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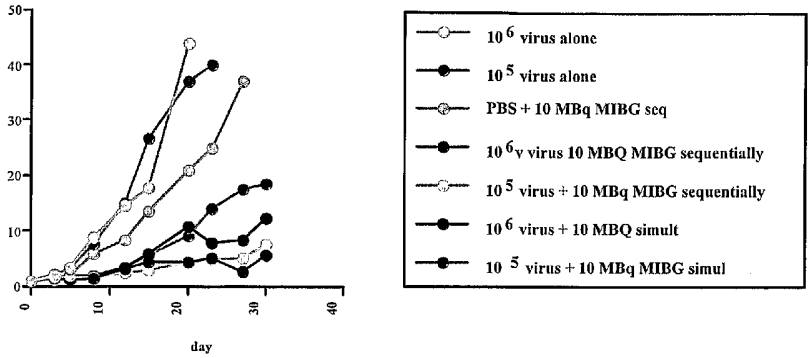
(54) Title: NOVEL HERPES SIMPLEX VIRUSES AND USES THEREOF



(57) Abstract: An herpes simplex virus is disclosed wherein the herpes simplex virus genome comprises nucleic acid encoding a monoamine transporter. Also disclosed are uses of the virus in the treatment of cancerous conditions. Such use may be in combination with a pharmaceutical capable of being transported by the monoamine transporter.

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Novel Herpes Simplex Viruses And Uses Thereof

Field of the Invention

The present invention relates to herpes simplex viruses and particularly, although not exclusively, to herpes simplex viruses wherein the herpes simplex virus genome comprises a nucleic acid sequence encoding a monoamine transporter and wherein the herpes simplex virus is non-neurovirulent. The invention further relates to compositions comprising, uses of and methods involving, such herpes simplex virus in combination with a pharmaceutical or radiopharmaceutical, said pharmaceutical or radiopharmaceutical capable of uptake to a cell by said monoamine transporter.

Background to the Invention

Tumours

Effective treatments for tumours are required to overcome the problems presented by existing chemotherapeutic and surgical resection approaches.

In particular, malignant gliomas are universally fatal even after aggressive conventional treatments such as tumour resection, chemotherapy and radiotherapy. The median patient survival time is one year. Therefore new treatment modalities are urgently required.

Herpes simplex virus (HSV)

The genomes of the two herpes simplex virus serotypes, HSV-1 and HSV-2, have been well characterised and genomic sequence information is available for a number of strains (e.g. the sequence of the HSV-1 strain 17 long repeat regions[11] or the HSV-2 strain HG52 complete genome sequence which is available under accession number NC_001798 (GI:9629267) from the NCBI database (www.ncbi.nlm.nih.gov)). Given the high degree of

characterisation, both HSV-1 and HSV-2 genomes can be manipulated by known genetic engineering techniques.

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

The HSV ICP34.5 (also γ34.5) gene, which has been extensively studied[29, 30, 31, 32], has been sequenced in HSV-1 strains F [28] and syn17+ [11] and in HSV-2 strain HG52[33]. One copy of the ICP34.5 gene is in the RL1 locus of each long repeat (R_L). Mutants inactivating both copies of the ICP34.5 gene (i.e. null mutants), e.g. HSV-1 strain 1716[34] or the mutants R3616 or R4009 in strain F[35], are known to lack neurovirulence, i.e. be avirulent, and have utility in the treatment of tumours by oncolysis.

Herpes simplex virus is capable of infecting a wide variety of cell types, including dividing and non-dividing tissues but does not normally replicate in vivo in non-neuronal peripheral tissues. Cellular infection with herpes simplex virus does not result in integration of the herpes simplex virus DNA into the genome of the host cell. Infection with virulent herpes simplex virus usually leads to peripheral and central nervous system infections, which in the case of many herpes simplex virus strains results in a virulent encephalitis with serious damage to the nervous system followed by death of the patient.

Due to its ability to selectively replicate in and lyse rapidly dividing tumour cells, e.g. cycling glioma cells, but not in growth arrested cells, terminally differentiated cells, quiescent neurons and supporting cells of the normal brain[8], selectively replication competent herpes simplex virus has shown promise as a therapeutic agent in the treatment of

malignant glioma[11, 28, 23, 22, 13]. These genetically engineered herpes simplex virus type 1 (HSV-1) variants fail to express the neurovirulence factor ICP34.5 and although capable of infecting cells at any stage in the cell cycle, discriminate between fully differentiated non-dividing cells and cycling malignant cells in their ability to replicate and produce an oncolytic response.

The ICP34.5 null mutant, HSV1716, is a specific variant of HSV-1 strain 17+ which is non-neurovirulent. It contains a 759bp deletion in the Bam HI s restriction fragment located in each of the terminal and internal repeats (TR_L and IR_L - map units 0-0.02 and 0.81-0.83 respectively). As a result both copies of the RL1 gene, which encodes the protein ICP34.5, code for a non-functional gene product[11, 21]. HSV1716 has been deposited under the Budapest Treaty at the European Collection of Animal Cell Cultures (ECACC), Porton Down, Salisbury, Wiltshire, United Kingdom under accession number V92012803.

HSV1716 and other ICP34.5 null mutants have demonstrated anti-tumour efficacy in a range of isogenic and xenograft models[19, 17]. In addition, an encouraging lack of toxicity has been demonstrated in Phase 1 clinical trials[23, 22, 20] and Phase II efficacy trials using mutant HSV are being conducted in patients suffering from glioma and other malignancies.

HSV1716 has a proven safety and efficacy profile in isogenic and xenograft tumour models[19, 17] and its safety in humans has been demonstrated in three Phase 1 clinical trials in glioma patients[23, 22] and one trial in melanoma patients[20]. It is also currently undergoing evaluation in patients with squamous cell carcinoma of the head and neck.

Targeted radiotherapy

As tumours are typically genetically heterogeneous and conventional therapy selects for resistant phenotypes, single

agents will not be universally applicable or completely effective for tumour erradication. While inhibition of tumour growth and improved survival of experimental animals have been observed in xenografted human tumours (reviewed by Varghese and Rabkin, 2002), only a fraction of the animals appear to be cured by the current forms of oncolytic herpes simplex virus. This could be due in part to the gene deletions which confer selective viral replication and the lack of a significant bystander effect, a process that can result in the killing of non-transduced neighbouring cells.

Targeted radiotherapy is the selective irradiation of tumour cells by radionuclides conjugated to tumour seeking molecules. One of the most promising, non-immunogenic, targeting molecules is radiolabelled meta-iodobenzylguanidine (MIBG) - an analogue of the adrenergic neurone blockers guanethidine and bretylium[26]. Because it has high affinity for the noradrenaline transporter (NAT)[15], [^{131}I]MIBG is used in the imaging and treatment of tumours derived from the neural crest, such as neuroblastoma and phaeochromocytoma. Plasmid-mediated transfer of the NAT gene into glioma cells, which do not express NAT, endowed these cells with the capacity for active [^{131}I]MIBG uptake and rendered them susceptible to cell kill in a dose-dependent manner[3, 4].

Several studies have investigated the toxicity of HSV-1 oncolytic therapy in combination with standard chemotherapeutic agents (Chahlavi et al, 1999, Toyoizumi et al, 1999) or with ionising radiation[1, 7]. Further studies have investigated modified oncolytic HSV vectors as gene delivery vectors combining the oncolytic activity of the virus with genes expressing prodrug activating enzymes and immunostimulatory antigens (reviewed by Varghese and Rabkin, 2002).

Summary of the Invention

The inventors have now determined that herpes simplex virus having an inactivating mutation in the RL1 locus, more specifically a mutation which inactivates the function of the ICP34.5 gene product, such that the herpes simplex virus does not produce a functional ICP34.5 gene product and is non-neurovirulent, can be used in the delivery to a cell of a gene encoding a gene product useful in targeted tumour therapy.

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[22, 23] 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[21].

The inventors have provided an engineered herpes simplex virus ICP34.5 null mutant which expresses the noradrenaline transporter (NAT) gene and provides for enhanced virus induced tumour cytotoxicity. This virus is designated HSV1716/NAT and combines NAT transgene delivery and [^{131}I]MIBG treatment[3, 4, 5] with the proliferation-specific, lytic capacity of HSV1716. In vitro, the inventors have demonstrated that the NAT gene, when introduced by HSV1716/NAT into glioma cell lines, is expressed 1 hour after viral infection, and enables active uptake of the radiopharmaceutical [^{131}I]MIBG.

The combination of viral oncolysis and induced radiopharmaceutical uptake resulted in significantly enhanced cytotoxicity compared to either agent alone and the response was dose and time dependent. The results demonstrate that the combination of oncolytic HSV therapy with targeted radiation provides effective tumour cell kill and a treatment for malignant glioma.

The inventors have demonstrated that HSV1716/NAT is a suitable vector for delivery and expression of the NAT gene and found that the level of tumour cell kill following the administration of [^{131}I]MIBG to cells infected with HSV1716/NAT was significantly greater than that achieved by either treatment alone, i.e. a synergistic effect in tumour cell kill was observed.

Transfection of tumour xenografts in mice together with administration of [^{131}I]MIBG also resulted in improved inhibition of tumour growth compared to virus alone. Tumour growth inhibition and [^{131}I]MIBG uptake were higher when [^{131}I]MIBG was administered sequentially at a time point of about a day after administration of HSV1716/NAT, compared with simultaneous administration of [^{131}I]MIBG and virus.

Combining herpes simplex virus HSV1716-mediated oncolysis with noradrenaline transporter gene transfer and targeted radiotherapy has yielded results exhibiting a surprising synergy and provides a novel therapeutic strategy for treatment of tumours such as glioma.

At its most general the present invention relates to a herpes simplex virus, wherein the herpes simplex virus genome comprises a nucleic acid sequence encoding a monoamine transporter.

The herpes simplex virus is preferably non-neurovirulent.

The present invention also relates to compositions comprising, uses of and methods involving, such herpes simplex virus in combination with a pharmaceutical or radiopharmaceutical, said pharmaceutical or radiopharmaceutical capable of uptake to a cell by said monoamine transporter. Accordingly, the pharmaceutical may be capable of being transported by the monoamine transporter and may be described as a transportable

substrate of the monoamine transporter. In this respect, 'transport' may comprise active movement of a molecule across a biological membrane, such as the plasma membrane.

According to a first aspect of the present invention there is provided a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a noradrenaline transporter in at least one of the long repeat regions (R_L).

According to a second aspect of the present invention there is provided a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a noradrenaline transporter and wherein the herpes simplex virus is non-neurovirulent.

According to a third aspect of the invention there is provided a composition comprising a said herpes simplex virus according to one of said first and second aspects and the radiopharmaceutical [^{131}I]MIBG.

According to a fourth aspect of the invention there is provided a composition comprising a said herpes simplex virus according to one of said first and second aspects and the radiopharmaceutical meta-[^{211}At]astatobenzylguanidine ([^{211}At]MABG).

According to a fifth aspect of the invention there is provided a composition comprising:

(i) a said herpes simplex virus according to one of said first and second aspects;

(ii) the radiopharmaceutical [^{131}I]MIBG; and

(iii) the radiopharmaceutical [^{211}At]MABG.

According to a sixth aspect of the present invention there is provided a herpes simplex virus, wherein the genome of said

virus comprises a nucleic acid sequence encoding a noradrenaline transporter in at least one of the long repeat regions (R_L) for use in the treatment of a tumour.

According to a seventh aspect of the present invention there is provided a herpes simplex virus for use in the treatment of a tumour, wherein the genome of said virus comprises a nucleic acid sequence encoding a noradrenaline transporter and wherein the herpes simplex virus is non-neurovirulent.

According to an eighth aspect of the present invention there is provided a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a noradrenaline transporter in at least one of the long repeat regions (R_L), for use in combination with the radiopharmaceutical [^{131}I]MIBG, in the treatment of a tumour.

According to a ninth aspect of the present invention there is provided a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a noradrenaline transporter and wherein the herpes simplex virus is non-neurovirulent, for use in combination with the radiopharmaceutical [^{131}I]MIBG, in the treatment of a tumour.

According to a tenth aspect of the present invention there is provided the radiopharmaceutical [^{131}I]MIBG in combination with a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a noradrenaline transporter in at least one of the long repeat regions (R_L), for use in the treatment of a tumour.

According to an eleventh aspect of the present invention there is provided the radiopharmaceutical [^{131}I]MIBG in combination with a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a noradrenaline

transporter and wherein the herpes simplex virus is non-neurovirulent, for use in the treatment of a tumour.

Suitably, in either or both of the tenth and eleventh aspects, a composition is provided comprising said radiopharmaceutical [^{131}I]MIBG and said herpes simplex virus, said composition for use in the treatment of a tumour.

According to a twelfth aspect of the present invention there is provided a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a noradrenaline transporter in at least one of the long repeat regions (R_L), for use in combination with the radiopharmaceutical [^{211}At]MABG, in the treatment of a tumour.

According to a thirteenth aspect of the present invention there is provided a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a noradrenaline transporter and wherein the herpes simplex virus is non-neurovirulent, for use in combination with the radiopharmaceutical [^{211}At]MABG, in the treatment of a tumour.

According to a fourteenth aspect of the present invention there is provided the radiopharmaceutical [^{211}At]MABG in combination with a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a noradrenaline transporter in at least one of the long repeat regions (R_L), for use in the treatment of a tumour.

According to a fifteenth aspect of the present invention there is provided the radiopharmaceutical [^{211}At]MABG in combination with a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a noradrenaline transporter and wherein the herpes simplex virus is non-neurovirulent, for use in the treatment of a tumour.

Suitably, in either or both of the fourteenth and fifteenth aspects, a composition is provided comprising said radiopharmaceutical [^{211}At]MABG and said herpes simplex virus, said composition for use in the treatment of a tumour.

According to a sixteenth aspect of the present invention there is provided the use of a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a noradrenaline transporter in at least one of the long repeat regions (R_L), in the manufacture of a medicament for the treatment of a tumour.

According to a seventeenth aspect of the present invention there is provided the use of a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a noradrenaline transporter and wherein the herpes simplex virus is non-neurovirulent, in the manufacture of a medicament for the treatment of a tumour.

Suitably, in either or both of the sixteenth and seventeenth aspects, the use of said herpes simplex viruses is in the manufacture of a medicament for treatment, in combination with the radiopharmaceutical [^{131}I]MIBG and/or the radiopharmaceutical [^{211}At]MABG, of a tumour. In one arrangement, the medicament may additionally comprise the radiopharmaceutical [^{131}I]MIBG and/or the radiopharmaceutical [^{211}At]MABG.

In an eighteenth aspect of the present invention there is provided a method for the treatment of a tumour comprising the steps of:

(i) administering to a patient in need of treatment a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a noradrenaline transporter in at least one of the long repeat regions (R_L); and

(ii) administering to said patient a therapeutically effective amount of the radiopharmaceutical [^{131}I]MIBG.

In a nineteenth aspect of the present invention there is provided a method for the treatment of a tumour comprising the steps of:

(i) administering to a patient in need of treatment a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a noradrenaline transporter and wherein the herpes simplex virus is non-neurovirulent; and

(ii) administering to said patient a therapeutically effective amount of the radiopharmaceutical [^{131}I]MIBG.

In a twentieth aspect of the present invention there is provided a method for the treatment of a tumour comprising the steps of:

(i) administering to a patient in need of treatment a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a noradrenaline transporter in at least one of the long repeat regions (R_L); and

(ii) administering to said patient a therapeutically effective amount of the radiopharmaceutical [^{211}At]MABG.

In a twenty first aspect of the present invention there is provided a method for the treatment of a tumour comprising the steps of:

(i) administering to a patient in need of treatment a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a noradrenaline transporter and wherein the herpes simplex virus is non-neurovirulent; and

(ii) administering to said patient a therapeutically effective amount of the radiopharmaceutical [^{211}At]MABG.

Suitably, administration of said herpes simplex virus and/or said radiopharmaceutical may comprise parenteral administration. Preferably administration of the herpes simplex virus is by injection, more preferably injection to the tumour which is to be treated. The radiopharmaceuticals [^{131}I]MIBG and [^{211}At]MABG may also be administered by injection, which may also comprise direct injection to the site of the tumour or may be intraperitoneal.

Alternatively injections may be intravenous. Alternative administration routes may comprise oral or nasal administration.

Administration of the herpes simplex virus and radiopharmaceutical may be simultaneous, e.g. by combining virus and radiopharmaceutical in a single composition, or be substantially simultaneous, e.g. one being administered immediately after the other. Alternatively, a predetermined time period may be provided between administration of the herpes simplex virus and radiopharmaceutical. The invention is not limited by the order of administration, but administration of the virus prior to the radiopharmaceutical may be preferred.

Thus a dosing regime may be provided in which administration of the virus and pharmaceutical are sequential with a time interval being provided between the two administrations. This time interval may be provided by the dosing regime, and may be provided in instructions that accompany the virus and/or pharmaceutical and may form part of a kit. A preferred time interval may be one that provides sufficient time for the virus to infect the target cells (e.g. tumour cells) and

express the monoamine transporter. The time interval selected may depend on the patient to be treated and may take into account factors such as sex, age and type of tumour. It is possible to adjust the time interval for each individual patient by monitoring infection of the target cells with virus and expression of the monoamine transporter in those cells in the patient concerned.

The time interval may be selected from any one or more of at least:

(a) 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59 or 60 minutes or a time interval in the range 1-60 minutes, 1-30 minutes or 30-60 minutes or a time interval in a range that starts with any one of the numbers recited under (a) and finishes with any other number recited under (a);

(b) 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167 or 168 hours or a time interval in a range that starts with any one of the numbers recited under (b) and finishes with any other number recited under (b);

(c) 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 days or a time interval in a range that starts with any one of the numbers recited under (c) and finishes with any other number recited under (c).

According to a twenty second aspect of the present invention there is provided a method of expressing in vitro or in vivo a noradrenaline transporter, said method comprising the step of infecting at least one cell or tissue of interest with a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a noradrenaline transporter in at least one of the long repeat regions (R_L).

According to a twenty third aspect of the present invention there is provided a method of expressing in vitro or in vivo a noradrenaline transporter, said method comprising the step of infecting at least one cell or tissue of interest with a non-neurovirulent herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a noradrenaline transporter.

The expressed noradrenaline transporter is preferably a functional protein and is preferably capable of uptake of a monoamine neurotransmitter (e.g noradrenaline) or one of the radiopharmaceuticals [^{131}I]MIBG or [^{211}At]MABG into a cell infected with said herpes simplex virus.

In a further aspect of the present invention in vitro or in vivo methods are provided for delivery of nucleic acid encoding a noradrenaline transporter to at least one cell or to a tissue of interest said method comprising the step of infecting said cell(s) or tissue with a herpes simplex virus according to the invention.

In another aspect of the invention, a kit of parts is provided comprising a first container in which a quantity of herpes

simplex virus according to the invention is provided and a second container in which a quantity of the radiopharmaceutical [^{131}I]MIBG is provided. Instructions for the administration, optionally including information on suitable dosages of herpes simplex virus and/or the radiopharmaceutical [^{131}I]MIBG, may also be provided with the kit.

In a further aspect of the invention, a kit of parts is provided comprising a first container in which a quantity of herpes simplex virus according to the invention is provided and a second container in which a quantity of the radiopharmaceutical [^{211}At]MABG is provided. Instructions for the administration, optionally including information on suitable dosages of herpes simplex virus and/or the radiopharmaceutical [^{211}At]MABG, may also be provided with the kit.

In yet another aspect of the invention, a kit of parts is provided comprising a first container in which a quantity of herpes simplex virus according to the invention is provided, a second container in which a quantity of the radiopharmaceutical [^{131}I]MIBG is provided and a third container in which a quantity of the radiopharmaceutical [^{211}At]MABG is provided. Instructions for the administration, optionally including information on suitable dosages of herpes simplex virus and/or one or both of the radiopharmaceuticals [^{131}I]MIBG and [^{211}At]MABG, may also be provided with the kit.

In another aspect of the present invention a method of making or producing a modified herpes simplex virus of the invention is provided comprising the step of introducing a nucleic acid sequence encoding a noradrenaline transporter at a selected or predetermined insertion site in the genome of a selected herpes simplex virus.

The nucleic acid sequence encoding the noradrenaline transporter is preferably an exogenous sequence, i.e. one not originating in the parent herpes simplex virus strain from which the herpes simplex virus of the invention is derived. The sequence of the noradrenaline transporter may be derived or obtained from any animal or microorganism including humans, non-human mammals and bacteria and may be selected from those sequences which are publicly available.

One suitable noradrenaline transporter is the bovine noradrenaline transporter[36]. The nucleotide sequence of which is publicly available from the NCBI database (www.ncbi.nlm.nih.gov) under accession number NM_174608 (Version NM_174608.2; GI:31341545). The amino acid sequence and nucleotide sequence available under accession number NM_174608 (Version NM_174608.2; GI:31341545) are reproduced herein as SEQ ID No.s 1 and 2 respectively (see Figures 7 and 8).

Another suitable noradrenaline transporter is the human noradrenaline transporter[36]. The nucleotide sequence of which is publicly available from the NCBI database (www.ncbi.nlm.nih.gov) under accession number M65105 (Version M65105.1; GI:189257). The amino acid sequence and nucleotide sequence available under accession number M65105 (Version M65105.1; GI:189257) are reproduced herein as SEQ ID No.s 3 and 4 respectively (see Figures 9 and 10).

Other suitable noradrenaline transporters may have an amino acid sequence or nucleotide sequence having a sequence identity of at least 60%, more preferably one of at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99%, to any one of SEQ ID No.s 1, 2, 3 or 4 or to a nucleic acid encoding the polypeptide of SEQ ID No.1 or 3.

Other suitable noradrenaline transporters may have a nucleic acid sequence encoding the noradrenaline transporter that hybrdises to a nucleic acid encoding the polypeptide of SEQ ID No.1 or 3 (which may be the nucleic acid having SEQ ID No.2 or 4) under high or very high stringency conditions.

The nucleic acid sequence encoding the noradrenaline transporter may form part of a nucleic acid cassette which is inserted in the genome of a selected herpes simplex virus by homologous recombination. Whether part of a cassette or not, the site of insertion may be in any genomic location selected. One preferred insertion site is in one of the long repeat regions (R_L), and one copy of the cassette is preferably inserted in each copy of the long repeat (R_L). More preferably the insertion site is in at least one RL1 locus and most preferably it is inserted in at least one of the ICP34.5 protein coding sequences of the HSV genomic DNA. It is preferred that the insertion occurs in identical or substantially similar positions in each of the two repeat regions, RL1 loci or ICP34.5 protein coding sequences.

Thus, the virus preferably has the monoamine transporter nucleic acid sequence inserted in both repeat regions, RL1 loci or ICP34.5 protein coding sequences. The inserted sequence may thereby disrupt the ICP34.5 protein coding sequence and cause loss of function or inactivation of the ICP34.5 gene product.

Viruses of the invention will therefore lack at least one expressible ICP34.5 gene. In some arrangements they may lack only one expressible ICP34.5 gene. However, most preferably both copies of the ICP34.5 gene are modified such that the virus cannot express functional ICP34.5 protein.

Insertion may be such as to produce a modified virus which is a non-neurovirulent mutant capable of expressing the encoded

noradrenaline transporter upon transfection into mammalian, more preferably human, cells in vivo and in vitro in a form which is functional to facilitate the uptake of [^{131}I]MIBG or [^{211}At]MABG to the cell.

The nucleic acid cassette preferably comprises a constitutive or inducible control or regulatory sequence, e.g. enhancer and/or promoter sequence (e.g. the constitutive cytomegalovirus (CMV) promoter) 5′ (upstream) of the noradrenaline transporter transcription initiation site. A polyadenylation (polyA) sequence, e.g. the Simian Virus 40 (SV40) polyA sequence may be located 3′ (downstream) of the noradrenaline transporter protein coding sequence.

The control or regulatory sequence may be operably linked to the nucleic acid encoding the noradrenaline transporter, wherein the control or regulatory sequence has a role in controlling transcription of the noradrenaline transporter.

Herpes simplex viruses according to the invention may further comprise a marker nucleotide sequence which may encode a marker protein such as GFP or may comprise a defined nucleotide sequence detectable by hybridisation under intermediate, high or very high stringency conditions using a corresponding labelled nucleic acid probe.

In one arrangement the cassette may further comprise an internal ribosome entry site (IRES), e.g. the encephalomyocarditis virus IRES (EMCV IRES), downstream (3′) of the noradrenaline transporter nucleic acid sequence followed by nucleic acid encoding a marker polypeptide downstream (3′) of the IRES. One suitable marker is the Green Fluorescent Protein (GFP) or Enhanced Green Fluorescent Protein (EGFP). In this arrangement the polyA sequence is preferably located downstream (3′) of the marker nucleic acid. The control sequence, noradrenaline transporter sequence, optional IRES

and GFP sequences and polyA sequences may be immediately adjacent their neighbouring sequence(s).

A transcription product of the cassette may be a bi- or polycistronic transcript comprising a first cistron encoding the noradrenaline transporter and a second cistron encoding the marker wherein the ribosome binding site is located between the first and second cistrons.

The nucleic acid cassette may be of any size, e.g. up to 5, 10, 15, 20, 25, 30, 35, 40 or 45Kbp, but is preferably up to 50Kbp in length.

Preferably, the herpes simplex virus contains at least one copy of the nucleic acid encoding the noradrenaline transporter in each long repeat region (R_L), i.e. in the terminal and internal long repeat (TR_L and IR_L) regions. In a preferred arrangement each exogenous sequence or cassette is located in an RL1 locus of the herpes simplex virus genome, more preferably in the DNA of the herpes simplex virus genome encoding the ICP34.5 gene or protein coding sequence. The herpes simplex virus thereby lacks neurovirulence.

The parent herpes simplex virus, from which a virus of the invention is derived may be of any kind, e.g. HSV-1 or HSV-2. In one preferred arrangement the herpes simplex virus is a variant of HSV-1 strain 17 and may be obtained by modification of the strain 17 genomic DNA. Suitable modifications include the insertion of the exogenous noradrenaline transporter nucleic acid sequence or exogenous cassette comprising said sequence into the herpes simplex virus genomic DNA. The insertion may be performed by homologous recombination of the exogenous nucleic acid sequence into the genome of the selected herpes simplex virus.

Although the non-neurovirulent phenotype of the herpes simplex virus of the invention may be the result of insertion of the exogenous nucleic acid sequence in the RL1 locus, herpes simplex viruses according to the present invention may be obtained by utilising a non-neurovirulent parent strain, e.g. HSV1716 deposited under the Budapest Treaty at the European Collection of Animal Cell Cultures (ECACC), Porton Down, Salisbury, Wiltshire, United Kingdom under accession number V92012803, and inserting the exogenous nucleic acid sequence at another location of the genome by standard genetic engineering techniques, e.g. homologous recombination. In this aspect the location selected for insertion of the noradrenaline transporter nucleic acid sequence or cassette containing said sequence may be a neutral location.

Herpes simplex viruses of the present invention may be variants of a known 'parent' strain from which the herpes simplex virus of the invention has been derived. A particularly preferred parent strain is HSV-1 strain 17. Other parent strains may include HSV-1 strain F or HSV-2 strain HG52. A variant comprises an HSV in which the genome substantially resembles that of the parent, contains the noradrenaline transporter nucleic acid sequence or cassette containing said sequence and may contain a limited number of other modifications, e.g. one, two or three other specific mutations, which may be introduced to disable the pathogenic properties of the herpes simplex virus, for example a mutation in the ribonucleotide reductase (RR) gene, the 65K trans inducing factor (αTIF) and/or a small number of mutations resulting from natural variation, which may be incorporated naturally during replication and selection in vitro or in vivo. Otherwise the genome of the variant will be that of the parent strain.

Herpes simplex viruses of the invention may be used alone, or in combination with one or both of the radiopharmaceuticals

[^{131}I]MIBG and [^{211}At]MABG in a method of medical treatment. This may be treatment of diseases associated with or involving the proliferation of cells, or cancers or tumours of any kind, herein referred to as cancerous conditions. Treatment may involve the selective lysis of dividing cells. This may be oncolysis, i.e. lysis of tumour cells. Tumours to be treated may be of any kind, may comprise cancers, neoplasms or neoplastic tissue and may be in any animal or human patient. Treatable tumour types may include primary or secondary (metastatic) tumours originating either in the central or peripheral nervous system, e.g. glioma, medulloblastoma, meningioma, neurofibroma, ependymoma, Schwannoma, neurofibrosarcoma, astrocytoma and oligodendroglioma, or originating in non-nervous system tissue e.g. melanoma, mesothelioma, lymphoma, hepatoma, epidermoid carcinoma, prostate carcinoma, breast cancer cells, lung cancer cells or colon cancer cells. Treatable metastatic tumours may be those of the central or peripheral nervous system which originated in a non-nervous system tissue.

The use of the virus described above may be use in the manufacture of a medicament for the treatment of a cancerous condition.

Herpes simplex viruses according to the present invention may be provided as a pharmaceutical composition or vaccine in combination with a pharmaceutically acceptable carrier, adjuvant or diluent. The pharmaceutical composition or vaccine may further comprise a radiopharmaceutical, e.g. one or both of the radiopharmaceuticals [^{131}I]MIBG and [^{211}At]MABG. The composition may be formulated for intratumoural, topical, parenteral, intravenous, intramuscular, intrathecal, intraocular, subcutaneous, oral or transdermal routes of administration which may include injection. Injectable formulations may comprise the selected compound in a sterile or isotonic medium.

Herpes simplex viruses of the invention may be used in 'gene delivery' methods in vitro or in vivo. Non-neurovirulent herpes simplex viruses of the invention are expression vectors and may be used to infect selected cells or tissues in order to express the noradrenaline transporter encoded by the herpes simplex virus genome.

In one arrangement, cells may be taken from a patient, a donor or from any other source, infected with a herpes simplex virus of the invention, optionally screened for expression and/or function of the encoded noradrenaline transporter, and optionally returned/introduced to a patient's body, e.g. by injection.

In aspects of the present invention the patient to be treated may be any animal or human. The patient may be a non-human mammal, but is more preferably a human patient. The patient may be male or female.

Delivery of herpes simplex viruses of the invention to the selected cells may be performed using naked virus or by encapsulation of the virus in a carrier, e.g. nanoparticles, liposomes or other vesicles.

In vitro cultured cells, preferably human or mammalian cells, transformed or infected with viruses of the present invention and preferably cells expressing the noradrenaline transporter protein as well as methods of transforming such cells in vitro with said viruses form further aspects of the present invention.

Many aspects of the present invention are concerned with the use of the radiopharmaceuticals [^{131}I]MIBG and/or [^{211}At MABG. The particular radioisotope used may be selected by the skilled person and may be other than ^{131}I or ^{211}At. For example,

in further aspects of the invention the radioisotopes [^{123}I] and [^{125}I] and, suitably, the radiopharmaceuticals [^{123}I]MIBG and/or [^{125}I]MIBG, may be used.

In any aspect of the invention, the pharmaceuticals MIBG and MABG may be used respectively in place of the radiopharmaceuticals [^{131}I]MIBG and [^{211}At]MABG.

In any aspect of the invention a radio-labelled pharmaceutical (a radiopharmaceutical) may be used that is capable of uptake by a monoamine transporter, e.g. a noradrenaline transporter. Thus, the pharmaceutical compound may be other than MIBG or MABG, provided it is capable of uptake by the monoamine transporter and can be radio-labelled with a suitable radioisotope.

The radio-labelled pharmaceutical is preferably capable of killing the target cell (e.g. tumour cell) into which it is taken up by the monoamine transporter.

In an alternative aspect of the invention, the noradrenaline transporter is a monoamine transporter which may be selected from a dopamine transporter (DAT) or serotonin transporter (SERT).

In this specification the term "operably linked" may include the situation where a selected nucleotide sequence and regulatory nucleotide sequence are covalently linked in such a way as to place the expression of a nucleotide coding sequence under the influence or control of the regulatory sequence. Thus a regulatory sequence is operably linked to a selected nucleotide sequence if the regulatory sequence is capable of effecting transcription of a nucleotide coding sequence which forms part or all of the selected nucleotide sequence. Where appropriate, the resulting transcript may then be translated into a desired protein or polypeptide.

Noradrenaline transporter (NAT)

The noradrenaline transporter, also called noradrenalin transporter or norepinephrine transporter, is a protein normally located in the presynaptic plasma membrane and capable of uptake of monoamine neurotransmitters. In this specification noradrenaline transporter relates to a protein capable of, or enabling, cellular uptake of one or both of the radiopharmaceuticals [^{131}I]MIBG and [^{211}At]MABG. One of the normal functions of noradrenaline transporters forming part of the present invention is the cellular uptake of one or more monoamine neurotransmitters such as noradrenaline (also called noradrenalin or norepinephrine). Cellular uptake may include the active or facilitated uptake of the relevant compound from an extracellular environment to the intracellular environment.

Herpes simplex viruses of the invention may comprise noradrenaline transporters of any kind. Preferred noradrenaline transporters include animal or insect noradrenaline transporters, more preferably human or mammalian noradrenaline transporters.

Examples of noradrenaline transporter nucleic acid sequences which may form part of a herpes simplex virus according to the present invention include the following which are referred to by their accession number for the NCBI database (www.ncbi.nlm.nih.gov):

- NM_001043 (GI 4557045) - Human;
- NM_031343 (GI 13786179)- Norway Rat;
- NM_204716 (GI 45382638)- Chicken;
- NM_174608 (GI:31341545)- Cow;
- M65105 (GI:189257) - Human.

Sequence identity

Percentage (%) sequence identity is defined as the percentage of amino acid residues in a candidate sequence that are identical with residues in the given listed sequence (referred to by the SEQ ID No.) after aligning the sequences and introducing gaps if necessary, to achieve the maximum sequence identity, and not considering any conservative substitutions as part of the sequence identity. Sequence identity is preferably calculated over the entire length of the respective sequences.

Where the aligned sequences are of different length, sequence identity of the shorter comparison sequence may be determined over the entire length of the longer given sequence or, where the comparison sequence is longer than the given sequence, sequence identity of the comparison sequence may be determined over the entire length of the shorter given sequence.

For example, where a given sequence comprises 100 amino acids and the candidate sequence comprises 10 amino acids, the candidate sequence can only have a maximum identity of 10% to the entire length of the given sequence. This is further illustrated in the following example:

(A)

Given seq: XXXXXXXXXXXXXXX (15 amino acids)

Comparison seq: XXXXXYYYYYYY (12 amino acids)

The given sequence may, for example, be that encoding bovine noradrenaline transporter (SEQ ID No.1) or human noradrenaline transporter (SEQ ID No.3).

% sequence identity = the number of identically matching amino acid residues after alignment divided by the total number of amino acid residues in the longer given sequence, i.e. (5 divided by 15) x 100 = 33.3%

Where the comparison sequence is longer than the given sequence, sequence identity may be determined over the entire length of the given sequence. For example:

(B)

Given seq:	XXXXXXXXXX	(10 amino acids)
Comparison seq:	XXXXXYYYYYYZZYZZZZZZ	(20 amino acids)

Again, the given sequence may, for example, be that encoding bovine noradrenaline transporter (SEQ ID No.1) or human noradrenaline transporter (SEQ ID No.3).

% sequence identity = number of identical amino acids after alignment divided by total number of amino acid residues in the given sequence, i.e. (5 divided by 10) x 100 = 50%.

Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways known to a person of skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software.

Identity of nucleic acid sequences may be determined in a similar manner involving aligning the sequences and introducing gaps if necessary, to achieve the maximum sequence identity, and calculating sequence identity over the entire length of the respective sequences. Where the aligned sequences are of different length, sequence identity may be determined as described above and illustrated in examples (A) and (B).

Hybridisation stringency

In accordance with the present invention, nucleic acid sequences may be identified by using hybridization and washing conditions of appropriate stringency.

Complementary nucleic acid sequences will hybridise to one another through Watson-Crick binding interactions. Sequences which are not 100% complementary may also hybridise but the strength of the hybridisation usually decreases with the decrease in complementarity. The strength of hybridisation can therefore be used to distinguish the degree of complementarity of sequences capable of binding to each other.

The "stringency" of a hybridization reaction can be readily determined by a person skilled in the art.

The stringency of a given reaction may depend upon factors such as probe length, washing temperature, and salt concentration. Higher temperatures are generally required for proper annealing of long probes, while shorter probes may be annealed at lower temperatures. The higher the degree of desired complementarity between the probe and hybridisable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so.

For example, hybridizations may be performed, according to the method of Sambrook et al., ("Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989) using a hybridization solution comprising: 5X SSC, 5X Denhardt's reagent, 0.5-1.0% SDS, 100 µg/ml denatured, fragmented salmon sperm DNA, 0.05% sodium pyrophosphate and up to 50% formamide. Hybridization is carried out at 37-42°C for at least six hours. Following hybridization, filters are washed as follows: (1) 5 minutes at room temperature in 2X SSC and 1% SDS; (2) 15 minutes at room temperature in 2X SSC and 0.1% SDS; (3) 30 minutes-1 hour at 37°C in 1X SSC and 1% SDS; (4) 2 hours at 42-65°C in 1X SSC and 1% SDS, changing the solution every 30 minutes.

One common formula for calculating the stringency conditions required to achieve hybridization between nucleic acid molecules is to calculate the melting temperature T_m (Sambrook et al., 1989):

$T_m = 81.5°C + 16.6Log [Na+] + 0.41(\% G+C) - 0.63 (\% formamide) - 600/n$

where n is the number of bases in the oligonucleotide.

As an illustration of the above formula, using [Na+] = [0.368] and 50% formamide, with GC content of 42% and an average probe size of 200 bases, the T_m is 57°C. The T_m of a DNA duplex decreases by 1 - 1.5°C with every 1% decrease in sequence complementarity.

Accordingly, nucleotide sequences can be categorised by an ability to hybridise to a target sequence under different hybridisation and washing stringency conditions which can be selected by using the above equation. The T_m may be used to provide an indicator of the strength of the hybridisation.

The concept of distinguishing sequences based on the stringency of the conditions is well understood by the person skilled in the art and may be readily applied.

Sequences exhibiting 95-100% sequence complementarity are considered to hybridise under very high stringency conditions, sequences exhibiting 85-95% complementarity are considered to hybridise under high stringency conditions, sequences exhibiting 70-85% complementarity are considered to hybridise under intermediate stringency conditions, sequences exhibiting 60-70% complementarity are considered to hybridise under low stringency conditions and sequences exhibiting 50-60%

complementarity are considered to hybridise under very low stringency conditions.

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

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.

Brief Description of the Figures

Figure 1. *Schematic representation of the HSV-1 genome (A) and the HSV1716/NAT genome (B).*

The HSV-1 genome consists of two regions of unique sequences designated unique long (U_L) and unique short (U_S), flanked by a set of repeat sequences designated terminal repeat long (TR_L) and terminal repeat short (TR_S), inverted long (IR_L) and inverted short (IR_S). Within the repeat sequences flanking the unique long segment (TR_L and IR_L) are two copies of the RL1 gene which codes for the neurovirulence factor ICP34.5. In HSV1716/NAT, the majority of both copies of this gene has been removed and replaced with an expression cassette consisting of CMV IE promoter (pCMV), upstream of the bovine noradrenaline transporter cDNA (NAT), the encephalomyocarditis virus internal ribosome entry site (IRES), the enhanced green fluorescent protein gene (EGFP) and the SV40 polyadenylation sequences (SV40 PolyA). HSV1716/NAT expresses NAT and EGFP but does not express ICP34.5.

Figure 2. *Growth kinetics of HSV-1 variants 17+, 1716, and 1716/NAT in various cell lines; BHK (A), 3T6 (B), MOG (C).*

Cells were infected at multiplicity of infection (MOI) of 0.1pfu/cell and at various times post-infection cells were harvested and cell associated virus released by sonication before titration on BHK cells.

Figure 3. *MIBG uptake in MOG glioma cell lines following HSV1716/NAT infection.*

(A) Various glioma cell lines were infected at a MOI of 0.1 and 1pfu/cell. Data represent the means of five experiments +/- standard deviation. Control wells (no virus) were mock infected, and treated with the same activity concentration of [^{131}I]MIBG as the virus infected cells;

(B) Assessment of time dependence of NAT gene expression, measured by [^{131}I]MIBG uptake at various times after treatment with HSV1716/NAT virus at a MOI of 5 pfu/cell of 1716/NAT virus.

Figure 4. *Cell survival following HSV1716/NAT and/or [^{131}I]MIBG administration.*

PN3 (A) and MOG (B) cell survival was determined following treatment with HSV1716/NAT, [^{131}I]MIBG or a combination of both virus and [^{131}I]MIBG. Cells were infected with a multiplicity of infection of 0.1 or 1pfu/cell of HSV-1 1716/NAT. One hour post-infection, the medium was removed and cells dosed with 1MBq/ml [^{131}I]MIBG which was incubated with the cells for 24 hours. At different times following [^{131}I]MIBG administration cell survival was determined by MTT assay. The data represent means and standard deviation of three experiments performed in triplicate.

(★and * indicate statistical significance as determined by Students t-test).

Figure 5. Graph showing percentage of injected dose of [^{131}I]MIBG taken up by tumour and non-tumour tissues in mice transfected with HSV1716/NAT.

Figure 6. Graph showing xenograft tumour size (as the ratio of tumour volume divided by starting tumour volume) over time in mice injected with HSV1716/NAT and [^{131}I]MIBG alone or in combination. The results show data for doses of 10^5 and 10^6 pfu and 10 MBq [^{131}I]MIBG. Combined doses were either given simultaneously or sequentially (10 MBq [^{131}I]MIBG administered 24 hours after virus).

Figure 7. Amino acid sequence of the bovine noradrenaline transporter[36] (SEQ ID No.1). This sequence is available from the NCBI database (www.ncbi.nlm.nih.gov) under accession number NM_174608 (Version NM_174608.2; GI:31341545).

Figure 8. Nucleotide sequence of the bovine noradrenaline transporter[36] (SEQ ID No.2). This sequence is available from the NCBI database (www.ncbi.nlm.nih.gov) under accession number NM_174608 (Version NM_174608.2; GI:31341545).

Figure 9. Amino acid sequence of the human noradrenaline transporter (SEQ ID No.3). This sequence is available from the NCBI database (www.ncbi.nlm.nih.gov) under accession number M65105 (Version M65105.1; GI:189257).

Figure 10. Nucleotide sequence of the human noradrenaline transporter (SEQ ID No.4). This sequence is available from the NCBI database (www.ncbi.nlm.nih.gov) under accession number M65105 (Version M65105.1; GI:189257).

Detailed Description of the Best Mode of the Invention

Specific details of the best mode contemplated by the inventors for carrying out the invention are set forth below,

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.

HSV1716 is a specific variant of HSV-1 strain 17 which is non-neurovirulent. It contains a 759bp deletion in the Bam HI s restriction fragment located in each of the terminal and internal repeats (TR_L and IR_L - map units 0-0.02 and 0.81-0.83 respectively) and has been deposited under the Budapest Treaty at the European Collection of Animal Cell Cultures (ECACC), Porton Down, Salisbury, Wiltshire, United Kingdom under accession number V92012803. In this specification, reference to HSV1716 or HSV1716/NAT is a reference to an HSV having the characteristics of non-neurovirulence of HSV1716 and may contain a modification in one or both of the long repeat regions (R_L) of the herpes simplex virus genome but is not necessarily identical to or derived from the deposited HSV1716 virus, although it may be.

Materials and methods

Reagents

Tissue culture media and supplements were purchased from Gibco BRL (Paisley, UK). All other reagents were obtained from Sigma-Aldrich Co Ltd (Dorset, UK), unless otherwise stated. [^{131}I]MIBG of specific activity 45-65 MBq/mg was obtained from Amersham.

Cell lines

Baby hamster kidney cells (BHK21/C13), human glioblastoma astrocytoma cells, (MOG-G-UVW - hereinafter designated as MOG) and 3T6 Swiss albino mouse fibroblast cells were obtained from the European Tissue Culture Collection. PN3 is a glioma cell line derived from MOG stably transfected with the bovine NAT gene under the control of the CMV immediate early promoter. BHK21/C13 cells were propagated in Glasgow modified Eagle's

medium (GMEM) supplemented with 5% (v/v) tryptose phosphate broth. MOG cells were grown in MEM and 3T6 cells in Dulbecco's modified medium (DMEM). PN3 cells were propagated in MEM in the presence of 100mg/ml geneticin. All media were supplemented with 10% (v/v) foetal calf serum, 2mM glutamine, 5% (v/v) fungizone, 100µg penicillin/streptomycin and maintained at 37°C in 5% CO_2.

Virus Constructs

HSV1716 was derived from HSV Glasgow strain 17+ as previously described (MacLean *et al.*, 1991). HSV1716 has been deposited under the Budapest Treaty at the European Collection of Animal Cell Cultures (ECACC), Porton Down, Salisbury, Wiltshire, United Kingdom under accession number V92012803.

The pREP9/NAT plasmid was constructed as detailed previously (Boyd *et al*, 1999), and the bovine noradrenaline transporter (NAT) cDNA was subcloned from this plasmid into the multiple cloning site of the pIRES2-EGFP vector (Clontech). The 5.4kb DNA fragment containing the CMV IE promoter upstream of NAT/IRES/EGFP, was excised from the pIRES2-EGFP and ligated into HpaI digested 'RL1.del' vector. RL1.del, is the pGEM 3zf(-) vector (Promega), into which has been cloned the entire ORF of RL1 (and flanking sequences), followed by deletion of the majority of RL1 (deleted- 'del') and insertion of a multiple cloning site (MCS). The sequence elements of the plasmids were confirmed by restriction enzyme digestion and clones which contained the CMV/NAT/IRES/EGFP/PolyA insert were linearised using SspI and co-transfected with HSV 17^+ DNA onto 80% confluent BHK cells using the $CaPO_4$ transfection method. Fluorescent, recombinant, viral plaques were purified and a stock - designated HSV1716/NAT - was grown and titrated in BHK21/C13 cells as previously described. The structure of HSV1716/NAT is shown in Figure 1.

Viral growth kinetics

Confluent monolayers containing approximately $2x10^6$ cells were infected with 0.1 plaque forming units (pfu) per cell of HSV17+, HSV1716, or HSV1716/NAT. After incubation for 1 hour at 37°C, cells were washed, overlaid with appropriate medium and the incubation continued at 37°C. At various times after infection, samples were harvested, progeny viruses were released by sonication, and applied to BHK21/C13 cells.

[^{131}I]MIBG Uptake

Cells were seeded at a density of $0.5x10^6$ cells per well in a 6 well tissue culture dish and cultured for 24 hours. Virus (multiplicity of infection (MOI) 1 or 0.1) was added in a 100µl volume and after 1 hour incubation, the cells were washed twice with PBS to remove any free virus and overlaid with 2ml of appropriate medium. At various times after infection, [^{131}I]MIBG uptake capacity of host cells was determined as previously described (Boyd *et al*, 1999).

Cell viability assays (MTT)

Cells were seeded into 96 well microtiter plates at a density of $1x10^4$ cells per well. After incubation for 24 hours, the cells were treated with various multiplicities of infection (MOI) of virus in a 100µl total volume. One hour after viral infection the medium was removed and replaced with fresh medium containing 1MBq/ml [^{131}I]MIBG. The cells were incubated with [^{131}I]MIBG for 24 hours. MTT assays were carried out on cells incubated with virus alone, [^{131}I]MIBG alone or a combination of both treatments. At a range of time points following HSV1716/NAT infection and/or [^{131}I]MIBG administration, medium was replaced with 100µl fresh medium containing 10µl MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide, Sigma Aldrich, Dorset, UK) per well (7mg/ml). After 4 h incubation, the formazan in the MTT was dissolved by addition of 100µl per well of 10% (w/v) SDS

in 0.1M HCl. After a further 16 hours incubation at 37°C, viable cell numbers were determined from measurement of the absorbance at 570 nm of well contents using an ELISA plate reader (MRX II, Dynex Technologies Inc., USA).

Bovine noradrenaline transporter cDNA

Bovine noradrenaline transporter cDNA was a kind gift from Professor Heinz Bonisch and Dr Michael Bruss, University of Bonn.

Results

Replication of HSV 1716 variants in human glioma cell lines.

BHK21/C13 cells are routinely used for growth and propagation of HSV-1. HSV Wild type strain 17+ and the ICP34.5 null mutant HSV1716 have been shown to grow with indistinguishable kinetics in this cell line. The genetically engineered variant, HSV 1716/NAT also grew with similar kinetics in BHK21/C13 cells (Figure 2A). Growth arrested mouse embryo fibroblast cells (3T6) have previously been shown to be permissive for growth of the wild type virus 17+ but not for HSV 1716. HSV 1716/NAT was also replication deficient in this cell line, (Figure 2B). In the glioma cell line MOG, the growth kinetics of HSV1716 and HSV1716/NAT were indistinguishable from those of wild type virus (Figure 2C). The growth patterns of HSV1716/NAT were indistinguishable from HSV1716 in the three cell lines suggesting that insertion of the NAT gene within the RL1 locus of HSV did not adversely affect the growth characteristics *in vitro.*

Expression of the noradrenaline transporter (NAT) and uptake of [^{131}I]MIBG.

Following incubation of HSV1716/NAT with the human glioma cell line MOG, which does not endogenously express NAT and therefore has no capacity for active uptake of [^{131}I]MIBG, uptake of [^{131}I]MIBG was assessed (Figure 3). 24 hrs after

virus infection there was a 5 and 16 fold increase in uptake of [^{131}I]MIBG when the MOG cells were infected at a MOI of 0.1 and 1pfu/cell respectively, compared to cells which had not been infected with the virus. Uptake was inhibited by DMI, an inhibitor of the active uptake of MIBG. In addition, PN3 cells which are stably transfected with the NAT transgene[3] took up significantly more [^{131}I] following infection with HSV1716/NAT (MOI 1) (Figure 3a). It therefore appears that cells endogenously expressing NAT may be induced to synthesise more NAT as a result of HSV1716 infection.

[^{131}I]MIBG uptake in MOG cells, measured at 24 hrs after infection with HSV1716/NAT, increased with increasing concentration of infectious virus (Figure 3a) and with increasing time following infection (Figure 3b).

Effect of combined virus and [^{131}I]MIBG treatment on glioma cells.

Tumour cell kill *in vitro* by HSV1716/NAT oncolytic activity, [^{131}I]MIBG and the combination of both treatments, were compared by MTT assays (Figures 4a and 4b).

After incubation of PN3 cells (which expressed the NAT prior to viral infection) with HSV1716/NAT at a MOI of 0.1pfu/cell, followed by treatment with [^{131}I]MIBG for 24 hours, a highly significant increase in cell kill at 48, 72 and 144 hours ($p<0.001$) compared to treatment with either[^{131}I] MIBG or HSV1716 NAT alone was observed (Figure 4a). A similar significantly enhanced cell kill with the dual treatment was observed when virus was added at higher concentrations (MOI 1).

The cytotoxic effect of the various treatments was examined in MOG cells which had no endogenous capacity for [^{131}I]MIBG uptake and therefore did not succumb to [^{131}I]MIBG mediated cytotoxicity (Figure 4b). As in the case of the treatment of

PN3 cells, the combination of HSV1716/NAT infection, at both 0.1 and 1 MOI, with [^{131}I]MIBG was highly statistically significantly more toxic ($p<0.001$) at 48, 72 and 144 hours after radiopharmaceutical treatment than treatment with virus alone. Furthermore, the toxicity to MOG cells of viral infection at 1pfu/cell combined with [^{131}I]MIBG treatment, at 24 hours after radiopharmaceutical administration, was significantly greater ($p<0.001$) than that resulting from either treatment alone.

Discussion

The inventors have investigated a novel combination therapy enabling tumour cell kill by the lytic activity of HSV1716 and by [^{131}I]MIBG treatment after expression of the NAT transgene. Introduction of the NAT cDNA into the RL1 locus of HSV strain 17+ resulted in a virus with growth characteristics indistinguishable from those of HSV1716. Expression of NAT from the HSV backbone did not alter the virus in terms of its selectively replication competent phenotype in a range of cell lines *in vitro*.

It has been previously demonstrated that plasmid mediated introduction of NAT into glioma cells, conferred upon them the capacity for active uptake of the radiopharmaceutical [^{131}I]MIBG [3, 4]. Similarly, treatment of MOG cells with HSV1716/NAT, resulted in expression of a functional transporter allowing the cells to actively concentrate [^{131}I]MIBG. MIBG uptake greater than background levels was apparent 1 hour after virus infection, suggesting rapid induction of transgene expression. The induced accumulation of radiopharmaceutical increased with time after infection and with increasing dose of HSV1716/NAT. These data demonstrate the effectiveness of HSV1716 as a transgene delivery vehicle.

The inventors examined cell kill induced by viral oncolytic activity and [^{131}I]MIBG-induced cell kill independently or in combination therapy in PN3 cells. These were derived from MOG cells by introduction of the NAT gene via plasmid mediated transfection. The combination of HSV1716/NAT infection at a MOI of 0.1 or 1pfu/cell followed by [^{131}I]MIBG treatment for 24 hours resulted in a highly statistically significant increase ($P < 0.001$) in cell death after 48 days, compared to that induced by virus or [^{131}I]MIBG treatment alone. When HSV1716/NAT and [^{131}I]MIBG were applied to MOG cells, which have no inherent capacity for uptake of the radiopharmaceutical, the combination therapy again resulted in a statistically significant increase in cell kill ($P < 0.001$) irrespective of the initial infecting dose of HSV1716/NAT. The MOG cell line, which is resistant to uptake of MIBG due to lack of endogenous expression of NAT, provides a more relevant model than PN3 cells of glioma in patients. Our results indicate that the combination of HSV 1716/NAT and [^{131}I]MIBG could be an effective tumour cell killing strategy.

Previous studies assessing the efficacy of tumour cell kill of HSV-1 attenuated vectors in various tumour cell lines *in vitro* have demonstrated variable time dependant survival of cells incubated with virus and/or higher levels of oncolysis at lower MOI. The efficiency of oncolysis in these systems appears to be cell type dependent (Cinatl et al, 2003; Coukos et al, 1999,2000; Bennett et al, 2002; Toyoizumi et al, 1999). In our study utilising the MOG cell line, viral titres were chosen which resulted in suboptimal cell kill resulting in approximately 10-20% cell survival during the 6 day time course after incubation with virus alone. Lower concentrations of virus were therefore used to allow survival of sufficient numbers of cells to examine the combined effect of viral oncolysis and targeted radiation damage which is readily demonstrable after 5-7 replicative cycles. It is expected that the effectiveness of HSV1716-induced lysis of

glioma cells in patients will be restrictive to cycling cells. This limitation to treatment, imposed by proliferative heterogeneity may be overcome by this combination strategy. Even in cells where complete cycles of virus replication do not take place, NAT will still be expressed allowing accumulation of [^{131}I]MIBG and targeted radiation cell kill. Further, as previously demonstrated utilising plasmid transfected three dimensional spheroid models, cells which have not been targeted to express NAT and therefore have no capacity for accumulation of [^{131}I]MIBG can be targeted by radiation cross fire and radiation mediated bystander effects[6].

The acute hypoxia of gliomas probably accounts in part for their resistance to treatment with radiation and anticancer drugs[16]. In recent decades, many efforts have been made to overcome hypoxia-induced resistance by increasing oxygenation, by using radiosensitizers or by the administration of agents which are especially toxic to hypoxic cells. Clinical studies have indicated that enhanced therapeutic benefit can be obtained by such schemes, but none has yet produced a significant, reproducible increase in the therapeutic ratio.

An alternative and potentially beneficial strategy is the tumour-targeted delivery of α-emitting radionuclides such as [^{211}At]astatine. The α-decay particles from this radiohalogen cause localised damage (having a mean range of only six cell diameters) and their high LET (linear energy transfer) quality ensures toxicity which is not compromised by low intracellular oxygen tension[27]. Furthermore, the short, 7.2h half-life of [^{211}At]astatine suggests that it may be particularly appropriate for glioma therapy following its intracerebral administration because most of the radionuclide will have decayed before gaining access to the systemic circulation. Recently, the first clinical study of the therapeutic use of ^{211}At commenced, in Duke Medical Centre, North Carolina. This phase I trial

involves the intra-cavitary injection of ^{211}At-labelled anti-tenascin antibody for the treatment of brain tumours.

The [^{211}At]astatinated benzylguanidine ([^{211}At]MABG) may also be effective in the selective eradication of glioma cells which have been transduced with the NAT gene via the HSV1716/NAT virus. It has been demonstrated that plasmid mediated NAT gene transfer induced similar enhancement of the uptake of [^{131}I]MIBG and [^{211}At]MABG [6]. However, in terms of tumour cell kill, [^{211}At]MABG was more effective than [^{131}I]MIBG by two to three orders of magnitude[25, 10, 6]. It is expected that the utilisation of [^{211}At]MABG rather than [^{131}I]MIBG following HSV1716/NAT administration, would be especially efficacious for treatment of hypoxic tumour regions, thereby improving tumour treatment.

Recent studies have reported synergy between viral mediated gene delivery and ionising radiation. The conditionally replicating ONYX-15 adenovirus, which is thought to replicate selectively in p53 deficient cells, reportedly had a synergistic effect in combination with radiation *in vitro* and *in vivo* [24, 12]. However multiple injections of ONYX-15 virus were required to achieve antitumour effects in xenografts models. This suggests low replication of this adenovirus compared to the proliferation rate of tumours and also that their capacity for efficient transgene expression is short lived[14].

Ionising radiation is a standard treatment option for many malignancies and previous studies have indicated that it increases the lytic activity of HSV1[1, 9, 2]. Several studies have shown enhanced dispersion of virus from inoculation sites and higher viral titres in recovered tumours which had been exposed radiation[1, 2, 7, 9]. Tumour xenografts treated with both radiation and recombinant HSV, also showed enhanced tumour regression compared to either modality alone[1, 2, 7]. Taken

together these results suggest a role for radiotherapy in enhancing the efficacy of HSV-mediated oncolytic therapy in human tumours.

The combination of HSV1716 with a transgene that allows tumour specific uptake of radiopharmaceutical, has a distinct advantage over external beam irradiation in terms of selectivity of damage. The present results *in vitro* for combination therapy provide an improved treatment for malignant glioma. Studies are underway to optimise this therapeutic approach using alternative radiohaloconjugates of benzylguanidine and fractionated radiotherapy.

In vivo validation

To confirm the applicability of this approach the inventors conducted further in vivo investigations. In these investigations various mice tumour xenografts and non-tumour tissues were transfected with HSV1716/NAT and the ability of the transfected tissue to uptake [^{131}I]MIBG was determined together with the therapeutic effect of the combination therapy on tumour cell kill. The results confirm that combination therapy of tumours using HSV1716/NAT and [^{131}I]MIBG provides an effective therapeutic treatment resulting in reduction in tumour size. Moreover, the results indicate that the therapeutic effect may be improved by administering the virus and radiopharmaceutical sequentially, i.e. where the virus is administered first and, after a predetermined time interval which is sufficient to allow the virus to stably infect the cell(s) of the tumour, the radiopharmaceutical is administered.

Biodistribution

This experiment was designed to determine whether HSV1716/NAT conferred the ability to uptake [^{131}I]MIBG to tumour xenografts in mice.

UVW cell lines are a radiation resistant human glioma cell line which does not express NAT[3].

UVW xenografts were prepared and were one of 3 sizes (3x3-4x4mm, 5x5-8x8mm, 8x8-11x11mm)

Xenografts were injected according to the following dosing regime:

Day 1: 10^6 pfu were injected intratumourly into xenografts in 50ml PBS;

One set of mice (subdivided into set 1 and set 2 for the purpose of measuring biodistribution - see below) were then selected for simultaneous treatment and were injected intraperitoneally with 2 MBq ca [^{131}I]MIBG on day 1.

Day 2: Another set of mice (subdivided into set 3 and set 4 for the purpose of measuring biodistribution - see below) were selected for sequential treatment and were injected intraperitoneally with 2 MBq ca [^{131}I]MIBG on day 2.

NB: ca = carrier added

Biodistribution was measured as follows:

- Set 1: simultaneous injection. Biodistribution measured at 24 hours post injection (day 2).
- Set 2: simultaneous injection. Biodistribution measured at 48 hours post injection (day 3).
- Set 3: sequential treatment. Virus injected day 1, [^{131}I]MIBG injected day 2, biodistribution measured 24 hours later (day 3).

- Set 4: sequential treatment. Virus injected day 1, [^{131}I]MIBG injected day 2, biodistribution measured 48 hours later (day 4).

The following material was then collected: Tumour either in its entirety (small tumours) or split into fragments; Liver; Spleen; Adrenal; Kidney; Lung; Heart; UVW only (tumour no virus).

All tissues collected were counted using a gamma counter on day 4 against standards to allow determination of % injected dose.

The results are shown in Figure 5 and show that HSV1716/NAT endows UVW cells in xenograft with the capacity to uptake [^{131}I]MIBG. Better uptake and therefore better NAT gene expression was achieved when the [^{131}I]MIBG was administered sequentially rather than simultaneously with the virus.

In vivo cell kill

This experiment was designed to determine the degree of cell kill obtained using virus + nca [^{131}I]MIBG as measured by xenografts size (a PBS control was included).

The intial experiment used 10^7 pfu intratumoural injection of HSV1716/NAT in 100µl PBS.

All xenografts were in the size range 4x4 -7x7mm. The dosing regime was as follows:

Cage 1: 10^7 pfu intratumour injection in 100µl PBS injected day 1.

Cage 2: 10^7 pfu intratumour injection in 100µl PBS injected day 1 and day 2.

Cage 3: 10^7 pfu intratumour injection in 100µl PBS injected day 1 + 10MBq nca [^{131}I]MIBG injected intraperitoneally on day 2.

Cage 4: 10^7 pfu intratumour injection in 100µl PBS injected day 1 and day 2 and 10MBq nca [^{131}I]MIBG injected intraperitoneally on day 2.

Cage 5: 100µl PBS intratumour injection only on day 1.

Cage 6: 100µl PBS intratumour injection day 1 and 10MBq nca [^{131}I]MIBG injected intraperitoneally on day 2.

Tumours were measured on day 1 and every 2-3 days thereafter.

NB: nca = no carrier added

Results:

We picked a titre of 10^7 pfu as biodistribution studies suggested NAT expression but only in isolated areas of the tumour. We wanted to have NAT expression throughout the tumour, and therefore considered that a higher viral titre would achieve this. However, at this titre, virus alone injections gave complete tumour disappearance in 5/6 mice. Therefore using this titre of virus we were unable to assess the results of the virus + [^{131}I]MIBG.

Therefore the experiment was repeated using lower viral titres of 10^6 and 10^5 pfu and the following groups:

1. 10^6 pfu virus day 1 alone.
2. 10^5 pfu virus day 1 alone.
3. 10^6 pfu virus followed by injection of 10MBq/ml [^{131}I]MIBG (this dose did not sterilise 100% NAT transfected cells) on the same day - simultaneous injection.
4. 10^5 pfu virus followed by injection of 10MBq/ml [^{131}I]MIBG (this dose did not sterilise 100% NAT transfected cells) on the same day - simultaneous injection.
5. 10^6 pfu virus followed by injection of 10MBq/ml [^{131}I]MIBG (this dose did not sterilise 100% NAT transfected cells)

24 hours after virus administration - sequential administration.

6. 10^5 pfu virus followed by injection of 10MBq/ml [^{131}I]MIBG (this dose did not sterilise 100% NAT transfected cells) 24 hours after virus administration - sequential administration.
7. PBS only on day 1 with [^{131}I]MIBG administered 24 hours later.

Results:

This titre of virus did not cause cure of tumour alone. Figure 6 shows the outcome. The two experiments in which virus and [^{131}I]MIBG were administered sequentially resulted in the largest inhibition of tumour growth. The data support the conclusion that the combination of HSV1716/NAT plus [^{131}I]MIBG at doses of each agent which do not cause tumour cure alone allows sterilisation and growth delay of the tumour xenografts, i.e. there is a synergistic effect.

The data also support the conclusion that sequential administration of virus provided an improved therapeutic strategy, and appears to be better than administering both virus and [^{131}I]MIBG at the same time.

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Claims:

1. An herpes simplex virus wherein the herpes simplex virus genome comprises nucleic acid encoding a monoamine transporter.

2. An herpes simplex virus as claimed in claim 1 wherein said monoamine transporter is a noradrenaline transporter.

3. An herpes simplex virus as claimed in claim 1 or 2 wherein said nucleic acid comprises SEQ ID No.2 or 4 or nucleic acid encoding the polypeptide of SEQ ID No.1 or 3.

4. An herpes simplex virus as claimed in claim 1 or 2 wherein said nucleic acid has at least 60% sequence identity to SEQ ID No.2 or 4 or to a nucleic acid encoding the polypeptide of SEQ ID No.1 or 3.

5. An herpes simplex virus as claimed in claim 4 wherein said degree of sequence identity is one of at least 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99%.

6. An herpes simplex virus as claimed in claim 1 or 2 wherein said nucleic acid hybridises to the nucleic acid of SEQ ID No.2 or 4, to the complement of SEQ ID No.2 or 4 or to a nucleic acid encoding the polypeptide of SEQ ID No.1 or 3 under high stringency conditions.

7. An herpes simplex virus according to any one of claims 1 to 6 wherein said herpes simplex virus genome further comprises a regulatory nucleotide sequence operably linked to said nucleic acid encoding a monoamine transporter, wherein said regulatory nucleotide sequence has a role in controlling transcription of said monoamine transporter.

8. An herpes simplex virus as claimed in claim 7 wherein said regulatory nucleotide sequence is inducible.

9. An herpes simplex virus as claimed in any preceding claim wherein the genome of the herpes simplex virus further comprises a marker nucleotide sequence.

10. An herpes simplex virus as claimed in any one of claims 1 to 9 wherein said nucleic acid is located in at least one RL1 locus of the herpes simplex virus genome.

11. An herpes simplex virus as claimed in any one of claims 1 to 10 wherein said nucleic acid is located in, or overlaps, at least one of the ICP34.5 protein coding sequences of the herpes simplex virus genome.

12. An herpes simplex virus as claimed in any one of claims 1 to 11 wherein the herpes simplex virus is a mutant of one of HSV-1 strains 17 or F or HSV-2 strain HG52.

13. An herpes simplex virus as claimed in any one of claims 1 to 11 wherein the herpes simplex virus is a mutant of HSV-1 strain 17 mutant 1716.

14. An herpes simplex virus as claimed in any one of claims 1 to 13 which is a gene specific null mutant.

15. An herpes simplex virus as claimed in any one of claims 1 to 14 which is an ICP34.5 null mutant.

16. An herpes simplex virus as claimed in any one of claims 1 to 13 which lacks at least one expressible ICP34.5 gene.

17. An herpes simplex virus as claimed in any one of claims 1 to 12 which lacks only one expressible ICP34.5 gene.

18. An herpes simplex virus as claimed in any one of claims 1 to 17 which is non-neurovirulent.

19. An herpes simplex virus as claimed in any one of claims 1 to 18 wherein said nucleic acid encoding a monoamine transporter forms part of a nucleic acid cassette integrated in the genome of said herpes simplex virus, said cassette encoding:

(a) said nucleic acid encoding a monoamine transporter; and nucleic acid encoding

(b) a ribosome binding site; and

(c) a marker,

wherein the nucleic acid encoding a monoamine transporter is arranged upstream (5') of the ribosome binding site and the ribosome binding site is arranged upstream (5') of the marker.

20. An herpes simplex virus according to claim 19 wherein a regulatory nucleotide sequence is located upstream (5') of the nucleic acid encoding a monoamine transporter, wherein the regulatory nucleotide sequence has a role in regulating transcription of said nucleic acid encoding a monoamine transporter.

21. An herpes simplex virus according to claim 19 or 20 wherein the cassette disrupts a protein coding sequence resulting in inactivation of the respective gene product.

22. An herpes simplex virus as claimed in any one of claims 19 to 21 wherein a transcription product of the cassette is a bi- or poly-cistronic transcript comprising a first cistron encoding the monoamine transporter and a second cistron encoding the marker wherein the ribosome binding site is located between said first and second cistrons.

23. An herpes simplex virus as claimed in any one of claims 19 to 22 wherein the ribosome binding site comprises an internal ribosome entry site (IRES).

24. An herpes simplex virus as claimed in any one of claims 9 or 19 to 23 wherein the marker is a defined nucleotide sequence encoding a polypeptide.

25. An herpes simplex virus as claimed in claim 24 wherein the marker comprises the Green Fluorescent Protein (GFP) protein coding sequence or the enhanced Green Fluorescent Protein (EGFP) protein coding sequence.

26. An herpes simplex virus according to any one of claims 9 or 19 to 23 wherein the marker comprises a defined nucleotide sequence detectable by hybridisation under high stringency conditions with a corresponding labelled nucleic acid probe.

27. An herpes simplex virus as claimed in any one of claims 19 to 26 wherein the cassette further comprises nucleic acid encoding a polyadenylation sequence located downstream (3′) of the nucleic acid encoding the marker.

28. An herpes simplex virus as claimed in claim 27 wherein the polyadenylation sequence comprises the Simian Virus 40 (SV40) polyadenylation sequence.

29. An herpes simplex virus as claimed in any one of claims 1 to 28 for use in a method of medical treatment.

30. An herpes simplex virus as claimed in any one of claims 1 to 28 for use in the treatment of a cancerous condition.

31. Use of an herpes simplex virus as claimed in any one of claims 1 to 28 in the manufacture of a medicament for the treatment of a cancerous condition.

32. Use in the manufacture of a medicament for the treatment of a cancerous condition of a herpes simplex virus as claimed in any one of claims 1 to 28 and a pharmaceutical capable of being transported by said monoamine transporter.

33. The use of claim 32 wherein said pharmaceutical is meta-[^{131}I]iodobenzylguanidine ([^{131}I]MIBG), [^{123}I]MIBG, [^{125}I]MIBG or meta-[^{211}At]astatobenzylguanidine ([^{211}At]MABG).

34. A method of treating a cancerous condition comprising the step of administering to a patient in need of treatment an herpes simplex virus as claimed in any one of claims 1 to 28.

35. A medicament, pharmaceutical composition or vaccine comprising an herpes simplex virus as claimed in any one of claims 1 to 28.

36. A medicament, pharmaceutical composition or vaccine as claimed in claim 35 further comprising a pharmaceutically acceptable carrier, adjuvant or diluent.

37. An herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a monoamine transporter in at least one of the long repeat regions (R_L).

38. An herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a monoamine transporter and wherein the herpes simplex virus is non-neurovirulent.

39. A composition comprising a herpes simplex virus according to claim 37 or claim 38.

40. A composition comprising a herpes simplex virus according to claim 37 or claim 38 and a pharmaceutical capable of being transported by said monoamine transporter.

41. A composition as claimed in claim 40 wherein said pharmaceutical is meta-[^{131}I]iodobenzylguanidine ([^{131}I]MIBG), [^{123}I]MIBG, [^{125}I]MIBG or meta-[^{211}At]astatobenzylguanidine ([^{211}At]MABG).

42. A kit of parts comprising a first container having a quantity of herpes simplex virus according to any one of claims 1 to 28, 37 or 38 and a second container having a quantity of a pharmaceutical capable of being transported by said monoamine transporter.

43. An herpes simplex virus for use in the treatment of a cancerous condition, wherein the genome of said virus comprises a nucleic acid sequence encoding a monoamine transporter in at least one of the long repeat regions (R_L).

44. An herpes simplex virus for use in the treatment of a cancerous condition, wherein the genome of said virus comprises a nucleic acid sequence encoding a monoamine transporter and wherein the herpes simplex virus is non-neurovirulent.

45. An herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a monoamine transporter in at least one of the long repeat regions (R_L), for use, in combination with a pharmaceutical capable of being transported by said monoamine transporter, in the treatment of a cancerous condition.

46. An herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a monoamine transporter and wherein the herpes simplex virus is non-neurovirulent, for use, in combination with a pharmaceutical capable of being transported by said monoamine transporter, in the treatment of a cancerous condition.

47. Use of an herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a monoamine transporter in at least one of the long repeat regions (R_L), in the manufacture of a medicament for the treatment of cancerous condition.

48. Use of an herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a monoamine transporter and wherein the herpes simplex virus is non-neurovirulent, in the manufacture of a medicament for the treatment of a cancerous condition.

49. Use in the manufacture of a medicament for the treatment of a cancerous condition of a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a monoamine transporter in at least one of the long repeat regions (R_L), and a pharmaceutical capable of being transported by said monoamine transporter.

50. Use in the manufacture of a medicament for the treatment of a cancerous condition of a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a monoamine transporter and wherein the herpes simplex virus is non-neurovirulent, and a pharmaceutical capable of being transported by said monoamine transporter.

51. Use of an herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a monoamine transporter in at least one of the long repeat regions (R_L) in the manufacture of a first medicament for administering sequentially or simultaneously with a second medicament comprising a pharmaceutical capable of being transported by said monoamine transporter in the treatment of a cancerous condition.

52. The use of claim 51, wherein the treatment comprises administering the first medicament to the patient and, following a predetermined time interval, sequentially administering the second medicament to the patient.

53. Use of a pharmaceutical capable of being transported by a monoamine transporter in the manufacture of a first medicament for administering sequentially or simultaneously with a second medicament comprising a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a monoamine transporter capable of transporting said pharmaceutical in at least one of the long repeat regions (R_L), in the treatment of a cancerous condition.

54. Use of a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a monoamine transporter and wherein the herpes simplex virus is non-neurovirulent, in the manufacture of a first medicament for administering sequentially or simultaneously with a second medicament comprising a pharmaceutical capable of being transported by said monoamine transporter, in the treatment of a cancerous condition.

55. The use of claim 52, wherein the treatment comprises administering the first medicament to the patient and,

following a predetermined time interval, sequentially administering the second medicament to the patient.

56. Use of a pharmaceutical capable of being transported by a monoamine transporter in the manufacture of a first medicament for administering sequentially or simultaneously with a second medicament comprising a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a monoamine transporter capable of transporting said pharmaceutical and wherein the herpes simplex virus is non-neurovirulent, in the treatment of a cancerous condition.

57. A method for the treatment of a cancerous condition comprising the steps of:

(i) administering to a patient in need of treatment a therapeutically effective amount of a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a monoamine transporter in at least one of the long repeat regions (R_L); and

(ii) administering to said patient a therapeutically effective amount of a pharmaceutical capable of being transported by said monoamine transporter.

58. A method for the treatment of a cancerous condition comprising the steps of:

(i) administering to a patient in need of treatment a therapeutically effective amount of a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a monoamine transporter and wherein the herpes simplex virus is non-neurovirulent; and

(ii) administering to said patient a therapeutically effective amount of a pharmaceutical capable of being transported by said monoamine transporter.

59. The use of claim 57 or 58 wherein step (ii) is performed at a predetermined time interval after step (i).

60. A method of expressing a monoamine transporter in vitro or in vivo, said method comprising the step of infecting at least one cell or tissue of interest with a herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a monoamine transporter in at least one of the long repeat regions (R_L) operably linked to a transcription regulatory sequence.

61. A method of expressing a monoamine transporter in vitro or in vivo, said method comprising the step of infecting at least one cell or tissue of interest with a non-neurovirulent herpes simplex virus, wherein the genome of said virus comprises a nucleic acid sequence encoding a monoamine transporter operably linked to a transcription regulatory sequence.

62. A cell, in vitro, infected with a herpes simplex virus according to any one of claims 1 to 28, 37 or 38.

63. A kit, herpes simplex virus, use or method according to any one of claims 37 to 61 wherein said monoamine transporter is a noradrenaline transporter.

64. A kit, herpes simplex virus, use or method according to any one of claims 37 to 61 wherein said pharmaceutical is meta-[^{131}I]iodobenzylguanidine ([^{131}I]MIBG), [^{123}I]MIBG, [^{125}I]MIBG or meta-[^{211}At]astatobenzylguanidine ([^{211}At]MABG).

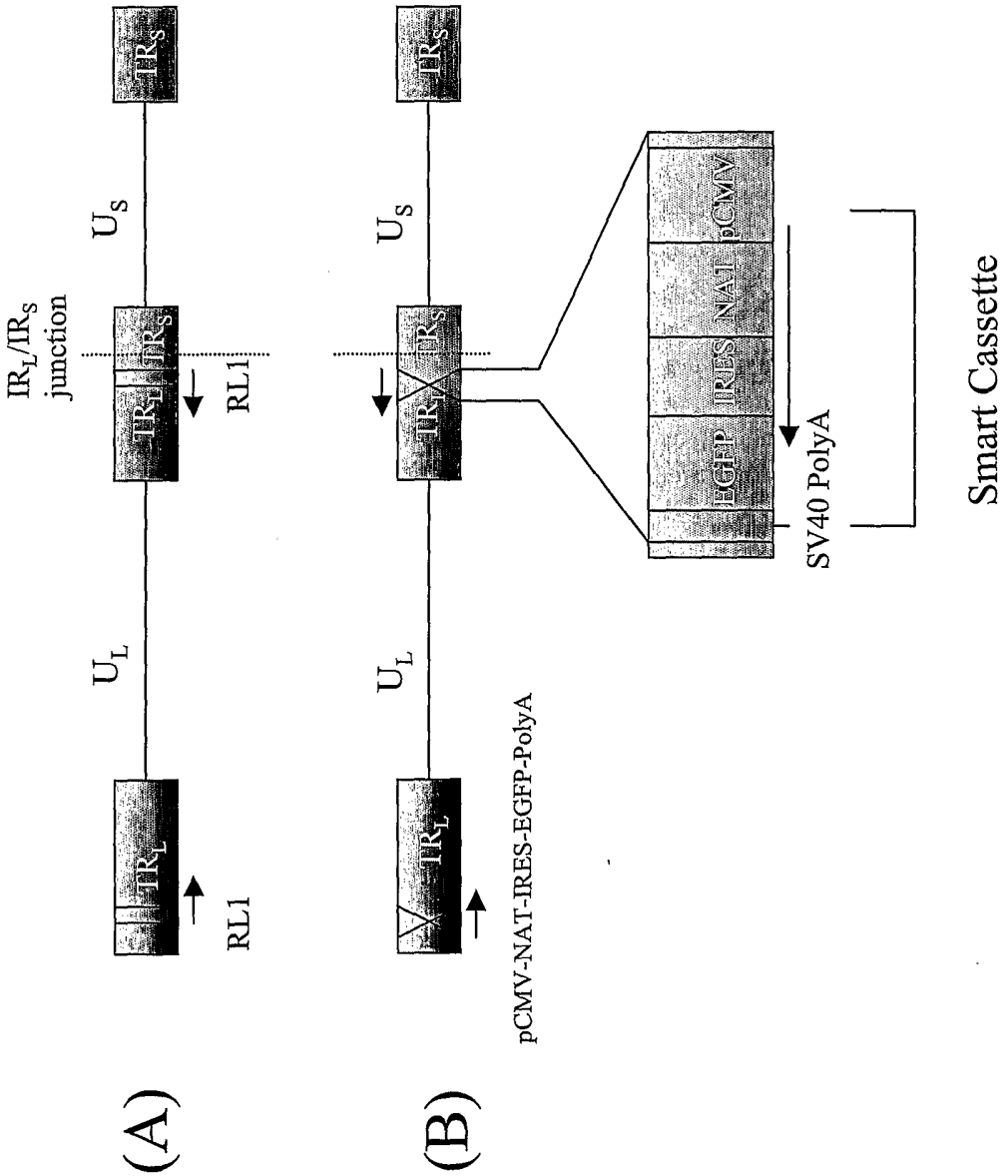
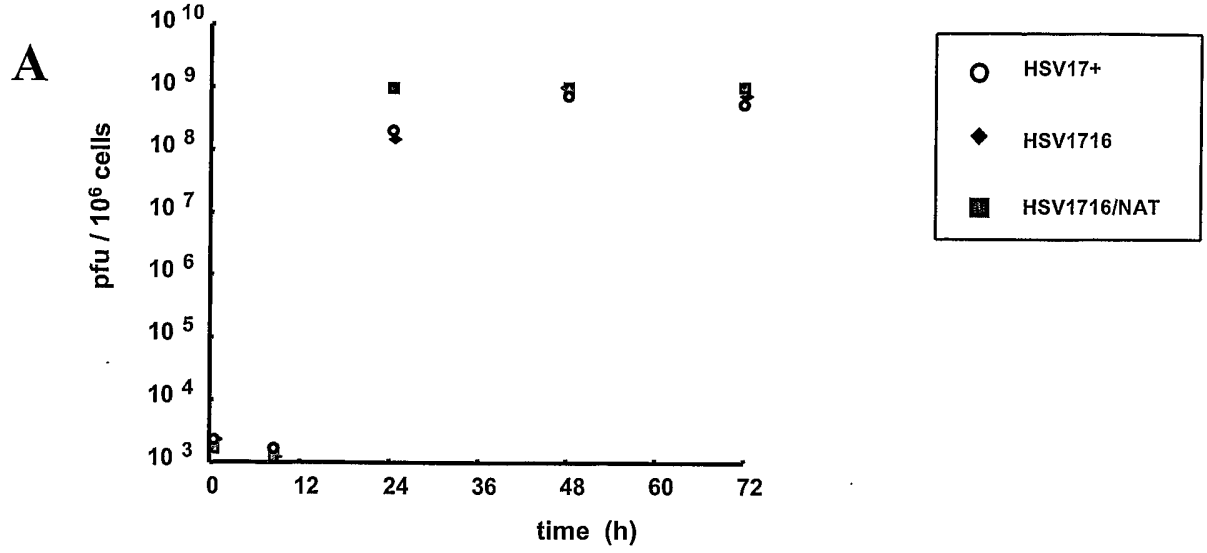

(A)
(B)
IR_L/IR_S junction
U_S
U_L
TR_S
IR_S
TR_L
IR_L
RL1
pCMV-NAT-IRES-EGFP-PolyA
pCMV
NAT
IRES
EGFP
SV40 PolyA
Smart Cassette

Figure 1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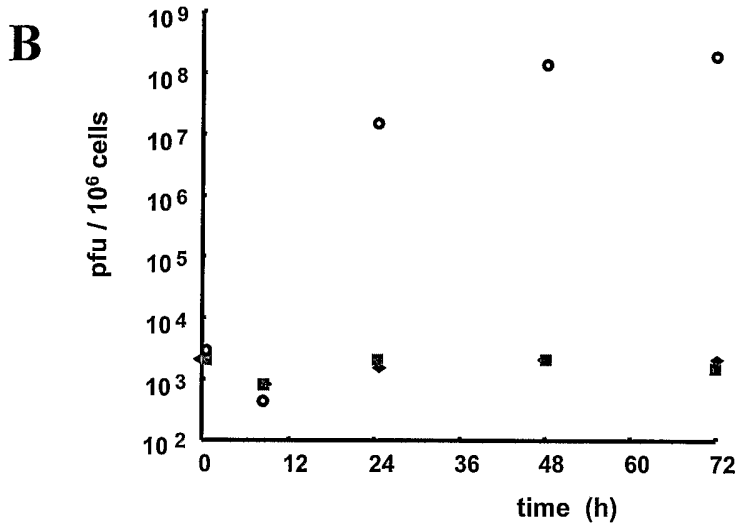
pfu / 10^6 cells
10^10
10^9
10^8
10^7
10^6
10^5
10^4
10^3
0
12
24
36
48
60
72
time (h)
HSV17+
HSV1716
HSV1716/NAT


B
pfu / 10^6 cells
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
0
12
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36
48
60
72
time (h)

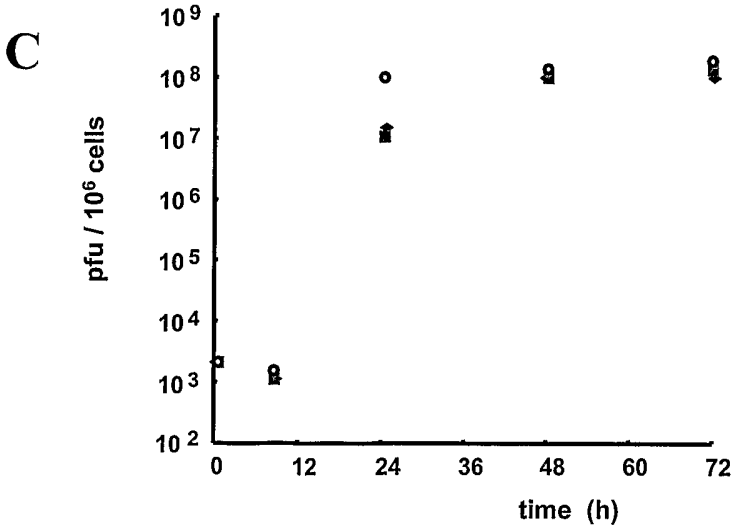
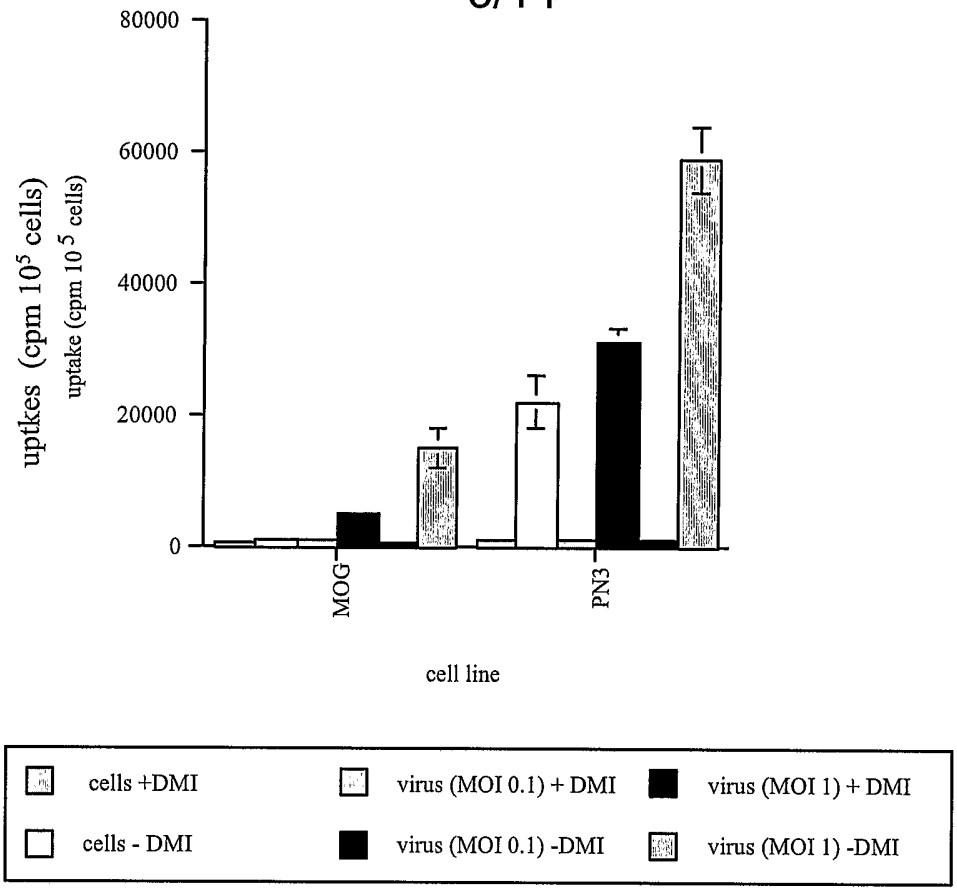

C
pfu / 10^6 cells
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
0
12
24
36
48
60
72
time (h)

Figure 2

A


80000
60000
40000
20000
0
uptkes (cpm 10^5 cells)
uptake (cpm 10^5 cells)
MOG
PN3
cell line
cells +DMI
cells - DMI
virus (MOI 0.1) + DMI
virus (MOI 0.1) -DMI
virus (MOI 1) + DMI
virus (MOI 1) -DMI

B

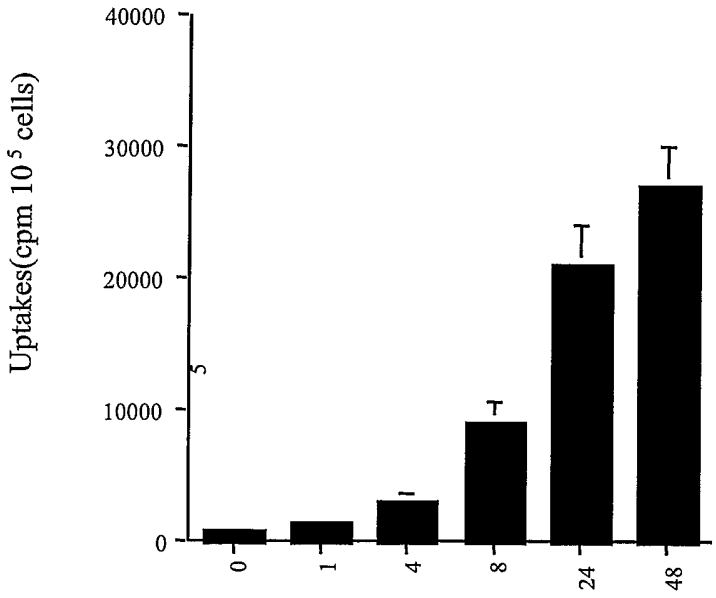

40000
30000
20000
10000
0
Uptakes(cpm 10^5 cells)
0
1
4
8
24
48
hours post-virus infection

Figure 3

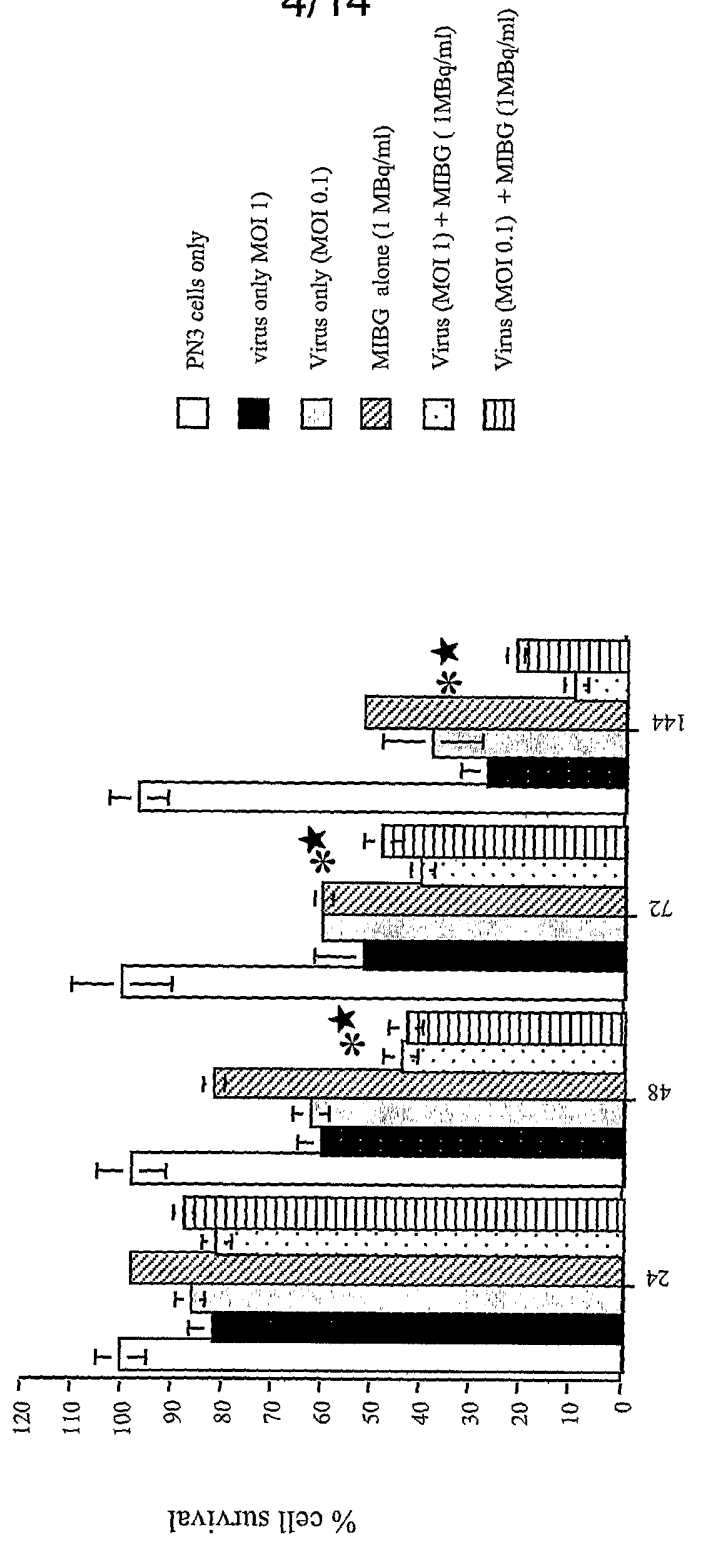

PN3 cells only
virus only MOI 1)
Virus only (MOI 0.1)
MIBG alone (1 MBq/ml)
Virus (MOI 1) + MIBG (1MBq/ml)
Virus (MOI 0.1) + MIBG (1MBq/ml)
% cell survival
120
110
100
90
80
70
60
50
40
30
20
10
0
24
48
72
144
time (hours)

Figure 4 (A)

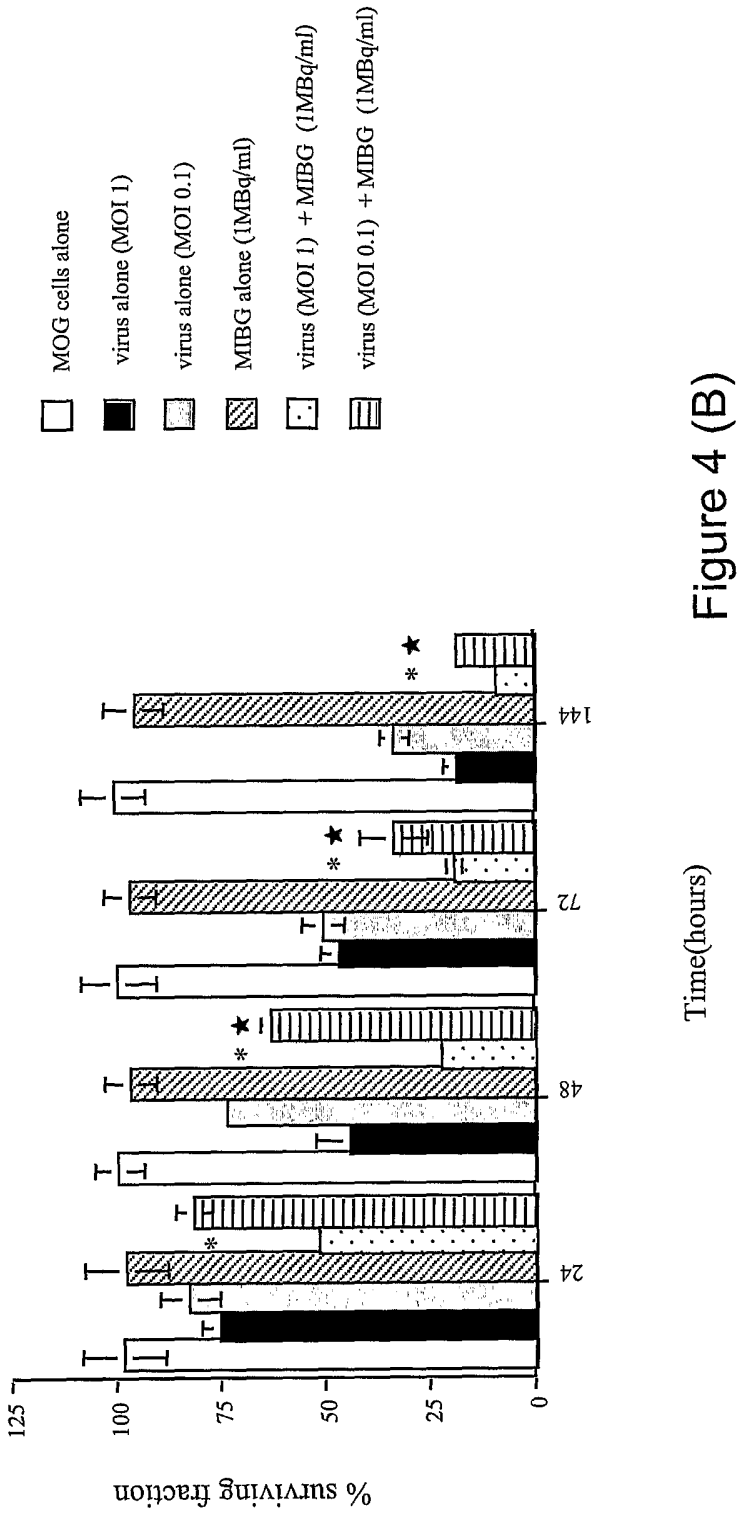

MOG cells alone
virus alone (MOI 1)
virus alone (MOI 0.1)
MIBG alone (1MBq/ml)
virus (MOI 1) + MIBG (1MBq/ml)
virus (MOI 0.1) + MIBG (1MBq/ml)
% surviving fraction
125
100
75
50
25
0
24
48
72
144
Time(hours)

Figure 4 (B)

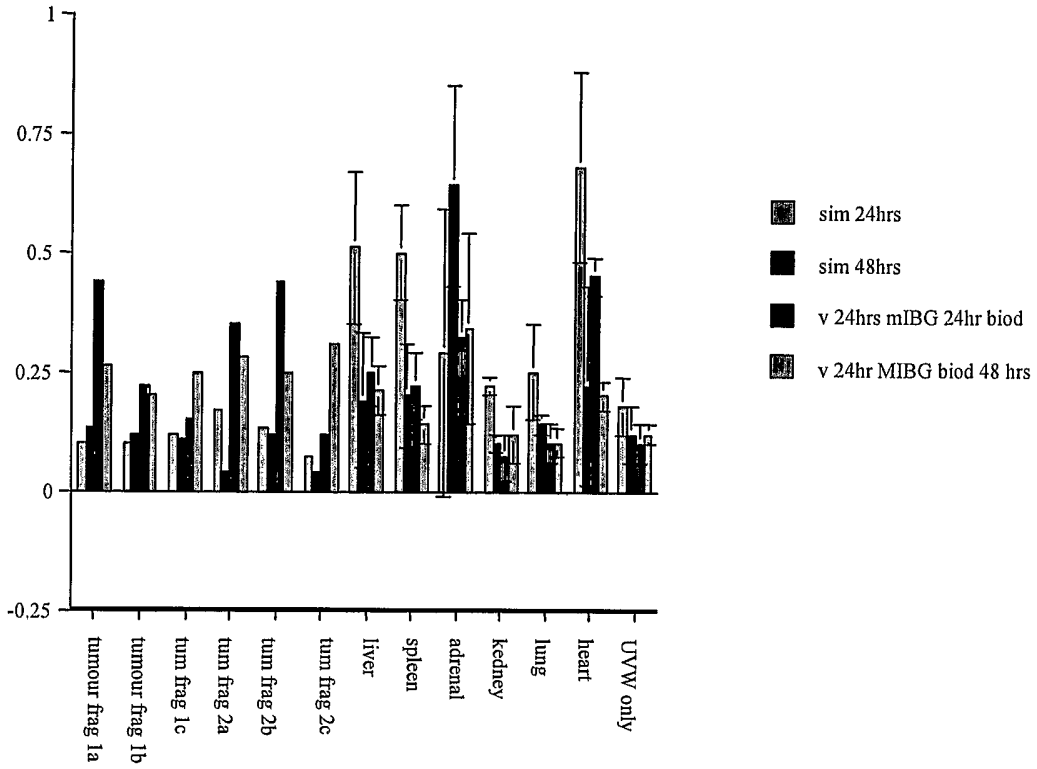

1
0.75
0.5
0.25
0
-0.25
tumour frag 1a
tumour frag 1b
tum frag 1c
tum frag 2a
tum frag 2b
tum frag 2c
liver
spleen
adrenal
kedney
lung
heart
UVW only
sim 24hrs
sim 48hrs
v 24hrs mIBG 24hr biod
v 24hr MIBG biod 48 hrs

Figure 5

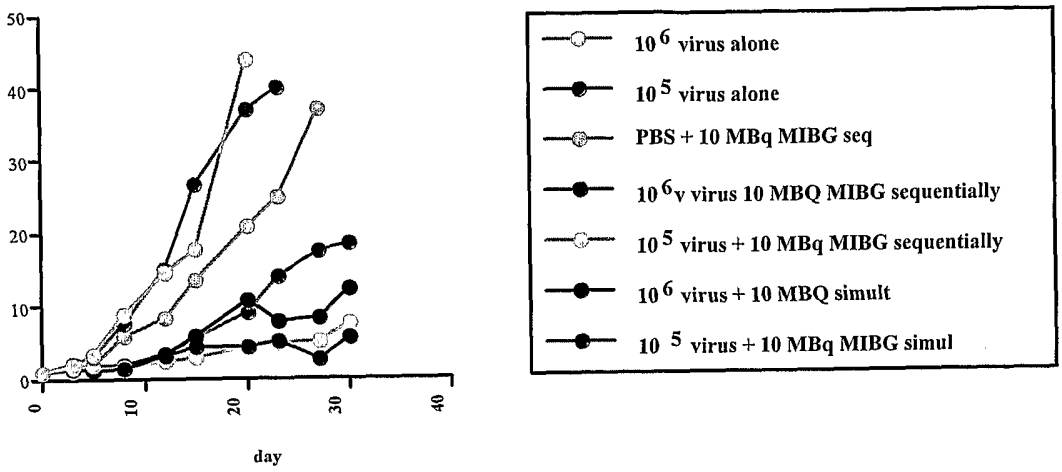

50
40
30
20
10
0
0
10
20
30
40
day
10 6 virus alone
10 5 virus alone
PBS + 10 MBq MIBG seq
10 6v virus 10 MBQ MIBG sequentially
10 5 virus + 10 MBq MIBG sequentially
10 6 virus + 10 MBQ simult
10 5 virus + 10 MBq MIBG simul

Figure 6

SEQ ID No. 1

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LFSSFTPTLPWTDCGHAWNSPNCTDPKLLNSSVLGNHTKYSKYKFTPAAEFYERGVLH

LHESSGIHDIGLPQWQLLLCLIIVVIVLFFSLWKGVKTSGKVVWITATLPYLVLFVLL

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Figure 7

SEQ ID No.2

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Figure 8

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Figure 8 continued

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Figure 8 continued

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Figure 9

SEQ ID No.4

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Figure 10