

In some embodiments detection of a Th1 response involves detection of an upregulation of a Th1 cell population or of Th1 cytokines.

Upregulation may be determined by comparing the level of a cell population or cytokine before and after a treatment, e.g. before and then after a period of treatment (optionally pre-treatment) with an herpes simplex virus. Levels of a cell population or of a cytokine may be quantitated for absolute comparison, or relative comparisons may be made.

In some embodiments upregulation may be considered to be present when the level of a cell population or cytokine in the test sample is at least 1.1 times that in the control sample (e.g. a sample obtained before treatment with herpes simplex virus). More preferably, the level may be selected from one of at least 1.2, at least 1.3, at least 1.4, at least 1.5, at least 1.6, at least 1.7, at least 1.8, at least 1.9, at least 2.0, at least 2.1, at least 2.2, at least 2.3, at least 2.4 at least 2.5, at least 2.6, at least 2.7, at least 2.8, at least 2.9, at least 3.0, at least 3.5, at least 4.0, at least 5.0, at least 6.0, at least 7.0, at least 8.0, at least 9.0, at least 10.0, at least 11.0, at least 12.0, at least 13.0, at least 14.0, or at least 15.0 times that in the control sample.

Detection of a Th1 response may be further confirmed by detection of an anti-tumor IgG response. This may be determined, for example, by immunoassay to detect the presence of IgG in a sample from a subject having received treatment with oncolytic herpes simplex virus. Comparison may be made to the absence of such a response in a patient not having received treatment with herpes simplex virus.

In addition to detection of a Th1 response, the presence of HSV DNA may be detected in a sample. HSV DNA may be detected by routine techniques such as qPCR or Real-Time PCR (e.g. *see* Kessler et al., J Clin Microbiol. 2000 Jul; 38(7):2638-2642; Pandori et al BMC Infectious Diseases 2006 6:104; Hong et al., BioMed Research International Vol 2014 (2014) Article ID 261947, 5 pages).

Sample

A sample may be obtained from a subject. Samples may be obtained before, during and/or after treatment with an herpes simplex virus. A sample obtained before treatment has commenced may provide reference values, e.g. for cytokine levels, cell surface proteins or receptors or proportion of $CD4^+$ or CD8+ T cells, which may allow for comparison with levels determined during or after treatment, the comparison enabling a determination of whether herpes simplex virus has reached the tumor or a Th1 response has been induced or elicited in the subject.

A sample may be taken or derived from any tissue, e.g. tumor tissue, or bodily fluid, and may be processed to isolate a cell population of interest, e.g. white blood cells, lymphocytes or T cells.

A sample may comprise or may be derived from: a quantity of blood; a quantity of serum derived from the individual's blood which may comprise the fluid portion of the blood obtained after removal of the fibrin clot and blood cells; saliva; other bodily fluids, pleural fluid, effusion fluid, ascites, or a fluid produced by a cancer.

In preferred arrangements the sample is taken from a bodily fluid, more preferably one that circulates through the body. Accordingly, the sample may be a blood sample or lymph sample.

In some embodiments the sample is a blood sample or blood-derived sample. The blood derived sample may be a selected fraction of a patient's blood, e.g. a selected cell-containing fraction or a plasma or serum fraction. A selected cell-containing fraction may contain cell types of interest which may include white blood cells (WBC), lymphocytes, peripheral blood mononuclear cells (PBC) and/or granulocytes, and/or red blood cells (RBC).

In some embodiments the sample is a biopsy, or is derived from a biopsy. In some embodiments the sample is a tumor tissue sample, e.g. obtained during surgery to remove tumor tissue. In some embodiments the sample may be obtained during surgical resection of a tumor. Solid tumors are particularly suitable for obtaining samples by biopsy or during surgical resection.

Subjects selected for treatment with herpes simplex virus may be indicated for surgical removal of tumor tissue (referred to herein as 'tumor resection'). For example, they may have a cancer considered, by a medical practitioner, operable to remove some or all of the tumor tissue. Treatment with herpes simplex virus may commence prior to tumor resection.

Tumor tissue removed during tumor resection may be analysed to determine whether herpes simplex virus has reached the tumor, e.g. by detecting *in vitro* the presence of HSV DNA in excised tumor tissue and/or the expression of HSV antigens by immunohistochemistry and/or to determine whether a Th1 response is present in the cancer, e.g. by detecting *in vitro* the presence of a Th1 response in excised tumor tissue.

In some embodiments the sample is a sample of effusion fluid, e.g. pleural fluid or ascites. Effusion fluid refers to an excess of fluid produced by a subject in direct or indirect response to the presence of a cancer in the subject. Effusion fluid may collect in a body cavity such that accumulation of effusion fluid may occur where the rate of production of the effusion fluid exceeds the rate of reabsorption. Pleural effusions (sometimes called malignant pleural effusions) lead to accumulation of fluid in the pleural cavity and occur in some lung cancers, e.g. mesothelioma. Effusion fluid collecting in the peritoneal cavity is commonly referred to as ascites, and can be a symptom of a number of types of cancer including cancer of the breast, lung, colon, stomach, pancreas, ovary, endometrium as well as lymphoma. Pericardial effusion is the abnormal accumulation of fluid in the pericardial cavity. The effusion fluid is preferably exudative.

Effusion fluid can be drained from a respective body cavity by well-known aseptic procedures (e.g. see Warren et al. Ann Thorac Surg 2008;85:1049-55; Warren et al. European Journal of Cardio-thoracic Surgery 33 (2008) 89-94). In some instances, a tube or catheter is inserted in the body cavity in order to drain effusion fluid. Drainage of effusion fluid is a common part of the diagnosis, treatment and management of many forms of cancer. Drainage of effusion fluid provides a means of obtaining a sample of a subject's effusion fluid for analysis.

Cancer

A cancer may be any unwanted cell proliferation (or any disease manifesting itself by unwanted cell proliferation), neoplasm or tumor or increased risk of or predisposition to the unwanted cell proliferation, neoplasm or tumor. The cancer may be benign or

malignant and may be primary or secondary (metastatic). A neoplasm or tumor may be any abnormal growth or proliferation of cells and may be located in any tissue. Examples of tissues include the adrenal gland, adrenal medulla, anus, appendix, bladder, blood, bone, bone marrow, brain, breast, cecum, central nervous system (including or excluding the brain) cerebellum, cervix, colon, duodenum, endometrium, epithelial cells (e.g. renal epithelia), gallbladder, oesophagus, glial cells, heart, ileum, jejunum, kidney, lacrimal glad, larynx, liver, lung, lymph, lymph node, lymphoblast, maxilla, mediastinum, mesentery, myometrium, nasopharynx, omentume, oral cavity, ovary, pancreas, parotid gland, peripheral nervous system, peritoneum, pleura, prostate, salivary gland, sigmoid colon, skin, small intestine, soft tissues, spleen, stomach, testis, thymus, thyroid gland, tongue, tonsil, trachea, uterus, vulva, white blood cells.

Tumors to be treated may be nervous or non-nervous system tumors. Nervous system tumors may originate either in the central or peripheral nervous system, e.g. glioma, medulloblastoma, meningioma, neurofibroma, ependymoma, Schwannoma, neurofibrosarcoma, astrocytoma and oligodendroglioma. Non-nervous system cancers/tumors may originate in any other non-nervous tissue, examples include melanoma, mesothelioma, lymphoma, myeloma, leukemia, Non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma, chronic myelogenous leukemia (CML), acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), cutaneous T-cell lymphoma (CTCL), chronic lymphocytic leukemia (CLL), hepatoma, epidermoid carcinoma, prostate carcinoma, breast cancer, lung cancer , colon cancer, ovarian cancer, pancreatic cancer, thymic carcinoma, NSCLC, haematologic cancer and sarcoma.

In the context of treatment of a metastatic cancer, treatment may be of cancers or tumors of a given cell type. The treatment may involve eliciting a systemic anti-tumor Th1 immune response in the subject, who may be at risk of developing single or multiple metastatic cancers or tumors of the given cell type. Administration of oncolytic herpes simplex virus may therefore induce a Th1 immune response that is specific for the tumor cell type and that kills cells of inoculated tumor and non-inoculated tumors.

In some embodiments the cancer may be a solid tumor. Solid tumors may, for example, be in bladder, bone, breast, eye, stomach, head and neck, germ cell, kidney, liver, lung, nervous tissue, ovary, pancreas, prostate skin, soft-tissues, adrenal gland, nasopharynx, thyroid, retina, and uterus. Solid tumors may include melanoma, rhabdomyosarcoma, Ewing sarcoma, and neuroblastoma.

The cancer may be a pediatric solid tumor, i.e. solid tumor in a child, for example osteosarcoma, chondroblastoma, chondrosarcoma, Ewing sarcoma, malignant germ cell tumor, Wilms tumor, malignant rhabdoid tumor, hepatoblastoma, hepatocellular carcinoma, neuroblastoma, melanoma, adrenocorticoid carcinoma, nasopharyngeal carcinoma, thyroid carcinoma, retinoblastoma, soft-tissue sarcoma, rhabdomyosarcoma, desmoid tumor, fibrosarcoma, liposarcoma, malignant fibrous histiocytoma, neurofibrosarcoma.

The cancer may be in a location that would require surgery or an invasive procedure in order to administer the virus by intratumoral injection. For example, the cancer may be a visceral cancer or visceral tumor, e.g. a cancer of the internal organs of the body such as within the chest (e.g. heart, lungs), abdomen, (e.g. liver, pancreas, stomach, intestines), body cavities (e.g. pleura, peritoneum) or brain. Cancers to be treated may be visceral lesions, e.g. metastatic visceral lesions.

In some embodiments the cancer is a head and neck cancer. In some optional embodiments the cancer is not a head and neck cancer.

In some preferred embodiments the cancer may be a mesothelioma, e.g. a malignant pleural mesothelioma.

The cancer may be one that is associated with effusion fluid. Such association may involve production of effusion fluid by the cancerous tissue, e.g. by cancer cells, or by normal cells near to or contained in the cancerous tissue, or it may involve overproduction of effusion fluid by other tissues (e.g. the lymphatic system) as a direct or indirect response to the presence of the cancer in the subject.

The cancer may be characterised by the collection of effusion fluid in one or more locations in the subject's body. Such locations may include one or more body cavities or tissue spaces. Body cavities (or serous cavities) may be formed by a serous membrane surrounding an organ or tissue and forming a sac in which fluid may collect.

For example, effusion fluid may collect in one or each (right or left) pleural cavity (the space between the visceral and parietal pleura). In another example, effusion fluid may accumulate in the peritoneal cavity (the space between the parietal peritoneum and

visceral peritoneum). In another example, fluid may accumulate in the pericardial cavity surrounding the heart (formed by the parietal and visceral pericardium). In another example, fluid may accumulate in the perimetrium surrounding the uterus.

Thus, in some embodiments the cancer is one in which pleural effusion, peritoneal effusion (ascites), pericardial effusion or perimetrial effusion occurs.

All types of cancer may be associated with production of effusion fluid, partly because all types of cancer can metastasize to any of the body's serous cavities and result in malignant effusion (Olopade CA-A Cancer Journal For Clinicians Vol.41, No.3 May/June 1991). Cancers in which production of effusion fluid is known to occur include cancers of the following type or tissues: lung cancers, pleural cancers, mesothelioma, malignant pleural mesothelioma, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), ovarian cancers, ovarian carcinoma, uterine cancer, endometrial cancer, heart cancer, breast cancer, colon cancer, stomach cancer, gastric cancer, pancreatic cancer, kidney cancer, liver cancer, lymphatic cancer (e.g. lymphoma, non-Hodgkin lymphoma), soft tissue sarcoma, osteosarcoma, adenocarcinoma, parotid cancer (e.g. parotid adenocarcinoma), thymic carcinoma, cancers of the reproductive tract (including cervical, fallopian tube, endometrium), gastrointestinal tract, or genitourinary tract, leukemia, larynx, prostate, bile duct, hypernephroma, sinus piriformis carcinoma, thyroid cancer, melanoma and cancers of unknown primary (CUP) origin.

The development of a malignant pleural effusion is a common complication of advanced malignancies of many types of cancer, especially breast, lung (including NSCLC and SCLC) and ovarian carcinoma (Warren et al. European Journal of Cardio-thoracic Surgery 33 (2008) 89-94). Pleural effusions are at least known to be associated with cancers of the following type or tissue: lung, breast, lymphoma, uterus, ovarian, female reproductive tract (e.g. cervical, fallopian tube, endometrium), leukemia, pancreas, kidney, colon, stomach (gastric), mesothelioma, sarcoma, larynx, prostate, bile duct, hypernephroma, sinus piriformis carcinoma, thyroid cancer, non-Hodgkin lymphoma, malignant melanoma, reproductive tract, gastrointestinal tract, genitourinary tract, (Warren et al. Ann Thorac Surg 2008;85:1049-55; Warren et al. European Journal of Cardio-thoracic Surgery 33 (2008) 89-94; Schulze et al. Ann Thorac Surg 2001;71:1809-12; Olopade CA-A Cancer Journal For Clinicians Vol.41, No.3 May/June 1991).

Peritoneal effusions (ascites) are at least known to be associated with cancers of the following type or tissue: ovarian, epithelial related ovarian, uterus, breast, colon, gastric, pancreatic, hepatic, colon, lymphoma, mesothelioma, and cancers of unknown primary (CUP) origin (Olopade CA-A Cancer Journal For Clinicians Vol.41, No.3 May/June 1991)

Pericardial effusions are at least known to be associated with cancers of the following type or tissue: lung, breast, leukemia, lymphoma, sarcoma, melanoma (Olopade CA-A Cancer Journal For Clinicians Vol.41, No.3 May/June 1991).

Optionally, in some preferred embodiments the cancer is not a melanoma. Optionally, in some preferred embodiments the cancer is not a cancer occurring in the skin. Optionally, in some preferred embodiments the cancer is not a primary melanoma. Optionally, in some preferred embodiments the cancer is not metastatic (secondary melanoma). Optionally, in some embodiments the cancer is not stage IIIb to stage IV melanoma.

Head and Neck Cancer

Some aspects and embodiments of the present invention concern the use of herpes simplex virus to treat head and neck cancer.

In some embodiments, the subject may receive a herpes simplex virus and an immune checkpoint inhibitor as part of the programme of treatment. During combined treatment, the herpes simplex virus and immune checkpoint inhibitor may be administered simultaneously, e.g. as a combined preparation or as separate preparations one administered immediately after the other. Alternatively, they may be administered separately and sequentially, where one agent is administered and then the other administered later after a predetermined time interval.

In one aspect of the present invention a herpes simplex virus is provided for use in a method of treating head and neck cancer, the method comprising administering to a subject having head and neck cancer said herpes simplex virus and an immune checkpoint inhibitor, wherein the herpes simplex virus lacks functional ICP34.5 genes.

In another aspect of the present invention an immune checkpoint inhibitor is provided for use in a method of treating head and neck cancer, the method comprising administering to a subject having head and neck cancer said immune checkpoint inhibitor and a herpes simplex virus, wherein the herpes simplex virus lacks functional ICP34.5 genes.

In another aspect of the present invention the use of a herpes simplex virus in the manufacture of a medicament for use in a method of treating head and neck cancer is provided, the method comprising administering to a subject having head and neck cancer said herpes simplex virus and an immune checkpoint inhibitor, wherein the herpes simplex virus lacks functional ICP34.5 genes.

In another aspect of the present invention the use of an immune checkpoint inhibitor in the manufacture of a medicament for use in a method of treating head and neck cancer is provided, the method comprising administering to a subject having head and neck cancer said immune checkpoint inhibitor and a herpes simplex virus, wherein the herpes simplex virus lacks functional ICP34.5 genes.

In another aspect of the present invention a method for the treatment of head and neck cancer is provided, the method comprising administering to a subject having head and neck cancer an immune checkpoint inhibitor and a herpes simplex virus, wherein the herpes simplex virus lacks functional ICP34.5 genes.

In preferred embodiments the herpes simplex virus is administered to the subject systemically, preferably to the blood. In a preferred embodiment administration is by intravenous administration, e.g. by intravenous infusion.

In preferred embodiments the head and neck cancer is stage III or stage IV.

In one aspect of the present invention method comprises administering to a patient with stage III or stage IV head and neck cancer a therapeutically effective amount of an immune checkpoint inhibitor and an herpes simplex virus, wherein the herpes simplex virus lacks functional ICP34.5 genes and the herpes simplex virus is administered to the patient systemically by intravenous infusion on one or more occasions.

In some embodiments the subject has a head and neck cancer which is suitable for surgical removal of some or all of the tumor tissue.

In some embodiments the subject may receive administration of one or a plurality (preferably a plurality) of doses of herpes simplex virus. Each dose of herpes simplex virus is preferably of greater than $2x10^6$ iu. Doses may be in a range selected from the

group consisting of: $2x10^6$ to $9x10^6$ iu, $2x10^6$ to $5x10^6$ iu, $5x10^6$ to $9x10^6$ iu, $2x10^6$ to $1x10^7$ iu, $2x10^6$ to $5x10^7$ iu, $2x10^6$ to $1x10^8$ iu, $2x10^6$ to $5x10^8$ iu, $2x10^6$ to $1x10^9$ iu, $5x10^6$ to $1x10^7$ iu, $5x10^6$ to $5x10^7$ iu, $5x10^6$ to $1x10^8$ iu, $5x10^6$ to $5x10^8$ iu, $5x10^6$ to $1x10^9$ iu, $5x10^6$ to $5x10^9$ iu, $1x10^7$ to $9x10^7$ iu, $1x10^7$ to $5x10^7$ iu, $1x10^8$ to $9x10^8$ iu, $1x10^8$ to $5x10^8$ iu. In some embodiments suitable doses may be in the range $2x10^6$ to $9x10^6$ iu, $1x10^7$ to $9 x10^7$ iu, or $1x10^8$ to $9 x10^8$ iu. In some embodiments suitable doses may be about $1x10^7$ iu or about $1x10^8$ iu. Dosage figures may optionally be +/- half a log value.

Doses of herpes simplex virus are preferably administered by intravenous infusion, which may take place over a period of several hours, e.g. about 30 minutes to about 4 hours. A subject will commonly receive a plurality of doses of herpes simplex virus as part of a course of treatment, preferably 3 or more doses. The doses may be administered in accordance with a dosing regime. For example, each dose of herpes simplex virus may be administered within 1 to 7, 1 to 14, or 1 to 21 days of the preceding dose.

Doses of herpes simplex virus may be administered at regular intervals, e.g. every 7 days, every 14 days, every 21 days, or every 28 days (+/- 1, 2 or 3 days). The number of doses of herpes simplex virus administered in a course of treatment may be any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 doses or more, preferably at least 3 or at least 4 doses, or up to 8 doses.

A dosing regime for a herpes simplex virus may be designed to continue dosing until: surgery is scheduled, toxicity or disease progression, a predetermined maximum number of doses is reached, e.g. 3, 4, 5, 6, 7, 8, 9 or 10 doses, or a fixed number of doses is reached, e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 doses, complete response, disease progression, or intolerance of the treatment.

The immune checkpoint inhibitor may be administered intravenously. The subject may receive a single intravenous administration of a combined preparation of herpes simplex virus and immune checkpoint inhibitor, or may receive separate intravenous administrations of herpes simplex virus and immune checkpoint inhibitor. Herpes simplex virus and immune checkpoint inhibitor may be administered on the same day, e.g. during the same hospital visit, or on separate days.

A subject will preferably receive a plurality of doses of immune checkpoint inhibitor during the course of treatment. The doses may be administered in accordance with a dosing

regime. For example, each dose of immune checkpoint inhibitor may be administered within 1 to 21 or 1 to 28 days of the preceding dose. Doses of immune checkpoint inhibitor may be administered at regular intervals, e.g. every 7 days, every 14 days, every 21 days, or every 28 days (+/- 1, 2 or 3 days).

Suitable doses of immune checkpoint inhibitor will vary depending on the immune checkpoint inhibitor selected and the prescribing information of the medical practitioner. By way of example, suitable doses may be one of 100mg, 200mg, 300mg or 400mg, or 1mg/kg, 2mg/kg, 3mg/kg or 4mg/kg.

Doses of immune checkpoint inhibitor may be administered by intravenous infusion, which may take place over a period of up to several hours, e.g. about 30 minutes to about 4 hours.

By way of example, pembrolizumab may be administered by intravenous infusion once every 3 weeks, optionally at a dose of about 200mg. In another example, pembrolizumab may be administered by intravenous infusion once every week, optionally at a dose of about 200mg, and optionally in combination with chemotherapy, e.g. in the form of platinum and/or 5-fluoruracil.

A combined dosing regime for a herpes simplex virus and immune checkpoint inhibitor may be designed to continue dosing until: toxicity or disease progression, a predetermined maximum number of doses of one or both agents is reached, e.g. 3, 4, 5, 6, 7, 8, 9 or 10 doses, a fixed number of doses of one or both agents is reached, e.g. 3, 4, 5, 6, 7, 8, 9, or 10 doses, complete response, disease progression, or intolerance of the treatment.

In some embodiments herpes simplex virus may be administered more regularly than the immune checkpoint inhibitor. Administration of herpes simplex virus may optionally precede, i.e. commence before, administration of the immune checkpoint inhibitor. For example, herpes simplex virus may be administered once weekly, whereas the immune checkpoint inhibitor may be administered once every 2, 3 or 4 weeks, optionally commencing in the first, second or third week of treatment. The dosing schedule may be designed such that administration of herpes simplex virus is on the same day as administration of the immune checkpoint inhibitor.

In another aspect of the present invention a herpes simplex virus is provided for use in a method of treating head and neck cancer, the method comprising administering: a herpes simplex virus by intravenous infusion at a dose of greater than $2x10^6$ iu at day 1 of week 1 followed by a dose of greater than $2x10^6$ iu at day 1 of week 2, and every week thereafter until surgery is scheduled, toxicity or disease progression, a predetermined maximum number of doses is reached or a fixed number of doses is reached, complete response, disease progression, or intolerance of the treatment, wherein the herpes simplex virus lacks functional ICP34.5 genes; and an immune checkpoint inhibitor by intravenous infusion every 3 weeks for at least 3 infusions beginning with or after the second or third dose of the herpes simplex virus.

In another aspect of the present invention an immune checkpoint inhibitor is provided for use in a method of treating head and neck cancer, the method comprising administering: a herpes simplex virus by intravenous infusion at a dose of greater than $2x10^6$ iu at day 1 of week 1 followed by a dose of greater than $2x10^6$ iu at day 1 of week 2, and every week thereafter until surgery is scheduled, toxicity or disease progression, a predetermined maximum number of doses is reached or a fixed number of doses is reached, complete response, disease progression, or intolerance of the treatment, wherein the herpes simplex virus lacks functional ICP34.5 genes; and an immune checkpoint inhibitor by intravenous infusion every 3 weeks for at least 3 infusions beginning with or after the second or third dose of the herpes simplex virus.

In another aspect of the present invention the use of a herpes simplex virus in the manufacture of a medicament for use in a method of treating head and neck cancer is provided, the method comprising administering: a herpes simplex virus by intravenous infusion at a dose of greater than $2x10^6$ iu at day 1 of week 1 followed by a dose of greater than $2x10^6$ iu at day 1 of week 2, and every week thereafter until surgery is scheduled, toxicity or disease progression, a predetermined maximum number of doses is reached or a fixed number of doses is reached, complete response, disease progression, or intolerance of the treatment, wherein the herpes simplex virus lacks functional ICP34.5 genes; and an immune checkpoint inhibitor by intravenous infusion every 3 weeks for at least 3 infusions beginning with or after the second or third dose of the herpes simplex virus.

In another aspect of the present invention the use of an immune checkpoint inhibitor in the manufacture of a medicament for use in a method of treating head and neck cancer is

provided, the method comprising administering: a herpes simplex virus by intravenous infusion at a dose of greater than $2x10^6$ iu at day 1 of week 1 followed by a dose of greater than $2x10^6$ iu at day 1 of week 2, and every week thereafter until surgery is scheduled, toxicity or disease progression, a predetermined maximum number of doses is reached or a fixed number of doses is reached, complete response, disease progression, or intolerance of the treatment, wherein the herpes simplex virus lacks functional ICP34.5 genes; and an immune checkpoint inhibitor by intravenous infusion every 3 weeks for at least 3 infusions beginning with or after the second or third dose of the herpes simplex virus.

In another aspect of the present invention a method for the treatment of head and neck cancer is provided, the method comprising administering: a herpes simplex virus by intravenous infusion at a dose of greater than $2x10^6$ iu at day 1 of week 1 followed by a dose of greater than $2x10^6$ iu at day 1 of week 2, and every week thereafter until surgery is scheduled, toxicity or disease progression, a predetermined maximum number of doses is reached or a fixed number of doses is reached, complete response, disease progression, or intolerance of the treatment, wherein the herpes simplex virus lacks functional ICP34.5 genes; and an immune checkpoint inhibitor by intravenous infusion every 3 weeks for at least 3 infusions beginning with or after the second or third dose of the herpes simplex virus.

The immune checkpoint inhibitor may be an inhibitor of at least one of PD-1, PD-L1, CTLA4, TIM-3 or LAG-3. The immune checkpoint inhibitor may be an anti-PD-1 antibody, anti-PD-L1 antibody, anti-CTLA4 antibody, anti-TIM-3 antibody or anti-LAG-3 antibody. The immune checkpoint inhibitor may be one of pembrolizumab, nivolumab or ipilimumab.

In some embodiments the head and neck cancer is selected from cancer of the larynx, thyroid, pharynx (e.g. throat, nasopharynx, oropharynx or hypopharynx), salivary glands, oral cavity (e.g. mouth, lips, tongue, gums, lining inside of the cheeks, floor of the mouth under the tongue, hard palate), nasal cavity, nose, paranasal sinuses, or skin cancers of the head and neck.

The head and neck cancer may be human papilloma virus (HPV)-positive and/or Epstein Barr Virus (EBV)-positive, or may be HPV-negative and/or EBV-negative.

In one aspect of the present invention a kit of parts is provided, the kit comprising a herpes simplex virus lacking functional ICP34.5 genes, and a package insert or label with directions to treat head and neck cancer by using a combination of the herpes simplex virus and an immune checkpoint inhibitor.

The directions may comprise instructions to administer to a patient with head and neck cancer: a herpes simplex virus administered by intravenous infusion at a dose of greater than $2x10^6$ iu at day 1 of week 1 followed by a dose of greater than $2x10^6$ iu at day 1 of week 2, and every week thereafter until surgery is scheduled, toxicity or disease progression, a predetermined maximum number of doses is reached or a fixed number of doses is reached, complete response, disease progression, or intolerance of the treatment; and an immune checkpoint inhibitor administered intravenously every 3 weeks for at least 3 infusions beginning with or after the second or third dose of the herpes simplex virus.

A method of manufacturing the kit is also provided.

In another aspect of the present invention a method of promoting a combination treatment comprising a herpes simplex virus lacking functional ICP34.5 genes and an immune checkpoint inhibitor, for the treatment of a patient with head and neck cancer is provided.

In some embodiments the promotion is by a package insert, wherein the package insert provides instructions to receive cancer treatment with a herpes simplex virus in combination with an immune checkpoint inhibitor. In some embodiments the promotion is by a package insert accompanying a formulation comprising the herpes simplex virus. In some embodiments the promotion is by written communication to a physician or health care provider. In some embodiments the promotion is by oral communication to a physician or health care provider. In some embodiments the promotion is followed by the treatment of the patient with the herpes simplex virus.

In another aspect of the present invention a method of instructing a patient with head and neck cancer by providing instructions to receive a combination treatment with a herpes simplex virus lacking functional ICP34.5 genes and an immune checkpoint inhibitor to extend survival of the patient is provided.

In some embodiments all copies of the ICP34.5 gene in the genome of the herpes simplex virus are modified such that the ICP34.5 gene is incapable of expressing a functional ICP34.5 gene product. As such the herpes simplex virus may be an ICP34.5 null mutant.

In some embodiments one or both of the ICP34.5 genes in the genome of the herpes simplex virus are modified such that the ICP34.5 gene is incapable of expressing a functional ICP34.5 gene product.

The herpes simplex virus may be an oncolytic herpes simplex virus.

In some embodiments the herpes simplex virus is a mutant of HSV-1 strain 17. In preferred embodiments the herpes simplex virus is HSV1716 (ECACC Accession No. V92012803). HSV1716 is also called SEPREHVIR®. In some embodiments the herpes simplex virus is a mutant of HSV-1 strain 17 mutant 1716.

In one aspect of the present invention a pharmaceutical composition comprising a herpes simplex virus and an immune checkpoint inhibitor is provided. The herpes simplex virus may be a mutant of HSV-1 strain 17. In preferred embodiments the herpes simplex virus is HSV1716.

In one aspect of the present invention a kit is provided, the kit comprising a predetermined amount of herpes simplex virus and a predetermined amount of an immune checkpoint inhibitor. The herpes simplex virus may be a mutant of HSV-1 strain 17. In preferred embodiments the herpes simplex virus is HSV1716. The kit may be provided together with instructions for the administration of the herpes simplex virus, and immune checkpoint inhibitor sequentially or simultaneously in order to provide a treatment for head and neck cancer.

In one aspect of the present invention products are provided containing therapeutically effective amounts of:

(i) HSV1716, and

(ii) an immune checkpoint inhibitor

for simultaneous or sequential use in a method of medical treatment, preferably treatment of head and neck cancer. The products may be pharmaceutically acceptable formulations and may optionally be formulated as a combined preparation for coadministration.

Optionally, in some embodiments the herpes simplex virus does not express GMCSF. Optionally, in some embodiments the herpes simplex virus encodes a functional ICP47 gene. Optionally, in some embodiments the herpes simplex virus is not a herpes simplex virus that lacks functional ICP34.5 genes and lacks a functional ICP47 gene and comprises a gene encoding human GM-CSF.

Optionally, in some embodiments the cancer is not a melanoma. Optionally, in some embodiments the cancer is not a primary melanoma. Optionally, in some embodiments the cancer is not a metastatic (secondary) melanoma. Optionally, in some embodiments the cancer is not stage IIIb to stage IV melanoma.

A cancer may be any unwanted cell proliferation (or any disease manifesting itself by unwanted cell proliferation), neoplasm or tumor or increased risk of or predisposition to the unwanted cell proliferation, neoplasm or tumor. A cancer may be benign or malignant and may be primary or secondary (metastatic). A neoplasm or tumor may be any abnormal growth or proliferation of cells and may be located in any tissue.

Head and neck cancers include cancers of the larynx, thyroid, pharynx (e.g. throat, nasopharynx, oropharynx or hypopharynx), salivary glands, oral cavity (e.g. mouth, lips, tongue, gums, lining inside of the cheeks, floor of the mouth under the tongue, hard palate), nasal cavity, nose, paranasal sinuses, or skin cancers of the head and neck.

Head and neck cancer may be oral cancer, e.g. of the tongue, the lip, the floor of the mouth, the salivary glands, or the gums; laryngeal cancer, e.g. of the larynx or trachea; pharyngeal cancer, e.g. cancer of the nasopharynx, the oropharynx, or the hypopharynx; nasopharyngeal cancer; oropharyngeal cancer, e.g. of the base of the tongue, the soft palate, and the area around the tonsils; hypopharyngeal cancer, e.g. of the uppermost portion of the oesophagus and surrounding the larynx; cancer of the nasal cavity or cancer of the paranasal sinus.

In some embodiments head and neck cancer may be squamous cell carcinoma of the head and neck (SCCHN or HNSCC); primary skin tumour (squamous cell carcinomas, basal cell carcinomas, melanomas, and Merkel cell carcinomas), lymphoma, or a malignant tumour of the salivary glands, soft tissues, and bones. In some embodiments

head and neck cancer may be a metastasis from tumours elsewhere in the body to the head and neck area.

A head and neck cancer may be at any stage, e.g. one of stages I, II, III, IV (i.e. one of IVA, IVB or IVC). In some preferred embodiments the head and neck cancer is stage III or IV. In some embodiments the head and neck cancer may be stage III or a higher stage. In some embodiments the head and neck cancer may be stage IV. In some embodiments the head and neck cancer may be stage IVA or a higher stage. In some embodiments the head and neck cancer may be stage IVB or a higher stage. In some embodiments the head and neck cancer may be stage IVC or a higher stage.

In some embodiments a subject having head and neck cancer may be treated with herpes simplex virus prior to surgery, e.g. surgical resection of tumor tissue. In some preferred embodiments a subject having head and neck cancer may be treated with herpes simplex virus during surgery of the head and neck cancer. For example, herpes simplex virus may be administered to the site of surgical resection during or following removal/ablation of tissue. In some embodiments a subject having head and neck cancer may be treated with oncolytic virus after surgery of the head and neck cancer, e.g. during a separate surgical procedure.

Optionally, the head and neck cancer may not be a cancer of one or more of the brain, central nervous system, oesophagus, glial cells, lacrimal gland, larynx, nasopharynx, oral cavity, parotid gland, salivary gland, skin, thyroid gland, tongue, tonsil, or trachea.

Types of Head & Neck Cancer

Cancer can develop in several different parts of the head and neck, including thyroid, salivary, and skin cancers, but by far the most common type of cancer is squamous cell carcinoma of the head and neck (SCCHN or HNSCC). Most HNSCC begins in the layer of flat cells (the epithelium) which line the structures of the upper aerodigestive tract, including the mouth (oral cavity), throat (pharynx), and voice box (larynx). Other malignancies in the head and neck area include primary skin tumours (squamous cell carcinomas, basal cell carcinomas, melanomas, and Merkel cell carcinomas), lymphomas, and malignant tumours of the salivary glands, soft tissues, and bones. Furthermore, metastases from tumours from elsewhere in the body may reveal in the head and neck area.

Some of the most common head and neck cancers include:

Oral Cancer: Cancer of the oral cavity is the most common type of head and neck cancer. Nearly 30,000 new cases of oral cancer are diagnosed in the US each year. Most oral cancers arise in the tongue, the lip, the floor of the mouth, and the minor salivary glands. The rest are found in the gums and other sites.

The main risk factors for oral cancer are smoking or chewing tobacco and excessive alcohol use. People who both smoke and drink heavily may be as much as 100 times more likely to develop oral cancer than those who neither smoke nor drink. Additional risk factors for oral cancer include infection with the human papillomavirus (HPV), although this risk is not as high as it is for pharyngeal cancer, and prolonged exposure to sunlight.

Many oral cancers are found incidentally during a routine dental examination. Most of these cancers can be cured if discovered early. The most common symptoms include a sore or lump on the lip or in the mouth that does not heal; a white and/or red patch on the gums, tongue, or cheeks (these white or red areas may also be a precancerous condition called dysplasia); unusual or persistent bleeding, pain, or numbness in the mouth; and swelling that causes dentures to fit poorly or become uncomfortable.

Laryngeal Cancer: Laryngeal cancer is the second most common type of head and neck cancer. An estimated 12,000 new cases of laryngeal cancer are diagnosed in the US each year. The vast majority of laryngeal cancers occur in men.

The larynx is located at the top of the trachea (windpipe) and is surrounded by the hypopharynx (the lower part of the throat where swallowing takes place). The larynx is visible on most men's throats as the Adam's apple. The larynx contains two bands of muscle called vocal cords, which vibrate as air passes through to make speech. The larynx also prevents food from entering the lungs. Tobacco and alcohol use, and especially the combination of the two, are the most common risk factors for laryngeal cancer. Additional risk factors include exposure in the workplace to wood and metal dusts, asbestos, paint fumes, and other chemical inhalants; a diet low in vitamins A and E; gastroesophageal reflux disease (GERD), which chronically exposes the throat to stomach acid; and infection with the human papillomavirus (HPV). People with aplastic anaemia, a blood disorder associated with certain hereditary conditions, also have a higher risk of developing laryngeal cancer.

The most common symptoms of laryngeal cancer include hoarseness, a lump in the neck (due to an enlarged lymph node), ear pain, and difficulty swallowing.

Pharyngeal (Throat) Cancer: Pharyngeal cancer arises in the hollow tube inside the neck that starts behind the nose and ends at the top of the oesophagus. Tumours in this region include cancer of the nasopharynx (the upper part of the throat behind the nose), the oropharynx (the middle part of the pharynx), and the hypopharynx (the bottom part of the pharynx). Each year in the US, an estimated 11,800 people develop pharyngeal cancers.

Nasopharyngeal Cancer: The nasopharynx, located behind the nose, includes two openings that lead to the ears. Nasopharyngeal cancer is much more common in Asia, especially southeast China, the Mediterranean area, and Africa than in the US, and is less commonly associated with tobacco and alcohol use than other head and neck cancers. Risk factors for this type of cancer include a diet high in salt-cured fish and infection with Epstein-Barr virus, a member of the herpesvirus family and one of the most common human viruses. The most common sign of nasopharyngeal cancer is a lump in the neck, caused by the spread of cancer to the lymph nodes. Other symptoms may include nasal congestion, pain or ringing in the ears, a persistent sore throat, or frequent nosebleeds.

Oropharyngeal Cancer: The oropharynx is located behind the mouth and includes the base of the tongue, the soft palate, and the area around the tonsils. Smoking and chewing tobacco and heavy alcohol use are the most common risk factors for oropharyngeal cancer, but there is evidence that a diet low in fruits and vegetables is linked to this form of head and neck cancer. Prior infection with HPV is also a particularly strong risk factor for this cancer site. Symptoms of oropharyngeal cancer may include a lump in the neck or throat, persistent sore throat, hoarseness, difficulty swallowing, and ear and/or jaw pain.

Hypopharyngeal Cancer: The hypopharynx is the uppermost portion of the oesophagus and surrounds the larynx (voice box). As with most other head and neck cancers, tobacco use and heavy alcohol consumption are the most common risk factors. Other risk factors for hypopharyngeal cancer may include a diet low in vitamins A and E; exposure in the work place to asbestos, wood dust, paint fumes, and other inhalants. Symptoms of

hypopharyngeal cancer may include a lump in the neck, hoarseness, difficulty swallowing, and ear pain.

Nasal Cavity & Paranasal Sinus Cancers: Each year, approx 2,000 people in the US are diagnosed with cancer in the mucus-producing tissues that line the nasal cavity (the space behind the nose through which air passes to the throat) and the paranasal sinuses (hollow areas in the facial bones near the nose). More than half of nasal cavity and paranasal sinus cancers occur in the maxillary sinuses (hollow spaces on either side of the nose and below the eyes); fewer cancers develop in the nasal cavity and in the ethmoid sinuses (sieve-like spaces made of thin bone and mucous tissues behind the bridge of the nose). These cancers arise more frequently in people who are exposed to wood and metal dusts, asbestos, paint fumes, and air pollution. Symptoms may include persistent nasal congestion, chronic sinus infections that do not respond to antibiotic treatment, frequent headaches or sinus pain, swelling of the eyes, and reduced sense of smell.

Staging

The tumor, node, metastasis (TNM) staging system allows clinicians to categorize tumors of the head and neck region in a specific manner to assist with the assessment of disease status, prognosis, and management. [4th edition of the Quick Reference Guide to TNM Staging of Head and Neck Cancer and Neck Dissection Classification, the American Academy of Otolaryngology—Head and Neck Surgery Foundation and American Head and Neck Society (2014)].

All available clinical information may be used in staging: physical exam, radiographic, intraoperative, and pathologic findings. Other than histopathologic analysis, biomarkers and molecular studies are not yet included in the staging of head and neck cancers.

Three categories comprise the system:

T: the characteristics of the tumor at the primary site (this may be based on size, location, or both);

N: the degree of regional lymph node involvement; and

M:the absence or presence of distant metastases.

The specific TNM status of each patient is then tabulated to give a numerical status of Stage I, II, III, or IV. Specific subdivisions may exist for each stage and may be denoted with an a, b, or c status.

Stage 0 head and neck cancer

A stage 0 head and neck cancer tumor means the cancer is only growing in the part of the head and neck where it started. No cancer cells are present in deeper layers of tissue, nearby structures, lymph nodes, or distant sites (Example: Tis, N0, M0 carcinoma *in situ*).

Stage I head and neck cancer

A stage I head and neck cancer tumor means the primary tumor is 2 cm across or smaller, and no cancer cells are present in nearby structures, lymph nodes, or distant sites (Example: T1, N0, M0).

Stage II head and neck cancer

A stage II head and neck tumor measures 2–4 cm across, and no cancer cells are present in nearby structures, lymph nodes, or distant sites (Example: T2, N0, M0).

Stage III head and neck cancer

A stage III head and neck tumor means one of the following:

- The head and neck tumor is >4 cm across, and no cancer cells are present in nearby structures, lymph nodes, or distant sites (Example: T3, N0, M0).
- The head and neck tumor is any size but has not grown into nearby structures or distant sites. However, cancer cells are present in one lymph node, which is located on the same side of the head or neck as the primary tumor and is <3 cm across (Example: T1–3, N1, M0).

Stage IV head and neck cancer

Stage IVA: One of the following applies –

- T4a, N0 or N1, M0: the head and neck cancer tumor is any size and is growing into nearby structures. Cancer cells may not be present in the lymph nodes, or they may have spread to one lymph node, which is located on the same side of the head or neck as the primary tumor and is <3 cm across. Cancer has not spread to distant sites; or

- T1–4a, N2, M0: the tumor is any size and may or may not have invaded nearby structures, it has not spread to distant sites, and one of the following is true:
    - cancer present in one lymph node, located on the same side of the head or neck as the primary tumor and measuring 3–6 cm across (N2a);
    - cancer present in one lymph node on the opposite side of the head or neck and measuring <6 cm across (N2b);
    - cancer present in 2 or more lymph nodes, all <6 cm across and located on either side of the head or neck (N2c);

Stage IVB: One of the following applies –

- T4b, any N, M0: the tumor has invaded deeper areas and/or tissues. It may or may not have spread to lymph nodes and has not spread to distant sites.

or

- Any T, N3, M0: the tumor is any size and may or may not have grown into other structures. It has spread to one or
- more lymph nodes >6 cm across, but has not spread to distant sites.

Stage IVC:

- Any T, Any N, M1: The head and neck cancer tumor is any size and may or may not have spread to lymph nodes.
- Cancer cells have spread to distant sites.

T4a disease indicates moderately advanced disease and is specific by subsite, but is still considered resectable. T4b disease is very advanced disease with findings— such as carotid artery encasement, prevertebral involvement, and skullbase involvement—that previously determined the disease to be unresectable. In general, early-stage disease is denoted as Stage I or II disease, and advanced stage disease as Stage III or IV disease. Of importance is that any positive metastatic disease to the neck will classify the disease as advanced, except in select nasopharynx and thyroid cancers. T4a disease is staged as IVa. T4b disease is staged as IVb, and any distant metastasis is staged as IVc.

Risk Factors

Risk factors for head and neck cancers include: tobacco use, heavy alcohol consumption, prolonged sun exposure, and certain viruses, including human papillomavirus (HPV) and Epstein-Barr virus (EBV). In particular, HPV infection is a risk factor for oropharyngeal cancer (cancer of the middle of the throat, including the tonsils and base of tongue). The

overall incidence of HPV-positive head and neck cancers is rapidly increasing in the US, while the incidence of HPV-negative (primarily tobacco- and alcohol-related) cancer is decreasing. While a strong causal relationship has been established between HPV type 16 and the development of oropharyngeal cancer, other HPV types have also been associated with oropharyngeal cancer. Human papillomavirus (HPV) is the most common sexually transmitted disease in the US, infecting 79 million Americans. HPV is known to play a major role in the development of head and neck cancers, which include cancers of the oral cavity, oropharynx, nose/nasal passages and larynx. Head and neck cancers associated with HPV account for nearly 3% of all cancers in the US and are twice as prevalent in men as in women. Incidence rates of HPV-caused head and neck cancers have been on the rise, especially HPV-associated oropharyngeal cancer in men, and are expected to continue growing. By 2025, researchers believe that HPV will be the causative factor of 90% of all head and neck cancers. HPV-related head and neck cancer has a unique risk factor profile, and a more favourable prognosis than tobacco or alcohol induced HNSCC. Unlike HPV-negative SCCHN, which is driven by stepwise mutations in the squamous epithelium, HPV-positive SCCHN is caused by two viral oncogenes that inactivate tumour suppressor genes and lead to malignant transformation of the squamous epithelium.

Treatment

Many cancers of the head and neck can be cured, especially if they are found early. Treatment varies according to the type, location, and extent of the cancer. In addition to treatment with hereps simplex virus and immune checkpoint inhibitors, accompanying treatment(s) may include a combination of surgery, radiation therapy, and chemotherapy.

Surgery

Surgery is the primary treatment for most cancers of the head and neck. Improvements in surgical techniques allow removal of many more tumours while preserving nearby structures involved in sensory and physical functioning. Some patients may require surgical examination of the lymph nodes in the neck to determine if any cancer cells have spread beyond their original site. New techniques allow surgeons to remove these lymph nodes while sparing nerves that are important for shoulder function. Complex surgery to remove tumours at the base of the skull, once considered nearly impossible, can now be safely performed. Reconstruction of bones and other structures is often possible immediately following surgery.

Minimally invasive surgical techniques are used when possible to remove tumours that are located near structures involved in sensory and physical functioning. In many cases, patients can recover more quickly when treated with minimally invasive surgery compared with traditional, open surgery.

Endoscopic Laser Surgery may be used to remove tumours in the larynx or pharynx while preserving the structures involved in speech and swallowing.

Robotic Surgery can be used for tumours of the tongue and tonsils can be removed with the aid of small robotic arms that are placed in the mouth, avoiding the need to make a large incision or to split the jawbone.

Radiation Therapy

Radiation therapy alone or in combination with chemotherapy is standard curative treatment for many patients with head and neck cancers. Which approach is used depends on the extent of the tumour; radiation and chemotherapy are used in combination when treating more advanced disease. In select situations, such as oral cavity tumours, the patient undergoes surgery followed by radiation therapy and/or chemotherapy. Radiation therapy, or a combination of radiation and chemotherapy, may be used to treat patients who would develop significant side effects from surgery, those with inoperable cancers, or those who have a poor prognosis after surgery.

Patients may be treated with one or both of the following types of radiation therapy: External-beam radiation therapy called intensity-modulated radiation therapy (IMRT), can be used which uses 3-D images from CT scans to deliver radiation to tumours with greater precision than conventional radiation therapy.

Brachytherapy uses tiny, radioactive seeds which are implanted into the tumour site, where they deliver the highest dose of radiation possible with minimal effect on nearby healthy tissue.

Proton Therapy: For some cases of head and neck cancer, proton therapy can be used to deliver high doses of radiation to tumours that may be resistant to conventional forms while minimising exposure to the surrounding healthy tissues thereby reducing the risk of treatment-related side effects.

Chemotherapy

Increasingly, chemotherapy, in combination with radiation therapy, is used to treat head and neck cancers that are difficult to reach surgically or that cannot be cured by surgery alone. This approach is also used to treat patients for whom surgery would cause significant functional or cosmetic disability, such as loss of the larynx, with its associated loss of natural voice and the need for a permanent stoma in the front of the neck. Chemotherapy has been shown to enhance the effectiveness of radiation therapy, improving cure rates compared to radiation therapy alone for advanced cancers such as those originating in the nasopharynx.

Chemotherapy is also used for patients with incurable disease, in an attempt to improve survival and decrease cancer-related symptoms. The most commonly used chemotherapy drugs include cisplatin, fluorouracil, methotrexate, carboplatin, paclitaxel, docetaxel, and, more recently, cetuximab.

There are many investigational agents currently in clinical trials for head and neck cancer. Early stage tumours are often cured by radiation or surgery alone. However, up to 60% of patients present as locally advanced disease (stages III and IVA/B) requiring multi-modality therapy.

Based on a pivotal phase III trial published in 2003, concurrent chemo-radiation using cisplatin has become the standard of care for patients with unresectable locally advanced disease. Cisplatin causes crosslinking of DNA followed by inhibiting mitosis, and also has anti-tumour immunomodulatory effects. These effects are induced by four distinguishing mechanisms, for example, upregulation of MHC class I, recruitment and proliferation of effector T cells and macrophages, enhancement of lytic activity in cytotoxic effector cells, and downregulation of MDSCs and Tregs. The three year overall survival was 23% in the radiation alone arm compared to 37% in the chemo-radiation arm (p value 0.014) [Adelstein DJ, Li Y, Adams GL, Wagner H,Jr, Kish JA, Ensley JF, et al. An intergroup phase III comparison of standard radiation therapy and two schedules of concurrent chemoradiotherapy in patients with unresectable squamous cell head and neck cancer. J Clin Oncol. 2003 Jan 1;21(1):92-8]. However, despite current therapy, many of these patients are faced with high rates of recurrence and progression necessitating the development of novel agents.

In patients that develop unresectable local recurrences and/or metastatic disease, the prognosis is poor. The 1-year survival rate is approximately 10-15% [Patel PR, Salama JK. Reirradiation for recurrent head and neck cancer. Expert Rev Anticancer Ther. 2012 Sep;12(9):1177-89]. Overall survivals (OS) were in the range of 5-6 months in these patients when treated with single agent chemotherapy [Jacobs C, Lyman G, Velez-Garcia E, Sridhar KS, Knight W, Hochster H, et al. A phase III randomized study comparing cisplatin and fluorouracil as single agents and in combination for advanced squamous cell carcinoma of the head and neck. J Clin Oncol. 1992 Feb;10(2):257-63 and Forastiere AA, Metch B, Schuller DE, Ensley JF, Hutchins LF, Triozzi P, et al. Randomized comparison of cisplatin plus fluorouracil and carboplatin plus fluorouracil versus methotrexate in advanced squamous-cell carcinoma of the head and neck: A southwest oncology group study. J Clin Oncol. 1992 Aug;10(8):1245-51] and up until recently, combination regimens did not significantly increase the OS over single agents.

In 2005, it was showed for the first time in a phase III study that adding the EGFR antibody, cetuximab, to cisplatin increased OS over cisplatin alone in the recurrent and metastatic disease setting [Burtness B, Goldwasser MA, Flood W, Mattar B, Forastiere AA, Eastern Cooperative Oncology Group. Phase III randomized trial of cisplatin plus placebo compared with cisplatin plus cetuximab in metastatic/recurrent head and neck cancer: An eastern cooperative oncology group study. J Clin Oncol. 2005 Dec 1;23(34):8646-54]. The response rate in the combination arm in the latter study was 26%. In the XTREME study that combined cisplatin plus 5FU chemotherapy with cetuximab showed a response rate of 36% that equated to an OS in these patients to 10.1 months [Vermorken JB, Mesia R, Rivera F, Remenar E, Kawecki A, Rottey S, et al. Platinum-based chemotherapy plus cetuximab in head and neck cancer. N Engl J Med. 2008 Sep 11;359(11):1116-27]. In another study, the alternate anti-EGFR monoclonal antibody, panitumumab in addition to cisplatin/5FU also showed an improvement in OS to 11.1 over control, but this benefit was limited to p16-negative tumours and was associated with an increase in serious adverse events [Vermorken JB, Stohlmacher-Williams J, Davidenko I, Licitra L, Winquist E, Villanueva C, et al. Cisplatin and fluorouracil with or without panitumumab in patients with recurrent or metastatic squamous-cell carcinoma of the head and neck (SPECTRUM): An open-label phase 3 randomised trial. Lancet Oncol. 2013 Jul;14(8):697-710].

Subjects

The subject to be treated may be any animal or human. The subject is preferably mammalian, more preferably human. The subject may be a non-human mammal, but is more preferably human. The subject may be male or female. The subject may be a patient. A subject may have been diagnosed with a cancer, or be suspected of having a cancer.

The subject may be a child, i.e. a human subject of age less than 18 years, or of age less than 16 years, or of age less than 14 years, or of age less than 12 years. The age may be determined at the point of first dose with oncolytic herpes simplex virus.

Subjects may optionally be indicated for surgical removal of tumor tissue (referred to herein as 'tumor resection'). For example, they may have a cancer considered, by a medical practitioner, operable to remove some or all of the tumor tissue.

Subjects may be selected for treatment as being subjects who have not mounted a clinical response to previous treatment with an immune checkpoint inhibitor as monotherapy.

A subject may be immunocompetent or immunocompromised.

Other Chemotherapeutic Agents

In addition to treating a cancer by using an oncolytic herpes simplex virus with or without an immune checkpoint inhibitor, subjects being treated may also receive treatment with other chemotherapeutic agents. For example, other chemotherapeutic agents may be selected from:

(i) alkylating agents such as cisplatin, carboplatin, mechlorethamine, cyclophosphamide, chlorambucil, ifosfamide;

(ii) purine or pyrimidine anti-metabolites such as azathiopurine or mercaptopurine;

(iii) alkaloids and terpenoids, such as vinca alkaloids (e.g. vincristine, vinblastine, vinorelbine, vindesine), podophyllotoxin, etoposide, teniposide, taxanes such as paclitaxel (Taxol™), docetaxel;

(iv) topoisomerase inhibitors such as the type I topoisomerase inhibitors camptothecins irinotecan and topotecan, or the type II topoisomerase inhibitors amsacrine, etoposide, etoposide phosphate, teniposide;

(v) antitumor antibiotics (e.g. anthracyline antibiotics) such as dactinomycin, doxorubicin (Adriamycin™), epirubicin, bleomycin, rapamycin;

(vi) antibody based agents, such as anti-VEGF, anti-TNFα, anti-IL-2, antiGpIIb/IIIa, anti-CD-52, anti-CD20, anti-RSV, anti-HER2/neu(erbB2), anti-TNF receptor, anti-EGFR antibodies, monoclonal antibodies or antibody fragments, examples include: cetuximab, panitumumab, infliximab, basiliximab, bevacizumab (Avastin®), abciximab, daclizumab, gemtuzumab, alemtuzumab, rituximab (Mabthera®), palivizumab, trastuzumab, etanercept, adalimumab, nimotuzumab,

(vii) EGFR inihibitors such as erlotinib, cetuximab and gefitinib

(viii) anti-angiogenic agents such as bevacizumab (Avastin®).

Routes of administration

Immune checkpoint inhibitors, chemotherapeutic agents, medicaments and pharmaceutical compositions according to aspects of the present invention may be formulated for administration by a number of routes, including but not limited to, parenteral, intravenous, intra-arterial, intramuscular, intratumoral and oral. Immune checkpoint inhibitors, chemotherapeutic agents, medicaments and pharmaceutical compositions may be formulated in fluid or solid form. Fluid formulations may be formulated for administration by injection to a selected region of the human or animal body.

In the present invention herpes simplex virus is administered to the blood, e.g. by intravenous administration (intravenous infusion) or intra-arterial administration, and is formulated for such administration.

In preferred embodiments the immune checkpoint inhibitor is administered to the blood, e.g. by intravenous administration (intravenous infusion), and is formulated for such administration.

Herpes simplex virus may be formulated as a suspension of virus in lactated Ringer's or in Hartmann;s solution. One litre of lactated Ringer's solution typically contains about 130 mEq of sodium ion (130 mmol/L), 109 mEq of chloride ion (109 mmol/L), 28 mEq of lactate (28 mmol/L), 4 mEq of potassium ion (4 mmol/L), and 3 mEq of calcium ion (1.5 mmol/L). One litre of Hartmann's solution typically contains about 131 mEq of sodium ion (131 mmol/L), 111 mEq of chloride ion (111 mmol/L), 29 mEq of lactate (29 mmol/L), 5 mEq of potassium ion (5 mmol/L), and 4 mEq of calcium ion (2 mmol/L).

Virus may be formulated for delivery in the clinic by mixing a small aliquot of virus with a specified volume of the chosen fluid carrier, e.g. lactated Ringer's or Hartmann's solution. Virus is supplied in fluid suspension at the specified dosage concentration, e.g. $1x10^7$ iu/ml and a small aliquot in the range 0.5 to 5ml, e.g. one of about 0.5ml, about 1ml, about 2ml, about 3ml, about 4ml or about ml, preferably about 1ml of virus, is mixed with the fluid carrier. The volume of fluid carrier to which the aliquot of virus is added may be one of about 100 ml, about 150ml, about 200ml, about 250ml, about 300ml, about 350ml, about 400ml, about 450ml, about 500ml. In some preferred embodiments the volume of fluid carrier is about 250ml. The fluid carrier may be provided in a bag suitable for use in intravenous or intra-arterial infusion. The viral suspension, fluid carrier and bag are all preferably sterile and the virus formulation is prepared in sterile conditions.

Infusion of the formulated viral composition to the blood may take between about 30 minutes and about 3 hours, for example about 1 hour, about 2 hours or about 3 hours.

Intravenous administration may comprise infusion into the venous system in close proximity to the location or locations of the cancer, e.g. head and neck cancer.

Infusion to the blood is preferably at a peripheral site, e.g. to a vein or artery near the surface of the skin and not within deep tissue. Examples of suitable peripheral locations are veins in the arm or leg. In some related embodiments, administration may be via a central venous line. Administration is preferably non-invasive, e.g. does not require a surgical, invasive or interventional radiological procedure in order to locate a specific vein or artery within deep tissue or proximal to internal organs. For example, administration is optionally not to the hepatic artery. The subject may have a peripheral venous device, catheter or cannula fitted in order to facilitate the administration. As such, administration can be performed in an out-patient setting in which the patient is connected to a drip.

Administration of oncolytic herpes simplex virus may be locoregional administration, e.g. to a localised region of the body in which the tumor is present. Locoregional administration may be achieved by use of chemoembolization in which administration of an oncolytic herpes simplex virus may be combined with other embolization (e.g. chemical embolization) of the tumor.

An example of a less preferred, and non-peripheral, route of administration, developed in the context of treatment of primary liver cancer, is trans-arterial chemoembolization (TACE).

TACE is normally performed by an interventional radiologist and involves accessing the hepatic artery with a catheter, which is possible by puncturing the common femoral artery in the right groin and passing a catheter through the abdominal aorta, through the celiac trunk and common hepatic artery, into the proper hepatic artery.

An arteriogram is performed to identify the branches of the hepatic artery supplying the tumor(s). Smaller catheters may then be threaded into these branches (so-called superselective positioning). This allows precision delivery of the active agents to the tumor tissue.

Once the catheter is in position, doses of the active agent (e.g. oncolytic herpes simplex virus, and/or chemotherapeutic agent and/or embolisation agent and/or contrast agent) are injected through the catheter. The total dose may be given to a single vessel, or if there are several tumor foci may be divided among several vessels supplying the tumors.

Because most liver tumors are supplied by the hepatic artery, arterial embolization interrupts the blood supply to the tumor and delays tumor growth. The focused nature of the administration of active agents enables delivery of a high therapeutic dose to the tissue requiring treatment whilst reducing systemic exposure and therefore toxicity. Embolization of the vessel assists this process in that the active agent(s) is not washed out from the tumor bed and the supply of nutrients to the tumor is decreased thereby promoting tumor necrosis.

TACE is widely used as a palliative treatment for surgically unresectable primary or metastatic HCC tumors.

In some optional embodiments, administration is not intraperitoneal.

Kits

In some aspects of the present invention a kit of parts is provided. In some embodiments the kit may have at least one container having a predetermined quantity of herpes simplex virus, e.g. predetermined viral dose or number/quantity/concentration of viral

particles. The herpes simplex virus may be formulated so as to be suitable for injection or infusion to a tumor or to the blood. In some embodiments the kit may further comprise at least one container having a predetermined quantity of immune checkpoint inhibitor. The immune checkpoint inhibitor may also be formulated so as to be suitable for injection or infusion to the tumor or to the blood, or alternatively may be formulated for oral administration. In some embodiments a container having a mixture of a predetermined quantity of herpes simplex virus and predetermined quantity of immune checkpoint inhibitor is provided, which may optionally be formulated so as to be suitable for injection or infusion to the tumor or to the blood.

In some embodiments the kit may also contain apparatus suitable to administer one or more doses of the herpes simplex virus and/or immune checkpoint inhibitor. Such apparatus may include one or more of a catheter and/or needle and/or syringe, such apparatus preferably being provided in sterile form.

The kit may further comprise instructions for the administration of a therapeutically effective dose of the herpes simplex virus and/or immune checkpoint inhibitor.

The invention includes the combination of the aspects and preferred features described except where such a combination is clearly impermissible or expressly avoided.

The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

Aspects and embodiments of the present invention will now be illustrated, by way of example, with reference to the accompanying figures. Further aspects and embodiments will be apparent to those skilled in the art. All documents mentioned in this text are incorporated herein by reference.

Throughout this specification, including the claims which follow, unless the context requires otherwise, the word “comprise,” and variations such as “comprises” and “comprising,” will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by the use of the antecedent "about," it will be understood that the particular value forms another embodiment.

Brief Description of the Figures

Embodiments and experiments illustrating the principles of the invention will now be discussed with reference to the accompanying figures in which:

Figure 1. Table showing changes in cytokine production in pleural fluid samples in human patients having malignant pleural mesothelioma in response to treatment with 1, 2 or 4 doses (each of $1x10^7$iu) of SEPREHVIR® given at weekly intervals. Changes are relative to patient levels of cytokine production prior to treatment with SEPREHVIR®. *No samples were available for testing from patient 06. + → +++++ = small to large increase over baseline level; - = no change; ↓= decrease; tba = sample yet to be analysed.

Figure 2. Table showing changes in IFN-γ production in pleural fluid samples in human patients having malignant pleural mesothelioma in response to treatment with 1, 2 or 4 doses (each of $1x10^7$iu) of SEPREHVIR® given at weekly intervals. No samples were available for testing from patient 06. + → +++++ = small to large increase over baseline level; - = no change; ↓= decrease; tba = sample yet to be analysed.

Figure 3. Table showing changes in IFN-α production in pleural fluid samples in human patients having malignant pleural mesothelioma in response to treatment with 1, 2 or 4 doses (each of $1x10^7$iu) of SEPREHVIR® given at weekly intervals. No samples were available for testing from patient 06. + → +++++ = small to large increase over baseline level; - = no change; ↓= decrease; tba = sample yet to be analysed.

Figure 4. Table showing changes in IL-1α production in pleural fluid samples in human patients having malignant pleural mesothelioma in response to treatment with 1, 2 or 4 doses (each of $1x10^7$iu) of SEPREHVIR® given at weekly intervals. No samples

were available for testing from patient 06. + → +++++ = small to large increase over baseline level; - = no change; ↓= decrease; tba = sample yet to be analysed.

Figure 5. Table showing changes in IL-2 production in pleural fluid samples in human patients having malignant pleural mesothelioma in response to treatment with 1, 2 or 4 doses (each of $1x10^7$iu) of SEPREHVIR® given at weekly intervals. No samples were available for testing from patient 06. + → +++++ = small to large increase over baseline level; - = no change; ↓= decrease; tba = sample yet to be analysed.

Figure 6. Table showing changes in IL-4 production in pleural fluid samples in human patients having malignant pleural mesothelioma in response to treatment with 1, 2 or 4 doses (each of $1x10^7$iu) of SEPREHVIR® given at weekly intervals. No samples were available for testing from patient 06. + → +++++ = small to large increase over baseline level; - = no change; ↓= decrease; tba = sample yet to be analysed.

Figure 7. Table showing changes in IL-6 production in pleural fluid samples in human patients having malignant pleural mesothelioma in response to treatment with 1, 2 or 4 doses (each of $1x10^7$iu) of SEPREHVIR® given at weekly intervals. No samples were available for testing from patient 06. + → +++++ = small to large increase over baseline level; - = no change; ↓= decrease; tba = sample yet to be analysed.

Figure 8. Table showing changes in IL-10 production in pleural fluid samples in human patients having malignant pleural mesothelioma in response to treatment with 1, 2 or 4 doses (each of $1x10^7$iu) of SEPREHVIR® given at weekly intervals. No samples were available for testing from patient 06. + → +++++ = small to large increase over baseline level; - = no change; ↓= decrease; tba = sample yet to be analysed.

Figure 9. Table showing changes in IL-12 production in pleural fluid samples in human patients having malignant pleural mesothelioma in response to treatment with 1, 2 or 4 doses (each of $1x10^7$iu) of SEPREHVIR® given at weekly intervals. No samples were available for testing from patient 06. + → +++++ = small to large increase over baseline level; - = no change; ↓= decrease; tba = sample yet to be analysed.

Figure 10. Table showing changes in IL-21 production in pleural fluid samples in human patients having malignant pleural mesothelioma in response to treatment with 1, 2 or 4 doses (each of $1x10^7$iu) of SEPREHVIR® given at weekly intervals. No samples

were available for testing from patient 06. + → +++++ = small to large increase over baseline level; - = no change; ↓= decrease; tba = sample yet to be analysed.

Figure 11. Table showing changes in TNF-α production in pleural fluid samples in human patients having malignant pleural mesothelioma in response to treatment with 1, 2 or 4 doses (each of $1x10^7$iu) of SEPREHVIR® given at weekly intervals. No samples were available for testing from patient 06. + → +++++ = small to large increase over baseline level; - = no change; ↓= decrease; tba = sample yet to be analysed.

Figure 12. Table showing changes in IP-10 production in pleural fluid samples in human patients having malignant pleural mesothelioma in response to treatment with 1, 2 or 4 doses (each of $1x10^7$iu) of SEPREHVIR® given at weekly intervals. No samples were available for testing from patient 06. + → +++++ = small to large increase over baseline level; - = no change; ↓= decrease; tba = sample yet to be analysed.

Figure 13. Table showing changes in MIG production in pleural fluid samples in human patients having malignant pleural mesothelioma in response to treatment with 1, 2 or 4 doses (each of $1x10^7$iu) of SEPREHVIR® given at weekly intervals. No samples were available for testing from patient 06. + → +++++ = small to large increase over baseline level; - = no change; ↓= decrease; tba = sample yet to be analysed.

Figure 14. Table showing changes in VEGF production in pleural fluid samples in human patients having malignant pleural mesothelioma in response to treatment with 1, 2 or 4 doses (each of $1x10^7$iu) of SEPREHVIR® given at weekly intervals. No samples were available for testing from patient 06. + → +++++ = small to large increase over baseline level; - = no change; ↓= decrease; tba = sample yet to be analysed.

Figure 15. Diagram illustrating Western Blotting procedure used to probe cell extracts for anti-tumor IgG response induced by intrapleural administration of SEPREHVIR®.

Figure 16. Western Blot showing results for sera taken from patients 01, 02, 03, 04 and 05 against MSTO-211H cells. Arrows indicates novel IgG anti-tumor response.

Figure 17. Western Blot showing results for sera taken from patients 06 and 07 against MSTO-211H cells. Arrows indicates novel IgG anti-tumor response.

Figure 18. Western Blot showing results for sera taken from patients 08 and 09 against MSTO-211H cells. Arrows indicates novel IgG anti-tumor response.

Figure 19. Table showing Th1 response and patient survival for one year or less or for greater than one year. Median survival is 12 months from diagnosis. *Only 7/9 patients were evaluated. No samples available from patient 06 and patient 09 is yet to reach 12 months from treatment. #10 patients, **patients 08 and 09 still alive, ***oldest treated patient was 84 yrs. Historical median survival for all malignant pleural mesothelioma patients is 9.5mths from diagnosis (Beckett et al (2015) Lung Cancer 88, 344).

Figure 20. Table showing cytokines and chemokines detected in pleural fluid samples at high levels (ng/ml) and low levels (pg/ml) and cytokines and chemokines not detected.

Figure 21. Seprehvir replicates and persists but patient 02 fails to mount a Th1 response. Charts show: HSV DNA detectable in pleural fluid samples taken from patient 02 at intervals post administration of Seprehvir; IFNγ response, MIG (CXCL9) response and IL-12 response.

Figure 22. Seprehvir is undetectable but patient 03 mounts a robust IFNγ response. Charts show: HSV DNA detectable in pleural fluid samples taken from patient 03 at intervals post administration of Seprehvir; IFNγ response, MIG (CXCL9) response and IL-12 response.

Figure 23. Table showing expression of pleural fluid cytokines and chemokines in nine human patients in response to treatment with Seprehvir.

Figure 24. Table showing cytokines and chemokines showing low or no response to treatment with Seprehvir in pleural fluid samples from nine human patients.

Figure 25. Table showing summary of patient Th1 cytokine and chomokine responses in nine human patients.

Figure 26. Charts showing immune cell recruitment post-Seprehvir is associated with Th1 cytokine response in two patients receiving two doses of Seprehvir (on days 1 and 8) and three patients receiving four doses of Seprehvir (on days 1, 8, 15 and 22). Day 0 data

point represents cell count prior to treatment with Seprehvir. Th1 responses were observed in patients 04, 07, 08 and 09. Pt = patient.

Figure 27. Charts showing Granzyme B is associated with Th1 cytokine response post Seprehvir treatment in three patients receiving one dose of Seprehvir, two patients receiving two doses of Seprehvir and three patients receiving four doses of Seprehvir. Th1 responses were observed in patients 01, 03, 04, 07, 08 and 09. Pt = patient. Granzyme B and perforin have been shown to induce CTL-mediated target cell DNA fragmentation and apoptosis. (Lord et al., Granzyme B: a natural born killer. Immun Rev. 2003 Jun 193:31-8).

Figure 28. C57BL/6 mice were injected with $5x10^6$ M3-9-M cells subcutaneously. Tumors were treated intra-tumorally (i.tu.) with Seprehvir when sizes reached 200~400 mm^3. Intra-peritoneal (i.p.) injection of anti-PD-1 antibody were given twice a week after last dose of virus treatment. Tumor growth was monitored twice a week. Mice were sacrificed when tumors reached 2,500 mm^3 in volume or grew over 2cm in length. pfu=Plaque Forming Unit.

Figure 29. Combination of Seprehvir with anti-PD-1 antibody significantly prolongs survival with several complete responses in male to female M3-9-M rhabdomyosarcoma tumor model. Female C57BL/6 mice were injected with $5x10^6$ M3-9-M cells subcutaneously. The effects of Seprehvir plus anti-PD-1 blockade on antitumor efficacy were evaluated by measuring tumor volumes over time. Survival data were evaluated for statistical significance with Log-rank (Mantel-Cox) test. Upper left chart: PBS/Ctrl Ab (n=10) and PBS/Anti-PD-1 antibody (n=11); Upper middle chart: PBS/Ctrl Ab (n=10) and Seprehvir/Ctrl (n=12); Upper right chart: PBS/Ctrl Ab (n=10) and Seprehvir/Anti-PD-1 antibody (n=11). Lower chart: PBS/Ctrl Ab (n=10) – dotted line, left-hand side; PBS/Anti-PD-1 antibody (n=11) – dashed and dotted line; Seprehvir/Ctrl (n=12) – dashed line; Seprehvir/Anti-PD-1 antibody (n=11) – solid line, right-hand side.

Figure 30. Combination of Seprehvir with anti-PD-1 antibody significantly prolongs survival in less immunogenic male to male M3-9-M tumor model. Male C57BL/6 mice were injected with $5x10^6$ M3- 9-M cells subcutaneously. The effects of Seprehvir plus anti-PD-1 blockade on antitumor efficacy were evaluated by measuring tumor volumes over time. Survival data were evaluated for statistical significance with Log-rank (Mantel-Cox) test. Upper left chart: PBS/Ctrl Ab (n=7) and PBS/Anti-PD-1 antibody (n=6); Upper

middle chart: PBS/Ctrl Ab (n=7) and Seprehvir/Ctrl (n=6); Upper right chart: PBS/Ctrl Ab (n=7) and Seprehvir/Anti-PD-1 antibody (n=7). Lower chart: PBS/Ctrl Ab (n=7) – dotted line, left-hand side; PBS/Anti-PD-1 antibody (n=6) – dashed and dotted line; Seprehvir/Ctrl (n=6) – dashed line; Seprehvir/Anti-PD-1 antibody (n=7) – solid line, right-hand side.

Figure 31. Checkpoint inhibition does not significantly alter intra-tumoral viral kinetics. Female M3-9-M tumor-bearing mice were treated with three doses of 10^8 pfu of Seprehvir intra-tumorally (i.tu.) followed by intra-peritoneal (i.p.) injection of anti-PD-1 or control antibody. Tumors were harvested 3, 24, 72 and 168 hours after intra-peritoneal antibody injection for plaque assay. Data are expressed as total plaque-forming units (pfu) per tumor.

Figure 32. Combination therapy induces more CD4+ and CD8+ T cells (A) but the increase in CD4+ T cells in both female (left) and male (right) is not due to higher numbers of CD25+CD4+ Treg cells (B). Female and male M3-9-M tumor-bearing mice received three doses of intra-tumoral (i.tu.) Seprehvir injection followed by intra-peritoneal (i.p.) injection of anti- PD-1 or control antibody. Immune cell infiltrates in tumors were evaluated by flow cytometry analyses 72 hours post intra-peritoneal antibody injection. . In each chart, columns from left to right are PBS/Control antibody, PBS/Anti-PD-1 antibody, Seprehvir/Control antibody and Seprehvir/Anti-PD-1 antibody.

Figure 33. Combination therapy induces higher inflammatory gene expression and lower immune suppressive gene expression. Female M3-9-M tumor-bearing mice received three doses of intra-tumoral (i.tu.) Seprehvir injection followed by intra-peritoneal (i.p.) injection of anti-PD-1 or control antibody. Tumors were harvested 72 hours post intra-peritoneal antibody injection. T-bet (Th-1-related gene), Foxp3 (Treg-related gene), IFNγ, IL-10, iNOS (M1 macrophage-related gene) and MRC-1 (M2 macrophage-related gene) were quantified by real-time. Date are represented as relative RNA expression to *gapdh*. In each chart, columns from left to right are PBS/Control antibody, PBS/Anti-PD-1 antibody, Seprehvir/Control antibody and Seprehvir/Anti-PD-1 antibody.

Figure 34. Tumour microenvironment is remodeled to Th1 away from Th2 by Seprehvir and combination with anti-PD-1. IFNγ and iNOS are used as Th1 markers and their proportion, relative to the Th2 markers IL-10 and MRC-1 are significantly increased by Seprehvir + anti-PD-1.

Figure 35. Charts showing results of re-challenge experiment in which mice cured of xenograft tumor are vaccinated against tumor re-challenge via development of memory anti-tumor immunity. Cured mice from Figure 30 (n=1 for anti-PD-1 alone, n=2 for Seprehvir alone and n=3 for the combination) were rechallenged by subcutaneous implantation of M3-9-M cells. Tumors failed to form in any animal but developed in 5/5 age-matched naive mice.

Figure 36. Seprehvir directly interacts and activates human PBMCs. Charts showing phenotypes of NK and CD4+ cells after treatment with reovirus (reo), HSV1716, steroid or Braf inhibitor or X-ray radiation treatment. PBMC were isolated from leukapheresis cones, seeded at 2 x 10^6 cells/ml and treated ± reovirus or HSV1716 (MOI 1); dexamethasone (0.2 mM); PLX4720 (2 µM); 2 Gy XRT; 8 Gy XRT. After 24 or 48 h culture the cells were harvested, stained for the markers indicated and analyzed by FACS. For each entry 24h data left bar shows 24h data and right bar shows 48h data.

Figure 37. Seprehvir directly interacts and activates human PBMCs. Charts showing phenotypes of CD8+ cells after treatment with reovirus (reo), HSV1716, steroid or Braf inhibitor or X-ray radiation treatment. PBMC were isolated from leukapheresis cones, seeded at 2 x 10^6 cells/ml and treated ± reovirus or HSV1716 (MOI 1); dexamethasone (0.2 mM); PLX4720 (2 µM); 2 Gy XRT; 8 Gy XRT. After 24 or 48 h culture the cells were harvested, stained for the markers indicated and analyzed by FACS. For each entry 24h data left bar shows 24h data and right bar shows 48h data.

Figure 38. Seprehvir directly interacts and activates human PBMCs. Charts showing phenotypes of CD14+ cells after treatment with reovirus (reo), HSV1716, steroid or Braf inhibitor or X-ray radiation treatment. PBMC were isolated from leukapheresis cones, seeded at 2 x 10^6 cells/ml and treated ± reovirus or HSV1716 (MOI 1); dexamethasone (0.2 mM); PLX4720 (2 µM); 2 Gy XRT; 8 Gy XRT. After 24 or 48 h culture the cells were harvested, stained for the markers indicated and analyzed by FACS. For each entry 24h data left bar shows 24h data and right bar shows 48h data.

Figure 39. Charts showing expression of cytokines in PBMC after treatment with reovirus (reo), HSV1716, steroid or Braf inhibitor or X-ray radiation treatment. PBMC were isolated from leukapheresis cones, seeded at 2 x 10^6 cells/ml and treated ± reovirus or HSV1716 (MOI 1); dexamethasone (0.2 mM); PLX4720 (2 µM); 2 Gy XRT; 8 Gy XRT.

After 24 or 48 h culture the cells were harvested, stained for the markers indicated and analyzed by FACS. (A) IL-6, IL-10, IFNα, IFNγ; (B) TNFα.

Figure 40. Chart showing FACs analysis and table showing GFP expression in tumor cell lines One58 (human mesothelioma), Ovcar3 (human ovarian cancer), T98 (human glioblastoma multiforme), Ln229 (human glioma). Human cancer cell lines were infected with HSV1716gfp (HSV1716 modified to express GFP) at moi 0.5 and cultured for 24 hours before addition of 10^6 human PBMCs. After 24 hours culture, PBMCs were decanted and cultured for 24-48 hours before analysed for expression of GFP. HSV1716 was found to infect and transfer to a monocyte/macrophage rich subset of PBMCs.

Figure 41. HSV1716 infected human cancer cell lines stimulate PBMC and Pleural Fluid Mononuclear Cell (PFMC) growth. Two separate experiments are shown and PBMC/PFMC were decanted and cultured for 48 hours before MTS assay. Left chart, experiment 1 shows result of MTS assay of PBMC/PFMC when added to human cancer cell lines infected with HSV1716; in each entry from left to right bars indicate T98 cells, One58 cells and Ovcar3 cells +/- virus infection. Right chart shows experiment 2 result of MTS assay following treatment of tumor cells lines Ln229, Ovcar3, T98 and One58 with HSV1716 followed by the addition of PBMC/PFMC; in each entry, from left to right bars indicate PBMC alone, PBMC+HSV1716, pleural fluid mononuclear cells, pleural fluid mononuclear cells+HSV1716.

Figure 42. Seprehvir preferentially infects monocytes in human PBMCs. Charts results of FACs analysis for human monocytes and lymphocytes infected with HSV1716gfp.

Figure 43. Seprehvir infects and polarises human macrophages. Charts show infection of macrophages with HSV1716gfp, macrophage cell death, expression of viral genes in macrophages: ICP0, ICP8, gB. All data were normalised to the house keeping gene GAPDH and 6 independent experiments were performed (n=6). X-axis 0 = macrophages (no virus).

Figure 44. Charts showing expression of FasL, Bcl-2, LC3B and Atg5 in macrophages 24 hours after infection with HSV1716 at moi of 5. All data were normalised to the house keeping gene GAPDH and 6 independent experiments were performed (n=6). X-axis 0 = macrophages (no virus).

Figure 45. Charts showing mRNA expression of markers of inflammation in human monocyte-derived macrophages 24 hours post infection. All data were normalised to the house keeping gene GAPDH and 6 independent experiments were performed (n=6). X-axis 0 = macrophages (no virus).

Figure 46. Charts showing mRNA expression of M1 macrophage markers (NOS2, CXCL10) and M2 macrophage marker (MRC1) in human monocyte-derived macrophages 24 hours post infection. All data were normalised to the house keeping gene GAPDH and 6 independent experiments were performed (n=6).

Figure 47. Chart showing HSV1716 infection of 7 day human monocyte derived macrophages significantly induces PCNA expression. All data were normalised to the house keeping gene GAPDH and 4 independent experiments were performed (n=4).

Figure 48. Chart showing oncolytic HSV therapy significantly delays 975A2 mNB (murine neuroblastoma) tumor growth.

Figure 49. Oncolytic HSV therapy recruits more T cells in 975A2 mNB tumor. Charts show cellular infiltrate into tumor of different cell types. In all charts pairs of data points are shown for treatment with PBS (left) and Seprehvir (right) for administration of 1 (1x) or 3 (3x) doses.

Figure 50. FACS analysis charts show expression of PD-L1 on 975A2 mNB cells.

Figure 51. Oncolytic HSV therapy induces PD-L1 expression in myeloid cells. Charts show PD-L1 expression in F4/80+ Macrophage cells, myeloid derive suppressor cells (MDSCs) and neutrophils. For bar charts, left bar = PBS, right bar = Seprehvir.

Figure 52. Charts showing mean tumor volumes from data shown for individual mice in Figure 29 (left) demonstrates that the combination of Seprehvir + anti-PD-1 is synergistic as the actual combination effect on tumor growth is greater than the predicted additive effect (right).

Figure 53. Diagrammatic illustration of treatment cycles: weekly, and twice weekly (one and two cycles).

Figure 54. Charts showing immune cell proliferation and IFNγ levels in patient 09 (Example 1).

Figure 55. Chart showing Seprehvir persistence in pleural fluids (Example 1).

Figure 56. Table showing summary of patient Th1, immune cell and cytokine responses (Example 1).

Figure 57. Table showing summary of patient anti-tumor IgG responses.

Figure 58. Table showing detection of HSV-1 DNA in patient blood samples. "Pos" = positive for HSV-1 DNA, "Neg" = negative for HSV-1 DNA.

Figure 59. Chart showing titration of human macrophages at various times after infection with 4 pfu/cell HSV1716 and culture in normoxia or hypoxia. Approximately 300,000 primary human macrophages were infected with 1,180,000 pfu HSV1716 with samples collected at various times post infection and HSV1716 titrated on Vero cells. Total titratable virus was graphed against time and the dotted line represents the amount of input virus.

Figure 60. Chart showing output (total pfu) from human macrophages after 72 hrs of normoxia infection with HSV1716 at various input moi. Approximately 300,000 primary human macrophages were infected with HSV1716 at moi 40, 4, 0.4 and 0.04 with samples collected at 72hrs post infection only and HSV1716 titrated on Vero cells.

Figure 61. Table showing detection of HSV-1 DNA in patient blood samples for 8 patients enrolled on NCT00931931. IV= intravenous administration of Seprehvir, ITu = intratumoral administration of Seprehvir, "Pos" = positive for HSV-1 DNA, "Neg" = negative for HSV-1 DNA, nd = not done.

Figure 62: Digital PET/CT images for patient HSV13 enrolled on NCT00931931 at day 14 and day 28 post intravenous administration of Seprehvir. Lesion is circled and SUV indicated.

Figure 63: Digital PET/CT images for patient HSV13 enrolled on NCT00931931 at day 14 and day 28 post intravenous administration of Seprehvir. Transverse image through body. Lesion is circled and SUV indicated.

Figure 64: Digital PET/CT image for patient HSV07 enrolled on NCT00931931 showing regions of tumor.

Figure 65: Digital PET/CT images for patient HSV07 enrolled on NCT00931931 prior to intratumoral administration of Seprehvir (top left), at day 14 post intratumoral administration of Seprehvir (top middle) two days prior to second intratumoral injection of virus on 6.27.14 (top right) and post second intratumoral injection (bottom row).

Figure 66. Table showing viability of SupT1, Toledo (TolB) or THP-1 cells 5 days after infection with either HSV1716 or HSV-1 17+.

Figure 67. Charts showing FACS analysis of fresh human PBMC fractions (monocytes or lymphocytes) mock infected (control) or infected with HSV1716gfp (GFP) at moi 1.

Figure 68. Chart showing stability of HSV1716 in PBS, whole blood, plasma or cell fraction. PBS = top, approximately horizontal line, whole blood = left most line at 2 minutes.

Figure 69. Chart showing HSV1716 released for infection from spiked whole blood, cell fraction or plasma during 72 hrs of incubation with Vero cells. The dotted line represents the yield from Vero cells infected with 10 pfu HSV1716. At each time point: whole blood = left bar, cell fraction = middle bar, plasma = right bar.

The details of one or more embodiments of the invention are set forth in the accompanying description below including specific details of the best mode contemplated by the inventors for carrying out the invention, by way of example. It will be apparent to one skilled in the art that the present invention may be practiced without limitation to these specific details.

Examples

Malignant pleural mesothelioma (MPM) remains a major challenge, with limited therapeutic options. Multifocal intrapleural disease can cause disabling symptoms of pain and breathlessness, in the absence of distant metastases, so an intrapleural treatment approach is attractive.

SEPREHVIR® (HSV1716) is a mutant oncolytic herpes simplex virus type 1 deleted in the RL1 gene which encodes the protein ICP34.5, a specific determinant of virulence. Mutants lacking the RL1 gene are capable of specific replication in cancer cells and inducing anti-tumor immune responses. Clinical studies with SEPREHVIR have been completed in adult glioma, melanoma, squamous cell head and neck cancer, and studies are ongoing in non-CNS solid tumors and MPM. In total, 98 patients have received SEPREHVIR and the virus is well-tolerated with no spread to surrounding normal tissue or no shedding in patients. SEPREHVIR selectivity for replication only in tumor cells and intimations of efficacy and immuno-stimulatory potential have been demonstrated.

Cytokines are secreted intercellular signalling molecules that regulate many different processes including inflammation, host defence and cell differentiation. Cytokine profiles may help understand changes in the pleural fluid samples in patients following SEPREHVIR® administration.

Upon activation, naive CD4+ helper T cells differentiate into distinct subsets. The development of the subsets is driven in part by the cytokine milleu. Type 1 (Th1) cells help drive cellular immunity against intracellular pathogens. IL-12 and IFNγ induce Th1 cell development. Th1 cells produce IFN-γ and IL-2, which provided a positive feedback loop to enhance Th1 cell differentiation and NK cell and CD8+ T cell cytolytic activity.

Th2 cells play a crucial role in the humoral response against extracellular pathogens. IL-4 drives development of Th2 cells, which subsequently produce IL-4, IL-5 and IL-13. These cytokines induce B cell proliferation, antibody production, IgE class switching and activate eosinophils respectively.

Another distinct helper T cell lineage, Th17 is important for mucosal immunity. De-regulation of Th17 may significantly contribute to the development of autoimmunity. IL-17 produced by Th17 cells induces secretion of proinflammatory cytokines IL-6, IL-8, GM-CSF and TNFα. Many of these molecules link innate and adaptive immunity through the recruitment and activation of innate immune cells.

Effective immune responses require finely tuned coordination between pro- and anti-inflammatory signals. Proinflammatory molecules play important roles in activating key immune players to fight infection. IL-8 induces granulocyte migration and activates neutrophil phagocytic activity. GM-CSF mobilizes monocytes into infected tissue and activates macrophage and neutrophils. TNFα is a multifunctional proinflammatory

cytokine involved with a number of processes including cell proliferation, differentiation and apoptosis.

Uncontrolled inflammation may damage surrounding host tissue. IL-10 is a prototypical anti-inflammatory cytokine that serves to terminate the acute inflammatory response by inhibiting Th1 cells function and pro-inflammatory cytokine production.

Example 1 – Cytokine responses following intrapleural administration of oncolytic HSV SEPREHVIR® in patients with malignant pleural mesothelioma

We are currently conducting a phase I/IIa trial at Cancer Clinical Trials Centre, Weston Park Hospital, Sheffield and Queen Elizabeth University Hospital, Glasgow, United Kingdom to determine the safety and potential for efficacy of SEPREHVIR® given intrapleurally to patients with malignant pleural mesothelioma (MPM). Patients receive $1x10^7$iu SEPREHVIR® through their pleural catheter on one, two or four occasions each dose given one week apart, in three separate patient cohorts. To date 11 patients have been treated, 3 in each 1 and 2 dose cohorts and 5 in the 4 dose cohort and SEPREHVIR® has been well-tolerated with few adverse events in any patients. An exploratory objective, to assess tumor responses by CT using modified RECIST criteria, has demonstrated disease stabilisation in 6/10 evaluable patients.

Pleural fluid and plasma samples have been collected pre- and post- treatment and analysed to assess patient responses to SEPREHVIR® administration.

1.1 HSV DNA

HSV DNA was detected in the pleural fluids of most patients and in some persisted for at least two or four weeks post-administration (Figure 55).

1.2 Cytokine Analysis

Pleural fluid samples were collected from patients following intrapleural administration of SEPREHVIR® and were analysed for changes in the levels of the following cytokines, or potential biomarkers: IFN-γ (Interferon-gamma), IFN- α (Interferon-alpha), the following Interleukins (IL): IL-1α, IL-2, IL-4, IL-6, IL-10, IL-12, IL-21, IP-10 (IFN-γ inducible protein 10), MIG (monokine induced by IFN-γ), TNF-α (Tumor necrosis factor alpha), and VEGF (Vascular Endothelial Growth Factor).

Changes in cytokine and chemokine levels may be indicative of a developing immune response in the pleural space and changes in potential biomarker levels may be indicative of patient responses to treatment.

1.2.1 Materials and Methods

Commercially available ELISA kits were used to measure the concentrations of these cytokines and potential biomarkers in biological fluids. ELISA kits for quantifying cytokines, chemokines and potential biomarkers in biological fluids were used exactly as specified in the manufacturer's instructions. For example, Novex® (Thermo Fisher) ELISA kits allow specific, quantitative measurements of cytokines, chemokines and disease-related proteins in various biological fluids. ELISA kits were selected on the basis that they are compatible with biological fluids such as serum or plasma.

For detection of human interferon-γ ELISA Kit Cat# KHC4021, 4022, 4021C (Invitrogen, Camarillo, CA, USA) was used. For detection of human VEGF ELISA Kit Cat# KHG0112, 0111 (Invitrogen, Camarillo, CA, USA) was used.

Pleural fluid samples from patients were delivered on dry ice, thawed and processed for subsequent analysis. 5-10 ml of each pleural fluid were stored at -70°C in 15ml centrifuge tubes for analysis of cytokines and potential biomarkers.

Prior to using an ELISA kit, its compatibility with pleural fluids and useful dilution range was tested. Two pleural fluids are used for this test, one sample prior and one post administration of SEPREHVIR® were diluted 1:10, 1:100 and 1:1000 using the dilution buffer provided with the kit. One strip of eight wells was removed from the kit and the undiluted, 1:10, 1:100 and 1:1000 dilutions for each samples were added to individual wells. The ELISA protocol was then followed exactly as specified by the manufacturer and the resultant OD450nm readings identify the most appropriate sample dilutions for use in the ELISA. The most appropriate dilutions should generate an OD450nm of between 0.5 - 1.5 within 15-30 mins. Pleural fluid samples were then analysed at this appropriate dilution.

1.2.2 Results

Detection of changes in levels of cytokines and biomarkers (see Figures 1 to 14).

Th1 associated cytokines

IL-2: Patients receiving 4 doses of SEPREHVIR® showed an increase in IL-2 production (Figure 5).

IL-12: Patients receiving 4 doses of SEPREHVIR® showed an increase in IL-12 production (Figure 9).

IL-12, produced by dendritic cells, macrophages and human B-lymphoblastoid cells, is known as a T cell stimulating factor and involved in the differentiation of naive T cells into Th1 cells. IL-12 is important within the immune response with various activities including mediating the enhancement of the cytotoxic activity of NK cells and CD8+ cytotoxic lymphocytes, stimulating production of IFN-γ, TNF-α from T-cells and reduces IL-4 mediated suppression of IFN-γ.

IL-12 has been shown to have anti-angiogenic abilities by increasing production of IFN-γ which causes the increased production of IP-10, which mediates an anti-angiogenic effect.

IFN-γ: IFN-γ levels were notably increased from low initial levels in patients receiving single and multiple doses of SEPREHVIR® (Figure 2).

IFN-γ functions include enhancing the cytotoxic activity, activation, growth and differentiation of T-cells, macrophages and NK cells. As well as the activation of other cells types such as B-cells and fibroblasts. IFN-γ production is a characteristic of Th1 differentiation and promotes a Th1 immune phenotype by causing naive CD4+ cells (Th0) to differentiate into Th1 cells while suppressing Th2 cell differentiation. IFN-γ further enhances the immune response by stimulating macrophages which upregulates antigen processing and presentation pathways, promoting CD4+T cell activation and cell-mediated immunity. Through upregulation of various cells, IFN-γ directs the flow of specific immune cells to the site of inflammation or infection (Boehm, U., Klamp, T., Groot, M., Howard, J. C. (1997) Cellular responses to interferon-gamma. Annu. Rev. Immunol. 15, 749-795).

IFN-γ produced by APC (antigen presenting cells) that secrete IFN-γ may stimulate the self-activation and activation of nearby cells. The production of IFN-γ is controlled by various cytokines, importantly IL-12 and IL-18 (Frucht, D. M., Fukao, T., Bogdan, C., Schindler, H., O'shea, J., Koyasu, S. (2001) IFN-gamma production by antigen-presenting cells: mechanisms emerge. Trends Immunol. 22, 556-560). These cytokines

serve roles within the innate immune response, IL-12 is secreted by macrophages which then attract NK cells to the site, while IL-12 continues to promote IFN- γ synthesis. IFN- γ is negatively regulated by IL-4 and IL-10.

IP-10: Patients receiving single and multiple doses showed a strong upregulation of IP-10 (Figure 12).

Interferon gamma-induced protein 10 (IP-10) is a chemokine secreted by various cell types including monocytes, endothelial cells and fibroblasts in response to IFN- γ. IP-10 has various roles within the immune system, arguably the most important of role is being a potent chemoattractant for monocytes/macrophages, T cells, NK cells and dendritic cells, IP-10 promotes anti-tumor activity and inhibition of angiogenesis (Dufour. J. H., Dziejman. M., Liu. M.T., Leung. J. H., Lane. T. E., Luster. A.D. (2002) IFN- γ-Inducible protein 10(IP-10) deficient mice reveal a role for IP-10 in effector T cell generation and trafficking. Jour Immunology. 168. 7. 3195-3204). IP-10 and other members of the chemokine family including MIG, CXCL9, CXCL11 and CXCL4 have been proposed as a therapeutic agent in the fight against cancer as they induce injury to established tumor associated vasculature and promote tumor necrosis (Homey, B., A. Müller, and A. Zlotnik. 2002. Chemokines: agents for the immunotherapy of cancer? Nat. Rev. Immunol. 2:175–184).

MIG: Analysis of pleural fluid cytokines by AbCam ELISA indicated that baseline levels of MIG (before treatment with SEPREHVIR®) were high. Samples were diluted 1:100 before assay. Patients receiving single and multiple doses showed a strong upregulation of MIG (Figure 13).

Monokine induced by gamma interferon (MIG), closely related to the chemokine CXCL10, is a T cell and NK cell bearing the chemokine receptor CXCR3 chemoattractant (Walser. C. T., Xinrong. M., Kundu. N., Dorsey. R., Goloubeva. W. O., Fulton. M. A. (2007) Immune-mediated Modulation of Breast Cancer Growth and Metastasis by the Chemokine Mig (CXCL9) in a Murine Model. J Immunother 2007;30:490–498). CXCR3 can regulate leukocyte trafficking, attracts Th1 cells and promotes Th1 cell maturation. MIG has been shown to have anti-tumor activity in a number of tumor models as well as stimulating T cells to the site of injury and having anti angiogenic properties (Saudemont A, Jouy N, Hotuin D, et al. NK cells that are activated by CXCL10 can kill dormant tumor cells that resist CTL-mediated lysis and can express B7-H1 that stimulates T cells. Blood.

2005;105:2428–2435). Furthermore, there is evidence to suggest NK cells that have been stimulated by MIG have the potential to kill dormant tumor cells that have previously been resistant to cell death (Saudemont. A., Jouy. N., Hetuin. D., Quesnel. B. (2005) NK cells that are activated by CXCL10 can kill dormant tumor cells that resist CTL-mediated lysis and can express B7-H1 that stimulates T cells. Blood. Vol 15. 6. 2428-2435).

TNF-α: Patients showed a small increase in TNF-α production (Figure 11).

Tumor necrosis factor alpha is a multifunctional inflammatory cytokine produced by macrophages/monocytes during inflammation and implicated in signalling events that lead to cell necrosis and apoptosis (Idriss. H. T and Naismith. H. J. (2000) TNFα and the TNF receptor subfamily: Structure-function relationship(s). Microscopy research and technique. 50. 184-195). Although the exact mechanism is unknown, TNFα is critical in efficient T cell immune responses, affecting T cell priming, proliferation, recruitment and function. The link between anti-TNFα therapies and increased incidence of malignancies in Rheumatoid Arthritis patients has suggested a link between TNFα in the development, progression and immune surveillance of tumors as well as potentially possessing anti-tumor properties (Calzascia T, Pellegrini M, Hall H, et al. TNF-α is critical for antitumor but not antiviral T cell immunity in mice. *The Journal of Clinical Investigation* 2007;117(12):3833-3845. doi:10.1172/JCI32567).

Proinflammatory cytokines

IL-6: Analysis of pleural fluid cytokines by ELISA indicated that baseline levels of IL-6 (before treatment with SEPREHVIR®) were high. Samples were diluted 1:1000 before assay. In most patients, even at multiple doses IL-6 levels did not rise notably compared to baseline levels (Figure 7).

Detection of high levels of IL-6 is consistent with previous reports of detection of IL-6 in patients having malignant pleural mesothelioma (T Nakano et al., Interleukin 6 and its relationship to clinical parameters in patients with malignant pleural mesothelioma. British Joumal of Cancer (1998) 77(6), 907-912; Siti N. Abdul Rahim et al., The role of interleukin-6 in malignant mesothelioma *Transl Lung Cancer Res* 2015;4(1):55-66).

IL-6 is a pro and anti-inflammatory cytokine which is produced by a variety of cells such as T cells, B cells monocytes, fibroblasts and keratinocytes and macrophages. IL-6

stimulates a broad range of cellular and physical responses in the event of infection or trauma. Recent research suggests IL-6 along with TNFα and IL-1, are major proinflammatory cytokines, IL-6 is an important modulator of CD4 T cell effector functions therefore impacting the immune response and contributing to inflammation (Dienz. O., Rincon. M. (2009). The effect of IL-6 on CD4 T cell responses. Clin Immunol. 130(1): 27–33). In response to PAMPS (pathogen-associated molecular patterns), which are located on the cell surface and intracellular compartments, IL-6 is produced by macrophages, causing a signalling cascade that produces an inflammatory cytokine production. IL-6 may protect CD4 T cells from undergoing apoptosis and stimulates T cell activation as well as T cell migration. A major function of IL-6 is antibody induction (Akira. S., Hirano. T., Taga. T., Kishimoto. T. (1990) Biology of multifunctional cytokines: IL6 and related moplecules (IL1 and TNF). The FASEB Journal. 4. 11. 2860-2867).

IL-1α: IL-1α levels were essentially unchanged in patients receiving single or multiple doses of SEPREHVIR® compared to baseline levels (Figure 4).

IL-1α possesses a strong proinflammatory effect. IL-1α is multifunctional and produced by tissue macrophages, monocytes, fibroblasts and dendritic cells. IL-1α enables transmigration of immunocompetent cells to sites of infection and considered a central cytokine in the regulation of immune responses. The release of IL-1α can induce activation of NFkB which will promote cell survival, proliferation and angiogenesis (Wolf. J. S., Chen. Z., Dong. G., Sunwoo. J. B., Bancroft. C. C., Capo. D. E., Yeh. N. T., Mukaida., Waes. C. V. (2001) IL (Interleukin)-1a Promotes Nuclear Factor-kB and AP-1-induced IL-8 Expression, Cell Survival, and Proliferation in Head and Neck Squamous Cell Carcinomas. Clin Cancer Res. 7. 1812-1820).

Th2 associated cytokines:

IL-4: IL-4 levels were essentially unchanged in patients receiving single of multiple doses of SEPREHVIR® compared to baseline levels (Figure 6).

IL-4 stimulates the differentiation of naive T cells (Th0 cells) to effector T cells (Th2 cells), subsequently Th2 cells produce additional IL-4 and have a role in a class switch response to IgG1 and IgE isotopes of B-cells (Kabsech. M., Schedel. M., Carr. D., Woitsch. B., Fritzsch. C., Weiland. S. K., Mutius. E. (2006) IL-4/IL-13 pathway genetics strongly

influence serum IgE levels and childhood asthma. Journal of Allergy and Clinical Immuno. Vol 117. 2. 269-274). One of the biological activities of IL-4 is the stimulation of activated B-cell and T-cell proliferation. IL-4 is considered a key regulator in humoral and adaptive immunity. IL-4 is known to decrease the production of Th1 cells, IFN gamma, macrophages and dendritic cell IL-12.

IL-10: Patients receiving 4 doses of SEPREHVIR® showed an increase in IL-10 production (Figure 8). Although IL-10 is associated with Th2 cells it acts to regulate the Th1 response, preventing an excessive Th1 response. Its upregulation in patients exhibiting a more pronounced Th1 response is consistent with this regulatory function and confirms the authenticity of the Th1 response.

IL-10 is an anti-inflammatory cytokine primarily produced by monocytes and to a lesser extent by Th2 lymphocytes, mastocytes and in certain activated T and B cells. IL-10 limits the production of proinflammatory cytokines (including IL-12, IL-6, IL-1α, TNFα, IL-8 and IP-10), resulting in the indirect inhibition of Th1 cells (Couper. K. N., Blount. D. G., Riley. E. M. (2008) IL-10: The master regulator of immunity to infection. Jour Immunol. 180. 5771-5777). However IL-10 can directly act on CD4+T cells causing an inhibition of proliferation and production of IL-2, IFN-y, IL-4, IL-5 and TNF α, allowing IL-10 to directly regulate the innate and adaptive Th1 and Th2 responses by limiting T cell activation while inhibiting pro inflammatory responses (Moore, K. W., R. de Waal Malefyt, R. L. Coffman, A. O'Garra. 2001. Interleukin-10 and the interleukin-10 receptor. Annu. Rev. Immunol. 19: 683-765).

During infection IL-10 both regulates and inhibits pro inflammatory cytokines to help prevent tissue damage which would result in the over production of pro inflammatory cytokines.

Other cytokines and biomarkers:

IFN-α: IFN-α levels were essentially unchanged in patients receiving single of multiple doses of SEPREHVIR® compared to baseline levels (Figure 3).

Classed within the type I IFN family of interferons, IFN–α are produced by dendritic cells in response to viral infection and have immunomodulatory functions which causes immune cell differentiation, activation and survival (Padovan, E., Spagnoli. G., Ferrantini. M., Heberer. M. (2002) IFN-α2a induces IP-10/CXCL10 and MIG/CXCL9 production in

monocyte-derived dendritic cells and enhances their capacity to attract and stimulate $CD8^+$effector T cells. Journal of Leukocyte Biologyvol. 71 no. 4 669-676).

VEGF: VEGF levels increased in some patients but it was notable that baseline levels of VEGF in these patients (before treatment with SEPREHVIR®) were high (Figure 14).

Vascular endothelial growth factor is a signal protein that stimulates angiogenesis and vasculogenesis, VEGF is considered to be an important factor in tumor growth (Carmeliet. P. (2005) VEGF as a key mediator of angiogenesis in caner. Oncology. 69. 3. 4-10). VEGF production can be induced in cells that are lacking oxygen, released VEGF triggers a tyrosine kinase pathway leading to angiogenesis, leading VEGF to be a potential target in the treatment of cancer (Ohm, J., Gabrilovich. D., Sempowski. G., Kisseleva. E., Parman. K., Nadaf. S., Carbone. D. (2003) VEGF inhibits T-cell development and may contribute to tumor-induced immune suppression. Blood. 101. 12). VEGF has been shown to promote monocytes/macrophage migration and increase the production of B cells, however VEGF has also been shown to inhibit T cell production and over all reducing immune cell function (Ferrara. N., Gerber H., LeCouter. J. (2003) The biology of VEGF and its receptors. Nature Medicine 9, 669 – 676).

IL-21: Some patients exhibited a small increase in IL-21 levels (Figure 10).

Induction of a Th1 response varies between patients

In some patients Seprehvir replicated and persisted in the pleural fluid but did not induce a Th1 response, e.g. patient 02 (Figures 21 and 27), patient 05 (Figures 26 and 27).

In other patients Seprehvir was undetectable in the pleural fluid but induced a robust Th1 response, e.g. patient 03 (Figures 22 and 27). The absence of detectable HSV DNA in the pleural fluid is not inconsistent with an HSV mediated treatment effect. The levels of the virus in the pleural fluids could be below the detection limit of 100pfu equivalents/ml or it could be persisting and spreading intracellularly. In other studies we have noted viral adsorbtion by cells and tissue within 1 hour of administration to patients such that virus was not detectable in blood for a period of time after which HSV DNA was detectable. This is consistent with adsorption of virus following administration and re-emergence later in fluid samples after build up of productive infection within the tumor tissue.

Proliferation of immune cells and IFNγ production was measured in pleural fluid samples from patient 09 on day 1 (pre-treatment) and at days 11, 17, 22 and 29 post-administration of Seprehvir (patient 09 received four doses of Seprehvir on days 1, 11, 15 and 22). Cells were stimulated in the presence of anti-CD3 antibody and the whole cell extract from an MPM cell line (MSTO211H). Control cells were incubated with the anti-CD3 antibody+PBS. Proliferation was measured by MTS assay and proliferation was calculated as MTS at 96hrs/MTS at 0Hrs. IFNγ was measured in culture supernatants by ELISA at 96hrs. Results are shown in Figure 54.

Comments

Analysis of pleural fluid cytokines by ELISA indicated that SEPREHVIR® administration was associated with Th1-type responses with increased levels of IFNγ, IP-10 and TNFα and accompanied by increased levels of IL-10 in most patients.

Analysis of pleural fluid cytokines by ELISA indicated that they were generally rich in IL-6, MIG and VEGF. Pleural fluids had high levels of IL-6 and IL-12 and, in most patients, there were moderate increases of both post SEPREHVIR® administration. Pleural fluids were also rich in VEGF and levels increased in 4/9 patients post SEPREHVIR® administration.

IL-1α, IL-4 and IFNα were not detected pre-treatment and showed no response to SEPREHVIR® administration.

Post SEPREHVIR administration there were increased levels of IFN-γ, IP-10, MIG, TNFα and IL-6 in most patients, including patients receiving only one dose of SEPREHVIR®. IL-2, and IL-12 increases were most notable in patients receiving 4 doses of SEPREHVIR®.

Overall, these responses are consistent with development of a Th1 response.

IL-1α, IL-4, IL-21, and IFNα were not detected pre-treatment and showed little or no response to SEPREHVIR® administration, consistent with lack of development of a Th2 response.

Example 2 - Novel anti-tumor serum IgG response

Patient serum samples were used to probe cell extracts in order to investigate the possibility of an anti-tumor antibody response to treatment with SEPREHVIR®. Cell

extracts were prepared from cell lines: MSTO-211H (mesothelioma; ATCC CRL-2081), SPC111 (mesothelioma), and HuH7 (hepatic carcinoma), and contacted with patient sera. IgG:target complexes were detected using an anti-human IgG antibody by standard Western Blotting procedures (Figure 15).

Analysis of plasma samples indicated a strong anti-HSV IgG responses post SEPREHVIR® administration, particularly after 2 and 4 doses. Intrapleural administration of SEPREHVIR® was found to induce a novel anti-tumor IgG response against an antigen present on MSTO-211H cells but not on SPC111 or HuH7 cells (Figures 16-18) more so in patients receiving 4 doses of Seprehvir.

Thus, SEPREHVIR® has immunotherapeutic potential capable of inducing novel anti-tumor immune responses in patients. This result is consistent with induction of IgG B cells directed to tumour antigens released during Seprehvir oncolysis and stimulated through a Th1 response.

Example 3 – Checkpoint Blockade Enhances Oncolytic Herpes Virotherapy in Immunosuppressive Sarcoma Models

Most solid tumors are characterized by an immunosuppressive microenvironment, limiting the effectiveness of antitumor immunotherapeutics. Programmed cell death protein (PD)-1-mediated T cell suppression via engagement of its ligand, PD-L1, is of particular interest due to recent successes in selected adult cancers. The utility of PD1- directed therapy for pediatric cancers is unknown, especially given the paucity of mutations and thus infrequent neoantigens in many types of childhood tumors. Oncolytic virotherapy induces tumor shrinkage via a multistep process including direct tumor cell lysis, induction of cytotoxic or apoptosis-sensitizing cytokines, and induction of antitumor T cell responses. We have demonstrated that intratumoral injection of an oncolytic herpes virus induced growth delays and in some cases durable remissions in several mouse models of rhabdomyosarcoma. The effects were T cell-mediated, as surviving mice were resistant to tumor rechallenge and efficacy was lost in athymic nude hosts. We found these tumor models express PD-L1, suggesting that T cell checkpoints may in part limit virus-induced antitumor immunity. Here we show the implantable C57BL/6 rhabdomyosarcoma model, M3-9-M, showed a moderate response to single-agent Seprehvir (HSV1716), a virus currently in pediatric clinical trials (NCT00931931). Single-agent PD-1 blockade also showed moderate but significant tumor growth delay with no complete responses. Combining these two therapies together substantially prolonged overall survival with

several complete responses post 60 days treatment. Interestingly, mice that received combination therapy showed more CD4+/CD8+ T cell recruitment to the tumor and displayed higher immune inflammatory responses and a less immunosuppressive microenvironment, as measured by the decreased proportion of CD4+/CD25+/Fox3P+ Tregs and suppressive cytokines. Overall, our data suggest the combination of PD-1 and oncolytic herpes virotherapy may be an effective treatment strategy for some cancers. Results are shown in Figures 28-33.

We observed that: combination of oHSV treatment with immune checkpoint inhibitor anti-PD-1 significantly prolongs survival in both male to male and male to female rhabdomyosarcoma models; greater antitumor efficacy was observed in male to female murine rhabdomyosarcoma, suggesting that combination therapy favors more immunogenic microenvironments; combination therapy resulted in more CD4+/CD8+ T cell recruitment but did not affect *in vivo* virus activity; combination therapy induces more inflammatory responses and, although CD4+ T cell numbers increased, CD25+/CD4+ Treg numbers were unchanged thus lowering the regulatory/suppressive tumor microenvironment.

Experimental Methods and Results

C57BL/6 mice were injected with $5x10^6$ M3-9-M cells subcutaneously. Tumors were treated intra-tumorally (i.tu.) with Seprehvir when sizes reached 200~400 mm^3. Intra-peritoneal (i.p.) injection of anti-PD-1 antibody [anti-PD1 antibody rat monoclonal RMP1-14 (AbCam plc)] were given twice a week after last dose of virus treatment. Tumor growth was monitored twice a week. Mice were sacrificed when tumors reached 2,500 mm^3 in volume or grew over 2cm in length.

Female C57BL/6 mice were injected with $5x10^6$ M3-9-M cells subcutaneously. The effects of Seprehvir plus anti-PD-1 blockade on antitumor efficacy were evaluated by measuring tumor volumes over time. Survival data were evaluated for statistical significance with Log-rank (Mantel-Cox) test. Figure 29 shows the combination of Seprehvir and anti-PD-1 antibody to significantly prolong survival with several complete responses in the male to female M3-9-M tumor model.

Male C57BL/6 mice were injected with $5x10^6$ M3- 9-M cells subcutaneously. The effects of Seprehvir plus anti-PD-1 blockade on antitumor efficacy were evaluated by measuring tumor volumes over time. Survival data were evaluated for statistical significance with

Log-rank (Mantel-Cox) test. Figure 30 shows the combination of Seprehvir and anti-PD-1 antibody to significantly prolong survival in the less immunogenic male to male M3-9-M tumor model.

Female M3-9-M tumor-bearing mice were treated with three doses of 10^8 pfu of Seprehvir intra-tumorally (i.tu.) followed by intra-peritoneal (i.p.) injection of anti-PD-1 or control antibody. Tumors were harvested 3, 24, 72 and 168 hours after intra-peritoneal antibody injection for plaque assay. Data are expressed as total plaque-forming units (pfu) per tumor. Figure 31 shows checkpoint inhibition does not significantly alter intra-tumoral viral kinetics.

Female M3-9-M tumor-bearing mice received three doses of intra-tumoral (i.tu.) Seprehvir injection followed by intra-peritoneal (i.p.) injection of anti- PD-1 or control antibody. Immune cell infiltrates in tumors were evaluated by flow cytometry analyses 72 hours post intra-peritoneal antibody injection. Figures 32A and 32B shows combination therapy induces more CD25+CD8+ memory T cells but less CD25+CD4+ Treg cells.

Female M3-9-M tumor-bearing mice received three doses of intra-tumoral (i.tu.) Seprehvir injection followed by intra-peritoneal (i.p.) injection of anti- PD-1 or control antibody. Tumors were harvested 72 hours post intra-peritoneal antibody injection. T-bet (Th-1-related gene), Foxp3 (Treg-related gene), IFNγ, IL-10, iNOS (M1 macrophage-related gene) and MRC-1 (M2 macrophage-related gene) were quantified by real-time PCR. Figure 33 shows combination therapy induces higher inflammatory gene expression and lower immune suppressive gene expression. Data are represented as relative RNA expression to *gapdh*.

Combination of oncolytic HSV treatment with immune checkpoint inhibitor anti-PD-1 significantly prolonged survival in both male to male and male to female rhabdomyosarcoma models.

Greater antitumor efficacy was observed in male to female murine rhabdomyosarcoma, suggesting that combination therapy favors more immunogenic microenvironments.

Combination therapy did not result in more T cell recruitment or affect *in vivo* virus activity.

Combination therapy induces more inflammatory responses with less immune regulatory/suppressive responses.

Example 4 - Seprehvir directly polarises PBMCs phenotype to Th1

When human PBMCs were exposed directly to Seprehvir, the virus induced a marked Th1 phenotype with increased production of IFNα and IFNγ and TNFα. IL-6 and regulatory IL-10 production were also stimulated and HSV was more effective than Reovirus, dexamethasone, PLX4720 and ionising radiation. Thus Seprehvir could influence these cells directly following their recruitment into the tumour microenvironment. Exposure of PBMCs to Seprehvir upregulated the expression of immune checkpoints in many different subsets including NK, CD4+, CD8+ and CD14+ (monocytes) cells (Figure 36 to 39).

Example 5 – Seprehvir infects and polarises human macrophages potentially inducing a Th1 response directly in human PBMCs

On Day 7 following infection with HSV1716 expressing gfp, human monocyte-derived macrophages demonstrated a significant increase in infection which correlated with an increase in cell death.

Infection was demonstrated via investigation of the expression of viral proteins immediate early (ICP0) and late (gB) genes indicating significant gene expression in macrophages (Figure 43).

Mechanism of cell death in human macrophages

HSV1716 kills macrophages via apoptosis and in a Fas dependent manner with both FasL and Bcl-2 gene expression up-regulated 24 hours after infection with HSV1716 at an MOI of 5.

Consistent with this observation, expression of genes involved in autophagy (Atg5 and LC3B) were not significantly altered (Figure 44).

HSV1716 infection induces an inflammatory phenotype in macrophages

HSV1716 infection of day 7 monocyte-derived macrophages significantly induces mRNA expression of typical markers of inflammation 24 hours post infection with significantly increased expression of IL-6, IL-8, TNFalpha. Expression of IL-10, TGFbeta and NFkappaB were not significantly enhanced (Figure 45).

HSV1716 infection induces an inflammatory phenotype in macrophages

HSV1716 infection of day 7 monocyte-derived macrophages significantly induces mRNA expression of typical inflammatory M1 macrophage markers (NOS2, and CXCL10) and significantly down regulated expression of the M2 marker MRC1 expressed by tumour-derived macrophages (Figure 46). There were no significant changes in two other M2 markers, Arg1 and VEGF.

HSV1716 infection induces PCNA expression in macrophages

HSV1716 infection of day 7 monocyte-derived macrophages significantly induces PCNA expression which therefore could be a potential mechanism for inducing viral replication, macrophage cell death and M2 to M1 switching in tumor-dwelling monocytes and other myeloid derived suppressor cells. Further studies are currently being pursued to investigate siRNA knockdown of PCNA prior to HSV1716 infection (Figure 47).

Taken together, examples 4 and 5 suggest that Seprehvir is capable of inducing Th1 responses leading to anti-tumor immunity by two separate but interdependent mechanisms. Oncolytic replication of cancer cells within the tumor microenvironment induces an inflammatory response which attracts circulating immune cells and initiates a Th1 response. Both tumor-resident and newly recruited immune cells, especially monocytes and other myeloid-derived cells, such as M2 macrophages (TAMs, tumor associated macrophages) are susceptible to Seprehvir infection in this localised inflammatory milieu. Their subsequent infection by Seprehvir progeny released from lytic replication of cancer cells amplifies this Th1 response as for example, macrophages are polarised to the aggressive anti-tumor M1 phenotype. Tumor antigens released by oncolysis and picked up by activated antigen presenting cells leads to the development of anti-tumor immunity.

Example 6 – A Phase I study investigating the safety, tolerability and efficacy of intravenous injections of the selectively replication-competent herpes simplex virus Seprehvir in patients with relapsed or refractory solid tumours

Summary of Clinical Experience

To date ninety eight patients have received Seprehvir, in the context of locally advanced disease, via a variety routes, mostly intra-tumoural (n= 83) and the remainder via

intrapleural (n=11) or intravenous (n=4) infusions, in the absence of any definitely attributable Seprehvir-related toxicity.

Forty seven patients with brain tumours have received a range of Seprehvir doses (10^3 to 2 x 10^6) intratumorally (n=35) or peri-tumorally post resection (n=12) in 4 clinical studies, three in primary or recurrent glioma and 1 in recurrent glioblastoma multiforme (GBM). No induction of encephalitis or any re-activation of latent wild type HSV was observed and no adverse clinical symptoms attributable to Seprehvir were identified.

Two further clinical studies of Seprehvir have been completed. The first of these, a study in melanoma patients involved five patients with metastatic melanoma and accessible soft tissue tumor nodules. No local or systemic toxicity associated with Seprehvir was observed.

The second of these studies involved 20 patients with resectable squamous cell carcinoma of the head and neck in which patients received a single preoperative intratumoral injection (either 1, 3 or 14 days prior to surgery) with Seprehvir at a dose of 10^5 i.u. (5 patients) or $5x10^5$ i.u. (15 patients). No toxicity was experienced by any of the patients and evidence of virus in tumor tissue was observed.

Two clinical studies are currently on-going.

A Phase I/IIa study in Malignant Pleural Mesothelioma is investigating the safety, tolerability and biological effect of single and repeat intra-pleural administration of Seprehvir at a dose of 1 x 10^7 iu. To date, three patients have received a single dose of Seprehvir through their IPC, three have received two doses and five have received four doses with recruitment of an additional one patient required at the four dose level to complete the trial. Seprehvir is well-tolerated with a limited number of transient possibly-related adverse events identified.

In the Phase I dose escalation study in non-CNS tumours, three patients have received a single intratumoral administration of $1x10^5$ i.u. Seprehvir, two patients have received a single intratumoral administration of $2x10^6$ i.u. Seprehvir, one patient has received a single intratumoral administration of $2x10^6$ i.u. Seprehvir on two separate occasions and three patients have received a single intratumoral administration of $1x10^7$ i.u. Seprehvir to date. The intratumoral arm of this study is now closed to recruitment.

Study Rationale

Seprehvir is an oncolytic virus that replicates in and lyses the dividing cells of tumours but fails to replicate in normal post-mitotic cells. Seprehvir also has anti-cancer vaccination potential with induction of anti-tumour immune responses observed in mesothelioma (MPM) patients

Based on this selective replication phenotype and the lack of attributable toxicity noted in preclinical systemic dosing models, coupled with the clinical safety profile demonstrated in 96 patients treated by localised Seprehvir delivery, a study in patients with recurrent/ metastatic advanced solid tumours is proposed. The starting dose will be 1 x 10^7 i.u. based on the current loco-regionally administered dose used in our MPM study and supported by a murine biodistribution studies and the maximum dose FDA-approved doses to be used in the systemic arm of the study in non-CNS solid tumours in children and adolescents.

Since it is considered highly relevant to analyse tumour tissue for evidence of Seprehvir replication and cell lysis, pre-tumour biopsies and post treatment biopsy or resection will be conducted for all patients.

Objectives and Endpoints

Primary objective: To evaluate the safety, tolerability and tumour localisation of repeat IV administration of Seprehvir in patients with relapsed or refractory solid tumours

Secondary objective: To evaluate the patient's immunological response post-Seprehvir administration

Primary endpoint: Safety and tolerability, in terms of the emergence of DLTs, will be assessed by conducting the following safety assessments at pre-defined time-points during the study:

- Physical examination, including vital signs
- ECG
- Analysis of laboratory parameters as follows.

  Haematology: full blood count including differential white cell count, haemoglobin, and haematocrit; coagulation parameters including prothrombin time (PT) and activated partial thromboplastin time (APPT)

  Biochemistry: urea, creatinine, sodium, potassium, total protein, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-

glutamyltranspeptidase, lactate dehydrogenase (LDH), alkaline phosphatase, albumin, calcium, phosphorus, glucose, creatine kinase

- Viral shedding in urine and buccal swab samples

Evidence of Seprehvir replication will be assessed using plasma/serum samples and tumour tissue by:

- PCR for the detection of Seprehvir genomes
- IHC of Seprehvir antigens in biopsy/resected tissue

Adverse events will be recorded throughout the study period.

Secondary endpoints: The immune response to Seprehvir administration will be assessed by conducting the following at pre-defined time-points during the study:

- Measurement of circulating anti-HSV IgG and IgM in plasma samples
- Analysis of circulating and tumour localised immune cells using immune cell profiling and emergence of anti-tumour immune responses
- Pharmacodynamic assessments in plasma/serum samples and tumour tissue
  - Tumour markers (CEA, Ca19-9, Ca15-3, Ca125, LDH, PSA as appropriate)
  - Biomarkers of Seprehvir activity to include but not limited to IFNgamma and related Th1 cytokines and chemokines, HMGB1, HSP70 and 90
  - Histology and immunohistochemistry for necrosis, apoptosis, immune infiltration

Study Design

This Phase I study will run at two sites in the UK.

This is a Phase I, open-label, dose-escalation study to evaluate the safety, tolerability and tumour localisation of Seprehvir, a selectively replication-competent herpes simplex virus, administered IV in 36-40 patients with histologically confirmed unresectable advanced or metastatic solid tumours that are refractory to standard therapy.

The study will follow a 3+3 design to explore the safety and tolerability and tumour localisation of up to 8 x IV administrations of Seprehvir, at 2 dose levels (1×10^7 iu and 1×10^8 iu).

The starting dose will be 1 x 10^7 iu, administered IV on 4 weekly occasions on days 1, 8, 15 and 22. The dose will then escalate to 1x10^8 iu and Seprehvir administered IV on 4 weekly occasions on days 1, 8, 15 and 22. Two other dosing regimen will be tested at 1x10^8 iu. Patients will receive either a single cycle of 4x IV Seprehvir on days 1, 5, 8 and 13 or two cycles of this dosing scheme one week apart.

The DLT assessment period will comprise the first 12 days after last IV dose. Recruitment into each cohort will be sequential, whereby the first patient to be treated must have successfully completed the DLT assessment period without experiencing a DLT, prior to the next patient being treated at that dose level. The twelve-day dosing interval will be observed for all subsequent patient(s). Initially three patients will be treated in a given cohort. If any of these 3 patients experience a DLT during their DLT assessment period, an additional 3 patients (total of six) will be treated at that dose level. Following completion of the DLT assessment period by the final patient in each cohort, all available adverse event and laboratory safety data will be collated and reviewed by the Principal Investigator and sponsor, and a decision made regarding progression to the next dose level.

Patients who do not complete the DLT assessment period for reasons other than toxicity will be replaced for the purpose of toxicity evaluation.

Dose Limiting Toxicities

The definition of Seprehvir DLT will be made according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events [NCI CTCAE Version 4].

Haematological DLT:

- Neutropenia < 0.5 × 10^9/L for > 5 days
- Neutropenia < 1 × 10^9/L with fever
- Thrombocytopenia < 25 × 10^9/L accompanied by bleeding or thrombocytopenia < 10 × 10^9/L

Non-Haematological DLT:

- Any Grade 3 or 4 toxicity that is not related to tumour progression with the exception of
    - Grade 3 'flu-like symptoms (including fever, chills and malaise) in the absence of appropriate prophylaxis
    - Grade 3 nausea, vomiting and abdominal pain unless persisting for > 2 days despite appropriate prophylaxis

- Isolated laboratory abnormalities ≥Grade 3 that resolve to ≤Grade 1 in ≤7 days without clinical sequelae or need for therapeutic intervention will not be considered a DLT

If a patient develops an absolute neutrophil count (ANC) <500/μL or a platelet count <25,000/μL, blood samples must be collected every 2 to 3 days and study treatment withheld until counts resolve or until ANC returns to >1000/μL and platelet counts return to >75,000/μL.

Study Population

Inclusion criteria:

1. Patients with histologically confirmed solid tumour who have exhausted all standard lines of therapy for advanced or metastatic disease and/or for whom no standard therapy exists
2. Previous treatment with anticancer agent(s), including chemotherapy, immunotherapy, biological or hormonal therapy (other than LHRH agonists), must be completed ≥ 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to administration of Seprehvir, and all associated toxicity must be resolved to ≤grade 1 prior to administration of Seprehvir
3. Previous radiation therapy must be completed ≥14 days prior to administration of Seprehvir, and all associated toxicity must be resolved to ≤Grade 1 prior to administration of Seprehvir
4. Prior major surgery must be completed within 4 weeks prior to Seprehvir administration
5. Age ≥18 years (at screening)
6. ECOG performance status 0 or 1 at screening
7. Life expectancy >12 weeks (at screening) as determined by the Principal Investigator/ Sub-Investigator
8. Ability to give written informed consent as evidenced by signature on the patient consent form, to communicate well with the investigator and to comply with the expectations of the study
9. Male and female patients of child-bearing potential must use an approved method of contraception during the study and for 3 months after the last dose of Seprehvir

Exclusion criteria:

A patient will be excluded from the study if **any** of the following apply:

1. Evidence of severe or uncontrolled systemic disease, congestive cardiac failure > New York Heart Association (NYHA) Class 2, myocardial infarction within 6 months, or any medical or surgical condition that is deemed significant by the Principal Investigator
2. Known hypersensitivity to any Seprehvir excipients
3. Brain metastases that are associated with a changing neurological deficit that has been documented to be stable for <3 months, or for which systemic corticosteroids are required
4. Laboratory values:
   a) ANC ≤1500/μL
   b) Platelet count ≤75,000/μL
   c) Haemoglobin<9 g/dL
   d) Serum bilirubin ≥1.5 × upper limit of normal (ULN) unless Gilbert's Disease (≥2 × ULN) is known to be the only underlying hepatic disorder
   e) Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≥2.5 × ULN (AST and ALT ≥5 × ULN for subjects with liver metastasis)
   f) Creatinine clearance within the local laboratory normal range
   g) >1+ proteinuria on consecutive testing at least 24 hours apart
5. Prior investigational agents for malignant or non-malignant disease within 4 weeks or 5 half-lives (whichever is shorter) prior to Day 1
6. Previous treatment with viral therapy of any kind within 8 weeks of entry to the study
7. Active systemic bacterial or clinically proven infection with hepatitis B (HBV) or C(HCV) or evidence of Human Immunodeficiency Virus (HIV) infection
8. Pregnancy or lactation
9. History of a second malignancy except those treated with curative intent >3 years previously in the absence of relapse and basal cell skin cancer or cervical cancer in situ

Treatment and Interventions

Patients will attend clinic study visits at screening and on Days 1, 8, 15 and 22 for IV Seprehvir administration at the first dose level of $1x10^7$ iu (Figure 43). The dose will then be escalated to $1x10^8$ iu and patients will attend clinic study visits at screening and on Days 1, 8, 15 and 22 for IV Seprehvir administration at this dose (Figure 43). Two other dosing regimen will be tested at $1x10^8$ iu. Patients will receive either a single cycle of 4x IV Seprehvir on days 1, 5, 8 and 13 or two cycles of this dosing scheme one week apart (Figure 43). Patients will then undergo biopsy or resection, 4-7 days after the final dose.

Screening Period: Tumour biopsy within 14 days before the first dose of Seprehvir (Day 1).

Treatment Cycle: Days 1 to 22, 1 to 13 or 1 to 32 (Figure 53).

Duration and Frequency

Seprehvir will be administered on Day 1 of each 4 to 8 dose cycle until development of severe toxicity or withdrawal of consent.

Evaluation

Physical examination, vital signs, ECG, routine blood panel (haematology, clinical chemistry coagulation), HSV immune response(IgG/IgM), uUrine sample and buccal swab for assessment of viral shedding taken at initiation of baseline assessment, followed by days +1, +8, +15, +22, +36 if weekly injections, days +1, +5, +8, +13, +26 if 2 injections/week or days +1, +5, +8, +13, +21, +25, +28, +32 and +46 if 2x2 injections per week.

HSV bloods (IgG/IgM) and immune cell profiling (FACS) taken at pre-screen, at time of biopsy/surgery, and at end of study visit.
Additional viremia assessment (HSV-1 PCR blood) conducted at 3, 6 and 24 hrs post administration.

Seprehvir replication and immune cell recruitment (HSV-1 PCR and IHC) - tumour tissue samples taken in pre-treatment biopsy and post treatment surgery or biopsy.

Follow up: The End of Study Visit is to occur 14 days after the subject has discontinued study treatment. All Seprehvir-related toxicities will be followed until the End of Study

Visit or until all treatment-related toxicities have resolved to ≤ Grade 2, stabilized, or returned to baseline.

Example 7 – Phase I trial of HSV1716 in Patients with Non-Central Nervous System (Non-CNS) Solid Tumors

Clinical trial NCT00931931 is an investigation into the use of HSV1716 in patients with non-central nervous system (non-CNS) solid tumors (typically sarcomas and neuroblastoma) and has a two part study design. Part 1 of the study specifies a single dose of virus. Participants who experience at least stable disease or relapse following a determination of stable disease, may qualify for subsequent doses in Part 2. There are two treatment arms: an intratumoral route in which participants with localised disease receive HSV1716 as an intratumoral injection; and an intravenous route in which participants with metastatic disease receive HSV1716 intravenously.

FDA approval for systemic administration of Seprehvir in clinical trial was supported by FDA-approved in vivo toxicology & biodistribution studies for IV Seprehvir and extensive preclinical efficacy studies in murine xenograft models.

Participants enrolled in the trial have now started to receive HSV1716. 9 patients are enrolled in the intravenous arm. Intravenous infusion has started at a conservative level with a single dose of 2x 10^6 i.u. HSV1716. Initial results from the first 4 patients demonstrate evidence that HSV1716 is reaching tumor and is replicating therein.

PCR analysis of blood samples obtained from the patients at several time points was used to identify the presence ("Pos") or absence ("Neg") of HSV-1 DNA. Results are shown in Figures 58 and 61 and indicate no evidence of HSV1716 in the circulation immediately following intravenous infusion (day 0 post infusion).

Blood samples were subjected to shell vial culture. All cultures for viable HSV1716 were also negative at all time points

However, by Day 4, it is notable that in 2/4 patients a signal reappears in blood samples collected and analysed for HSV DNA by PCR. This signal is consistent with an initial burst of HSV1716 replication in tumor post administration and shedding of HSV1716 DNA back into the circulation. In 1 of 4 patients, the signal persisted to Day 14. This is

encouraging given the treatment involved a single dose at low titre. In 1 patient, the signal did not materialise until day 28.

This data shows that herpes simplex virus administered to the blood is immediately absorbed such that intact viral particles are not detectable in the blood. Viral DNA is also not detectable immediately following administration but is detectable several days after administration. This supports the theory that HSV1716 is quickly absorbed by cells or is neutralised following intravenous administration, but is able to reach tumor tissue where it may infect, replicate and lyse cells, lysis of tumor cells releasing viral DNA which is detectable in the blood. Similar PCR results have been seen at Day 4 following image-guided intratumoral administration of HSV1716 in some patients in this study and the similarity in pattern between the PCR bloods by both intratumoral injection and IV infusion is significant.

Pharmacokinetic data from the first two patients treated with intravenous Seprehvir indicates an initial loss of the input signal during the first 24hrs post IV infusion with subsequent re-emergence of signal at day 4. The fourth IV patient had a positive signal on day 28 (Figure 61). Supportive of intratumoural replication (intratumoral patients have also shown this pattern of HSV emergence in the circulation).

To date, no virus has been detected in the circulation of third IV patient.

Case study – patient HSV13.

This is the fourth patient to receive Seprehvir by intravenous administration. The patient is a 25 year old Caucasian male diagnosed with Ewing's Sarcoma (primary lesion in tibia, metastatic lesion in lung) and enrolled on the study on April 21, 2016. HSV13 received a single dose of $2x10^6$ pfu Seprehvir by intravenous infusion.

PET/CT screening (Figures 62 and 63) revealed a low level of standardized uptake value (SUV) at prescreen (no digital PET available), day 14 post Seprehivir administration a flare up (pseudo-progression) was noted with an increased SUV. At day 28 post Seprehivir administration a return to low level SUV was noted. The effect of "pseudo-progression" shortly after treatment is acknowledged for biologic agents.

A similar pattern has been seen in patient HSV06 (receiving intratumoral Seprehvir).

Case study – patent HSV07

This patient received Seprehvir by intratumoral administration. Patient is an 8 year old male with a diagnosis of recurrent rhabdomyosarcoma (13 cm Stage III eRMS) in a retroperitoneal location. Prior treatment includes surgery, radiation (41.4 Gy tumor bed / 36 Gy LN), chemotherapy (VAC per D9803 – remission; Relapse: VI, Cyclo/Topo, IE per ARST0121; PD: Vinorelbine, oral cytox, temsirolimus; PD: Vinorelbine, oral cytox, Avastin). Complications include AKI from obstructive uropathy and ureteral stents, nephrostomies.

HSV07 showed interesting PET/CT findings (Figures 64 and 65). A pre-injection PET on 28th May 2014 identified a site in tumour mass for injection. PET scan day 14 post 1st injection indicated stable disease at injection site – 2nd injection administered. PET hot spot at site distant from 1st injection on day 28. Hot spot chosen as site for 2nd injection on 27th June 2014. PET hot spot gone on 24th July 2014.

Discussion

HSV is being detected in patients receiving a single intravenous low dose ($2x10^6$ pfu) of Seprehvir. This is a low dose, similar to doses normally used in experiments with mice. Experiments in mice typically use a dose of $1x10^6$ or greater, meaning that following scale up for human administration (considering human mass and blood volume) the expected dose required would be about $1x10^9$ pfu or higher. Such a dose would provide new challenges to (i) prove the safety of such a high dose and (ii) manufacture sufficient quantities of virus. The finding that an effect is present at a dose as low as $1x10^6$ pfu means that intravenous administration of doses in the range $1x10^7$ to $1x10^8$ represents a viable approach to treatment of tumors in human patients. Results from our study of patients having mesothelioma (Example 1) are also consistent with multiple systemic doses of Seprehvir leading to a sustained Th1 response.

No HSV is being detected immediately following administration of virus but, surprisingly, an HSV signal is re-emerging in 3 out of 4 patients after at least several days. The re-emergence of signal is consistent with results seen in patients receiving intra-tumoral administration of Seprehvir by image guided technology (see compare IV and ITu arms in Figure 61).

The levels of HSV DNA detected by quantitative PCR are approximately equivalent to the administered dose, which is a clear indicator that virus is replicating. Our experiments on the stability of Seprehvir in human blood (Examples 11 to 13) show that Seprehvir has a

short half-life in human blood. Our observation is therefore consistent with sufficient virus reaching the tumor and replicating therein.

Our intravenous infusion protocol has been well tolerated, with no adverse reactions so far.

These observations are significant. Contrary to the established view (e.g. *see* Russell et al., (Oncolytic virotherapy. Nature Biotechnology Vol. 30 No.7 July 2012) and Seymour and Fisher (British Journal of Cancer (2016) 114, 357-361)), the data emerging from this trial indicates that Seprehvir can successfully circumvent the innate obstacles presented by human blood and the human immune system, can replicate and expand the viral population to therapeutically effective levels and reach tumor tissue. This opens the door to an alternative treatment of tumors that are difficult to access by intratumoral injection.

Description of NCT00931931

Official title: A Phase I Dose Escalation Study of Intratumoral or Intravenous Herpes Simplex Virus-1 Mutant HSV1716 in Patients with Refractory Non-Central Nervous System (Non-CNS) Solid Tumors.

Purpose

Patients with relapsed solid tumors such as sarcomas and neuroblastoma have a poor survival, generally < 20%. There is an urgent need for new treatments that are safe and effective.

HSV1716, an oncolytic virus, is a mutant herpes simplex virus (HSV) type I, deleted in the RL1 gene which encodes the protein ICP34.5, a specific determinant of virulence. Mutants lacking the RL1 gene are capable of replication in actively dividing cells but not in terminally differentiated cells - a phenotype exploited to selectively kill tumor cells. In previous clinical studies, HSV1716 has been shown to be safe when injected at doses up to 10^5 plaque forming units (pfu) directly into human high-grade glioma and into normal brain adjacent to tumour, following excision of high-grade glioma. In an extension study, HSV1716 has been shown to be safe when injected at a dose of up to 10^6 pfu directly into brain tumours.

Replication of HSV1716 in human glioblastoma in situ has been demonstrated. Following a single administration of HSV1716 by direct injection into active recurrent tumor or brain

adjacent to tumor, some patients have lived longer than might have been expected. In part, this study seeks to evaluate the safety of a single injection of HSV1716 in the treatment of extracranial solid tumors in adolescents and young adults.

HSV1716 has also proved safe when given by direct intra-tumoural injection in patients with squamous carcinoma of the head and neck, and in patients with malignant melanoma.

Replication of HSV mutants in human sarcomas and neuroblastoma in cultured cells and human xenograft models has been demonstrated. This study is designed in two parts. PART 1 of the study specifies a single dose of virus. Participants who experience at least stable disease or relapse following a determination of stable disease, may qualify for subsequent doses in PART 2. PART 2 requires signing of a separate consent.

Primary Outcome Measures:
To determine whether intratumoral injection or intravenous infusions of HSV1716 is safe in adolescents and young adults with non-CNS solid tumors.

Secondary Outcome Measures:
To measure antiviral immune response in patients with refractory cancer treated with HSV1716.

Treatment Arms
Intratumoral route – participants with localized disease receive HSV1716 as an intratumoral injection.

Intravenous route – participants with metastatic disease receive HSV1716 intravenously.

Condition
Participants may have one of the following conditions: Rhabdomyosarcoma, Osteosarcoma, Ewing Sarcoma, Soft Tissue Sarcoma, Neuroblastoma, Wilms Tumor, Malignant Peripheral Nerve Sheath Tumor, Clival Chordoma, Non-CNS Solid Tumors.

Eligibility

Ages Eligible for Study:	7 Years to 30 Years
Genders Eligible for Study:	Both

Accepts Healthy Volunteers: No

Inclusion Criteria:

Inclusion of Women and Minorities: The study is open to all participants regardless of gender or ethnicity.

Inclusion for intratumoral injection: Subject must have 1-3 lesions amenable to HSV1716 administration by needle if superficial; by needle and/or catheter if deep or pulmonary, via interventional radiology without undue risk. Lesion(s) must meet specific size criteria.

Inclusion for intravenous administration: Subject must have metastatic disease or a lesion not deemed suitable for direct injection.

Age: Subjects must be greater than or equal to 7 years and less than or equal to 30 years of age at the time of signing consent (study entry).

Histologic Diagnosis: Subjects must have had histologic verification of a non-CNS solid tumor at original diagnosis. The tumor must be amenable to HSV1716 administration without undue risk. Disease must be considered refractory to conventional therapy or for which no conventional therapy exists.

Metastatic Disease: Subjects who have metastasis to the brain are eligible for the intratumoral arm of this study; however, no metastatic sites within the brain will be considered for injection. Subjects who have metastasis to the brain are eligible for the intravenous arm of this study only if those metastases have been treated and are no longer active.

Performance Level: Karnofsky greater than or equal to 50. Subjects who are unable to walk because of paralysis, but who are up in a wheelchair will be considered ambulatory for the purpose of assessing the performance score.

Subjects must have fully recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, or radiotherapy prior to entering this study;

Myelosuppressive chemotherapy: Must not have received within 28 days of entry onto this study (42 days if prior nitrosourea) accompanied by hematopoietic recovery, or 14 days of stopping non-myelosuppressive therapy as long as hematopoietic requirements are met;

Biologic (anti-neoplastic agent): Must not have received within 7 days of entry onto this study (21 days if prior VEGF-Trap and at least 3 half lives after last dose of a monoclonal antibody). For biologic agents that have known adverse events occurring beyond 7 days after administration, this period must be extended beyond the time during which adverse events are known to occur;

No Radiation Therapy greater than or equal to 14 days for local palliative XRT (small port): greater than or equal to 6 months must have elapsed if prior craniospinal XRT or if greater than or equal to 50% radiation of pelvis; greater than or equal to 42 days must have elapsed if other substantial bone marrow radiation;

Immunoablative or myeloablative Stem Cell Transplant (SCT): greater than or equal to 6 months must have elapsed from prior autologous transplant. Subjects must not have graft versus host disease post autologous transplant;

Investigational agent: greater than or equal to 28 days must have elapsed from treatment with a different phase I agent;

Subjects with seizure disorder may be enrolled if on anticonvulsants and well controlled. At the time of enrollment, specified CNS conditions must be less than or equal to Grade II toxicity per CTCAE 3.0 criteria;

All subjects must have adequate blood counts defined as: peripheral absolute neutrophil count (ANC) greater than or equal to 750/uL, Platelet count greater than or equal to 100,000/uL (may be a post transfusion value), Hemoglobin greater than or equal to 9.0 gm/dL (may be a post transfusion value)

Adequate renal function defined as: Serum creatinine less than or equal to 1.5 x upper limit of normal (ULN) for age or creatinine clearance or radioisotope GFR greater than or equal to 70 ml/min/1.73 m2;

Adequate liver function defined as: Total bilirubin less than or equal to 2.0 x ULN for age, and SGPT (ALT) less than or equal to 2.5 x ULN for age and albumin greater than or equal to 2g/dL, GGT < 2.5 x ULN

Adequate cardiac function as defined by: Shortening fraction >25% by echocardiogram or ejection fraction above the institutional lower limit of normal by MUGA, No focal wall motion abnormalities as determined by either of the above studies, EKG without evidence of ischemia or significant arrythmia

Adequate coagulation as defined by:PT/INR and PTT <1.5 x ULN for age;

Infectious Disease: Documented evidence of negative tests for the presence of Hepatitis B surface antigen, Hepatitis C antibody, HIV1 and HIV2 antibodies within the three months preceding study entry. Subjects who do not have such evidence must undergo appropriate testing prior to virus administration;

Exclusion Criteria:

Stem cell transplant: No subjects who have received an allogeneic hematopoietic stem cell transplant are eligible;

Pregnancy or Breast-Feeding: There is no available information regarding human fetal or teratogenic toxicities. Pregnant women are excluded and pregnancy tests must be obtained in girls who are post-menarchal. Males or females of reproductive potential may not participate unless they have agreed to use an effective contraceptive method from the time of study entry to a period of no less than four months post the finalHSV1716 injection. For the same period of time, women who participate in this study must agree not to breast feed;

Consent: Unable or unwilling to give voluntary informed consent / assent;

Leukemia: Subjects with leukemia are not eligible for study participation;

Infection or any other severe systemic disease or medical or surgical condition deemed significant by the principal investigator;

Administration of any unlicensed or investigational agent within 4 weeks of entry to the study;

Growth factor(s): No PEG-GCSF within 14 days of virus injection (day 0);

Anti-HSV antivirals: Subjects whose physicians determine that anti-HSV antiviral therapy (such as acyclovir, ganciclovir, foscarnet, etc.) cannot be safely discontinued from 2 days prior to the injection to 28 days following the injection should not be in the study.

Subjects who have other conditions which in the opinion of the investigator contra-indicate the receipt of HSV1716 or indicate subject's inability to follow protocol requirements.

Example 8 - A Phase Ib/2 open-label evaluation of the safety and efficacy of intravenous administration of oncolytic herpes simplex virus HSV1716 and pembrolizumab compared to pembrolizumab alone and HSV1716 alone in subjects with Stage III or Stage IV head and neck cancer.

In the Phase Ib part of this study, the objective of the study is to demonstrate tumor targeting of HSV1716 when administered by intravenous administration in patients with any operable head and neck cancer who are indicated to receive a tumor resection. Each patient will receive up to 4 doses of HSV1716 by intravenous infusion at two dose levels ($1x10^7$ i.u. and $1x\ 10^8$ i.u.). Each dose will be administered within 1 to 7 days of the previous dose. The final dose will be administered within 1 to 14 days of the tumor resection. Patient tumor material will be collected during the procedure and will be stored for analysis to confirm evidence of HSV1716 localisation to tumor and anti-tumor

immunological or biological effect. Analysis will involve shell vial culture, immunohistochemical analysis of tumor tissue, qPCR to detect HSV DNA, and detection of immunological response, e.g. infiltrating immune cells, cytokine response.

In addition, the safety and tolerability of the two dose levels will be carefully monitored and compared during the period up to tumor resection to confirm the maximum tolerated dose (MTD) for the Phase II part of the Study. 3 patients will be recruited to each dose level but in the event of a single Dose Limiting Toxicity at any dose level, the cohort will be expanded to 6 patients according to the usual "3+3" dose-escalation design.

In the Phase 2 part of this study, the objectives of this study are to evaluate the following measures in an open-label, multi-center, controlled study. Approximately 180 patients are to be recruited and randomized 1:1:1 across each of the 3 treatment arms:

Arm 1: pembrolizumab alone;
Arm 2: HSV1716 alone;
Arm 3: HSV1716 and pembrolizumab.

Pembrolizumab (also known as MK-3475; lambrolizumab, Keytruda™; Merck, USA) is a humanised antibody that binds PD-1.

Primary Outcome Measures:

- Progression-free Survival (PFS) per immune related response criteria ("irRC") for All Participants
- Overall Survival (OS) for All Participants

Secondary Outcome Measures:

- PFS per irRC in Participants with PD-L1-Positive Expression
- OS in Participants with PD-L1-Positive Expression
- Objective Response Rate (ORR) per irRC in All Participants
- ORR per irRC in Participants with PD-L1-Positive Expression
- Time to Tumor Progression (TTP) per irRC in All Participants
- TTP per irRC in Participants with PD-L1-Positive Expression
- Percentage of Participants Experiencing Grade 3-5 AEs
- Time to First Grade 3-5 Adverse Event (AE)

- Percentage of Participants Experiencing Viral Shedding of HSV1716
- Percentage of Participants Experiencing an Anti-viral immune response to HSV1716

In Arm 1, pembrolizumab is administered intravenously at a dose of 200mg on day 1 of each 3 week cycle.

In Arms 2 and 3, HSV1716 is administered by intravenous infusion at a dose of up to $1x10^8$ i.u. on each occasion or at the dose of HSV1716 established as the MTD in the Phase 1b part of the study. For intravenous infusion of HSV1716, vials of HSV1716 will be diluted into 250 mL lactated Ringer's solution and administered over one hour. Virus will be infused via peripheral IV access. Standard hospital contact and respiratory precautions will be followed, per institutional standards of operations for this type of product. The dosing schedule for HSV1716 commences on day 1 and continues every week thereafter until up to 8 doses have been administered (i.e. Days 1, 8, 15, 22, 29, 36, 43 and 50).

In Arm 3, pembrolizumab at a dose of 200mg and HSV1716 at up to $1x10^8$ i.u. are administered according to the following schedule. The treatment may occur on the same day. Where a delay in commencement of pembrolizumab is clinically justified, 1 cycle of HSV1716 may be given prior to commencement of pembrolizumab.

Agent/Day	1	8	15	22	29	36	43	50
HSV1716	+	+	+	+	+	+	+	+
Pembrolizumab		+			+			+

In Arms 1 and 3, subjects shall continue dosing with pembrolizumab therapy until a predetermined number of doses is reached, dose limiting toxicity is observed or disease progression is observed.

Times specified above are all subject to a tolerance of +/- 3 days.

Results may be stratified by stage of disease, PD-L-1 status of tumor, treatment cycles and anti-viral immune response.

Subjects are treated in each arm of the study until the first to occur of: complete response; disease progression as per the irRC; or intolerance of study treatment. For intravenous infusion of HSV1716, vials of HSV1716 will be diluted into 250 mL lactated Ringer's and administered over one hour. Virus will be infused via peripheral IV access. Standard hospital contact and respiratory precautions will be followed, per institutional standards of operations for this type of product.

Example 9 – Method for selecting patients for treatment with a combination of HSV1716 and pembrolizumab

Patients with head and neck cancer who are indicated for surgery may receive up to 4 doses of HSV1716 by intravenous infusion prior to surgical resection. Tumor tissue from the resection may be analysed for evidence of HSV1716 targeting the tumor and for an immunological or biological activity in response to oncolytic immunotherapy. Patients demonstrating such activity may be selected for cycles of HSV1716 therapy following surgery with the aim of targeting residual tumor cells at the site of surgical resection and/or metastatic disease.

Example 10 – Preparation of a vial of HSV1716 for intravenous infusion

The total virus dose for each patient will be diluted into 250 mL lactated Ringer's and administered over one hour according to the following instructions. Virus will be infused via peripheral IV access. Standard hospital contact and respiratory precautions will be followed, per institutional standards of operations for this type of product.

Frozen vials of HSV1716 will be dispensed from the Pharmacy. Preparation for intravenous administration will be performed within an appropriate 'clean' room. If transport to a 'clean' room is required, vials will be placed into a secondary container, labeled appropriately and transported on dry ice. The label will include "Route of administration – intravenous".

A 250mL bag of lactated Ringer's solution for intravenous infusion will also be dispensed from the Pharmacy and transported as needed to the 'clean' room in preparation for intravenous administration. The lactated Ringer's solution to be maintained at room temperature.

Defrost the vials of HSV1716 according to the manufacturer's instructions. Once the vials are defrosted, they must be used immediately.

Place the re-suspended vials and the bag containing 250mL of lactated Ringer's solution (IV bag) in a biosafety cabinet to prepare the HSV1716 final drug product for intravenous administration,

Aspirate 1mL of the virus suspension from each vial into a syringe ready for injection into the 250mL bag of lactated Ringer's via the inlet port. Gently mix the contents of the IV bag using a backwards and forwards rocking motion.

Immediately following dilution of the investigational product in the 250ml of lactated Ringer's solution, label the IV bag containing the HSV1716 final investigational drug product for intravenous administration according to institutional policies and applicable state and federal regulations. Immediately transfer to the Principal Investigator or other staff as appropriate for use.

Intravenous administration must be completed within a three hour time period following preparation of the HSV1716 final drug product.

Following the preparation of HSV1716 for intravenous use, immediately place used vials on ice and return to the study biosafety team for appropriate research purposes or deactivation.

Example 11 – HSV1716 and human primary macrophages

1) In an initial study human macrophages were infected with HSV1716 at approximately 4 pfu/cell and the cells were then incubated under normal and hypoxic conditions. Samples were removed at various time points after infection (+1.5hr, +24hrs, +48hrs and +72hrs) and titrated (Figure 59).

Within 1 hour 90% of the virus had been adsorbed by the macrophages and then no virus was detectable at 24 or 48 hrs in either normoxia or hypoxia (detection limit of titration is 100pfu/ml).

Significantly, virus was detectable at 72 hrs but the amounts at this time were similar in the normoxic vs hypoxic macrophages. This emergent virus is of significant interest as it

could either be the original input which had entered some transient latent state or represent the first wave of replication in the macrophages.

2) Macrophages were infected with decreasing HSV1716 moi (40, 4, 0.4 and 0.04) and samples were titrated after 72 hrs only. Virus was detected from the macrophages infected at moi 40, 4 and 0.4 but not from those infected at 0.04 moi (Figure 59). Interestingly, the ratio of virus detected after 72 hrs relative to the input pfu was approximately the same and similar to those from the two other 72hr normoxia/hypoxia time points shown in Figure 60.

In summary, human primary macrophages were found to have a high capacity to adsorb HSV1716, and active virus can be recovered from the macrophages after 48 hrs in culture.

This data evidences that white blood cell components, specifically monocyte derived cells, can rapidly absorb HSV1716 and furthermore that HSV1716 is able to kill monocyte derived cells after 96 hours from infection.

Accordingly, the inventors' have observed that following infection of monocyte derived cells virus is not detected in the infected cells but presence of virus is re-established upon prolonged culture (Figure 59). This is consistent with productive infection of the cells, i.e. involving replication and cell lysis by viral progeny, although the invention is not bound by such theory. The finding that infection with oncolytic Herpes Simplex Virus leads to cell death of monocyte derived cells means that infected monocytes or monocyte derived cell may be used to deliver the oncolytic Herpes Simplex Virus to the diseased tissue, including to hypoxic areas of a tumor, subsequently allowing the release of virus directly to the diseased tissue as the cell dies.

The inventors also found that oncolytic Herpes Simplex Virus replication in, and subsequent cell death (e.g. lysis) of, monocyte derived cells is actually greater in hypoxic conditions. This indicates that death of the monocyte derived cells occurs (apparently preferentially) in hypoxic tumor environments and will directly release the oncolytic Herpes Simplex Virus to the hypoxic parts of a tumor that are otherwise difficult to access.

The data in Figures 58 and 59 support a hypothesis in which intravenous administration of HSV1716 exposes the virus to a population of macrophages or other monocyte derived cells that transport the virus to hypoxic parts of the tumor.

Example 12 – HSV1716 infects but does not replicate in human PBMC

Three human white blood cell lines were tested for HSV1716 infection and replication using HSV1716gfp, an HSV1716 variant expressing gfp. SupT1 (ATCC, CRL-1942) is a T-cell lymphoma cell line derived from the malignant cells collected from the malignant pleural effusion of an 8 year old child with T-Cell Lymphoblastic Lymphoma. THP-1 (ATCC, TIB-202) cells are monocytic cells derived from a patient with acute monocytic leukaemia. Toledo (CRL-2631) is a B-cell lymphoma cell line established from peripheral blood leukocytes of a patient that originally had a diffuse large cell lymphoma (DLCL). HSV1716gfp at moi 1 readily infected each of these cell lines but failed to affect survival of the cells when assessed by Trypan blue exclusion 5 days after virus infection (Figure 66). Wild-type HSV-1 17+ had some toxicity in SupT1 and THP-1 cells compared to HSV1716.

Fresh human PBMC and monocytes were infected at moi 1 with HSV1716gfp and green fluorescence was observed after 60 hrs of infection using fluorescence microscopy. Light microscopy indicated that only a subset of the PBMC were infected and there was no green fluorescence in uninfected cells. Similar observations with monocytes indicated that all of the monocytes were infected with HSV1716gfp. Although the gfp fluorescence was much weaker in these primary cells, all of the monocyte aggregates were positive for green fluorescent and FACS analysis confirmed HSV1716 principally infected the monocyte fraction of PBMC (Figure 67).

HSV1716 is able to infect but not replicate in human leukaemic cell lines. In the PBMC fraction of human blood, HSV1716 associates principally with monocytes. Human primary macrophages have a high capacity to adsorb HSV1716 with no obvious morphological signs of virus cytopathic effects at high moi. Live virus was recovered from the macrophages after 48 hrs in culture.

Example 13 – HSV1716 stability in human blood.

Human blood is perceived as a very hostile environment to oncolytic viruses and therefore there are apparent, major limitations to intravenous delivery of oncolytic viruses. Hostile factors to oncolytic virus persistence in blood include immune attacks against the viruses mediated via complement, cytokines or probably most critically, by neutralising antibodies in seropositive patients. Therefore, most oncolytic virus studies to date favour intratumoural or loco-regional delivery.

The stability of HSV1716 was assessed in human blood by spiking samples of whole blood, plasma and cell fraction with a known amount of HSV1716 and removing aliquots for titration at various times after virus was added. An additional aliquot was removed at each time point and added to Vero cells plated out in 60mm dishes. The Vero cells were then incubated for 72 hrs and the cells and medium were harvested, subjected to one freeze/thaw cycle (-70°C) and titrated. This additional Vero cell culture step was used to identify HSV1716 which was not available immediately for infection, as in a titration assay, but is released and becomes available to infect the Vero cells during the 72 hrs of incubation. For example, HSV1716 may bind to or be taken up by cells in the blood and released at later times or could be transiently associated with plasma proteins that block infection.

5ml each of whole blood, plasma and cell fraction were obtained from a volunteer and were spiked with 100ul clinical grade HSV1716 (November fill, vial no 96, CRU-01A@ $2x10^6$ pfu/ml) which is equivalent to 200,000 pfu HSV1716 in each sample. The expected titre in each of these samples is $4x10^4$ pfu/ml and, assuming that the average human blood volume is 5,000 ml, this equates to a systemic dose of $2x10^8$ pfu HSV1716.

Additionally, 5ml of PBS were spiked also with 200,000 pfu HSV1716 and samples were titrated in parallel. The titre of this spiked 5ml of PBS spiked at the start of the experiment was $4.8x10^4$ pfu/ml which is within the acceptable range (+/- $0.5\log_{10}$) for the expected titre of $4x10^4$ pfu/ml.

The anti-HSV-1 IgG levels in the volunteer's plasma were tested using a commercially available anti-HSV-1 IgG ELISA kit (EUROIMMUN, D-23560 Lubeck, Seekamp 31). The kit was used exactly as directed by the manufacturer and the anti-HSV-1 IgG levels are determined in relative units/ml (RU/ml) with values derived from a standard curve using samples of known anti-HSV-1 IgG levels provided with the ELISA. The volunteer was

seropositve for HSV-1 IgG with a high plasma concentration of anti-HSV-1 IgG of 600RU/ml.

Two aliquots of 100ul were removed from the spiked whole blood, plasma, cell fraction and PBS at 2 mins, 5 mins, 10 mins, 20 mins, 60 mins, 120 mins, 180 mins and 240 mins after spiking with HSV1716. The first 100ul aliquot was titrated immediately for half-life determination and the percentage virus remaining at each sampling point was calculated using the titre of the PBS at the start of the experiment ($4.8x10^4$ pfu/ml) as 100%. Results are presented graphically in Figure 68 for samples collected up to 10 mins after spiking. Titres of HSV1716 remain stable in PBS during this time but decrease rapidly in whole blood, plasma and the cell fraction during the first 5 minutes of incubation with virus undetectable by titration within 10 mins. The estimated half-lives for HSV1716 in whole blood, plasma or cell fractions are approximately 2 mins 40 secs, 3 mins 10 secs or 3 mins 20 secs for whole blood, plasma or cell fraction respectively. As it takes approximately one minute for blood to complete a circuit in the human circulation systems then at least two complete circuits will be completed within the half-life of HSV1716. The other 100ul aliquot was added to Vero cells in a 60mm plate and the plate returned to the incubator and left for 72 hrs. HSV1716 always became available to infect Vero cells from the 100ul aliquot of whole blood, plasma or cell fraction during the 72 hour incubation period as indicated by titration of the harvested Vero cell extract (Figure 69). Titres of between 200,000 to 400,000 pfu/ml were detected in the Vero cell extracts after the Vero cells had been incubated with 100ul spiked whole blood, plasma or cell fraction indicating the availability of HSV1716 within these fractions to infect cells. Titres obtained from Vero cells which received whole blood or cell fraction tended to be higher than those from plasma suggesting that more virus was available for release in these fractions.

In parallel, Vero cells were infected with 10 or 100 pfu HSV1716 diluted in PBS and these yielded 33,000 pfu (dotted line in Figure 69) or 420,000 pfu respectively indicating that between 10-100 pfu are released for infection of Vero cells from whole blood, cell fraction or plasma during the 72 hrs incubation. It should be noted that this probably represents an underestimation of the amount of virus released from the 100ul whole blood, cell fraction or plasma added to the Vero cells as these may exert some neutralisation effects on progeny virus propagated from the initial Vero cell infections. The titre of the spiked whole blood, cell fraction and plasma was approximately 40,000 pfu/ml HSV 1716 and, as the 100ul added to the Vero cells will contain 4000 pfu, then the 10-100 pfu that infects the Vero cells during the 72 hrs incubation suggests that between 0.25-2.5% of the input

virus is available for infection. This equates to $2.5x10^5 – 2.5x10^6$ pfu from a $1x10^8$ pfu dose.

Results indicate that although HSV1716 has a short half life in human blood, a small but significant proportion of the input dose is not irreversibly neutralised and is continuously available for infection. This proportion of the input HSV1716 may be bound to or be taken up by cells in the blood and released at later times or could be transiently associated with plasma proteins that block infection.

Claims:

1. An oncolytic herpes simplex virus for use in a method of treating cancer in a human subject, the method comprising administering to the human subject at least one dose of oncolytic herpes simplex virus by infusion to the blood, wherein the oncolytic herpes simplex virus reaches cells of the cancer in which it replicates.

2. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to claim 1, wherein upon infusion oncolytic herpes simplex is absorbed by cells or is neutralised, but is able to reach cancer cells where it infects, replicates and lyses cancer cells, lysis of cancer cells releasing viral DNA which is detectable in the blood.

3. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to claim 1 or 2, wherein oncolytic herpes simplex virus DNA is not detectable in a sample of the subject's blood within about 24 hours of infusion, but is detectable in a sample of the subject's blood taken after one of about 72 hours, about 96 hours, about 120 hours, about 144 hours, about 168 hours, about 2 weeks, about 3 weeks or about 4 weeks.

4. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 3, wherein the method comprises determining whether oncolytic herpes simplex virus DNA is present in a sample of the subject's blood.

5. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 4, wherein the method comprises taking a blood sample from the subject, and determining whether herpes simplex virus DNA is present in the blood sample.

6. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 5, wherein the method comprises determining whether oncolytic herpes simplex virus is present in a sample of the subject's tumor tissue.

7. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 6, wherein the method comprises

taking a sample of tumor tissue from the subject and determining whether oncolytic herpes simplex virus is present in a sample of the subject's tumor tissue.

8. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 7, wherein the oncolytic herpes simplex virus is a mutant of HSV-1 strain 17 or is HSV1716.

9. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 8, wherein the cancer is a solid tumor, a recurrent or metastatic solid tumor, or a non-CNS solid tumor.

10. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 9, wherein the dose of oncolytic herpes simplex virus administered is at least $1x10^6$ iu.

11. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 10, wherein a dose of oncolytic herpes simplex virus is administered over a period of 3 hours or less.

12. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 11, wherein the administered oncolytic herpes simplex virus is formulated as about 0.5ml to about 5ml of a suspension of virus in about 200ml to about 300ml of lactated Ringer's solution.

13. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 12, wherein the method comprises administering to the human subject at least one treatment cycle of oncolytic herpes simplex virus, wherein a treatment cycle comprises, or consists, of at least two doses of oncolytic herpes simplex virus, each dose administered by infusion to the blood wherein the second and subsequent doses are each administered within about 17 days of the preceding dose, each dose of oncolytic herpes simplex virus being in the range about $1x10^6$ iu to about $1x10^8$ iu.

14. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to claim 13, wherein one dose of oncolytic herpes simplex virus is administered per week.

15. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to claim 13 or 14, wherein two doses of oncolytic herpes simplex virus are administered per week.

16. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 13 to 15, wherein each dose of oncolytic herpes simplex virus is in the range about 1 x 10^7 iu to about 1 x 10^8 iu.

17. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 13 to 16, wherein the treatment cycle further comprises, or consists of, administration of a therapeutically effective amount of an immune checkpoint inhibitor.

18. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 13 to 17, wherein the treatment cycle further comprises, or consists, of administration of a therapeutically effective amount of an immune checkpoint inhibitor selected from the group consisting of an inhibitor of PD-1, PD-L1, CTLA4, TIM-3 or LAG-3.

19. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 13 to 18, wherein the subject receives two or more treatment cycles.

20. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 19, wherein the method comprises determining the presence of a Th1 response in the subject.

21. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 20, wherein the level of IL-2 and/or IL-12 and/or IFN-γ in the subject's blood is upregulated for more than 7 days after one or more treatment cycles.

22. An oncolytic herpes simplex virus for use in a method of treating cancer in a human subject, the method comprising administering to the human subject at least one treatment cycle of oncolytic herpes simplex virus, wherein a treatment cycle comprises, or

consists, of at least two doses of oncolytic herpes simplex virus, each dose administered by infusion to the blood wherein the second and subsequent doses are each administered within about 17 days of the preceding dose, each dose of oncolytic herpes simplex virus being in the range about $1x10^6$ iu to about $1x10^8$ iu.

23. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to claim 22, wherein a treatment cycle comprises up to 4 doses, or 4 doses, of up to 8 doses, or 8 doses, of oncolytic herpes simplex virus.

24. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to claim 22 or 23, wherein the second and subsequent doses are each administered within about 14 days of the preceding dose

25. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to claim 22 or 23, wherein the second and subsequent doses are each administered within about 7 days of the preceding dose

26. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 22 to 25, wherein one dose of oncolytic herpes simplex virus is administered per week.

27. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to claim 26, wherein administration of each weekly dose of oncolytic herpes simplex virus is separated by 7±1 or 7±2 days.

28. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 22 to 25, wherein two doses of oncolytic herpes simplex virus are administered per week.

29. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to claim 28, wherein administration of each twice weekly dose of oncolytic herpes simplex virus is separated by 4±1 or 4±2 days.

30. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 22 to 29, wherein each dose of oncolytic herpes simplex virus is in the range about 1×10^7 iu to about 1×10^8 iu.

31. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 22 to 30, wherein the treatment cycle further comprises, or consists, of administration of a therapeutically effective amount of an immune checkpoint inhibitor.

32. An oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 22 to 31, wherein the method comprises administering to the human subject at least one treatment cycle of oncolytic herpes simplex virus, wherein a treatment cycle comprises, or consists, of:

(i) four doses of oncolytic herpes simplex virus over a period of about four weeks, one dose administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu; or

(ii) four doses of oncolytic herpes simplex virus over a period of about two weeks, two doses administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu.

33. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to claim 32, wherein administration of each weekly dose of oncolytic herpes simplex virus in is separated by 7±1 or 7±2 days.

34. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to claim 32, wherein administration of each twice weekly dose of oncolytic herpes simplex virus is separated by 4±1 or 4±2 days.

35. An oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 22 to 34, wherein the method comprises administering to the human subject an oncolytic herpes simplex virus and a therapeutically effective amount of an immune checkpoint inhibitor, wherein the method comprises at least one treatment cycle of oncolytic herpes simplex virus comprising, or consisting, of:

(i) four doses of oncolytic herpes simplex virus over a period of about four weeks, one dose administered per week by infusion to the blood, each dose of

oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu; or

(ii) four doses of oncolytic herpes simplex virus over a period of about two weeks, two doses administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu.

36. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to claim 35, wherein administration of each weekly dose of oncolytic herpes simplex virus in is separated by 7±1 or 7±2 days.

37. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to claim 35, wherein administration of each twice weekly dose of oncolytic herpes simplex virus is separated by 4±1 or 4±2 days.

38. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 35 to 37, wherein the method comprises administering an immune checkpoint inhibitor according to at least one treatment cycle of immune checkpoint inhibitor, wherein the periods of time of the oncolytic herpes simplex treatment cycle and immune checkpoint inhibitor treatment cycle overlap.

39. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 35 to 38, wherein the method comprises at least one treatment cycle of immune checkpoint inhibitor comprising, or consisting, of 1 or 2 doses of immune checkpoint inhibitor administered within a period of about 3 or 4 weeks.

40. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 35 to 39, wherein the method comprises at least one treatment cycle of immune checkpoint inhibitor comprising, or consisting, of a period of about 3 weeks in which 1 dose of immune checkpoint inhibitor is administered.

41. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 35 to 40, wherein the immune checkpoint inhibitor is an inhibitor of at least one of PD-1, PD-L1, CTLA4, TIM-3 or LAG-3.

42. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 35 to 41, wherein the immune checkpoint inhibitor is selected from an anti-PD-1 antibody, anti-PD-L1 antibody, anti-CTLA4 antibody, anti-TIM-3 antibody or anti-LAG-3 antibody.

43. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 35 to 42, wherein the immune checkpoint inhibitor is selected from the group consisting of pembrolizumab, nivolumab or ipilumab.

44. An oncolytic herpes simplex virus for use in a method of treating cancer in a human subject, the method comprising administering:

a herpes simplex virus by intravenous infusion at a dose of greater than $2x10^6$ iu at day 1 of week 1 followed by a dose of greater than $2x10^6$ iu at day 1 of week 2, and every week thereafter until surgery is scheduled, toxicity or disease progression, a predetermined maximum number of doses is reached, a fixed number of doses is reached, complete response, disease progression, or intolerance of the treatment, wherein the herpes simplex virus lacks functional ICP34.5 genes; and

an immune checkpoint inhibitor by intravenous infusion every 3 weeks for at least 3 infusions beginning with or after the second or third dose of the herpes simplex virus.

45. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 22 to 44, wherein a dose of oncolytic herpes simplex virus is administered over a period of 3 hours or less.

46. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 22 to 45, wherein the administered oncolytic herpes simplex virus is formulated as about 0.5ml to about 5ml of a suspension of virus in about 200ml to about 300ml of lactated Ringer's solution.

47. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 22 to 46, wherein the administered oncolytic herpes simplex virus is formulated as about 1.0ml of a suspension of virus in about 250ml of lactated Ringer's solution.

48. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 22 to 47, wherein the subject receives two or more treatment cycles.

49. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 48, wherein the subject receives two or more treatment cycles consecutively.

50. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 49, wherein the subject receives two or more treatment cycles, each treatment cycle separated by a break from treatment.

51. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to claim 50, wherein the break from treatment is about 1, 2, 3, or 4 weeks.

52. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 49 to 51, wherein the first 1, 2 or 3 treatment cycles comprises administration of a dose of oncolytic herpes simplex virus that is lower than the dose administered in later treatment cycles.

53. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 52, wherein the method comprises determining the presence of a Th1 response in the subject.

54. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 53, wherein the method comprises measuring the level of IFNγ, IL-2 and/or IL-12 in a sample obtained from a subject.

55. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to of any one of claims 1 to 54, wherein the level of IL-2 and/or IL-12 and/or IFN-γ in the subject's blood is upregulated for more than 7 days after one or more treatment cycles.

56. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 55, wherein the oncolytic herpes simplex virus is a mutant of HSV-1 strain 17 or is HSV1716.

57. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 56 wherein the cancer is a solid tumor.

58. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 57, wherein the cancer is a recurrent or metastatic solid tumor.

59. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 58, wherein the cancer is a non-CNS solid tumor.

60. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 59, wherein the cancer is in a child.

61. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 60, wherein the oncolytic herpes simplex virus does not encode (or is not further modified to contain nucleic acid encoding) a cytokine or chemokine, an interleukin, an interferon, a tumor necrosis factor, a colony stimulating factor, an immune modulator, a member of the CC family, a member of the CXC family or a member of the CXC family.

62. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 61, wherein the oncolytic herpes simplex virus does not express GMCSF.

63. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 62, wherein the oncolytic herpes simplex virus encodes a functional ICP47 and/or ICP6 gene.

64. The oncolytic herpes simplex virus for use in a method of treating cancer in a human subject according to any one of claims 1 to 63, wherein the cancer is not a melanoma.

65. A kit comprising a herpes simplex virus lacking functional ICP34.5 genes, and a package insert or label with directions to treat cancer by using a combination of the herpes simplex virus and an immune checkpoint inhibitor.

66. The kit of claim 65, wherein the directions comprise instructions to administer to a human subject with cancer at least one treatment cycle of oncolytic herpes simplex virus, wherein a treatment cycle comprises, or consists, of at least two doses of oncolytic herpes simplex virus, each dose administered by infusion to the blood wherein the second and subsequent doses are each administered within about 17 days of the preceding dose, each dose of oncolytic herpes simplex virus being in the range about $1x10^6$ iu to about $1x10^8$ iu.

67. The kit of claim 66, wherein the directions comprise instructions to administer to a human subject with cancer at least one treatment cycle of oncolytic herpes simplex virus, wherein a treatment cycle comprises, or consists, of:

(i) four doses of oncolytic herpes simplex virus over a period of about four weeks, one dose administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu; or

(ii) four doses of oncolytic herpes simplex virus over a period of about two weeks, two doses administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu.

68. The kit of claim 66, wherein the directions comprise instructions to administer to a human subject with cancer an oncolytic herpes simplex virus and a therapeutically effective amount of an immune checkpoint inhibitor, wherein the instructions for administration of the oncolytic herpes simplex virus comprise instructions to administer at least one treatment cycle of oncolytic herpes simplex virus comprising, or consisting, of:

(i) four doses of oncolytic herpes simplex virus over a period of about four weeks, one dose administered per week by infusion to the blood, each dose of oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu; or

(ii) four doses of oncolytic herpes simplex virus over a period of about two weeks, two doses administered per week by infusion to the blood, each dose of

oncolytic herpes simplex virus being in the range about 1 x 10^7 iu to about 1 x 10^8 iu.

69. The kit of claim 66, wherein the directions comprise instructions to administer to a patient with cancer a herpes simplex virus administered by intravenous infusion at a dose of greater than 2x10^6 iu at day 1 of week 1 followed by a dose of greater than 2x10^6 iu at day 1 of week 2, and every week thereafter until surgery is scheduled, toxicity or disease progression, a predetermined maximum number of doses is reached, a fixed number of doses is reached, complete response, disease progression, or intolerance of the treatment; and an immune checkpoint inhibitor administered intravenously every 3 weeks for at least 3 infusions beginning with or after the second or third dose of the herpes simplex virus.

70. A method of manufacturing the kit of any one of claims 65 to 69.

71. A method of promoting a combination treatment comprising a herpes simplex virus lacking functional ICP34.5 genes and an immune checkpoint inhibitor, for the treatment of a human patient with cancer.

72. A method of instructing a human patient with cancer by providing instructions to receive a combination treatment with a herpes simplex virus lacking functional ICP34.5 genes and an immune checkpoint inhibitor to extend survival of the patient.

		Th1 response						Pro-inflammatory		Th2 response				
Patient	**No. doses**	**IL-2**	**IL-12**	**IFN-γ**	**IP-10**	**MIG**	**TNF-α**	**IL-6**	**IL-1α**	**IL-4**	**IL-10**	**IFN-α**	**VEGF**	**IL-21**
01	1	↓	-	++	+++	+++	++	+	-	-	+	-	↓	-
02	1	↓	↓	-	↓	-	+/↓	↓	+	-	+	-	+++	+
03	1	↓	++	++	+++	+++	+	+	-	-	-/↓	-	+++	↓
04	2	↓	-	+++++	+++	++++	+++	+	-	-	+	-	↓	-
05	2	↓	-	-	-	-	↓	↓	-	-	-/↓	-	++	+
06*	2													
07	4	++	+++	+++++	++++	+++	+	+	-	-	++	-	-	↓
08	4	++	++	+++++	+++	+++	+	+++	-	↓	+++	+	↓	+
09	4	++	++	++	+++	+++	tba	+	tba	+++	+	tba	+++	+

Figure 1

IFN-γ			
Patient	**No. of doses**	**Level at baseline**	**Response**
01	1	<0.5pg/ml	+
02		<0.5pg/ml	-
03		<0.5pg/ml	++
04	2	<0.5pg/ml	+++++
05		1pg/ml	-
06		No samples	tba
07	4	<0.5pg/ml	++++
08		<0.5pg/ml	++++
09		<0.5pg/ml	++

Figure 2

IFN-α			
Patient	**No. of doses**	**Level at baseline**	**Response**
01	1	<50pg/ml	-
02		<10pg/ml	-
03		<50pg/ml	-
04	2	<50pg/ml	-
05		<50pg/ml	-
06		No samples	
07	4	<50pg/ml	-
08		<50pg/ml	+
09		tba	

Figure 3

IL-1α			
Patient	**No. of doses**	**Level at baseline**	**Response**
01	1	<3.9pg/ml	-
02		5pg/ml	+
03		<3.9pg/ml	-
04	2	<3.9pg/ml	-
05		<3.9pg/ml	-
06		No samples	
07	4	<3.9pg/ml	-
08		<3.9pg/ml	-
09		tba	

Figure 4

IL-2			
Patient	**No. of doses**	**Level at baseline**	**Response**
01	1	876pg/ml	-
02		461pg/ml	-
03		463pg/ml	-
04	2	481pg/ml	-
05		587pg/ml	-
06		No samples	
07	4	387pg/ml	++
08		441pg/ml	++
09		327pg/ml	++

Figure 5

IL-4			
Patient	**No. of doses**	**Level at baseline**	**Response**
01	1	0pg/ml	-
02		0pg/ml	-
03		0pg/ml	-
04	2	0pg/ml	-
05		0pg/ml	-
06		No samples	
07	4	0pg/ml	-
08		0pg/ml	+
09		tba	

Figure 6

IL-6			
Patient	**No. of doses**	**Level at baseline**	**Response**
01	1	187ng/ml	+
02		340ng/ml	-
03		55ng/ml	+
04	2	89ng/ml	+
05		193ng/ml	-
06		No samples	
07	4	53ng/ml	+
08		60ng/ml	+++
09		59ng/ml	+

Figure 7

IL-10			
Patient	**No. of doses**	**Level at baseline**	**Response**
01	1	18pg/ml	+
02		17pg/ml	+
03		21pg/ml	-
04	2	21pg/ml	+
05		26pg/ml	-
06		No samples	
07	4	30pg/ml	++
08		40pg/ml	+++
09		17pg/ml	+

Figure 8

IL-12			
Patient	**No. of doses**	**Level at baseline**	**Response**
01	1	108pg/ml	-
02		136pg/ml	↓
03		297pg/ml	++
04	2	330pg/ml	-
05		61pg/ml	-
06		No samples	
07	4	126pg/ml	+++
08		165pg/ml	++
09		60pg/ml	++

Figure 9

IL-21			
Patient	**No. of doses**	**Level at baseline**	**Response**
01	1	188pg/ml	-
02		187pg/ml	+
03		220pg/ml	↓
04	2	242pg/ml	-
05		204pg/ml	+
06		No samples	
07	4	462pg/ml	↓
08		211pg/ml	+
09		334pg/ml	+

Figure 10

TNF-α			
Patient	**No. of doses**	**Level at baseline**	**Response**
01	1	<0.5pg/ml	+
02		4pg/ml	+/-
03		2.5pg/ml	+
04	2	3pg/ml	+++
05		<0.5pg/ml	-
06		No samples	
07	4	<0.5pg/ml	+
08		<0.5pg/ml	+
09		tba	

Figure 11

IP-10			
Patient	**No. of doses**	**Level at baseline**	**Response**
01	1	222pg/ml	+++
02		182pg/ml	-
03		11,903pg/ml	+++
04	2	237pg/ml	+++
05		371pg/ml	-
06		No samples	
07	4	5149pg/ml	++++
08		49pg/ml	+++
09		24.2pg/ml	+++

Figure 12

MIG			
Patient	**No. of doses**	**Level at baseline**	**Response**
01	1	21,572pg/ml	+++
02		25,141pg/ml	-
03		47,219pg/ml	+++
04	2	34,449pg/ml	++++
05		31,083pg/ml	-
06		No samples	
07	4	46,667pg/ml	+++
08		29,791pg/ml	+++
09		30,844pg/ml	++++

Figure 13

VEGF			
Patient	**No. of doses**	**Level at baseline**	**Response**
01	1	1660pg/ml	-
02		2500pg/ml	+++
03		330pg/ml	+++
04	2	1070pg/ml	-
05		2000pg/ml	++
06		No samples	
07	4	210pg/ml	-
08		1060pg/ml	-
09		75pg/ml	+++

Figure 14

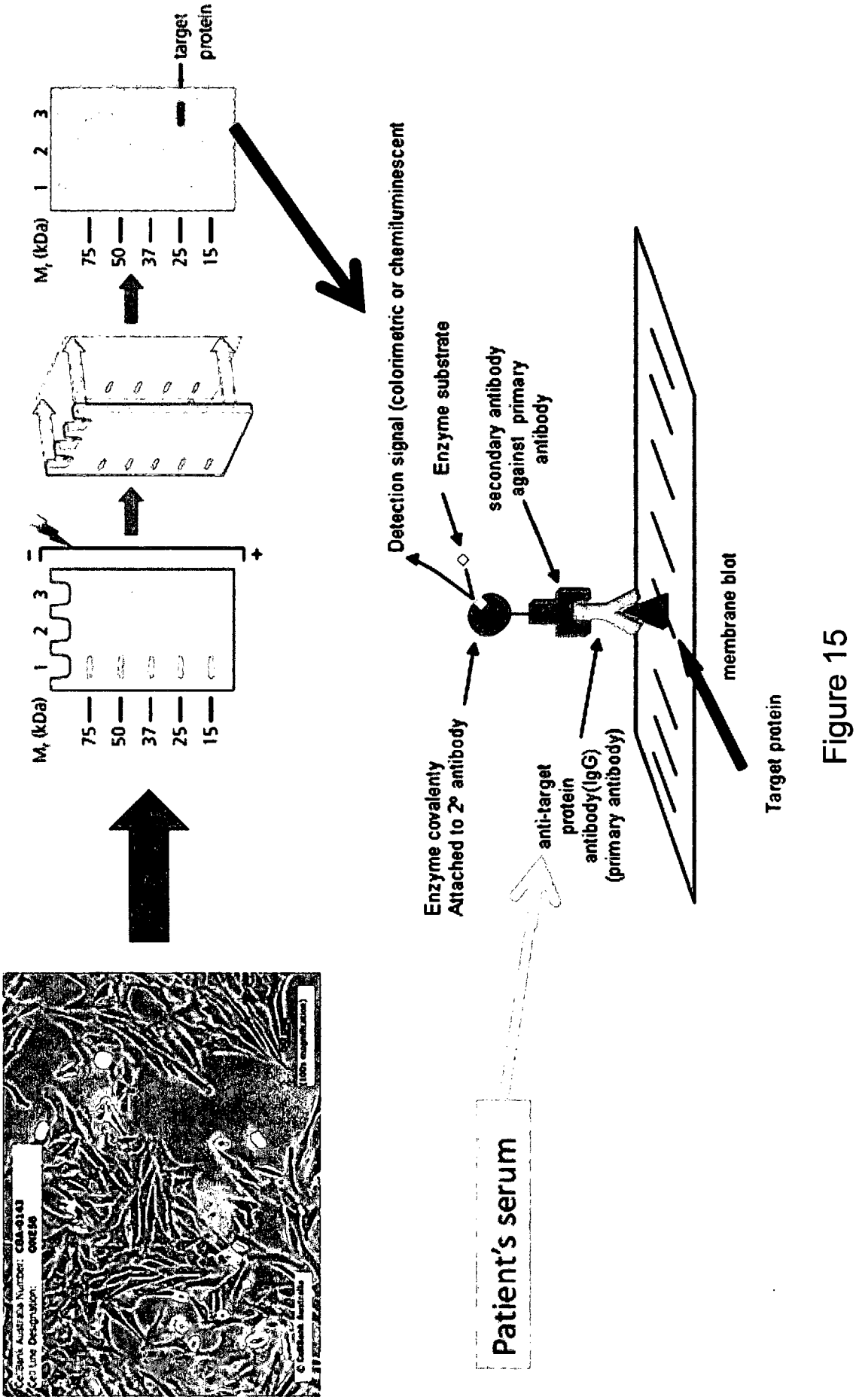

target protein
1 2 3
M_r (kDa)
75
50
37
25
15
Detection signal (colorimetric or chemiluminescent
Enzyme substrate
secondary antibody against primary antibody
membrane blot
Enzyme covalently Attached to 2° antibody
anti-target protein antibody(IgG) (primary antibody)
Target protein
Patient's serum

Figure 15

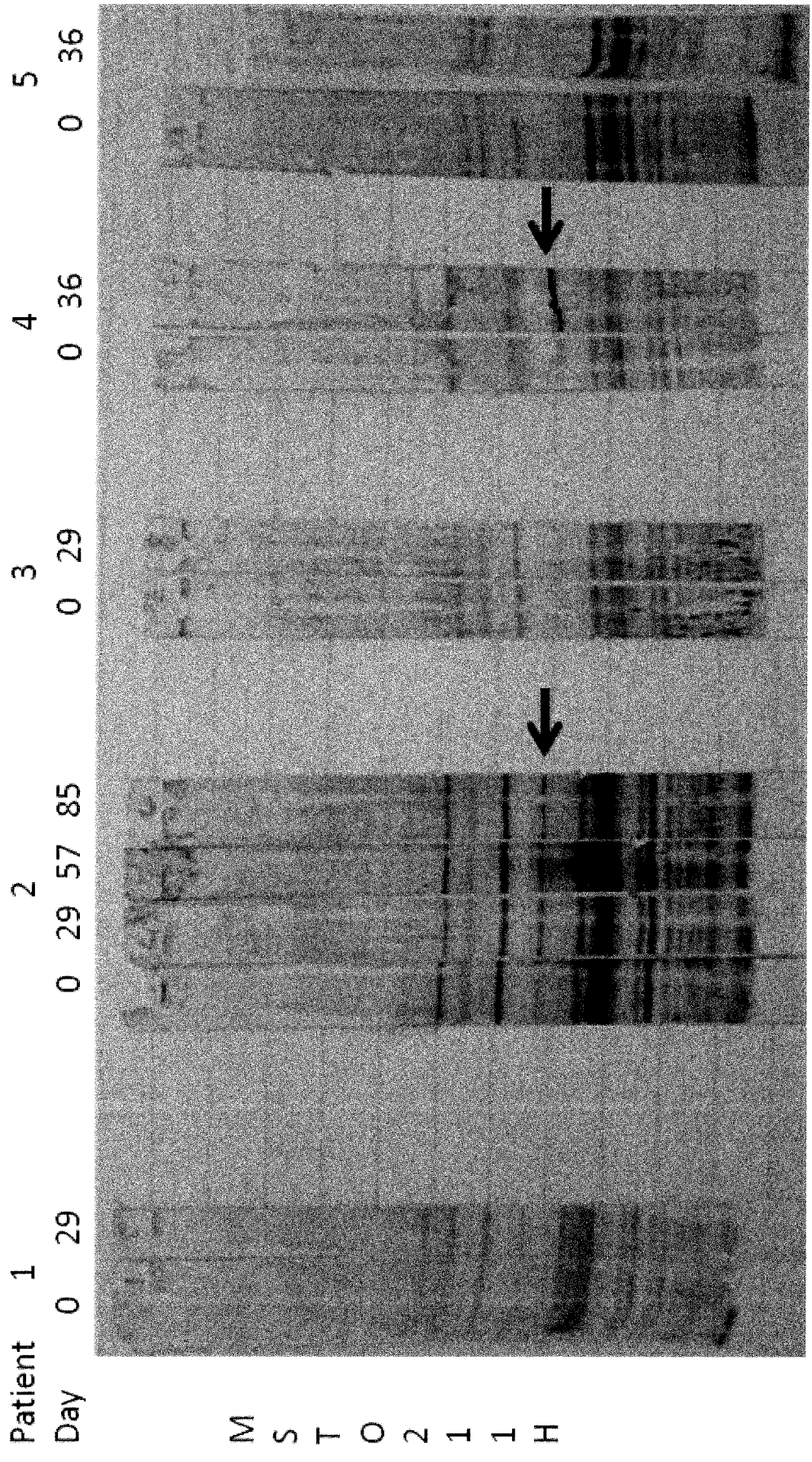


Figure 16

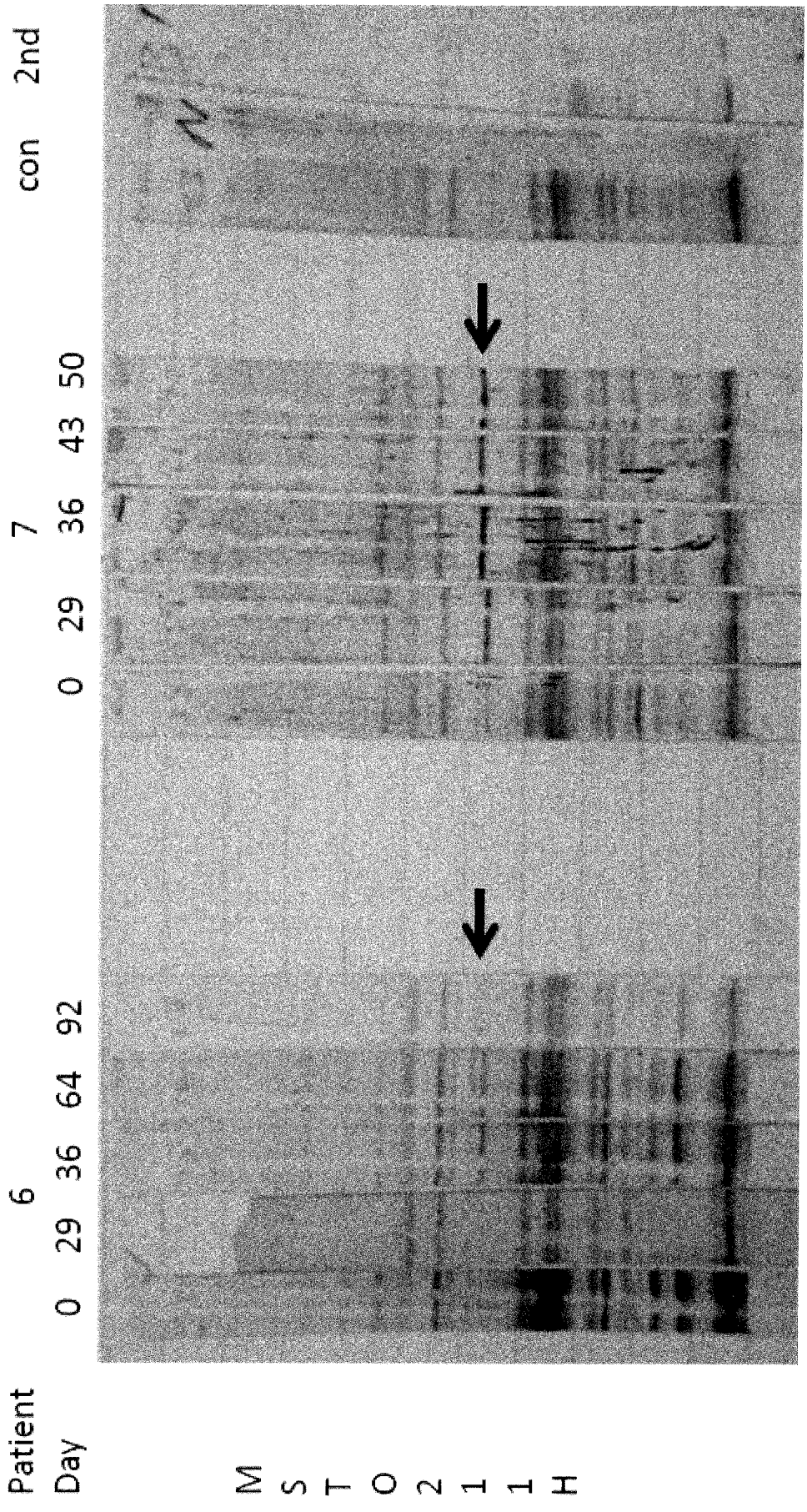


Figure 17

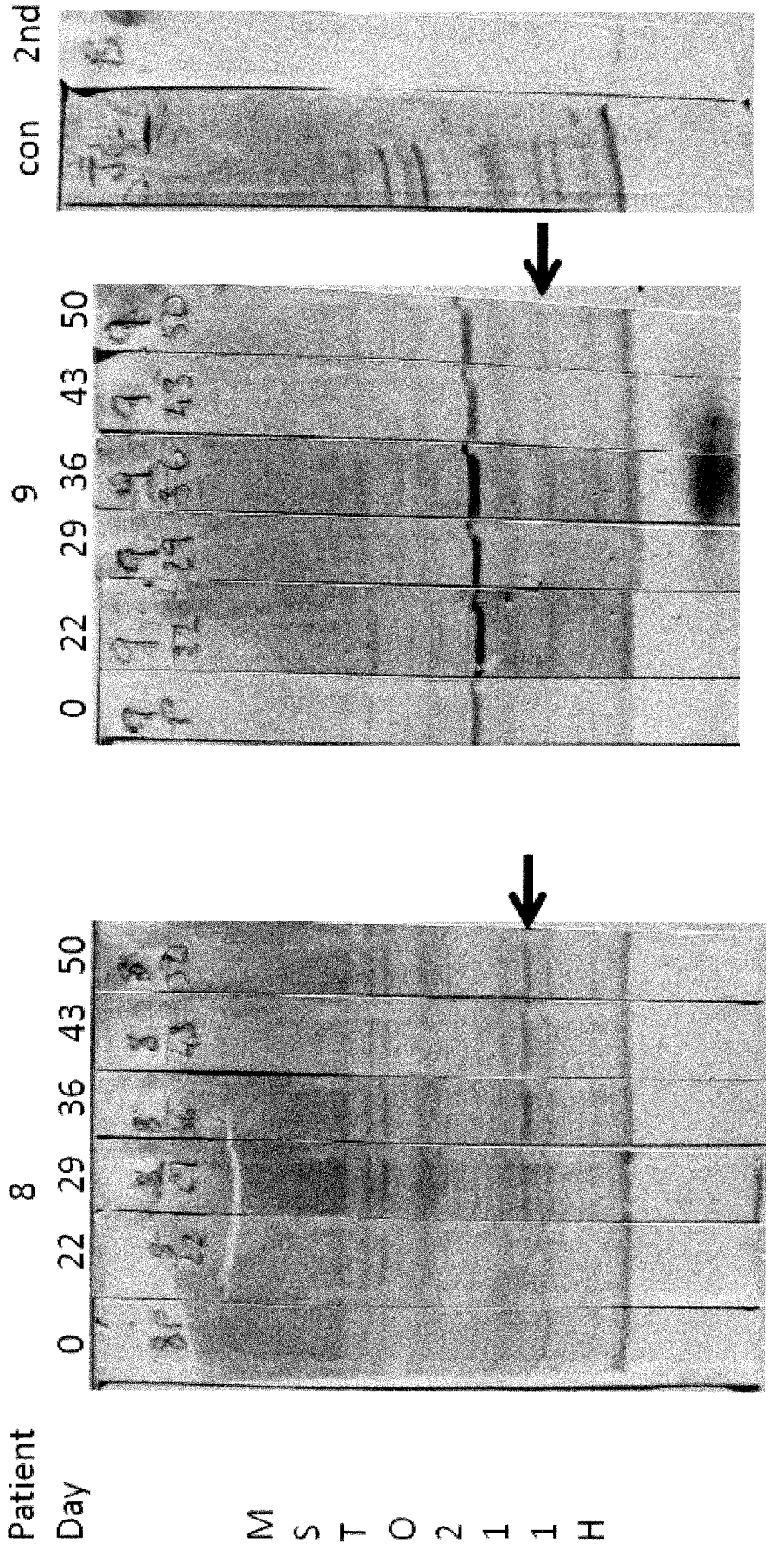


Figure 18

	Cytokine Th1 response versus patient survival				
		Survival ≥1 yr		Survival <1yr	
		Th1 response	No Th1 response	Th1 response	No Th1 response
Data at 20 July 2015	No of patients*	4 (mean =16.75 mths)	1 (13 mths)	1 (10 mths)	1 (4 mths)
Data at 25 May 2016	No of patients#	6** (median = 19 months)	1 (13 mths)	1*** (10 mths)	2 (4 mths)

Figure 19

High (ng/ml)	Low (pg/ml)	undetected
IL-6, IL-8, IL-27, CXCL9 (MIG), VEGF	IL-2, IL-10, IL-12, IL-21, CXCL10 (IP10), CXCL11 (I-TAC)	IFNγ, IFNα, IL-1α, IL-4, TNFα, GM-CSF

Figure 20

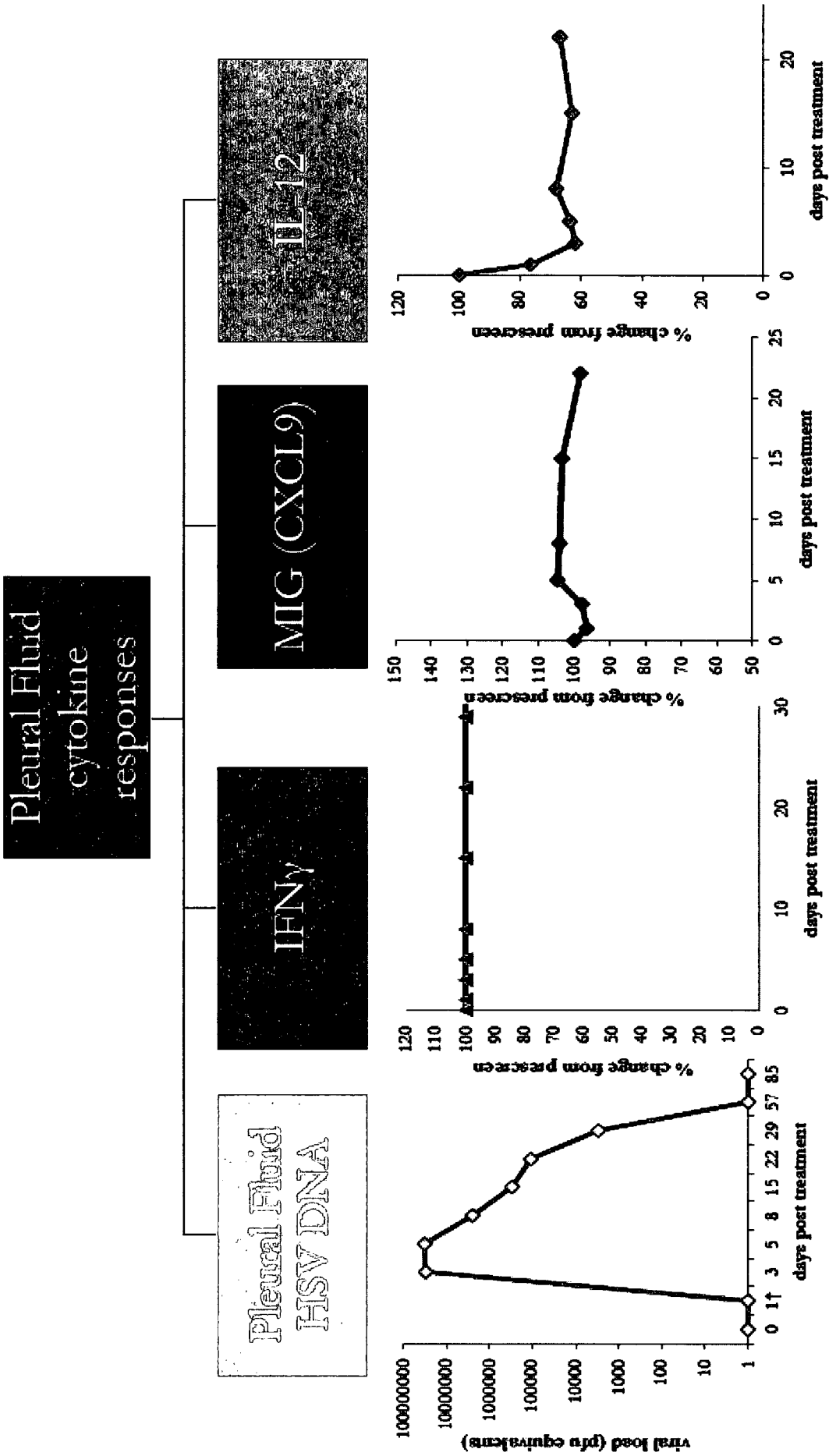

Pleural Fluid cytokine responses
Pleural Fluid HSV DNA
IFNγ
MIG (CXCL9)
IL-12
viral load (pfu equivalents)
% change from prescreen
days post treatment

Figure 21

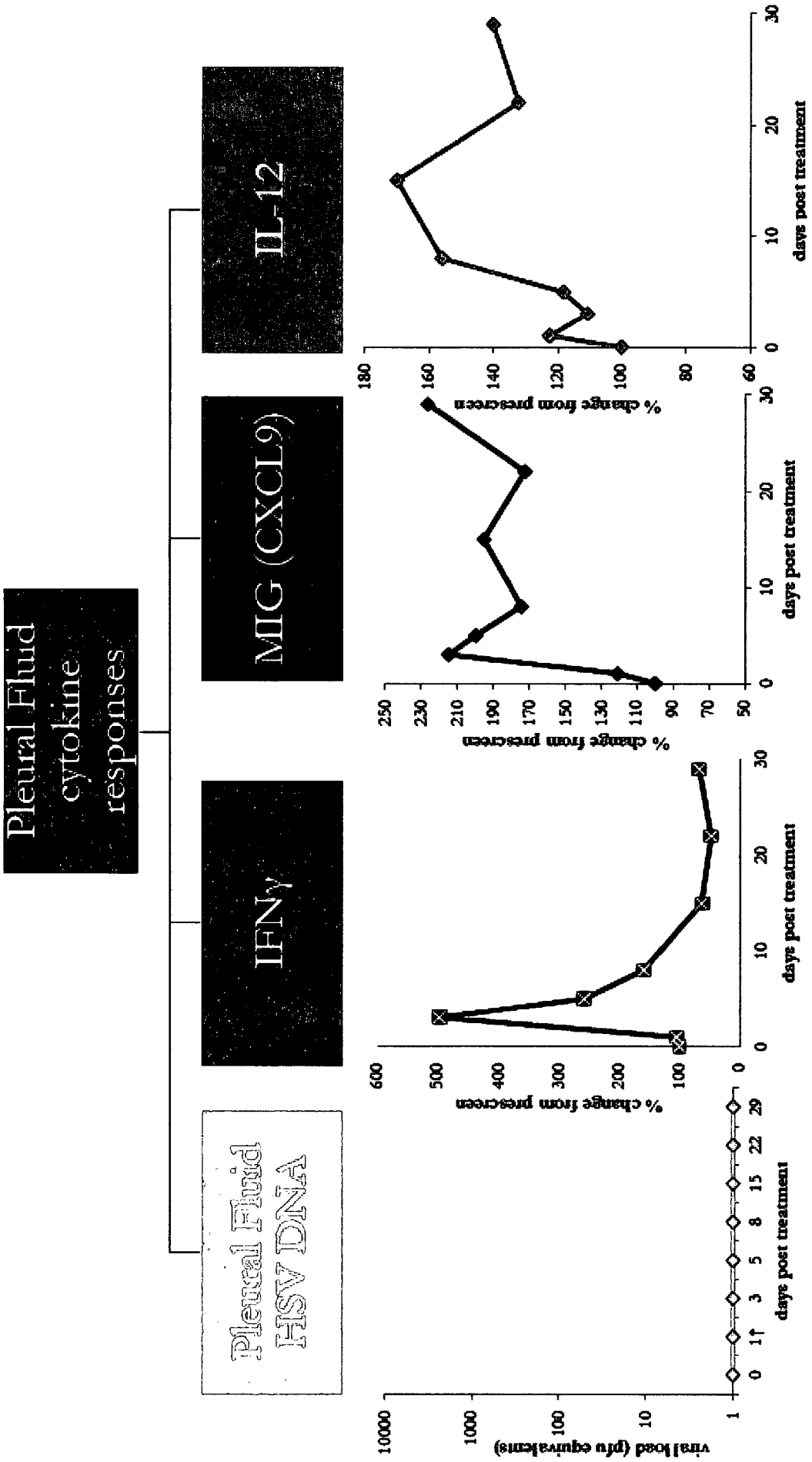

Pleural Fluid cytokine responses
Pleural Fluid HSV DNA
IFNγ
MIG (CXCL9)
IL-12
viral load (pfu equivalents)
% change from prescreen
days post treatment

Figure 22

	doses	IFNγ	IP10	MIG	I-TAC	VEGF	TNFα	IL2	IL6	IL8	IL10	IL12
Pt 01	1	++	+++	+++	+++	↓	+++	↓	+	+	+	-
Pt 02		-	↓	-	-	+++	↓	↓	↓	↓	+	↓
Pt 03		++	+++	+++	++	+++	++	↓	+	+	-/↓	++
Pt 04	2	++++	+++	++++	+++	↓	+++	↓	+	↓	+	+
Pt 05		-	-	-	+	++	↓	↓	↓	-	-/↓	-
Pt 06		-	+++	+++	nd	++	-	-	↓	-	-	-
Pt 07	4	+++++	++++	+++	++++	-	++	++	+	+++	++	+++
Pt 08		+++++	+++	+++	++++	↓	++	++	+++	+++	+++	++
Pt 09		++	+++	+++	++	+++	-	++	+	++	+	++

= cytokine/chemokine associated with aTh1 response - = unchanged, + = weak to +++++ = strong response, ↓ = decrease, nd = not done

Figure 23

	Doses	IFNα	IL1α	IL4	IL21	IL27	GM-CSF
Pt 01	1	-	-	-	-	↓	-
Pt 02		-	+	-	+	↓	-
Pt 03		-	-	-	↓	↓	-
Pt 04	2	-	-	-	-	-	-
Pt 05		-	-	-	+	+	-
Pt 06		++	-	nd	nd	nd	-
Pt 07	4	-	-	-	↓	++	-
Pt 08		+	-	+	+	-	-
Pt 09		-	+	-	+	-	-

nd = not done, + = increase, - = unchanged, ↓ = decrease

Figure 24

Pt no	No of doses	status	HSV DNA in pleural fluids	Pleural fluid Th1 response
01001		seropositive	-	+++
01002	1	seropositive	+++++	-
01903		seropositive	-	++
01004		seropositive	-	++++
01005	2	seropositive	+	-
01006		seropositive	+++	++
01007	4	seropositive	+++	+++
01008		seronegative	++++	+++++
01009		seropositive	+	++

- = unchanged, + = weak to +++++ = strong response

Figure 25

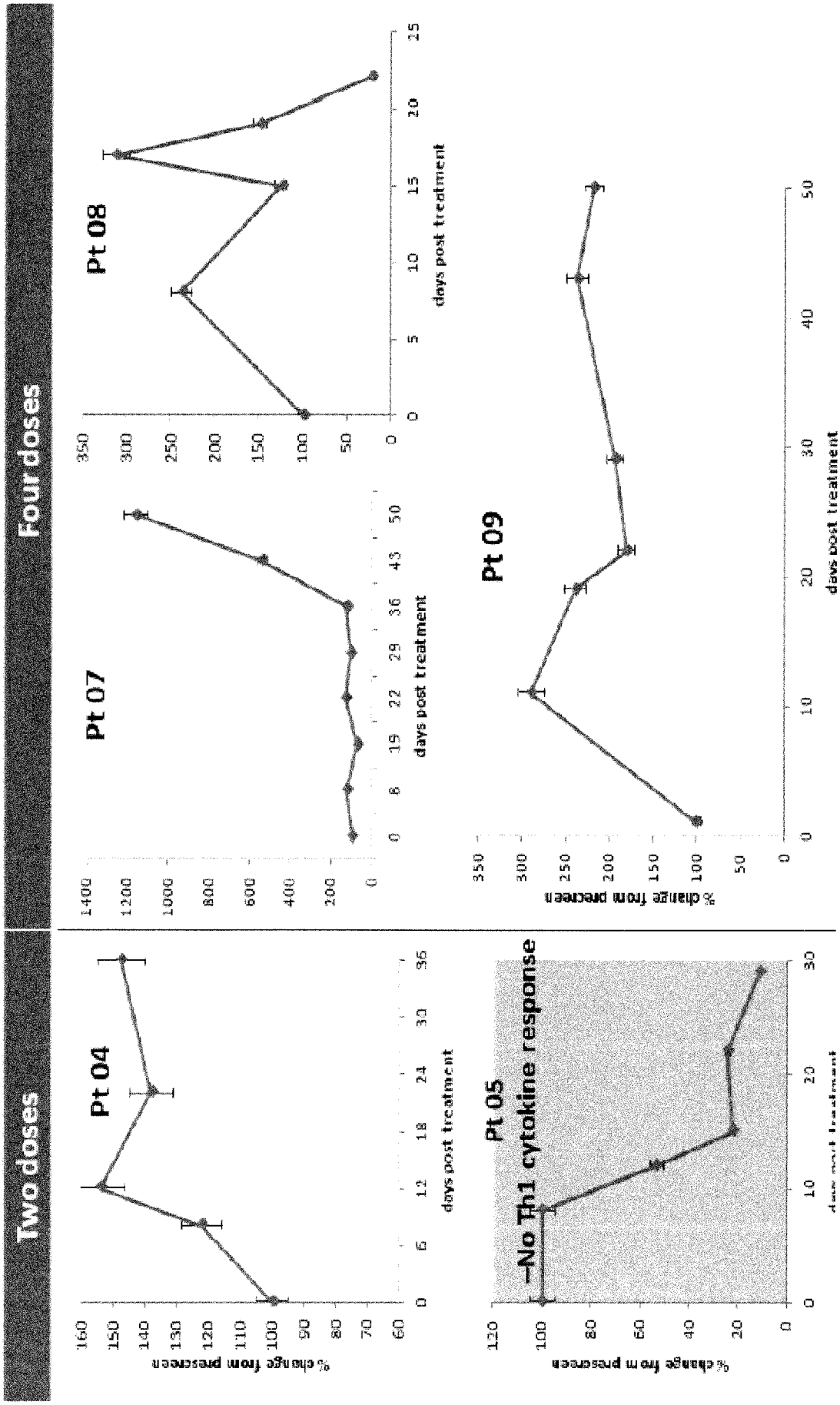

Pleural Fluid immune cell counts pre- and post treatment
Two doses
Four doses
Pt 04
Pt 07
Pt 08
Pt 09
Pt 05
–No Th1 cytokine response
% change from prescreen
days post treatment

Figure 26

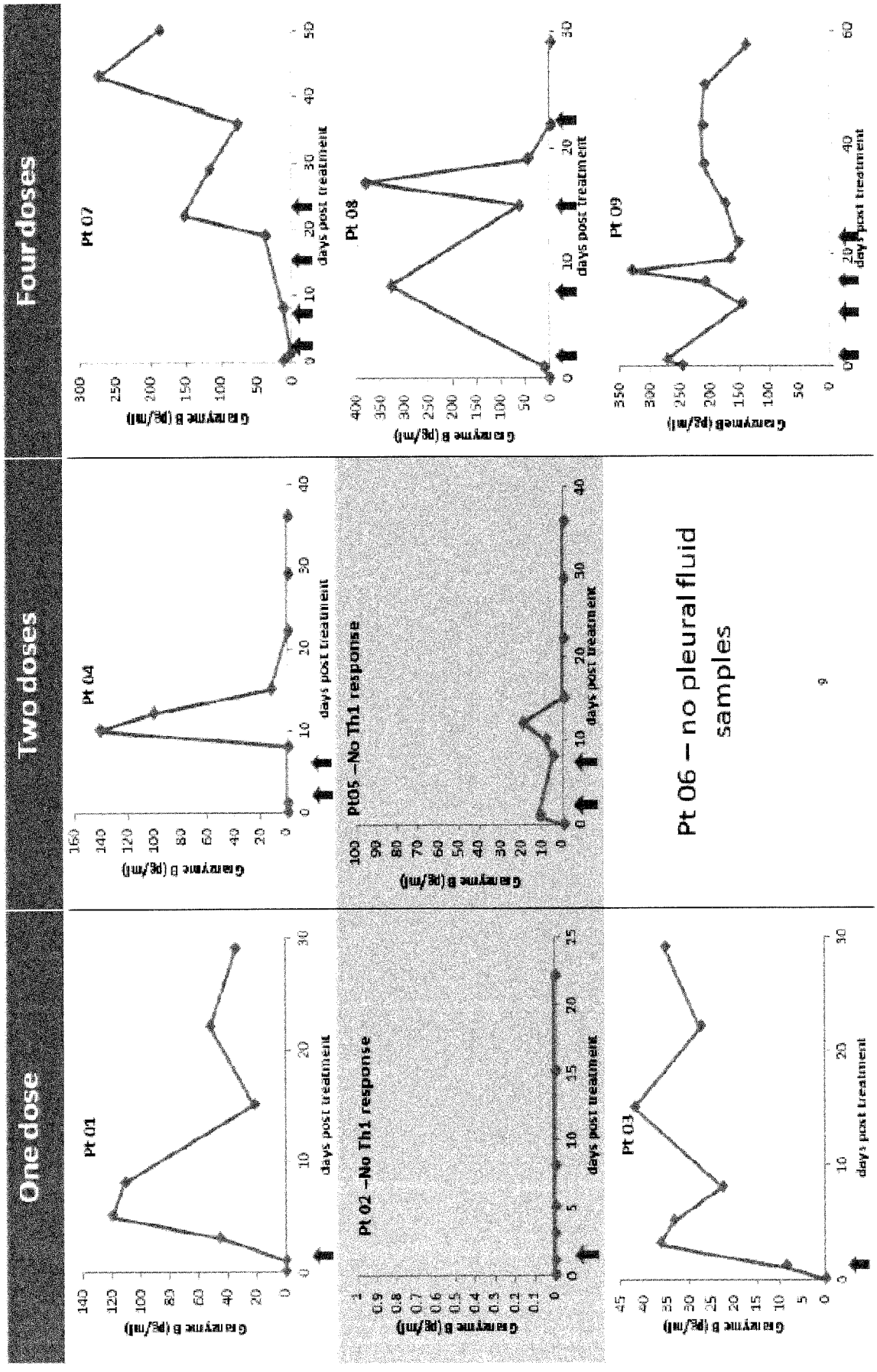

One dose
Two doses
Four doses
Pt 01
Pt 04
Pt 07
Pt 02 –No Th1 response
Pt05 –No Th1 response
Pt 08
Pt 09
Pt 06 – no pleural fluid samples
Pt 03
Gamzyme b (pg/ml)
days post treatment

Figure 27

Treatment regimen for virotherapy
i.tu. 1E8 pfu Seprehvir
Tumor size
(200~400 mm3)
0
+2
+4
+7
+9(Day)
250ug of Anti-PD-1 Ab (i.p.)
and tumor measurement twice a week

Figure 28

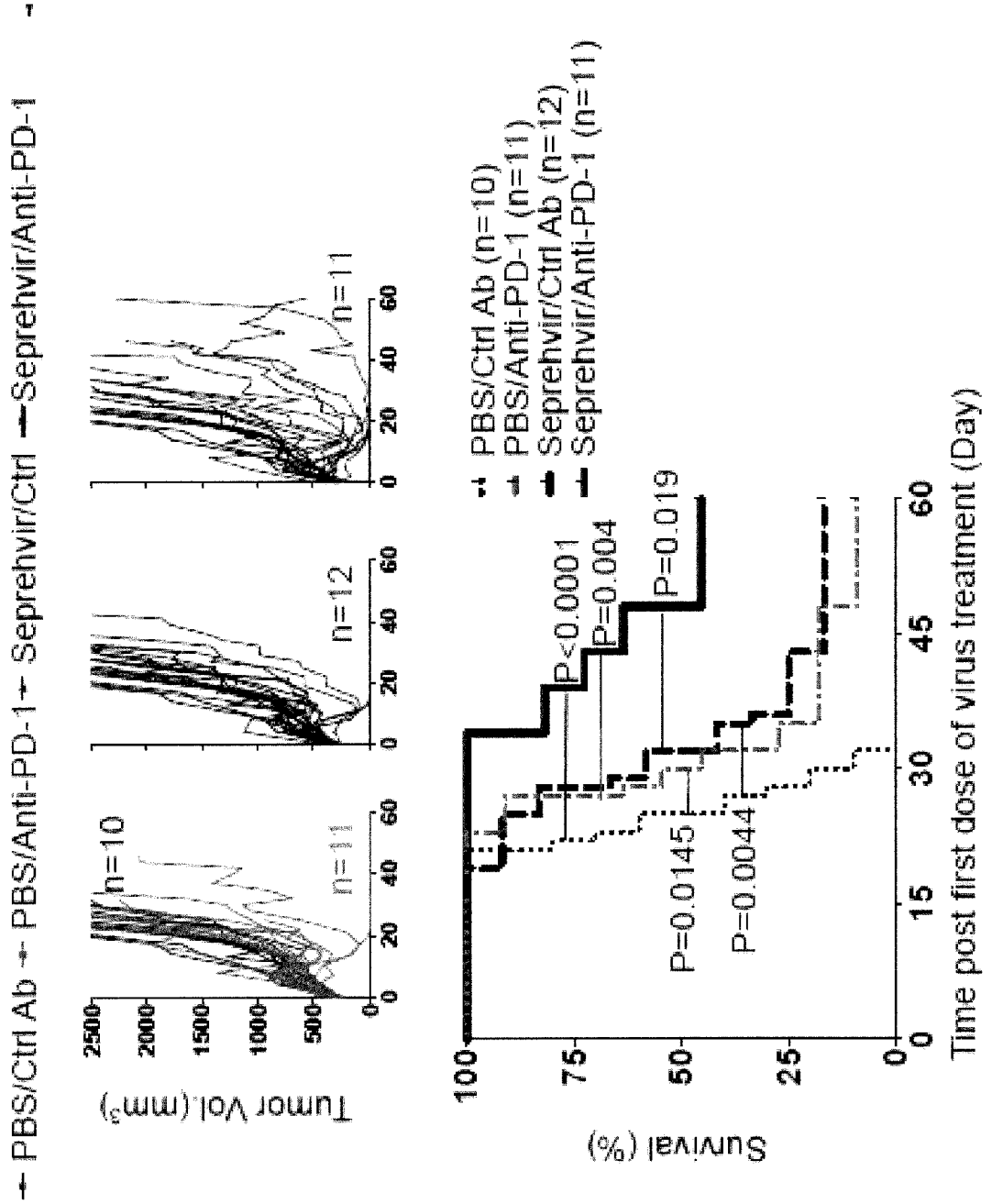

PBS/Ctrl Ab
PBS/Anti-PD-1
Seprehvir/Ctrl
Seprehvir/Anti-PD-1
Tumor Vol.(mm³)
n=10
n=11
n=12
n=11
PBS/Ctrl Ab (n=10)
PBS/Anti-PD-1 (n=11)
Seprehvir/Ctrl Ab (n=12)
Seprehvir/Anti-PD-1 (n=11)
P<0.0001
P=0.004
P=0.019
P=0.0145
P=0.0044
Survival (%)
Time post first dose of virus treatment (Day)

Figure 29

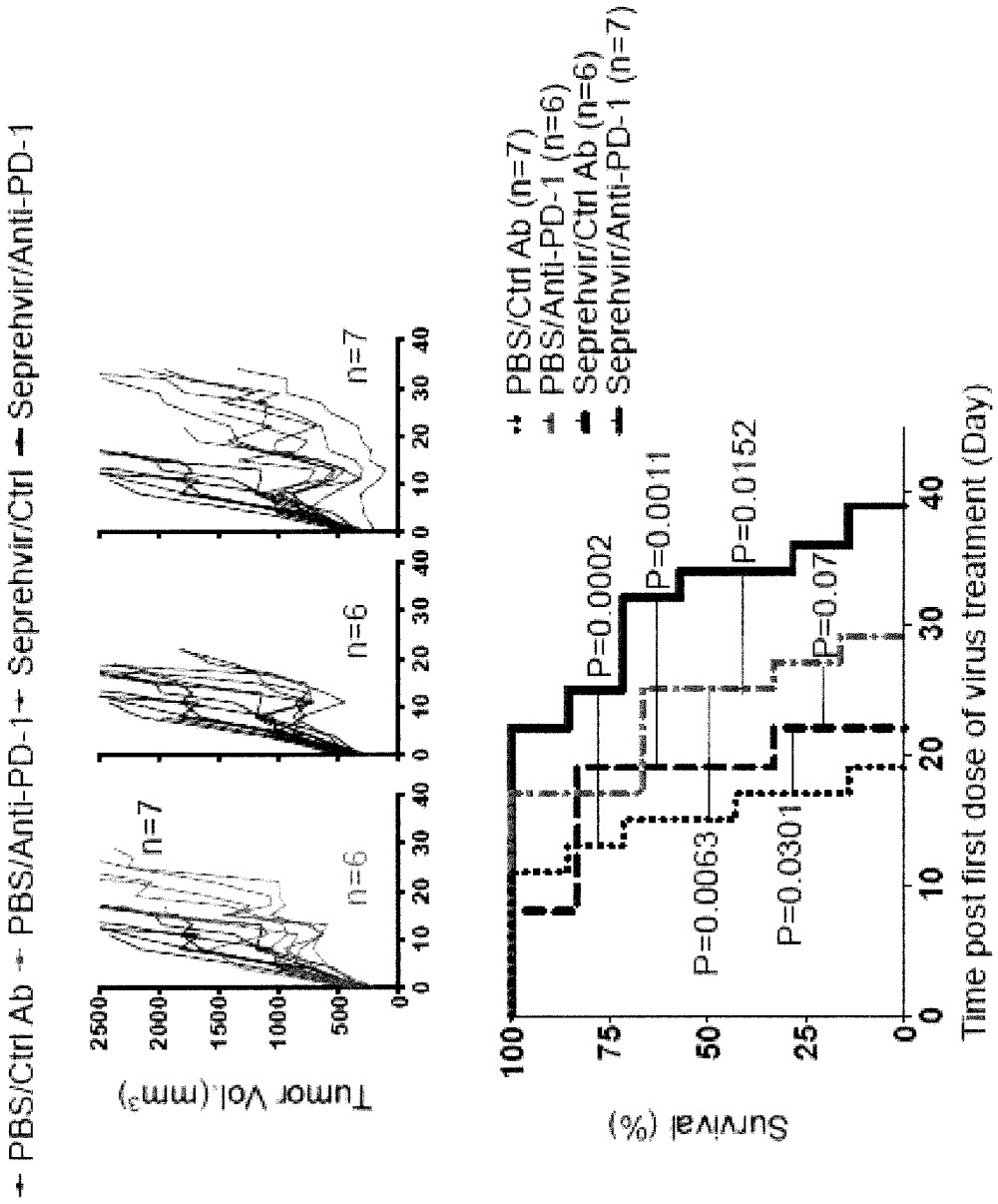

PBS/Ctrl Ab
PBS/Anti-PD-1
Seprehvir/Ctrl
Seprehvir/Anti-PD-1
Tumor Vol.(mm³)
n=7
n=6
n=6
n=7
Survival (%)
Time post first dose of virus treatment (Day)
P=0.0002
P=0.0011
P=0.0152
P=0.07
P=0.0063
P=0.0301
PBS/Ctrl Ab (n=7)
PBS/Anti-PD-1 (n=6)
Seprehvir/Ctrl Ab (n=6)
Seprehvir/Anti-PD- 1 (n=7)

Figure 30

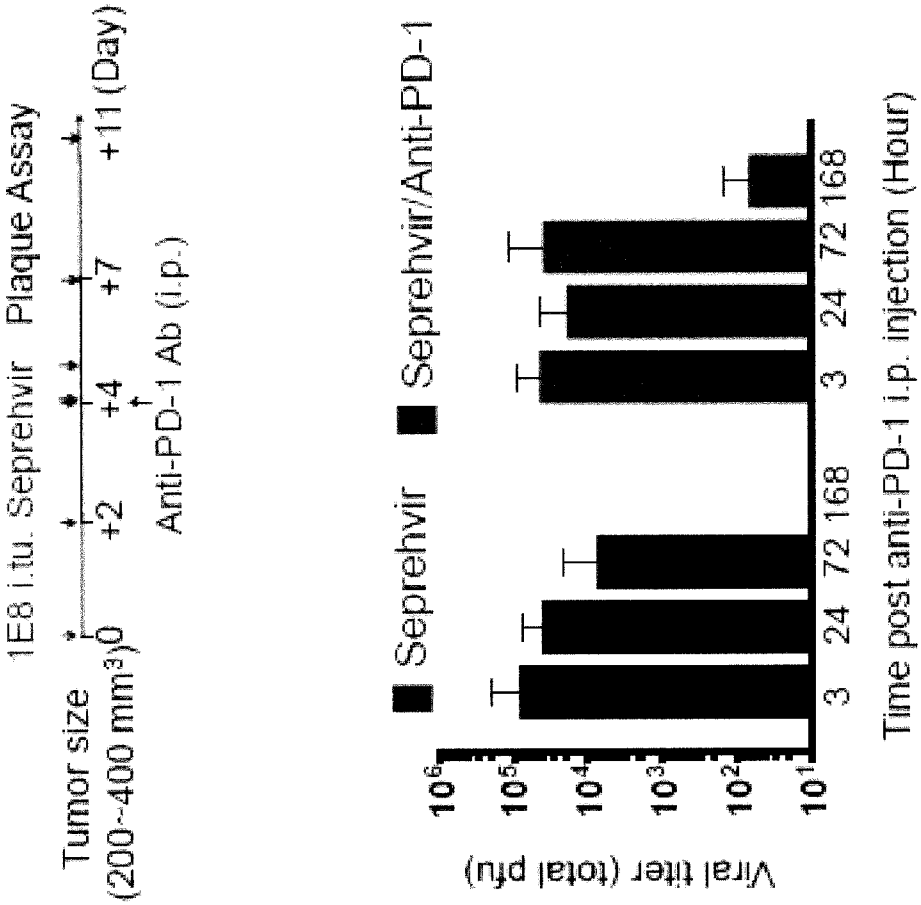

1E8 i.tu. Seprehvir Plaque Assay
Tumor size
(200~400 mm3)
0
+2
+4
+7
+11 (Day)
Anti-PD-1 Ab (i.p.)
Seprehvir
Seprehvir/Anti-PD-1
Viral titer (total pfu)
10^6
10^5
10^4
10^3
10^2
10^1
3
24
72
168
3
24
72
168
Time post anti-PD-1 i.p. injection (Hour)

Figure 31

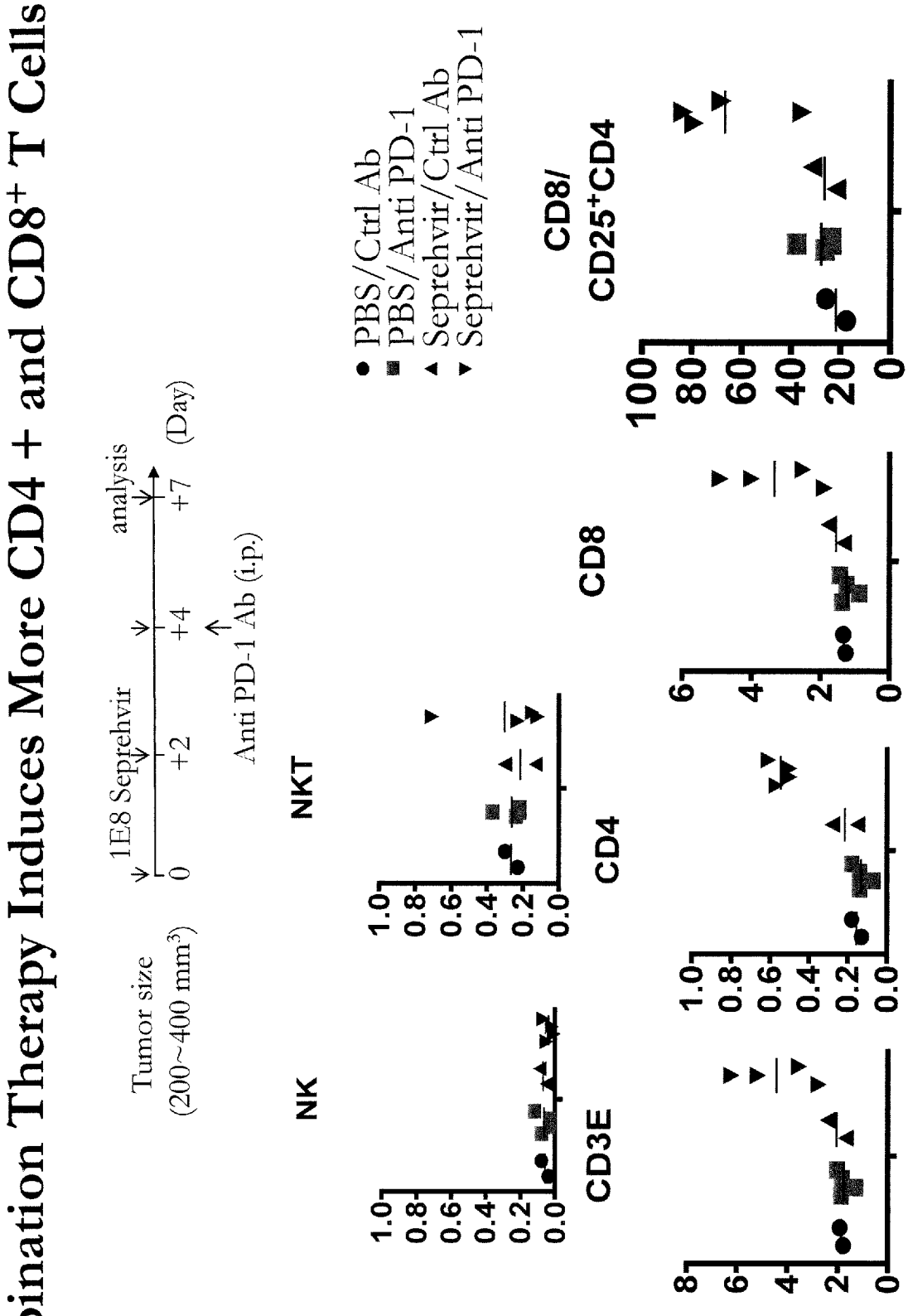

Combination Therapy Induces More CD4 + and CD8+ T Cells
Tumor size
(200~400 mm3)
1E8 Seprehvir
analysis
0
+2
+4
+7
(Day)
Anti PD-1 Ab (i.p.)
PBS/Ctrl Ab
PBS/Anti PD-1
Seprehvir/Ctrl Ab
Seprehvir/Anti PD-1
NK
NKT
CD8/
CD25+CD4
CD3E
CD4
CD8
Cellular infiltrate (% of live cells)

Figure 32A

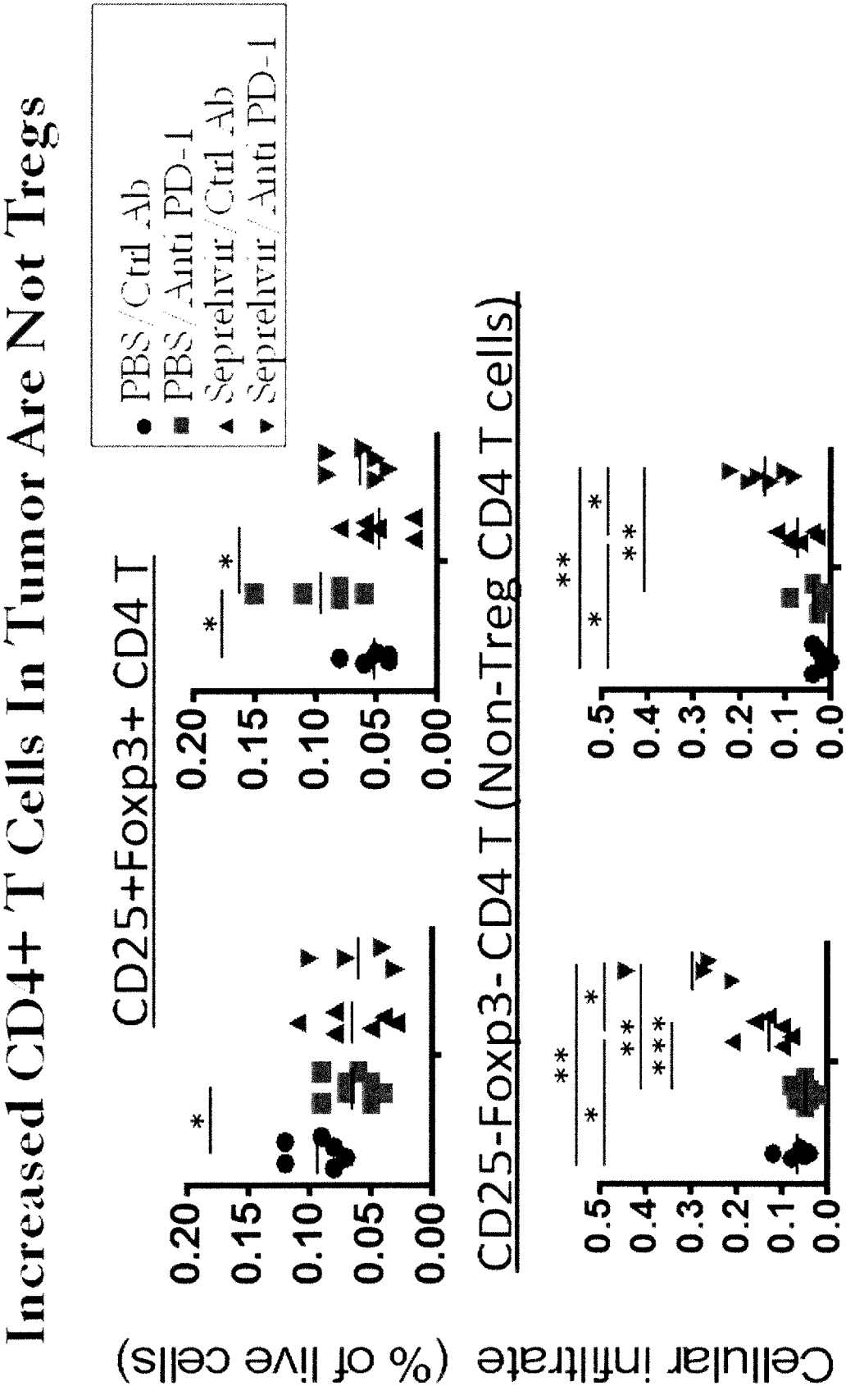

Increased CD4+ T Cells In Tumor Are Not Tregs
PBS / Ctrl Ab
PBS / Anti PD-1
Sepretivir / Ctrl Ab
Sepretivir / Anti PD-1
CD25+Foxp3+ CD4 T
CD25-Foxp3- CD4 T (Non-Treg CD4 T cells)
Cellular infiltrate (% of live cells)
0.20
0.15
0.10
0.05
0.00
0.5
0.4
0.3
0.2
0.1
0.0

Figure 32B

1E8 Seprehvir Gene analysis
Tumor size (200-400 mm³)
0 +2 +4 +7 (Day)
Anti-PD-1 Ab (i.p.)
PBS/Ctrl Ab
PBS/Anti-PD-1
Seprehvir/Ctrl Ab
Seprehvir/Anti-PD-1
Relative RNA expression to gapdh
ICP-0
T-bet
IFNg
iNOS
Gc
Foxp3
IL-10
MRC-1
T-bet/Foxp-3
IFN-g/IL-10
iNOS/MRC-1

Figure 33

Seprehvir and Combination Therapy Alter The Microenvironment Towards Th1 Immunity
PBS/Ctrl Ab
PBS/Anti PD-1
Seprehvir/Ctrl Ab
Seprehvir/Anti PD-1
IFN-g/IL-10
iNOS/MRC-1
Gene Expression (Fold relative to gapdh)
0
5
10
15
20
25

Figure 34

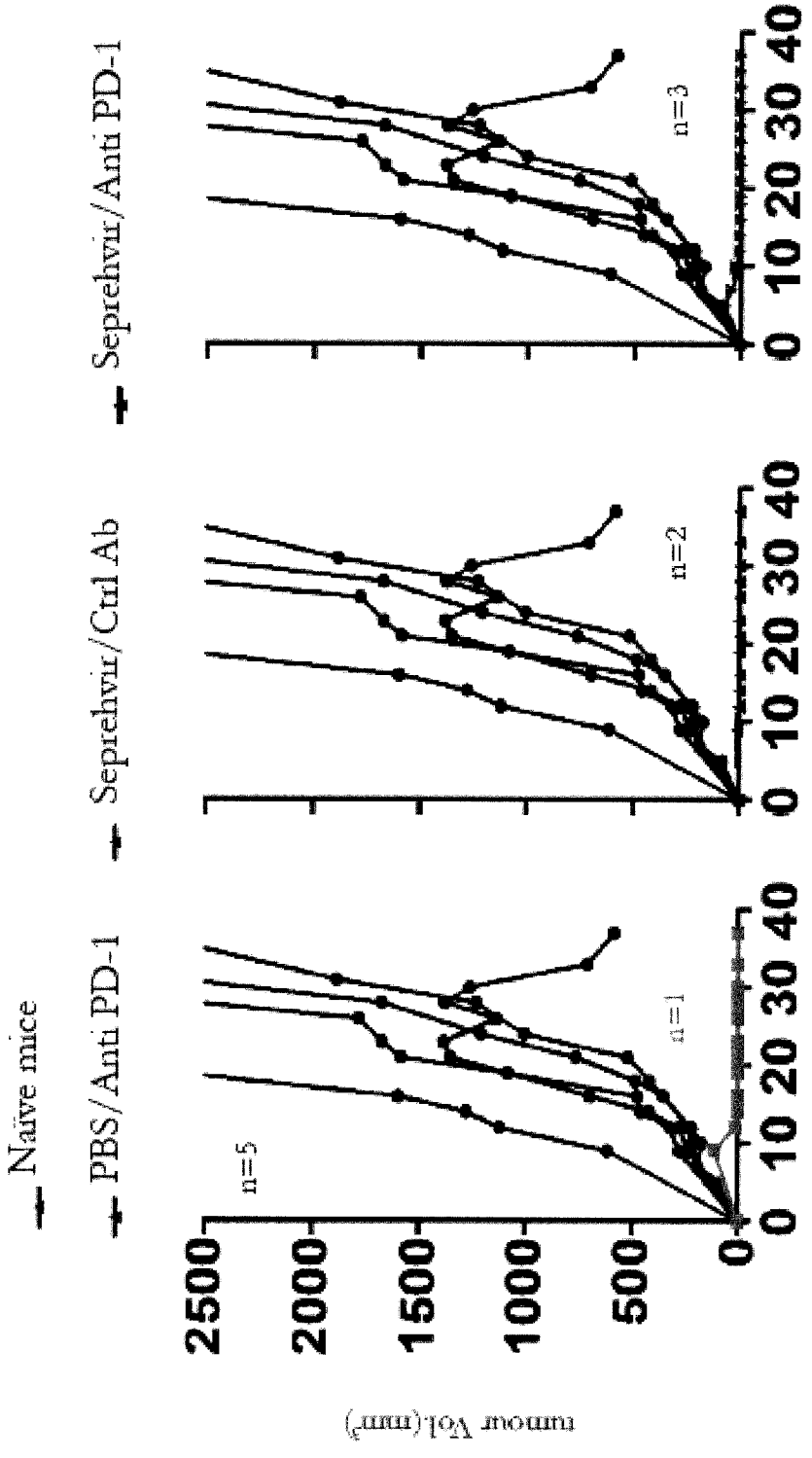

Naïve mice
PBS/Anti PD-1
Seprehvir/Ctrl Ab
Seprehvir/Anti PD-1
tumour Vol.(mm³)
2500
2000
1500
1000
500
0
n=5
n=1
n=2
n=3
0 10 20 30 40
days

Figure 35

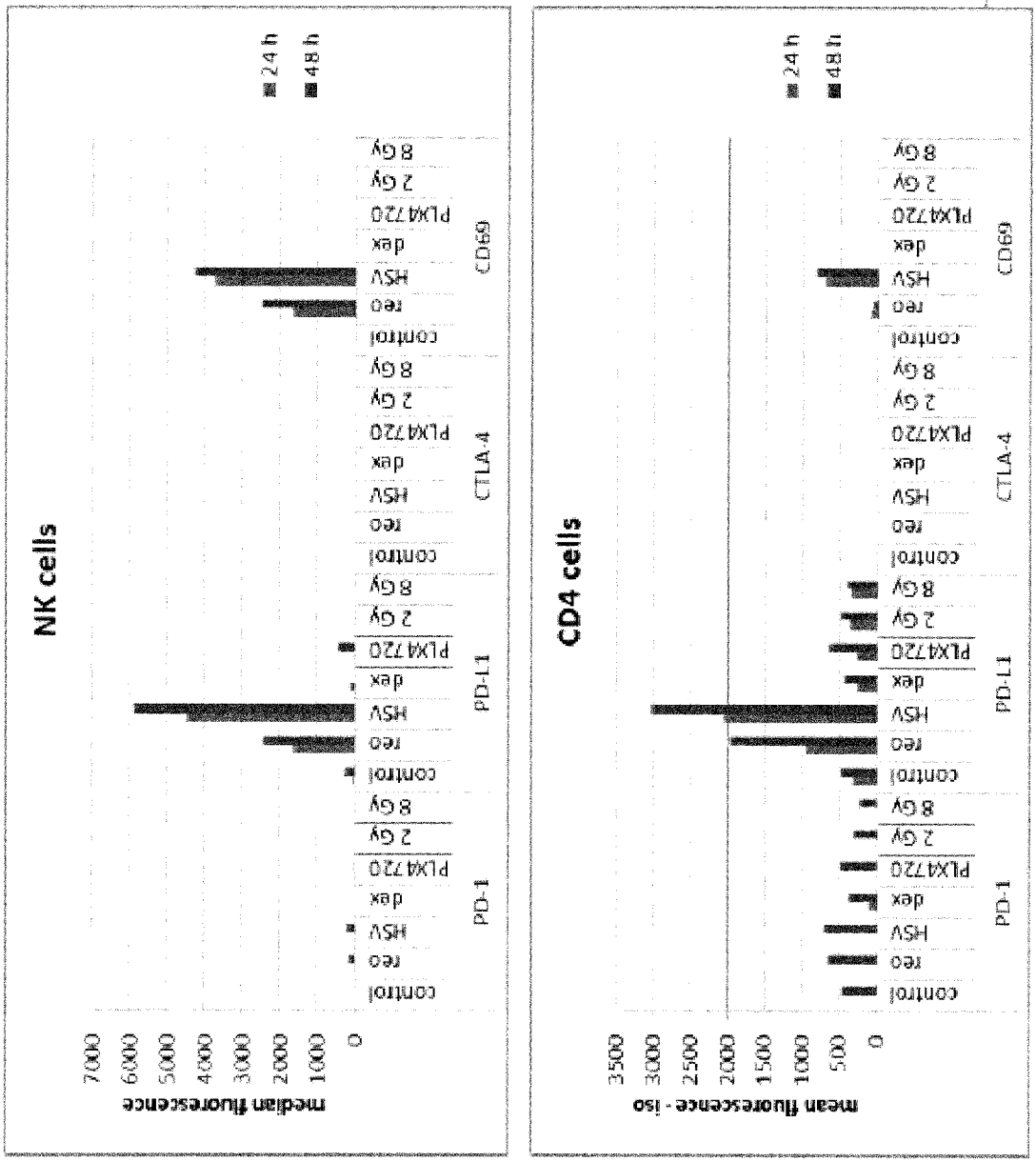

NK cells
median fluorescence
0
1000
2000
3000
4000
5000
6000
7000
24 h
48 h
control
reo
HSV
dex
PLX4720
2 Gy
8 Gy
PD-1
PD-L1
CTLA-4
CD69
CD4 cells
mean fluorescence - iso
0
500
1000
1500
2000
2500
3000
3500

Figure 36

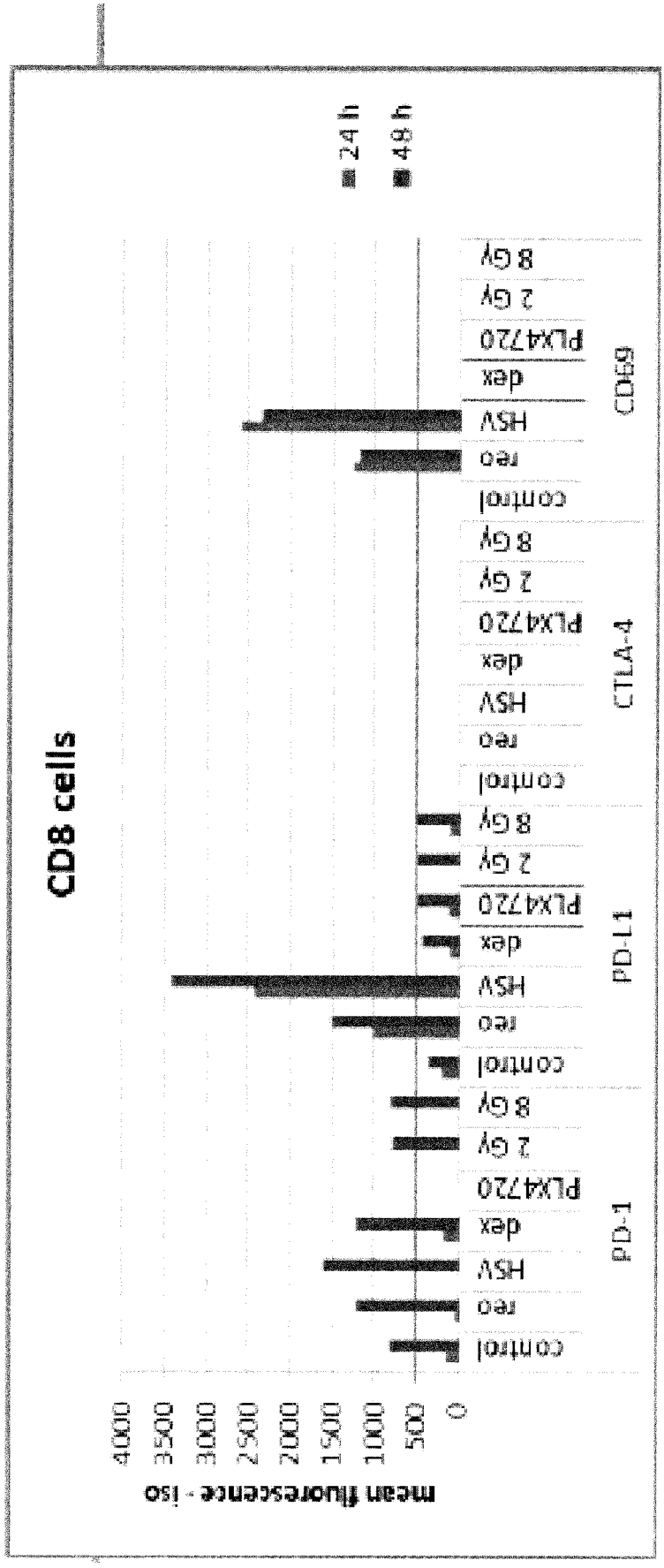

CD8 cells
24 h
48 h
mean fluorescence - iso
4000
3500
3000
2500
2000
1500
1000
500
0
control
reo
HSV
dex
PLX4720
2 Gy
8 Gy
PD-1
control
reo
HSV
dex
PLX4720
2 Gy
8 Gy
PD-L1
control
reo
HSV
dex
PLX4720
2 Gy
8 Gy
CTLA-4
control
reo
HSV
dex
PLX4720
2 Gy
8 Gy
CD69

Figure 37

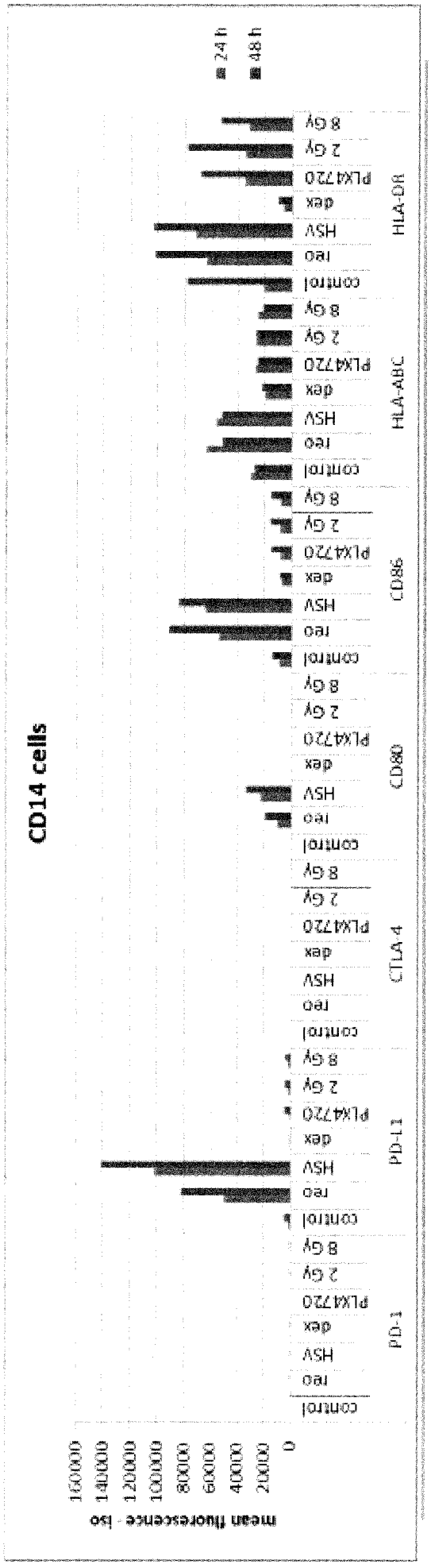

CD14 cells
mean fluorescence - Iso
160000
140000
120000
100000
80000
60000
40000
20000
0
24 h
48 h
PD-1
PD-L1
CTLA-4
CD80
CD86
HLA-ABC
HLA-DR
control
reo
HSV
dex
PLX4720
2 Gy
8 Gy

Figure 38

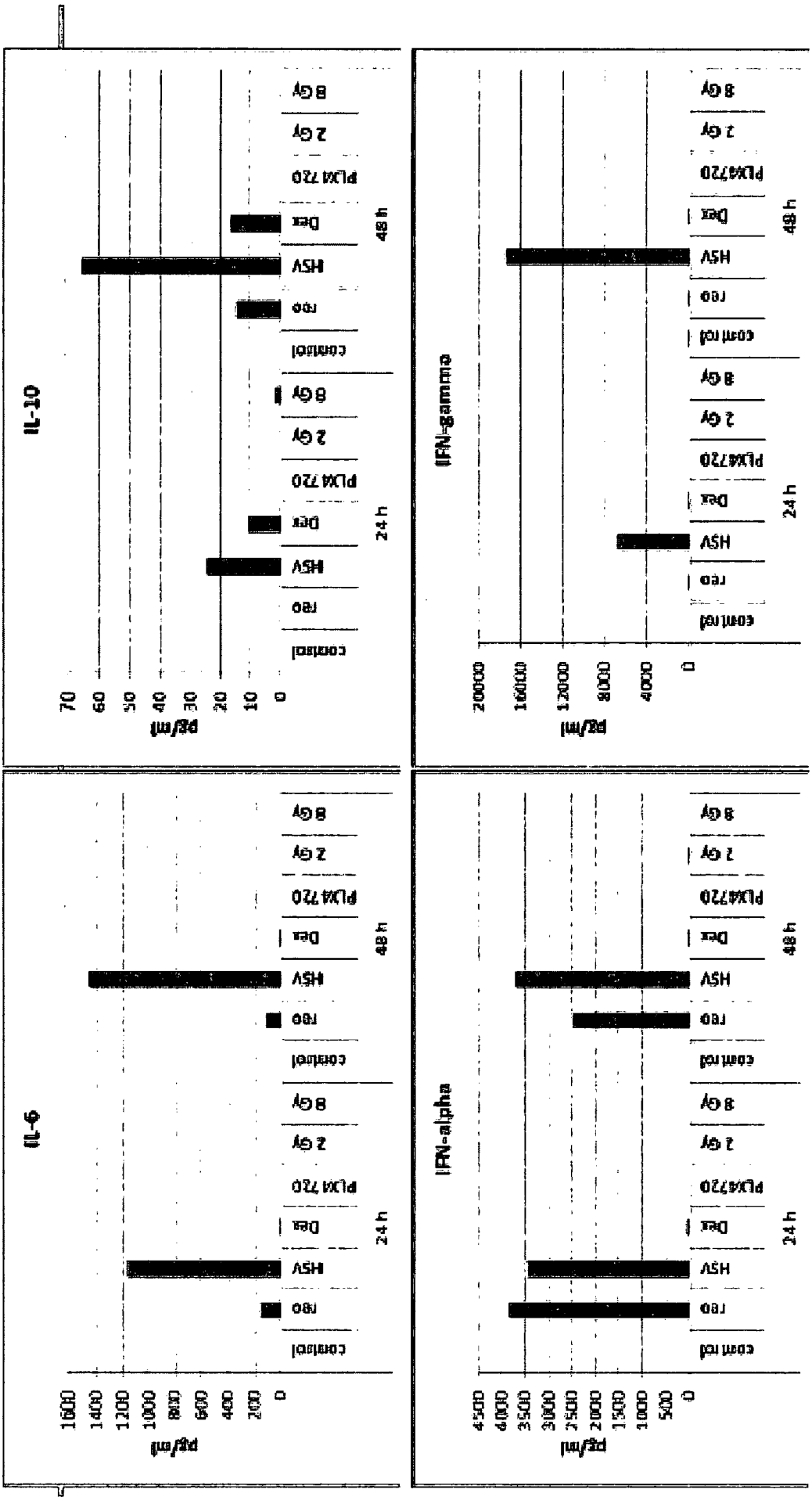

IL-10
IFN-gamma
IL-6
IFN-alpha
pg/ml
control
RBO
HSV
Dex
PLX4720
2 Gy
8 Gy
24 h
48 h

Figure 39A

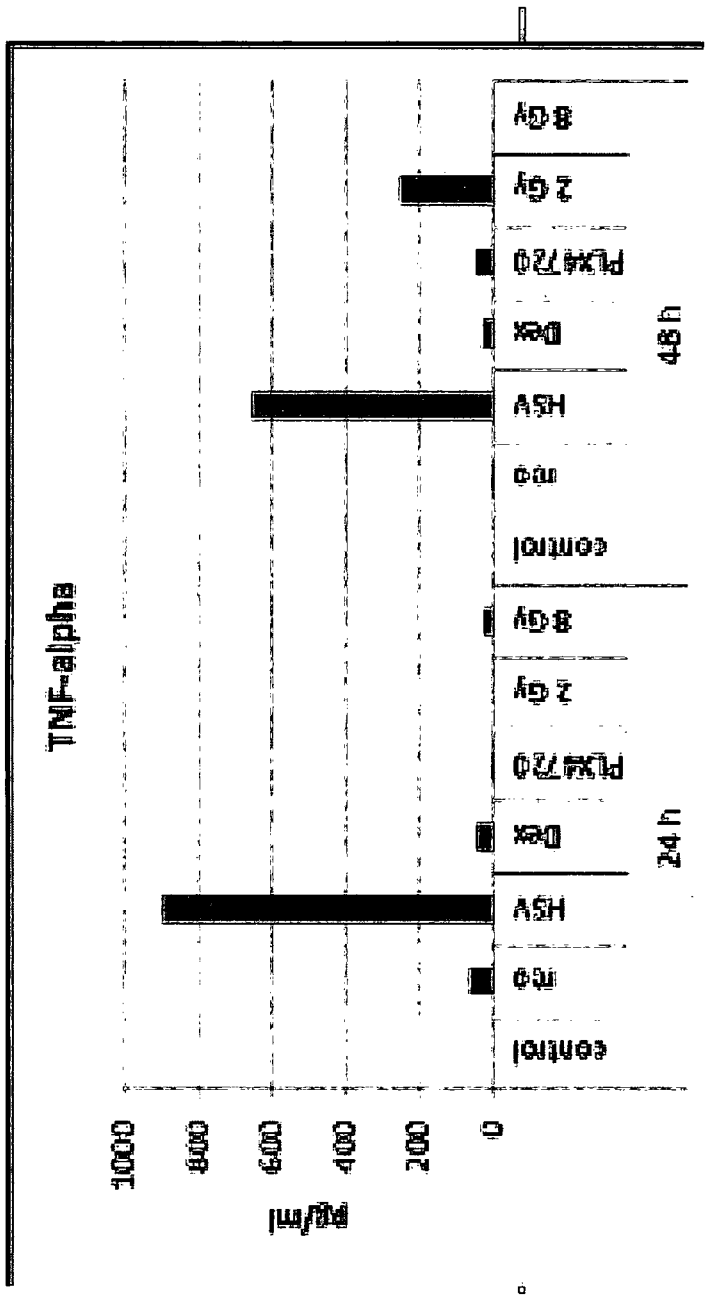
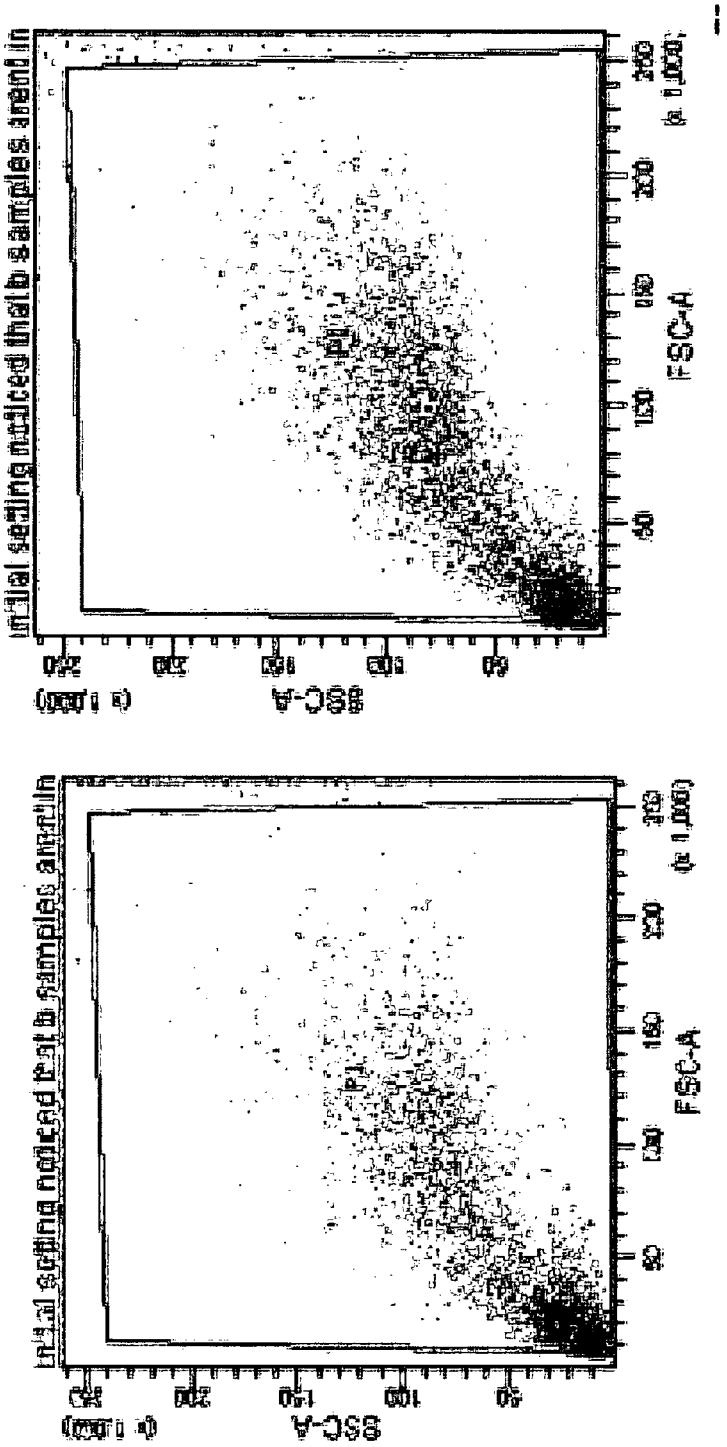

TNF-alpha
pg/ml
10000
8000
6000
4000
2000
0
48 h
24 h
control
mtd
HSV
Dex
PLX4720
2 Gy
8 Gy

Figure 39B



Cell line	GFP positive
One58	45.5%
Ovcar3	50.6
T98	39.8
Ln229	44

Figure 40

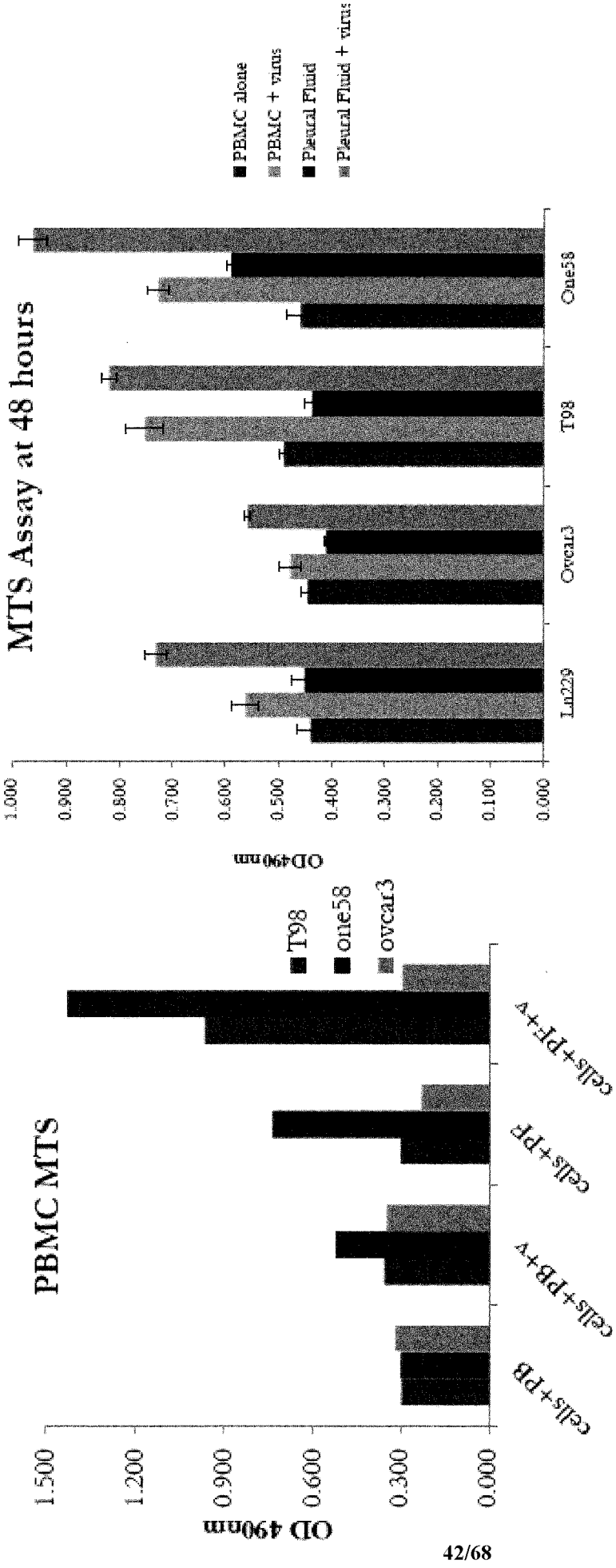

PBMC MTS
OD 490nm
1.500
1.200
0.900
0.600
0.300
0.000
cells+PB
cells+PB+v
cells+PF
cells+PF+v
T98
one58
ovcar3
MTS Assay at 48 hours
OD490nm
1.000
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Lu229
Ovcar3
T98
One58
PBMC alone
PBMC + virus
Pleural Fluid
Pleural Fluid + virus

Figure 41

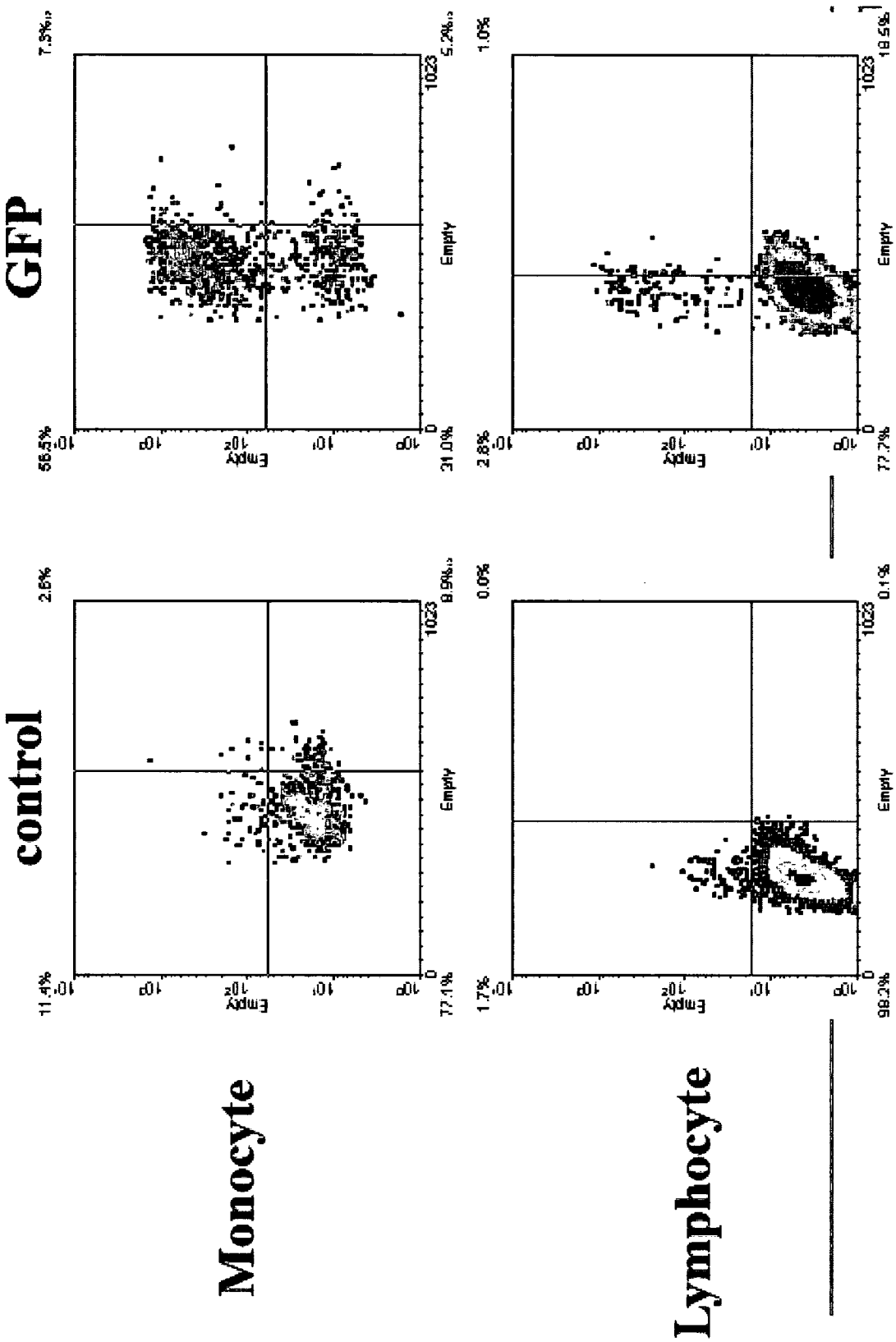

GFP
control
Monocyte
Lymphocyte
Empty
1023
0
7.3%
55.5%
31.0%
2.6%
11.4%
77.1%
1.0%
2.8%
77.7%
0.0%
1.7%
98.2%

Figure 42

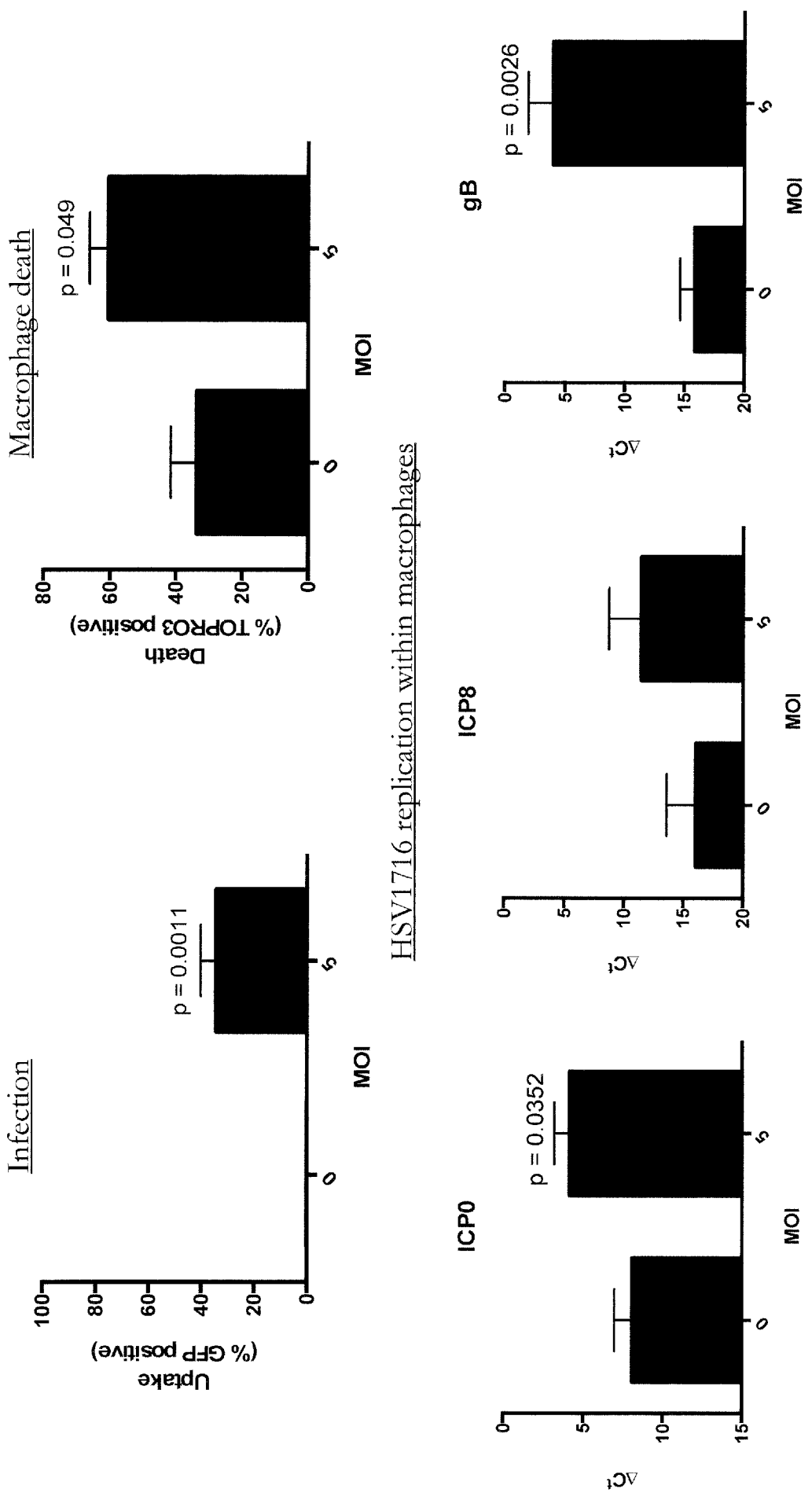
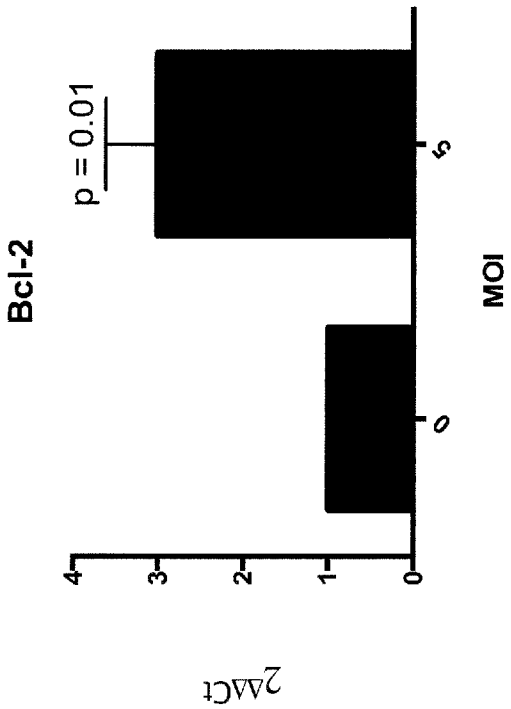
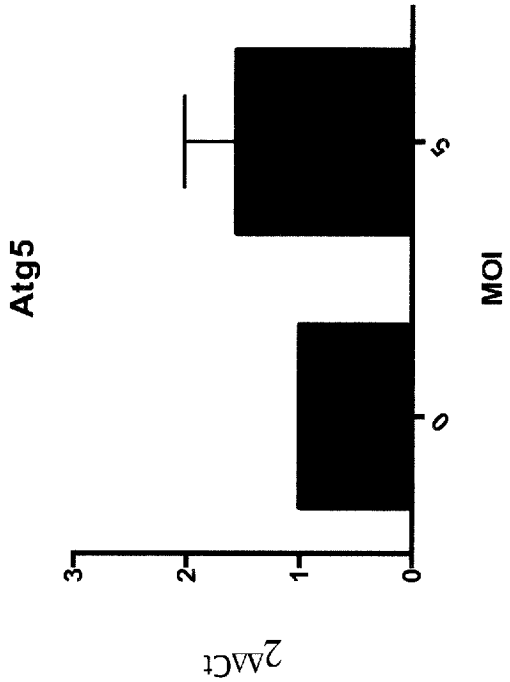
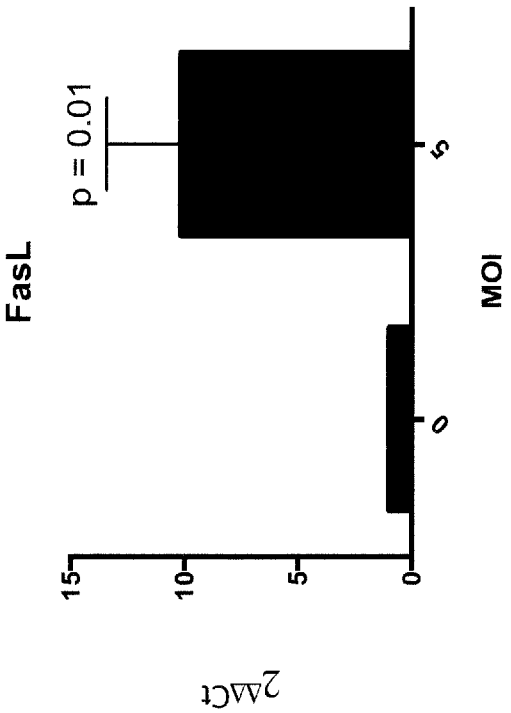

Infection
Uptake
(% GFP positive)
100
80
60
40
20
0
p = 0.0011
0
5
MOI
Macrophage death
Death
(% TOPRO3 positive)
80
60
40
20
0
p = 0.049
0
5
MOI
HSV1716 replication within macrophages
ICP0
ΔCt
0
5
10
15
p = 0.0352
0
5
MOI
ICP8
ΔCt
0
5
10
15
20
0
5
MOI
gB
ΔCt
0
5
10
15
20
p = 0.0026
0
5
MOI

Figure 43


Bcl-2
p = 0.01
2ΔΔCt
MOI
0
5


Atg5
2ΔΔCt
MOI
0
5


FasL
p = 0.01
2ΔΔCt
MOI
0
5

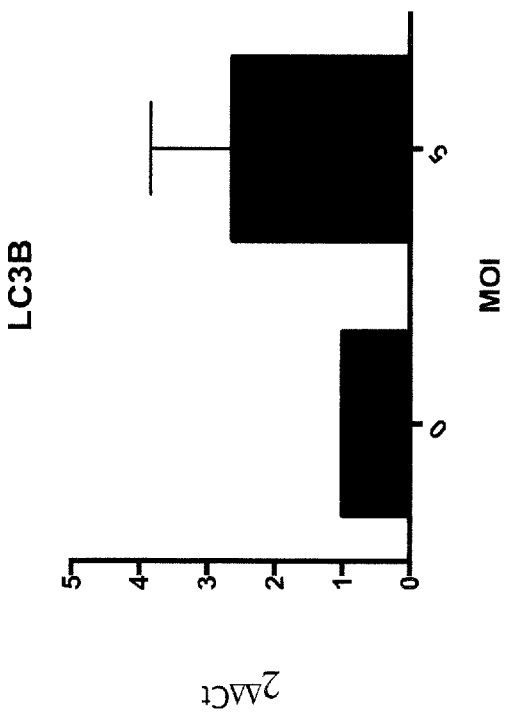
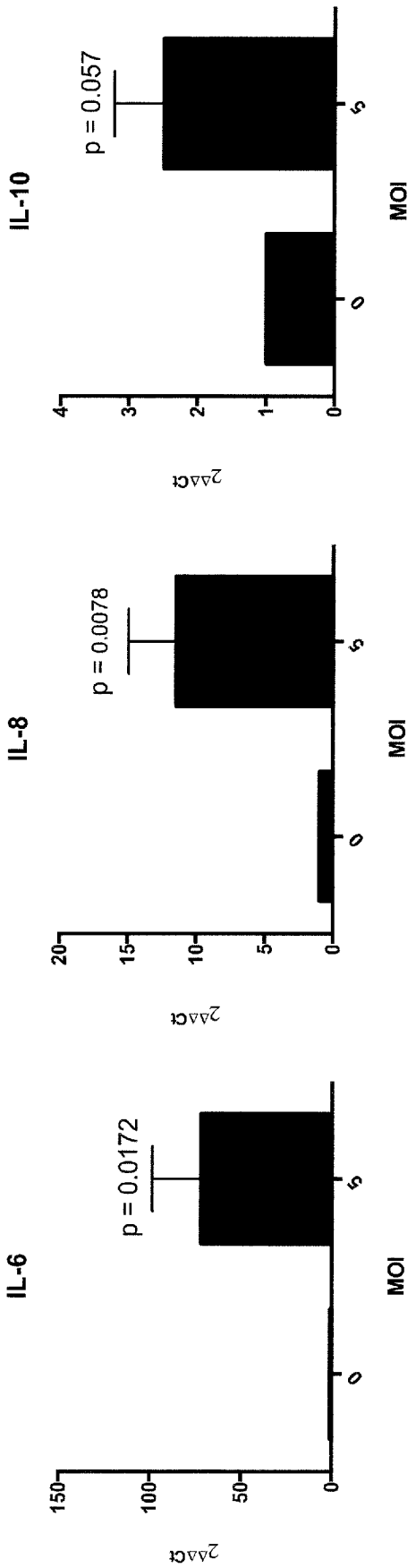
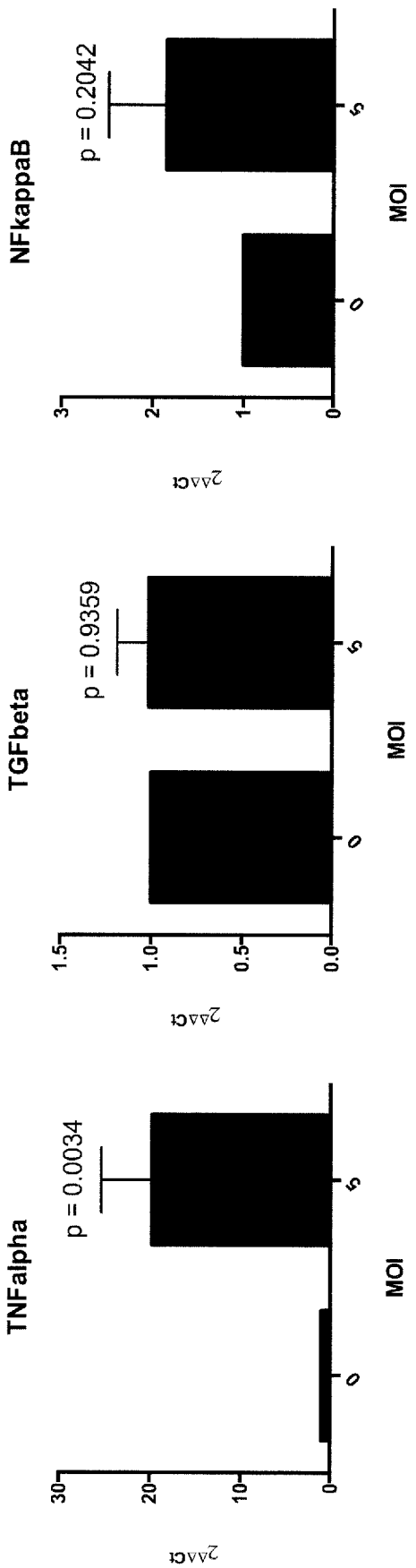

LC3B
2ΔΔCt
MOI
0
5

Figure 44


IL-6
p = 0.0172
150
100
50
0
0
5
MOI
2^-ΔΔCt

IL-8
p = 0.0078
20
15
10
5
0
0
5
MOI
2^-ΔΔCt

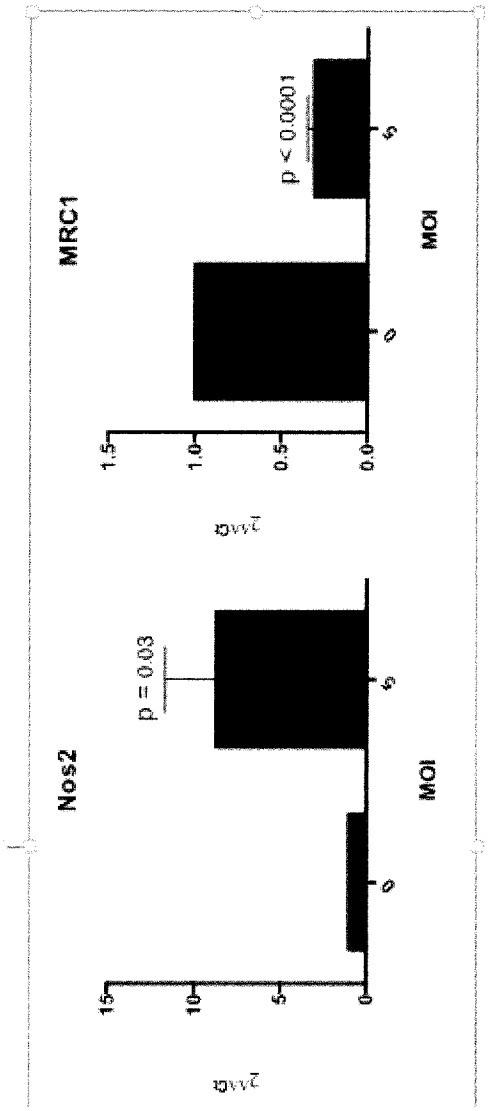
IL-10
p = 0.057
4
3
2
1
0
0
5
MOI
2^-ΔΔCt


TNFalpha
p = 0.0034
30
20
10
0
0
5
MOI
2^-ΔΔCt

TGFbeta
p = 0.9359
1.5
1.0
0.5
0.0
0
5
MOI
2^-ΔΔCt

NFkappaB
p = 0.2042
3
2
1
0
0
5
MOI
2^-ΔΔCt

Figure 45


MRC1
p < 0.0001
MOI
0
5
0.0
0.5
1.0
1.5
2-ΔΔCt
Nos2
p = 0.03
MOI
0
5
0
5
10
15
2-ΔΔCt

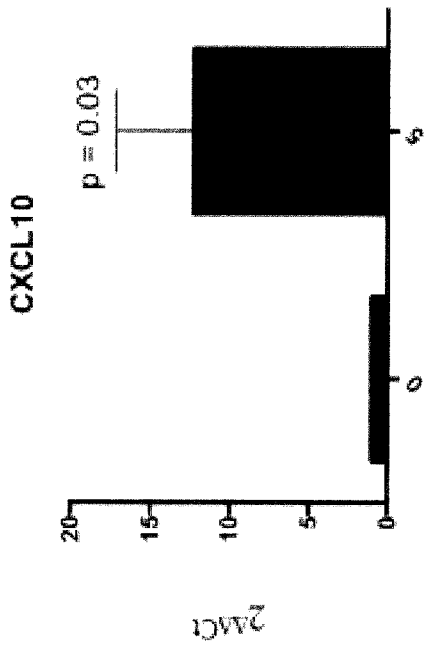

CXCL10
p = 0.03
MOI
0
5
0
5
10
15
20
2-ΔΔCt

Figure 46

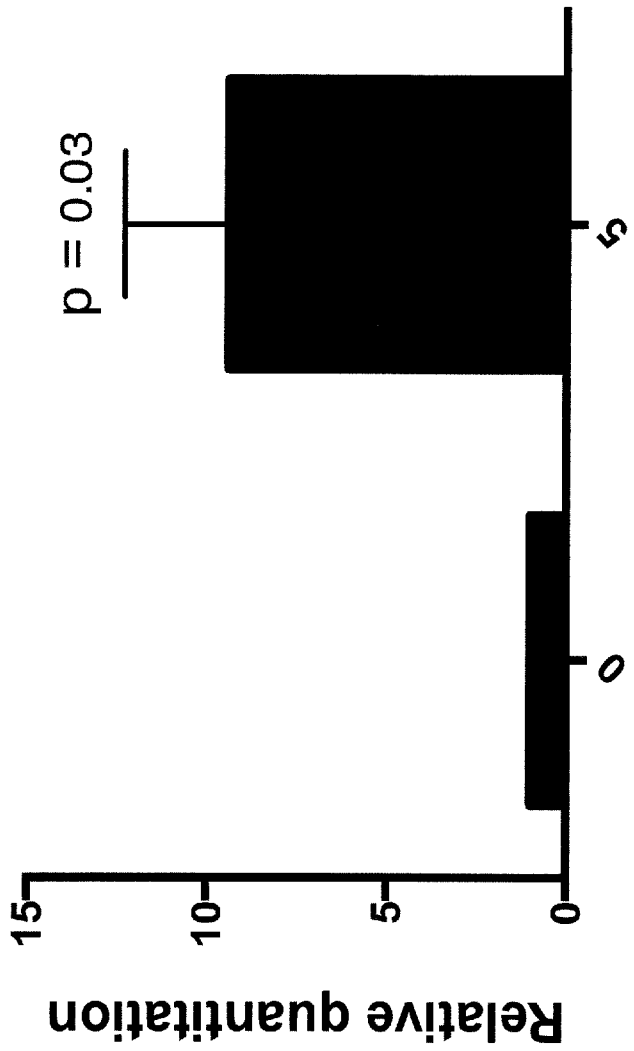


Figure 47

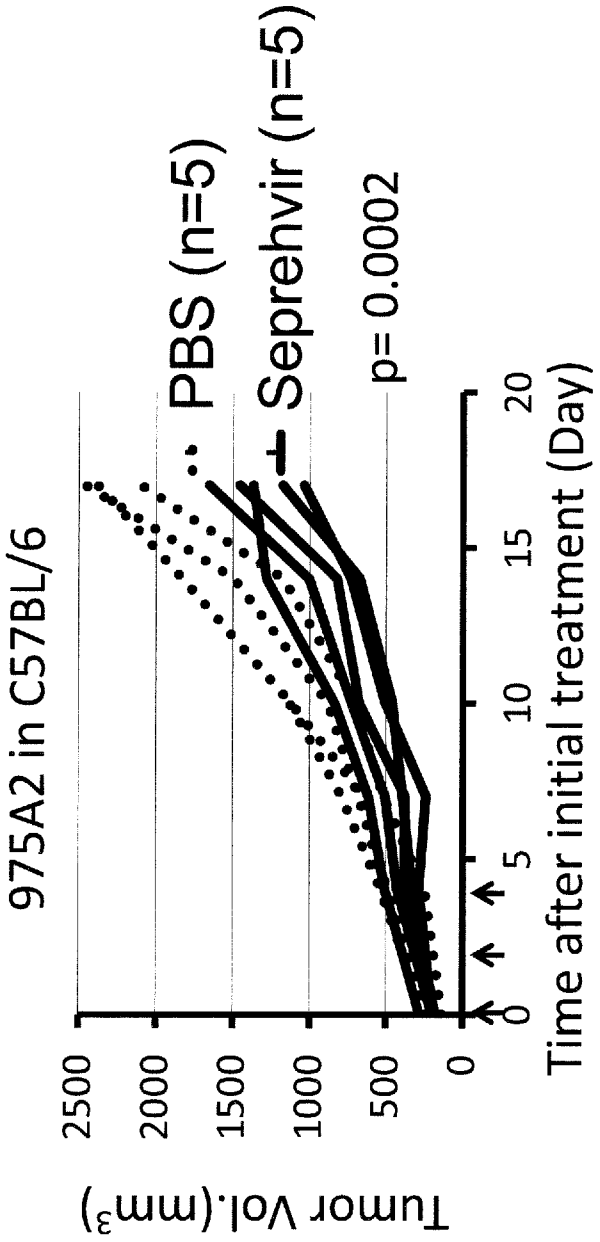

975A2 in C57BL/6
PBS (n=5)
Seprehvir (n=5)
p= 0.0002
Tumor Vol.(mm³)
2500
2000
1500
1000
500
0
0
5
10
15
20
Time after initial treatment (Day)

Figure 48

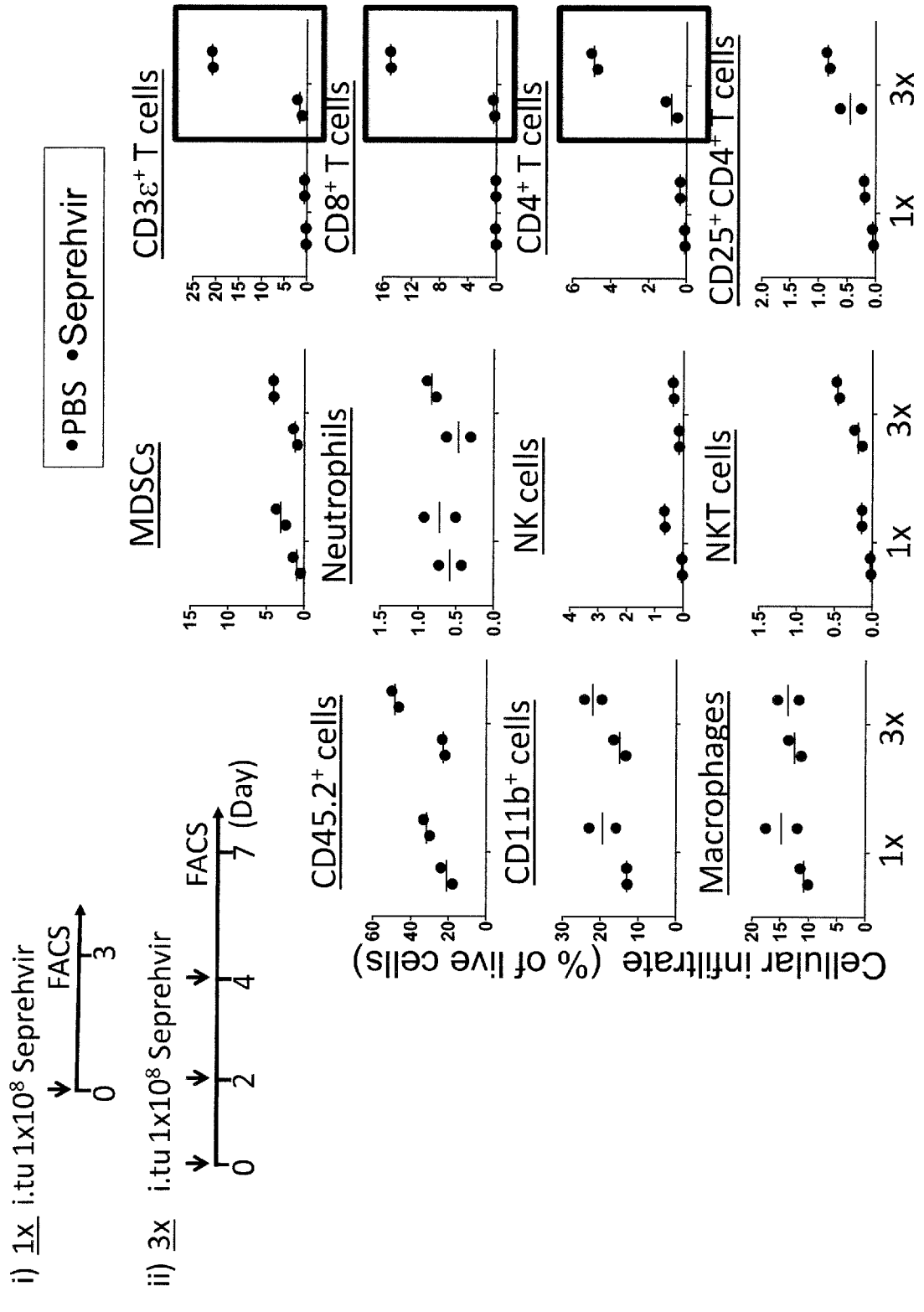

i) 1x i.tu 1x10^8 Seprehvir
FACS
0
3
ii) 3x i.tu 1x10^8 Seprehvir
FACS
0
2
4
7 (Day)
•PBS •Seprehvir
Cellular infiltrate (% of live cells)
CD45.2+ cells
CD11b+ cells
Macrophages
MDSCs
Neutrophils
NK cells
NKT cells
CD3ε+ T cells
CD8+ T cells
CD4+ T cells
CD25+CD4+ T cells
1x
3x

Figure 49

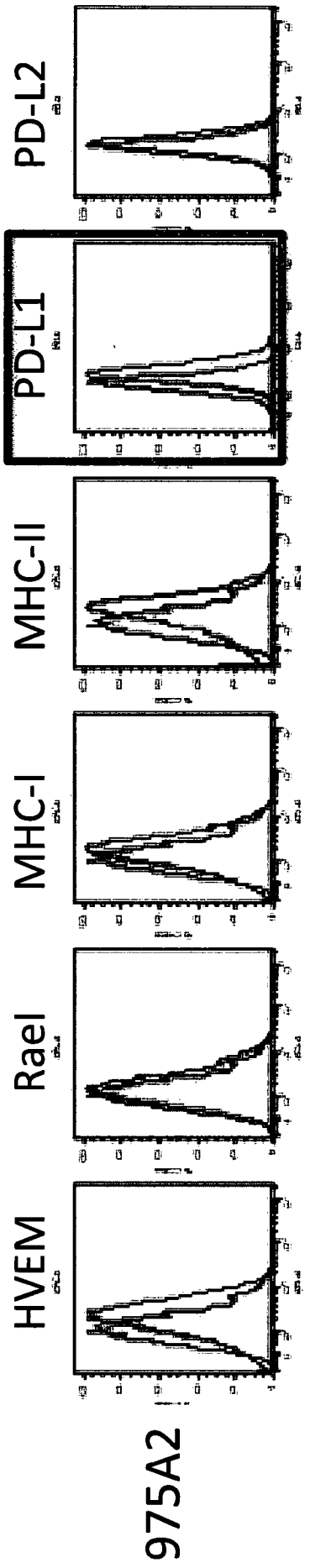


Figure 50

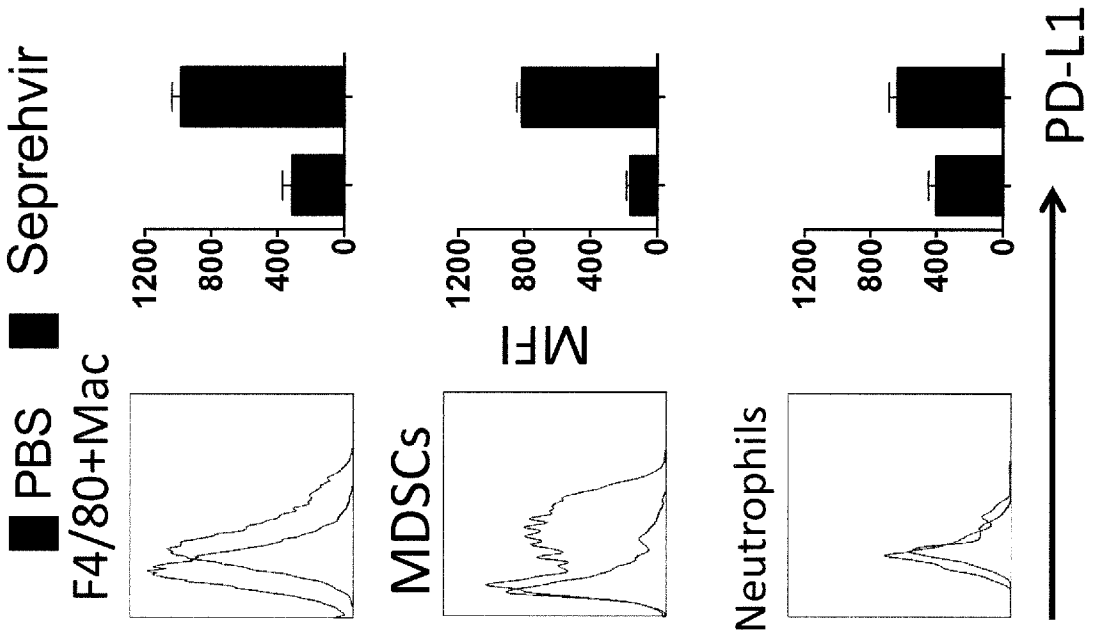

PBS
Seprehvir
F4/80+Mac
MDSCs
Neutrophils
MFI
1200
800
400
0
PD-L1

Figure 51

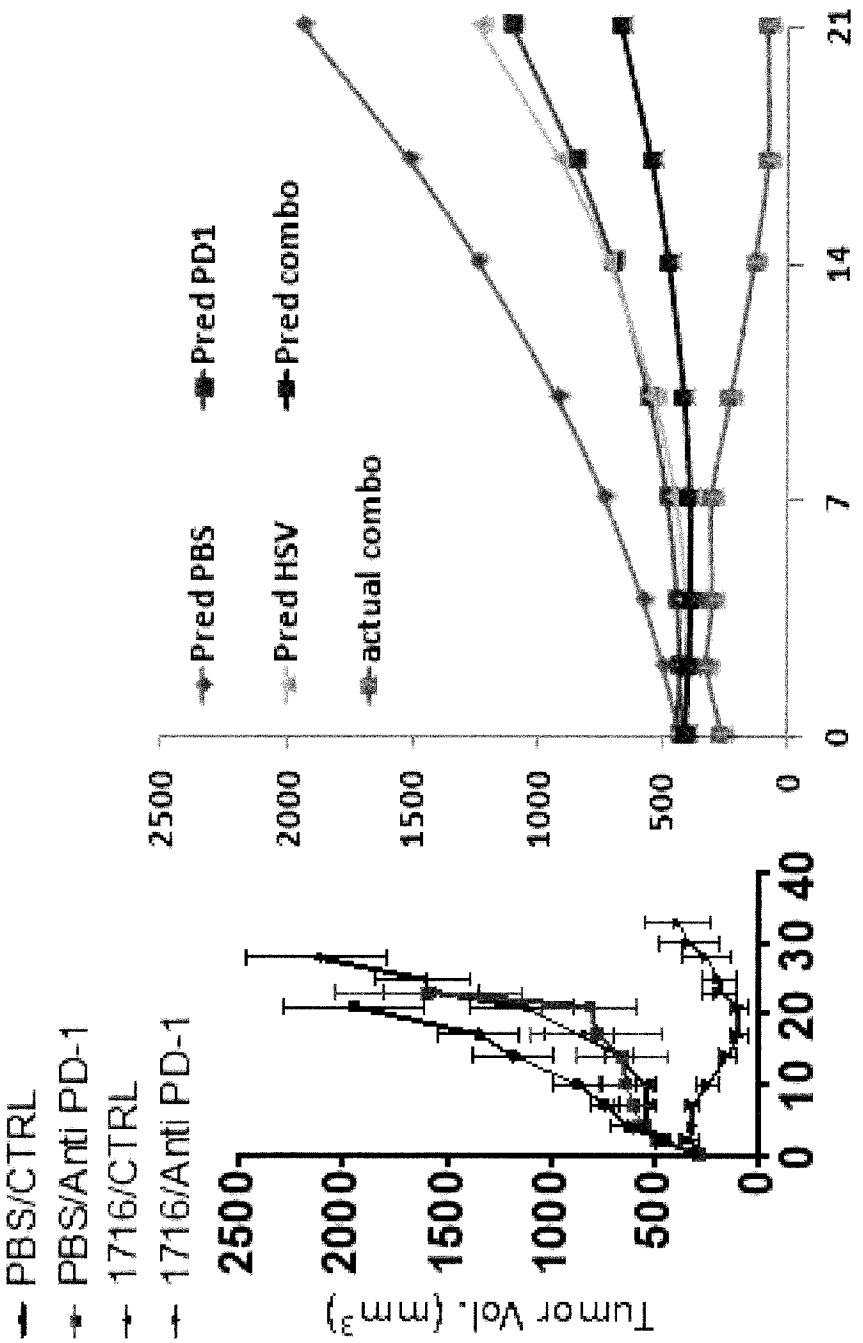

PBS/CTRL
PBS/Anti PD-1
1716/CTRL
1716/Anti PD-1
Tumor Vol. (mm3)
2500
2000
1500
1000
500
0
0 10 20 30 40
Time post first I.Tu. Injection (Days)
Pred PBS
Pred HSV
actual combo
Pred PD1
Pred combo
2500
2000
1500
1000
500
0
0
7
14
21

Figure 52

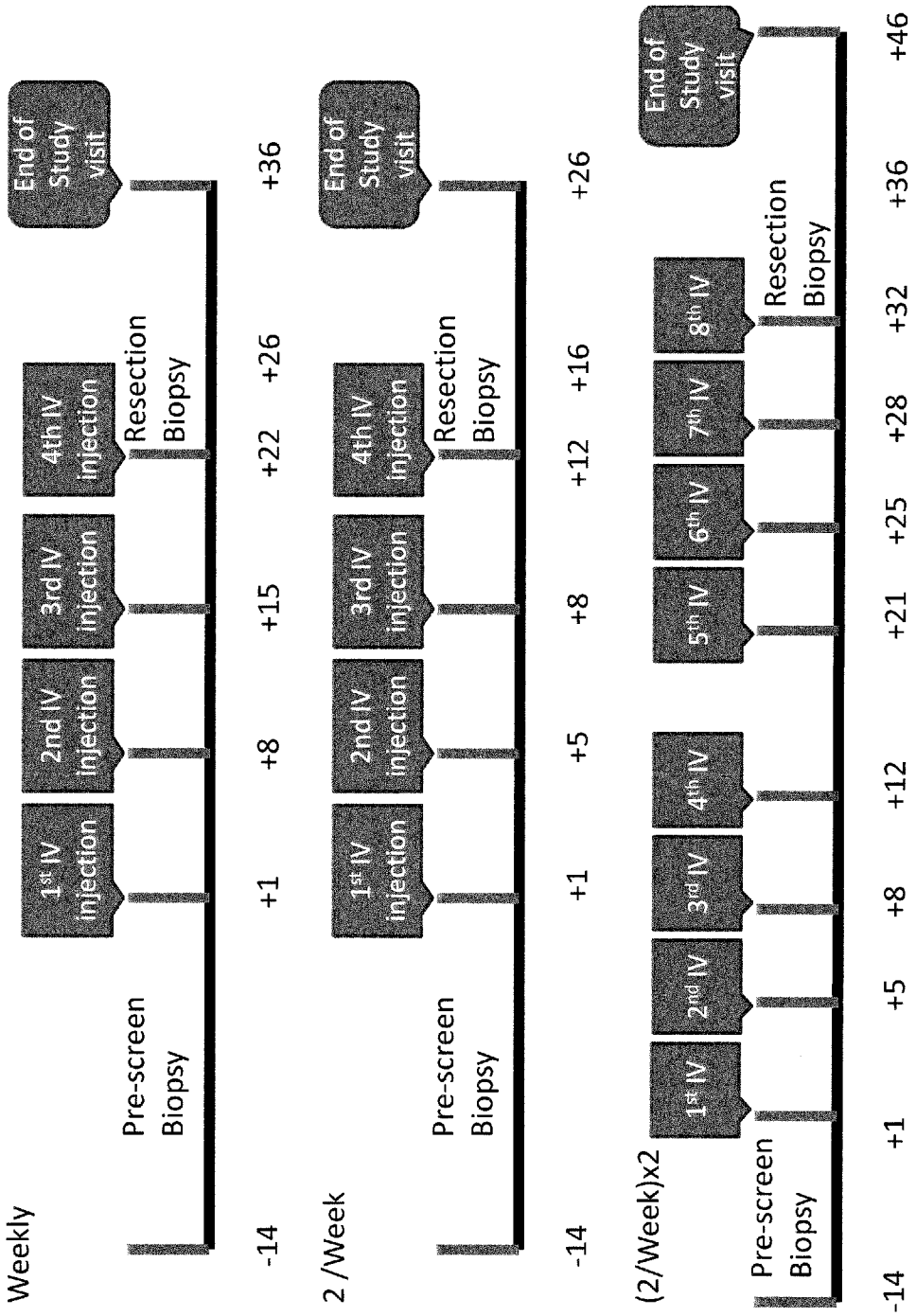

Weekly
Pre-screen Biopsy
1st IV injection
2nd IV injection
3rd IV injection
4th IV injection
Resection Biopsy
End of Study visit
-14
+1
+8
+15
+22
+26
+36
2 /Week
Pre-screen Biopsy
1st IV injection
2nd IV injection
3rd IV injection
4th IV injection
Resection Biopsy
End of Study visit
-14
+1
+5
+8
+12
+16
+26
(2/Week)x2
Pre-screen Biopsy
1st IV
2nd IV
3rd IV
4th IV
5th IV
6th IV
7th IV
8th IV
Resection Biopsy
End of Study visit
-14
+1
+5
+8
+12
+21
+25
+28
+32
+36
+46

Figure 53

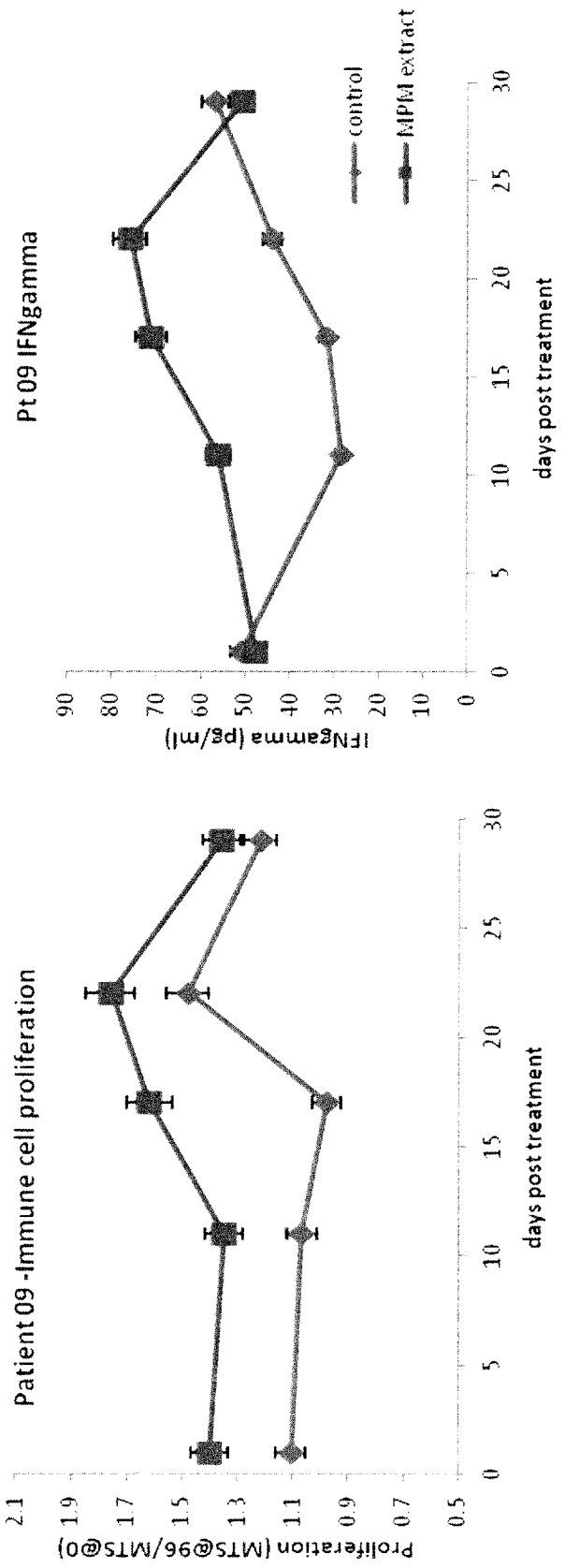

Patient 09 -Immune cell proliferation
Proliferation (MTS@96/MTS@0)
2.1
1.9
1.7
1.5
1.3
1.1
0.9
0.7
0.5
0
5
10
15
20
25
30
days post treatment
Pt 09 IFNgamma
IFNgamma (pg/ml)
90
80
70
60
50
40
30
20
10
0
control
MPM extract
days post treatment

Figure 54

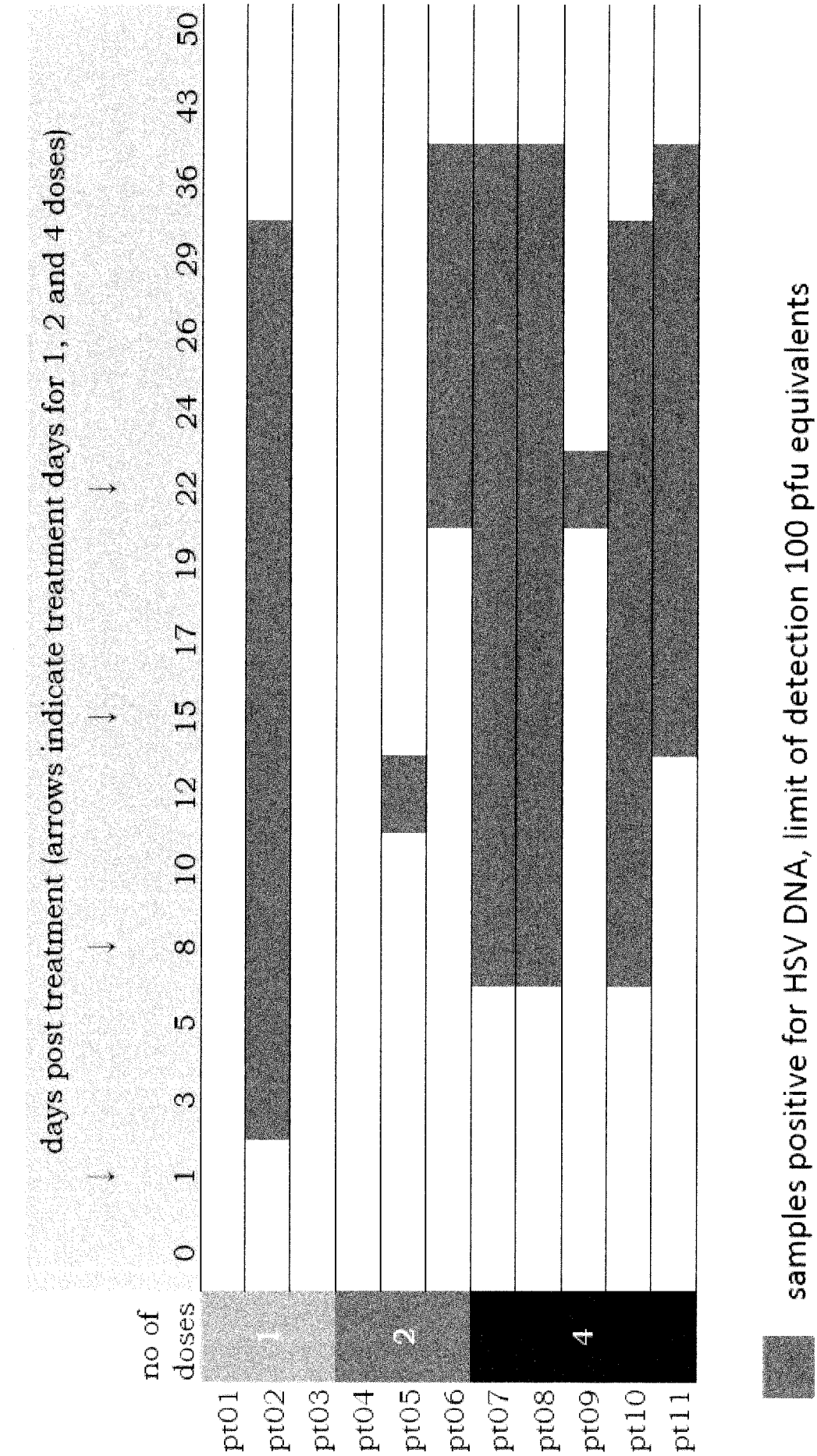

days post treatment (arrows indicate treatment days for 1, 2 and 4 doses)
0
1
3
5
8
10
12
15
17
19
22
24
26
29
36
43
50
no of doses
1
2
4
pt01
pt02
pt03
pt04
pt05
pt06
pt07
pt08
pt09
pt10
pt11
samples positive for HSV DNA, limit of detection 100 pfu equivalents

Figure 55

	Doses	HSV DNA in pleural fluids	Pleural Fluid Th1	Immune cells	Granzyme B
Pt 01	1	-	+++	nd	+++
Pt 02		+++++	-	nd	-
Pt 03		-	++	nd	+++
Pt 04	2	-	++++	++	+++
Pt 05		+	-	↓	-
Pt 06		+++	++	nd	++++
Pt 07	4	+++	+++	+++	++++
Pt 08		++++	+++++	+++	++
Pt 09		+	++	++	+

- = unchanged, + = weak to +++++ = strong response, ↓ = decrease, nd = not determined

Figure 56

Patient no.	No. of doses	Pleural fluid Th1 response	Novel anti-tumour IgG response
01001	1	+++	-
01002		-	-
01903		++	-
01004	2	++++	+++
01005		-	-
01006		++	++
01007	4	+++	+++
01008		+++++	++
01009		++	+

Figure 57

Time	Patient HSV06	Patient HSV09	Patient HSV10
Baseline	Neg	Neg	Neg
Day 0 Pre Infusion	Neg	Neg	Neg
Day 0 Post Infusion			
3 hours	Neg	Neg	Neg
6 hours	Neg	Neg	Neg
18 hours	Neg	Neg	Neg
24 hours	Neg	Neg	Neg
Day 4	Pos	Pos	Neg
Day 7	Pos	Neg	Neg
Day 14	Pos	Neg	Neg

Figure 58

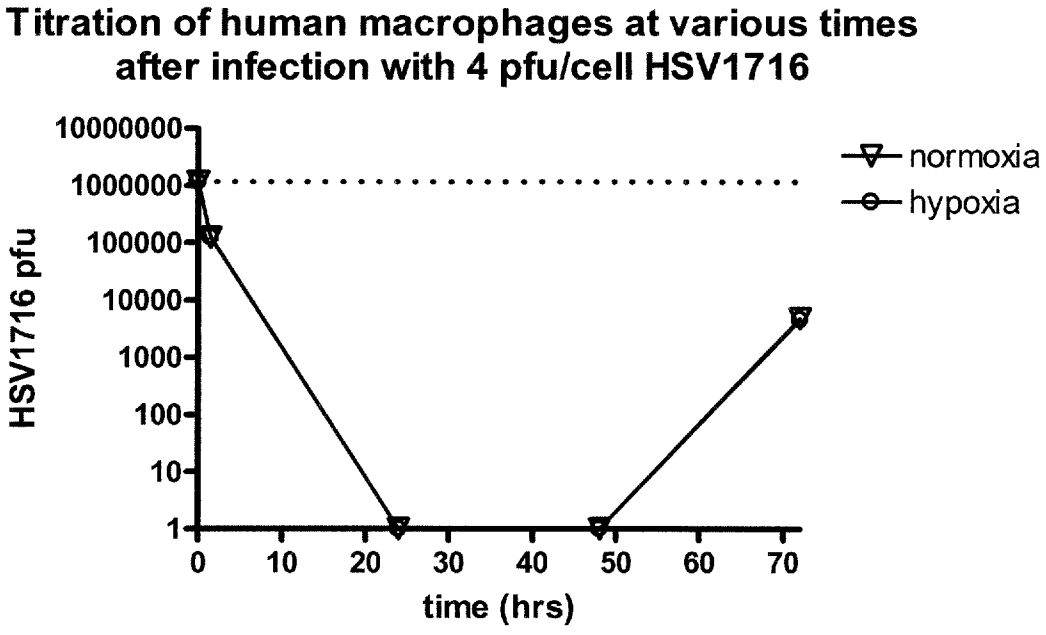


Figure 59

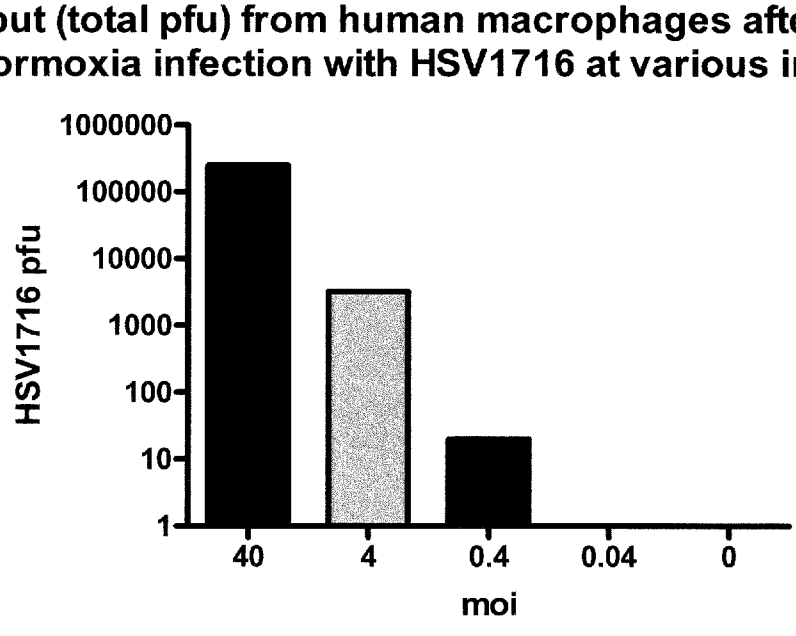
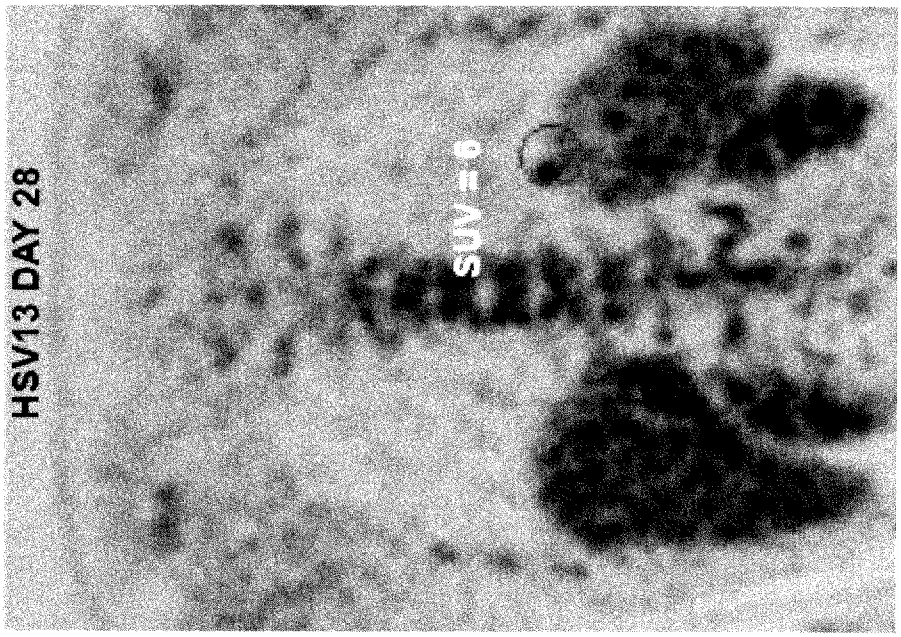


Figure 60

	HSV06	HSV09	HSV10	HSV13	HSV07	HSV08	HSV11	HSV12
Route of administration	IV	IV	IV	IV	ITu	ITu	ITu	ITu
Baseline	neg	neg	neg	neg	neg	neg	neg	neg
Day +1	neg	neg	neg	neg	neg	neg	neg	neg
Day +4	Pos	Pos	neg	neg	Pos	Pos	Pos	Pos
Day +7	Pos	neg	neg	neg	neg	nd	Pos	Pos
Day +14	Pos	neg	nd	neg	neg	nd	neg	nd
Day +28				Pos				

Figure 61



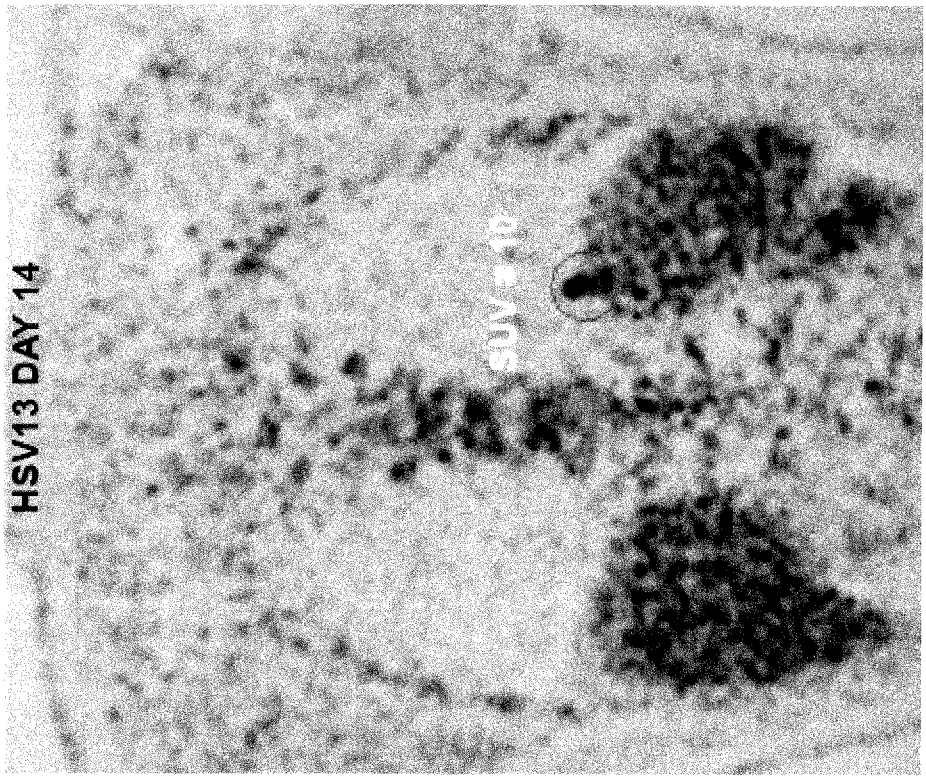


Figure 62

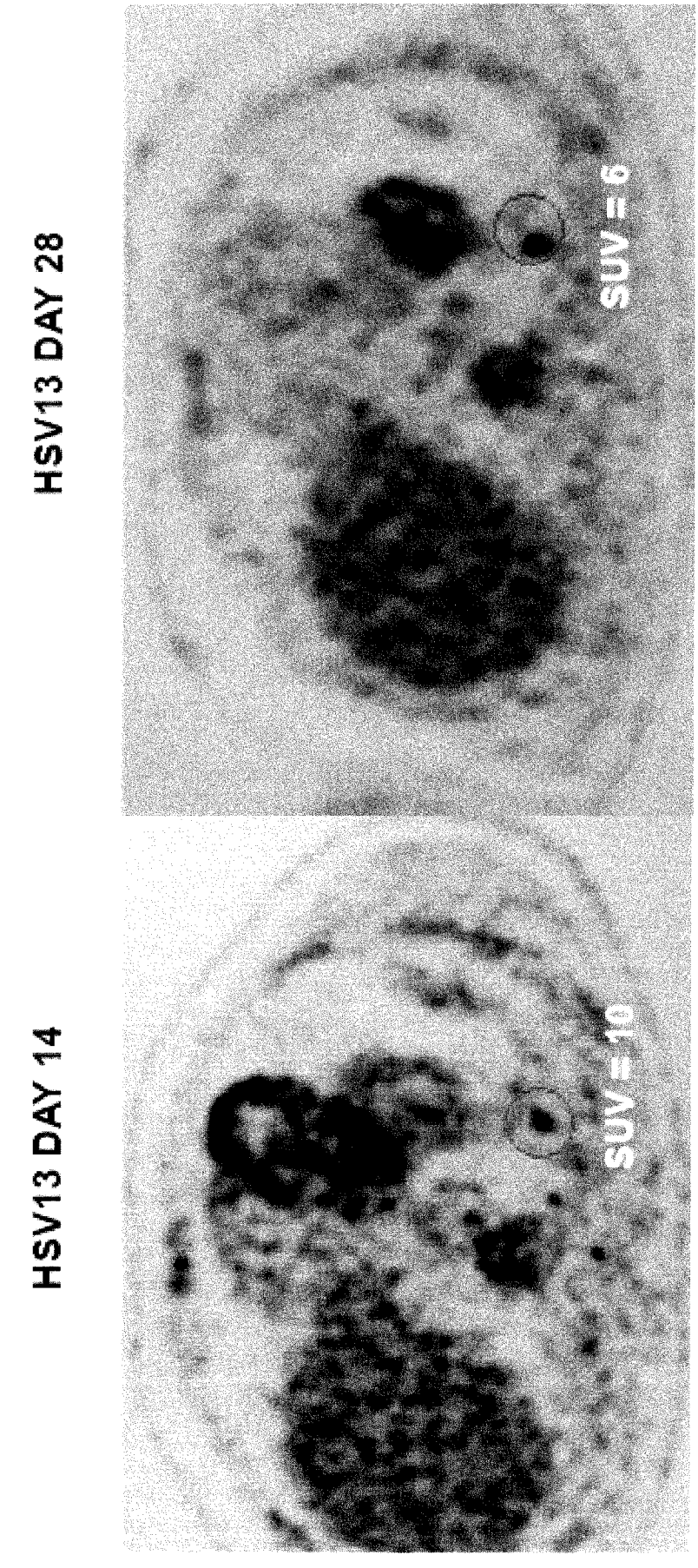


Figure 63

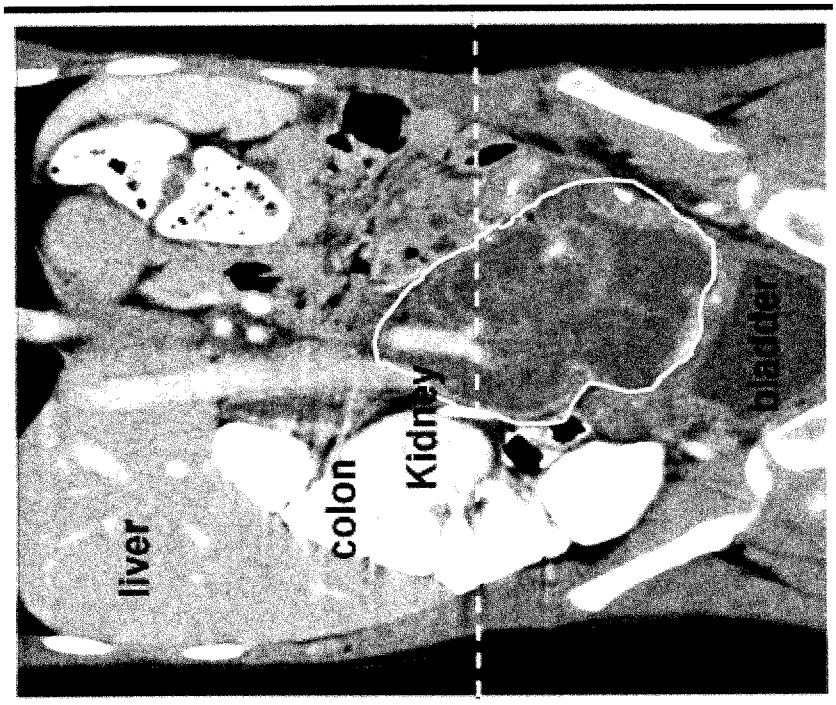


Figure 64

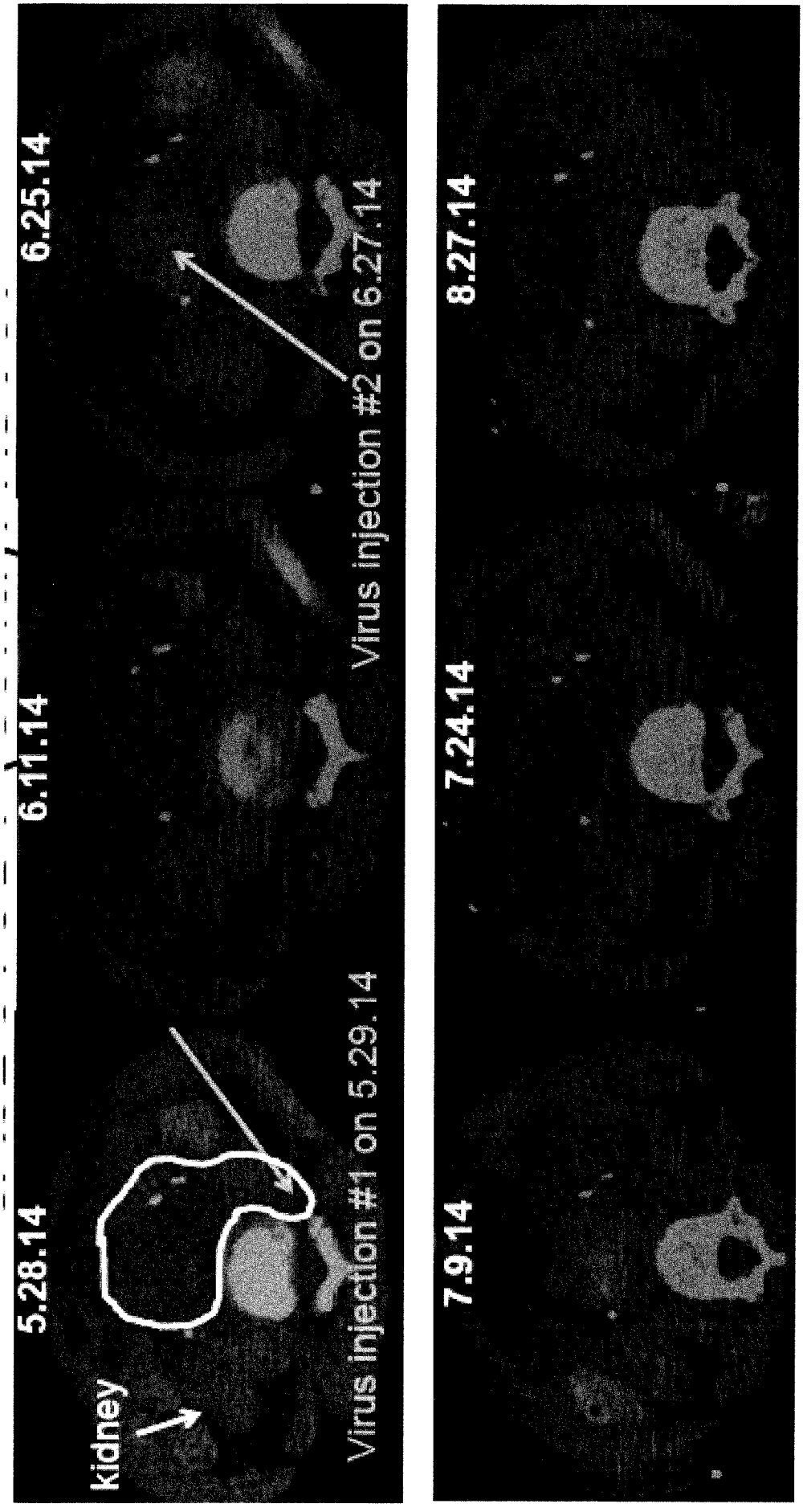

5.28.14
6.11.14
6.25.14
Kidney
Virus injection #1 on 5.29.14
Virus injection #2 on 6.27.14
7.9.14
7.24.14
8.27.14

Figure 65

Cell Line	Virus	Viable Cells	Non Viable Cells	Total Cell Number	% Non Viable Cell Population
SUPT	None	315	23	338	6.80
SUPT	1716	231	13	244	5.33
SUPT	17+	264	41	305	13.44
TOLB	None	165	8	173	4.62
TOLB	1716	103	13	116	11.21
TOLB	17+	114	9	123	7.32
THP1	None	427	36	463	7.78
THP1	1716	413	15	428	3.50
THP1	17+	303	103	406	25.37

Figure 66

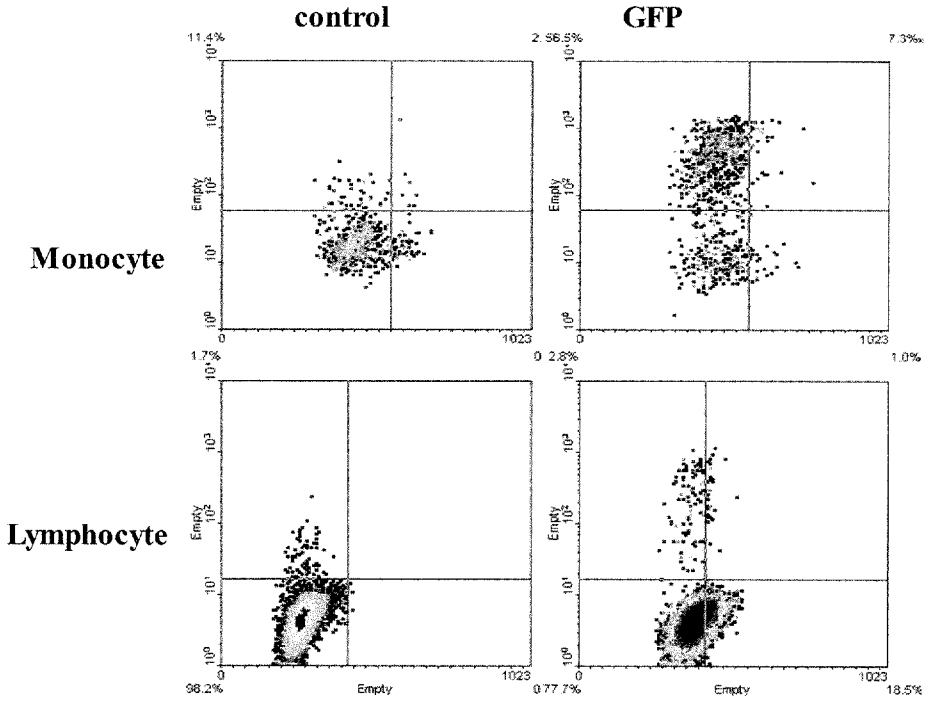


Figure 67

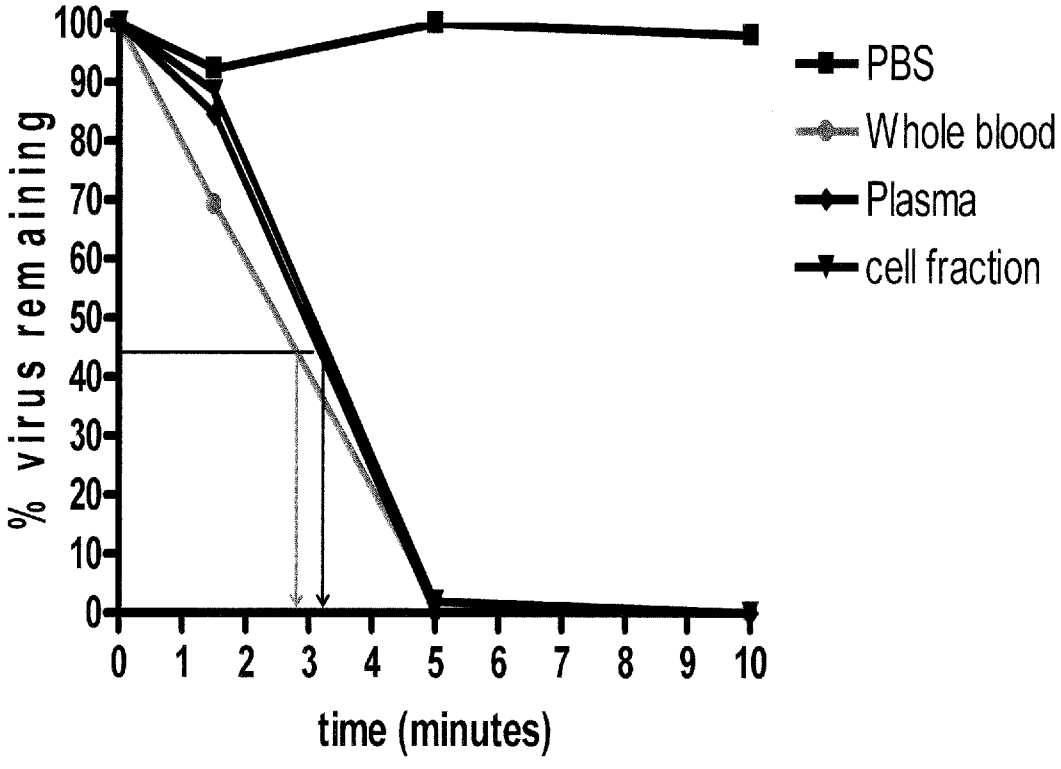


Figure 68

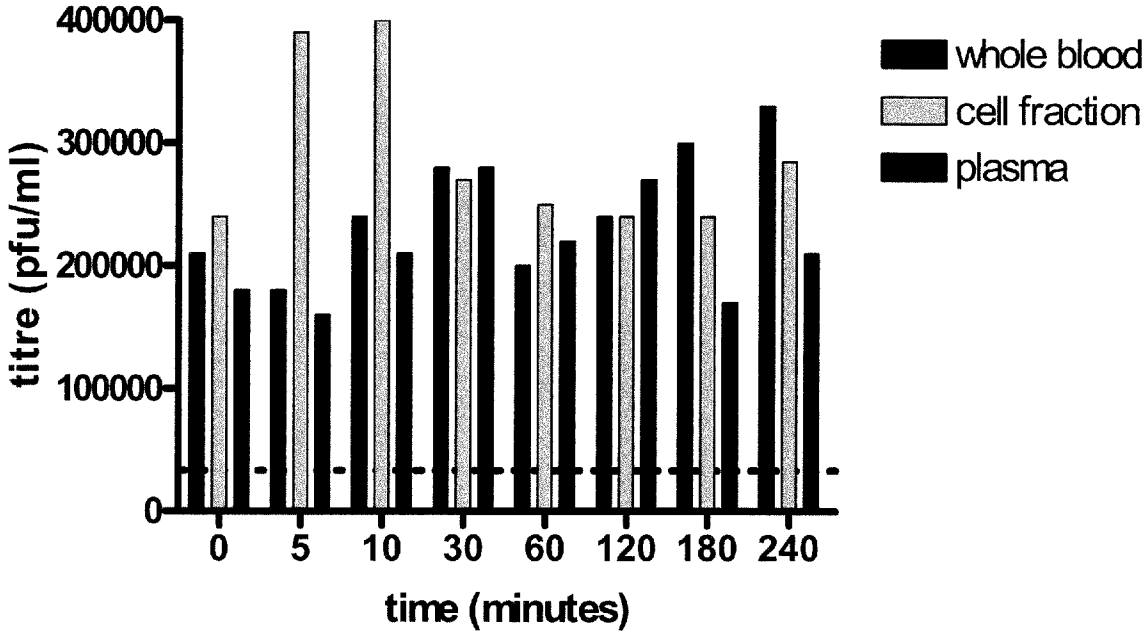

400000
300000
200000
100000
0
titre (pfu/ml)
0
5
10
30
60
120
180
240
time (minutes)
whole blood
cell fraction
plasma

Figure 69

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2016/052177

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K35/763 A61K9/00 A61K39/395 A61K35/768 A61K45/06
A61P35/00
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/036412 A2 (AMGEN INC [US]) 6 March 2014 (2014-03-06) see claims 1-15, pages 6, 10-12 and example -----	65-72
Y	SAHA DIPONGKOR ET AL: "Immunovirotherapy Combined with Immune Checkpoint Inhibitors for Treating Glioblastoma", MOLECULAR THERAPY, vol. 23, no. Suppl. 1, 1 May 2015 (2015-05-01), page S248, XP002761905, & 18TH ANNUAL MEETING OF THE AMERICAN-SOCIETY-OF-GENE-AND-CELL-THERAPY (ASGCT); NEW ORLEANS, LA, USA; MAY 13 -16, 2015 the whole document ----- -/--	65-72

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 September 2016	21/10/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Merckling-Ruiz, V

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2016/052177

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2014/047350 A1 (MORNINGSIDE TECHNOLOGY VENTURES LTD [MC]; NOLIN WESTLEY B [US]) 27 March 2014 (2014-03-27) see claims, paragraphs [005], [109]-[112], examples 4-10	1-72
A	Timothy P ET AL: "HSV1716 (SEPREHVIR)", NIH RAC, Meeting of 11 June 2013, 11 June 2013 (2013-06-11), XP055302840, Retrieved from the Internet: URL:http://osp.od.nih.gov/sites/default/files/738_Updt_Cripe.pdf [retrieved on 2016-09-15] the whole document	1-72
A	Anonymous: "NCT02272855. A study of combination treatment with HF10 and ipilimumab in patients with unresectable or metastatic melanoma.", NIH , 27 April 2015 (2015-04-27), XP002761906, Retrieved from the Internet: URL:https://clinicaltrials.gov/archive/NCT02272855/2015_04_27 [retrieved on 2016-04-14] the whole document	1-72
A	LI H ET AL: "Virotherapy with a type 2 herpes simplex virus-derived oncolytic virus induces potent antitumor immunity against neuroblastoma", CLINICAL CANCER RESEARCH, vol. 13, no. 1, 1 January 2007 (2007-01-01), pages 316-322, XP002761907, US ISSN: 1078-0432 see abstract and page 318	1-72
A	WANG JIANI ET AL: "Oncolytic herpes simplex virus treatment of metastatic breast cancer", INTERNATIONAL JOURNAL OF ONCOLOGY, vol. 40, no. 3, March 2012 (2012-03), pages 757-763, XP002761908, ISSN: 1019-6439 see abstract and page758	1-72

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2016/052177

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOHNSON DOUGLAS B ET AL: "Talimogene laherparepvec (T-VEC) for the treatment of advanced melanoma", IMMUNOTHERAPY, vol. 7, no. 6, 22 June 2015 (2015-06-22), pages 611-619, XP002761909, the whole document	65-72
Y	NIH: "NCT02263508. Pembrolizumab (MK-3475) With or Without Talimogene Laherparepvec in Unresected Melanoma", , 22 June 2015 (2015-06-22), XP002761910, Retrieved from the Internet: URL:https://clinicaltrials.gov/archive/NCT02263508/2015_06_22 [retrieved on 2016-04-14] the whole document	65-72
A,P	WO 2016/100364 A1 (AMGEN INC [US]) 23 June 2016 (2016-06-23) see claims 31-34 and paragraphs [107]-[113]	1-72
A	Learmonth K. et al.: "Neutralisation effects of pleural fluids do not predict the persistence of the oncolytic HSV Seprehvir following intrapleural administration in patients with malignant pleural mesothelioma", VirttuBiologics , 13 June 2015 (2015-06-13), XP002762696, Retrieved from the Internet: URL:http://www.virttu.com/files/Boston%20OV%20meeting%202015-neutralisation%20print%20version.pdf [retrieved on 2016-09-19] the whole document	1-72
A	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; December 2014 (2014-12), CRIPE TIMOTHY ET AL: "A phase I dose escalation study of intratumoural or intravenous herpes simplex virus-1 mutant HSV1716 in pediatric/young adult patients with refractory non-central nervous system solid tumours", XP002761994, Database accession no. PREV201500194291 abstract	1-72

-/--

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	& HUMAN GENE THERAPY, vol. 25, no. 12, December 2014 (2014-12), pages A5-A6, 8TH INTERNATIONAL CONFERENCE ON ONCOLYTIC VIRUS THERAPEUTICS; OXFORD, UK; APRIL 10 -13, 2014 ISSN: 1043-0342(print) -----	
A	BRAIDWOOD LYNNE ET AL: "Potent efficacy signals from systemically administered oncolytic herpes simplex virus (HSV1716) in hepatocellular carcinoma xenograft models.", JOURNAL OF HEPATOCELLULAR CARCINOMA 2014, vol. 1, 2014, pages 149-161, XP002761995, ISSN: 2253-5969 abstract -----	1-72

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/GB2016/052177

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014036412 A2	06-03-2014	AU 2013308595 A1	05-03-2015
		CA 2881851 A1	06-03-2014
		CN 104704002 A	10-06-2015
		EA 201590451 A1	31-05-2016
		EP 2890714 A2	08-07-2015
		HK 1211601 A1	27-05-2016
		JP 2015526525 A	10-09-2015
		KR 20150047512 A	04-05-2015
		US 2015202290 A1	23-07-2015
		WO 2014036412 A2	06-03-2014
WO 2014047350 A1	27-03-2014	US 2015250837 A1	10-09-2015
		WO 2014047350 A1	27-03-2014
WO 2016100364 A1	23-06-2016	NONE	