Antitumor Activity of a Selectively Replication Competent Herpes Simplex Virus (HSV) with Enzyme Prodrug Therapy

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Abstract. *Background: HSV1790 is an oncolytic virus generated by inserting the enzyme nitroreductase (NTR) into the virus HSV1716. NTR converts the prodrug CB1954 into an active alkylating agent. Materials and Methods: In vitro, 3T6 cells (non permissive to HSV) were used in order to distinguish between virus-induced cytopathic effect and cell death due to activated prodrug. In vivo, xenograft models were injected with HSV1790 (10^5-10^9 PFU) with or without CB1954 (max 80mg/kg) and tumor volume recorded regularly. Biodistribution of HSV1790 was determined immunohistochemically and by PCR. Results: HSV1790 + CB1954 in vitro was more effective at killing tumor cells than the virus or the prodrug alone. In vivo, the combination reduced tumor volume and increased survival compared to treatment with HSV1790 or CB1954 alone. Following systemic administration of HSV1790, viral replication was detected in tumors, but not organs. Conclusion: HSV1790 + prodrug enhances tumor cell killing in vitro and reduces tumor volume and increases survival in vivo.*

Herpes simplex virus type 1 (HSV-1) has a number of pertinent characteristics that support its use in cancer therapy. It infects a broad range of cell types, it is cytolytic by nature and it has a well characterized and, in the case of Glasgow strain 17+, a fully sequenced genome (1). Furthermore, its large genome (152 kb) contains non essential genes that can be replaced by therapeutic transgenes of up to 30 kb (2).

HSV1716 is a selectively replication competent mutant of HSV-1 in which both copies of the *RL1* gene have been deleted (3). The *RL1* gene encodes the virulence factor ICP34.5. *ICP34.5* null mutants of HSV are selectively replication competent in, and lyse, rapidly dividing tumor cells but not growth arrested or terminally differentiated cells (4). HSV1716 has demonstrated selective tumor cell killing, with minimal toxicity, and its administration has resulted in improved survival in a number of xenograft tumor models in mice, including glioma (5), melanoma (6-8), mesothelioma (9), ovarian (10), lung (11, 12) and breast (13) carcinomas. Clinical trials of intra-lesional administration of HSV1716 in patients with glioma, melanoma and squamous cell carcinoma of the head and neck have been performed (14-17) and have demonstrated the safety of this approach, and provided evidence that the virus is capable of directly destroying human tumor cells while leaving normal cells intact.

One potential limitation of HSV1716 for the treatment of human tumors is that the virus may not infect and destroy all cells within the tumor. One strategy to overcome this limitation is to engineer the HSV1716 virus to express genes with therapeutic potential. Enhanced antitumor activity with HSV viruses engineered to express thymidine kinase (18) or cytosine deaminase (19) have been reported.

A second-generation virus (HSV1790) has been generated and characterized which contains the *Escherichia coli* nitroreductase (*ntr*) gene inserted into the RL1 locus of the HSV1716 genome. *E. Coli* nitroreductase (NTR) converts the pro-drug CB1954 [5 (aziridin-1-yl)-2,4-dinitrobenzamide] from a mono-functional alkylating agent, which is poorly metabolized in human cells and has low toxicity, into its active form which is a functional cytotoxic alkylating agent that introduces poorly repaired inter-strand cross-links into DNA resulting in cell killing independently of the cell cycle state (20). In addition, the active metabolite is diffusible and membrane permeable and this results in an efficient bystander effect (21, 22).

It is hypothesized that the selectively replicating HSV engineered to express *ntr* would activate CB1954 within the tumor to produce a cytotoxic effect of the active metabolite, and would achieve bystander tumor cell killing, in addition to the oncolytic effects of the virus itself, thereby enhancing antitumor efficacy.

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This study reports that the combination of HSV1790 and the prodrug CB1954 enhanced tumor cell killing *in vitro* and increased tumor reduction and improved survival *in vivo* compared to the oncolytic effects of HSV1790 alone. Preliminary evidence which suggests that the virus localises to the tumor after systemic administration is also presented.

Materials and Methods

Construction of HSV-1 recombinant virus expressing ntr. The plasmid pPS949, containing the *ntr* gene downstream of the CMV IE promoter (pCMV-NTR) in a pLNCX (Clonetech) backbone, was a donation from Professor Lawrence Young (University of Birmingham, UK). The pCMV-NTR fragment was excised from pPS949 and cloned into Bg1II digested, CIP treated RL1.dIRES-GFP and recombinant virus produced as previously described (23).

Western Blotting. Cell lines to be infected with HSV 1716 and HSV1790 were plated out in 35 mm plates and incubated for 24 h at 37˚C in 5% CO_2 before infection. For harvesting, cells were washing once with PBS and whole cell extracts obtained by the direct addition of 0.2 ml SDS-PAGE sample buffer. After SDS-PAGE and transfer to nitrocellulose membranes, blots were probed with the polyclonal NTR-specific antibody at 1/1,000. The NTR antibody was kindly donated by Professor Lawerence Young.

Cell culture. In all, nine different cell lines were used. BHK cells were grown in Eagle's medium with 10% NCS and 10% (v/v) TPB. For virus titrations and plaque purification EMC10 (Eagle's medium containing 1.5% methylcellulose and 10% NCS) was used to overlay the cells. Mouse embryo fibroblast 3T6 cells were obtained from the ECACC, C8161 human metastatic melanoma cell line was supplied by Professor Rona MacKie, the VM spontaneous mouse astrocytoma cell line was supplied by Dr E. McKie, both from the University of Glasgow, UK. The ovarian cell lines A2780 and CP70 (obtained from ECACC) were cultured in RPMI-1640 medium + 10% FCS. Cell cultures were incubated at 5% CO_2 / 95% O_2 at 37˚C. The squamous cell carcinoma cell line A431, cervical carcinoma cell line C33a and the glioma line U373MG (all obtained from the ATCC), 3T6, VM and C8161 were cultured in DMEM +10% FCS and incubated at 10% CO_2 / 90% O_2 at 37˚C. All cultures were tested for *Mycoplasma* using VenorGeM® Mycoplasma PCR Detection Kit (Cambio, Cambridge, UK). Virus stocks were grown and titrated in BHK cells as previously described (24).

In vivo tumor reduction and biodistrubution studies. Female 6- to 8-week-old athymic nude mice (Charles River Labs, UK) were maintained under specific pathogen-free conditions. Actively growing cells were harvested and resuspended in PBS. Cells (1×10^7 for A2780 and A431, 5×10^6 for CP70 in 200 μL PBS) were injected subcutaneously into the right flank. The mice were monitored regularly for signs of tumor formation. Once the tumors reached approximately 5 mm in diameter (10-20 days post cell injection depending on the cell line) the mice were randomized into groups to commence treatment. Virus was given by either intratumoural (1×10^6 PFU in 100 μL PBS on 2 occasions) or intravenous injection (1×10^7 PFU in 100 μL). CB1954 (Sigma) was resuspended in 10% acetone/90% Arachais oil and injected intraperitoneally at 80 mg/kg on up to 3 occasions).

Statistical analysis. Data are expressed as means±s.e.m. Statistical significance of differences was determined by unpaired Student's *t*-tests. *P* values of <0.05 were considered significant.

Immunohistochemistry. Selected internal organs and xenograft tumor samples were removed immediately after sacrifice of the mice and fixed for at least 24 h before embedding and sectioning using standard procedures. Briefly, paraffin embedded sections were dewaxed, dehydrated and endogenous peroxidase was quenched. Non-specific binding was reduced with 10% normal goat serum before incubation with primary antibody (HSV-1 polyclonal, Dako 1:1,000). A biotinylated secondary antibody (Vector anti rabbit Elite kit 1: 500) and avidin biotin complex (ABC) solution (Elite Kit, Vector Laboratories, Peterborough, UK) were used and DAB (Vector Laboratories) was used as the chromogen. The slides were counterstained, dehydrated, mounted and visualized using a light microscope. Omission of primary antibody and uninfected tumor sections constituted the negative controls.

Extraction of RNA and DNA from tissues. *RNA:* Tissue samples were collected from selected internal organs and tumors at the time of sacrifice of the mice and frozen at –70˚C. A small (<0.5 g) tissue sample was resuspended in buffer and the cells were disrupted with a Retsch MM200 homogenieizer. RNA was extracted using the Promega (SV) RNA extraction kit following the manufacturers recommended procedure (Promega, Southampton, UK). The reverse transcriptase reaction was preformed using ImProm Reverse transcriptase kit (Promega) under the manufacturer's recommended conditions.

DNA: Tissue (<0.5 g) samples were homogenized using a Retsch MM200 homogenizer and DNA extracted using Nucleon ST DNA extraction Kit (Thistle Scientific Ltd, Glasgow, UK) following the manufacturer's instructions.

Polymerase Chain Reaction. PCRs were carried out with Reddy-Mix (Abgene, Surrey, UK) and 1 μL of template in a 20 μL reaction volume. For HSV PCR, the primers HS13 (ACG ACG ACG TCC GAC GGC GA) and HS14 (GTG CTG GTG CTG GAC GAC AC) (34) were used. PCR conditions were 94˚C for 2 mins then 34 cycles of 94˚C for 15 s; 72˚C for 1 min; 72˚C for 1 minute and a final extension step at 72˚C for 2 minutes. The PCR product was 278 bp. For NTR: Upstream primer 5-CTTTCACATTGAGTC ATTATGG-3 and downstream primer 5-TTACACTTCGGTTAA GGTGATG-3 were used (25). Following initial denaturation at 94˚C for two minutes, PCR conditions were 95˚C for 30 s, 55˚C for 30 s and 72˚C for 60 s, for 32 cycles.

Results

To demonstrate that HSV1790 expresses the NTR protein, a Western blot was performed. Although the recombinant virus strongly expressed GFP (observed during the plaque purification process) indicating that it should express NTR, this was not considered to be conclusive proof. Four cell lines: BHK, C8161, VM and 3T6: were infected with 10 pfu/cell HSV1716, HSV1790, $HSV17^+$ or were mock infected. Sixteen hours post infection, the cells were harvested and protein extracts analyzed in a Western blot using a polyclonal NTR-specific antibody. Significant NTR

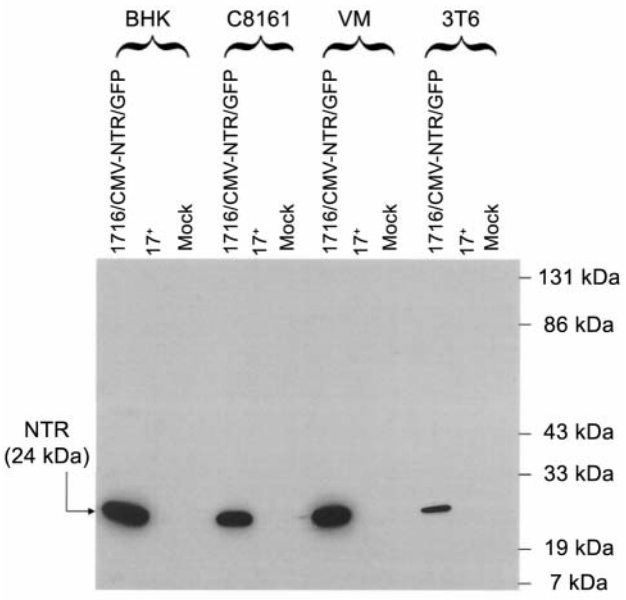


Figure 1. *Western blot analysis of NTR expression in HSV1716/CMV-NTR/GFP infected cell lines.*

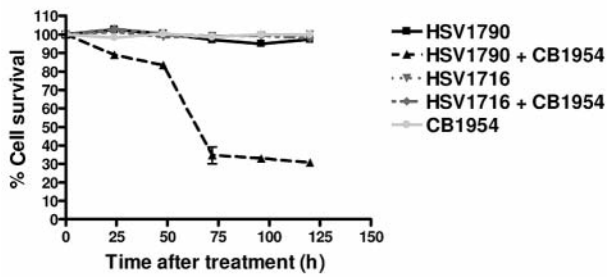


Figure 2. *Effect of HSV1790 and HSV1716 with or without CB1954 (50 μM) on confluent 3T6 cells.*

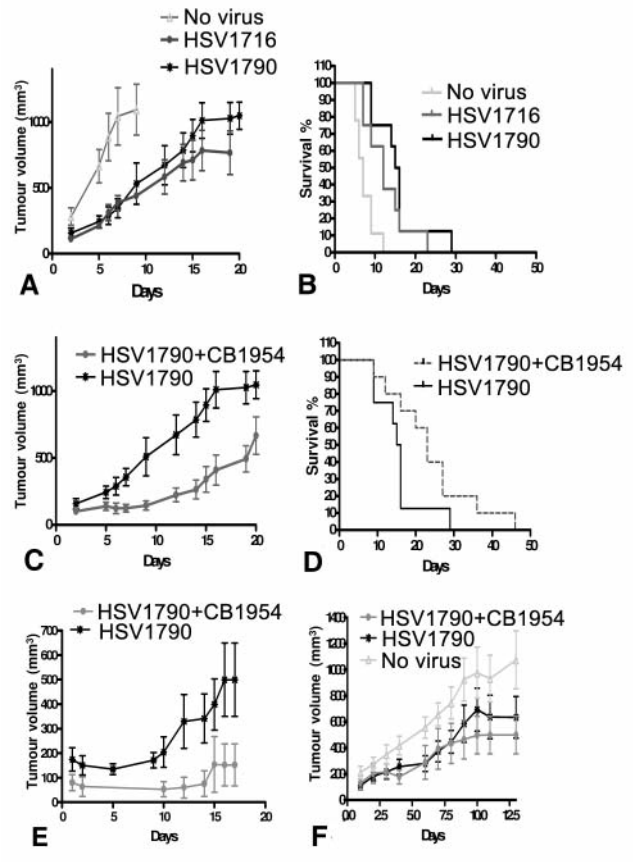
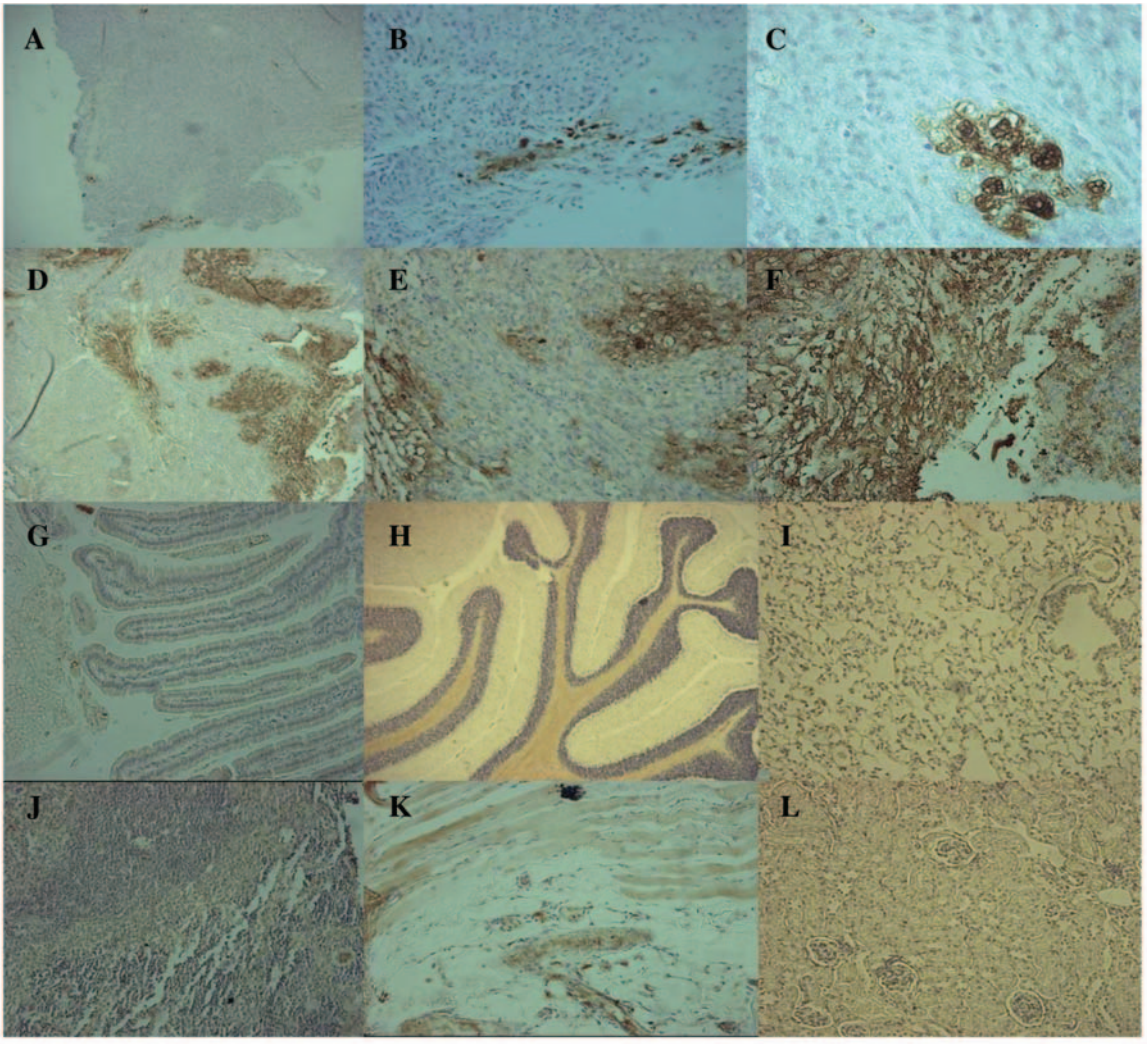


Figure 3. *Oncolytic potential of HSV1790. (A) A431 human tumour xenograft model mice were treated with HSV1790, HSV1716or PBS only as a control. (B) KM survival curve of A431 tumour-bearing mice treated with HSV1716, HSV1790 or PBS only control. (C) Tumour growth curve of A431 human xenograft tumours treated with HSV1790 ± CB1954. (D) KM survival curve of A431 tumour-bearing mice treated with either HSV1790 or HSV1790 + CB1954. (E) Tumour growth curve of A2780 human xenograft tumours treated with HSV1790 ± CB1954. Mice treated with HSV1790 and prodrug (CB1954) had smaller tumour burdens to mice treated with HSV1790 alone (n=3). (F) Tumour growth curve of CP70 human xenograft tumours treated with HSV1790 ± CB1954. Mice treated with HSV1790 and prodrug (CB1954) had similar tumour burdens to mice treated with HSV1790 alone.*

expression was detected in all the HSV1716/CMV-NTR/GFP infected cells. No NTR expression was detected in the mock or HSV17^{+} infected cells. Figure 1 shows that the 24 kDa NTR protein was expressed in all the cell lines infected with HSV1790.

Enhanced cell kill in vitro in HSV1790 infected cells treated with CB1954. Previous experiments had shown that the replication kinetics of HSV1790 were identical to those of the parental strain (P. Dunn, Ph. D. thesis, University of Glasgow, 2003). To determine whether NTR protein expression would result in enhanced cell killing after HSV1790 infection and addition of the prodrug CB1954, cytotoxicity assays were performed in 3T6 cells, a cell line in which the parental HSV1716 did not replicate efficiently (24) and which would allow the distinction of cell killing due to viral replication from cell lysis due to virus-directed enzyme prodrug activation. As almost no HSV1790 replication occurs in 3T6 cells infected at a low MOI (>0.1) and addition of 50μM CB1954 alone had previously been shown to result in less than 5% cell death (data not shown), any significant cell death observed in the cells infected with HSV1790 and with the subsequent addition of CB1954 would be due to the activation of CB1954 into its active form by NTR. Figure 2 shows the effect of HSV1790 and HSV1716 with or without CB1954 on confluent 3T6 cells. Confluent 3T6 cells were infected with 1 pfu/cell HSV1790, HSV1716 or mock infected. After 45 minutes, the infected



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	HSV DNA Day 1	HSV DNA Day 7	NTR DNA Day 1	NTR DNA Day 7	HSV RNA Day 1	HSV RNA Day 7	NTR RNA Day 1	NTR RNA Day 7
Tumour	+	+++	-	+++	++	+++	-	++
Blood	-	-	-	-	NI	NI	-	NI
Brain	-	-	-	-	-	-	-	-
Skin	-	-	-	-	-	-	-	-
Lung	-	-	-	-	-	-	-	-
Kidney	-	-	-	-	-	-	-	-
Gut	-	-	-	-	-	-	-	-
Spleen	+++	-	-	-	-	-	-	-
Heart	-	-	-	-	-	-	-	-
Liver	-	-	-	-	-	-	-	-

Figure 4. *Immunohistochemical staining of UVW xenograft tumours using an HSV antibody. (A) ×5 Tumour day 1 post i.v. viral injection, (B) ×20, (C) ×40. (D) ×5 magnification Day 7 post i.v. HSV1790 injection. (E, F) ×20 magnification of areas from (D). (G) Gut villi. (H) Cerebellar cortex. (I) Lung. (J) Spleen. (K) Skin. (L) Liver. (M) PCR results from samples of major organs and xenograft tumours following i.v injection of HSV1790. Key: +++ very strong band, ++ strong band, + weak band, - no band (RNA only), NI non informative, sample contaminated with DNA.*

cells were overlaid with media containing CB1954 or with media alone and incubated at 37˚C. At 24, 48, 72, 96 and 120 hours, % cell survival (y axis) was determined relative to that of mock infected cells without prodrug using CellTiter 96 Aqueous Solution Cell Proliferation Assay (Promega, UK). The experiment was performed in triplicate and the mean of 3 values±SEM is shown. One pfu/cell of either HSV1716 or HSV1790 alone failed to result in significant cell death. However, when 50 μM CB1954 was included in the overlay, significant cell death was observed in cells infected with HSV1790 (75% at 120 hours) but not in cells infected with HSV1716 (2% at 120 hours).

HSV1790 toxicity and efficacy in vivo. To examine the oncolytic potential of HSV1790 compared to the parental virus, HSV1716, either HSV1716 or HSV1790 (1×10^6 pfu) was administered intra-tumorally on 2 occasions to mice bearing A431 xenografts. Administration of either virus resulted in significantly smaller tumors than the PBS control [day 9: HSV1716, mean volume = 440±26 mm^3 (p=0.013); HSV1790, mean volume = 508±142 mm^3 (p=0.017); PBS, mean volume = 1092±193 mm^3; n=6] (Figure 3A) and improved survival (p<0.05, log rank test) (Figure 3B). There was no discernable difference between the antitumor efficacy of HSV1790 and HSV1716.

To determine the efficacy of HSV1790 + CB1954 in treating established subcutaneous xenografts, serial tumor volume measurements were taken regularly after intra-tumoral administration of HSV1790 (1×10^6 pfu on two occasions), HSV1790 + CB1954 (80 mg/kg intra-peritoneally on three occasions) or control vehicle (PBS alone). Administration of CB1954 alone was not performed as previous experiments had shown that CB1954 administration as a single agent has no antitumoral effect (data not shown). CB1954 was administered by intraperitoneal injection 48 h after administration of virus (max. 80 mg/kg in 200 μL on 3 occasions). Administration of HSV1790 and CB1954 had no effect on body weight (data not shown) and there were no signs of toxicity in the mice. In mice bearing A431 (Figure 3C) xenografts there was a significant reduction in tumor volume following administration of HSV1790 + CB1954 compared to HSV1790 alone (p-value at day 9=0.014: HSV1790 + CB1954, mean volume=144±34 mm^3; HSV1790 alone, mean volume=508±142 mm^3, n=7). Furthermore, the HSV1790 + CB1954 treatment group survived significantly longer than the group treated with HSV1790 only (Figure 3D) (p<0.05 log rank test: median survival HSV1790+ CB1954=23 days; mean survival, HSV179 =15.5days.)

A similar reduction in tumor volume following administration of both HSV1790 + CB1954 compared to HSV1790 alone was observed in athymic mice bearing A2780 tumours (Figure 3E) (HSV1790+CB1954, mean volume=152±85 mm^3; HSV1790, mean volume=499±149 mm^3). However, because of the low tumor take rate with this cell line, this difference did not reach statistical significance due to the small sample size in each group (n=3). In mice bearing CP70 tumor xenografts, administration of HSV1790 reduced tumor volume compared to control mice (Figure 3F) but the addition of CB1954 had no further anti-tumor effect (HSV1790+CB1954, mean volume=551± 159 mm^3); HSV1790, mean volume=662±157 mm^3; [n=6]).

Tumor and organ distribution of HSV1790 after systemic (intravenous) administration. To explore the tumor and organ distribution of HSV1790 after systemic (*i.v.*) administration, 1×10^7 pfu of HSV1790 was administered by tail vein injection to athymic nude mice bearing established UVW and A431 tumor xenografts overlying their right hind flank. Mice were sacrificed on day 1 or day 7 and blood samples, tumor samples and major organs were harvested. Samples of tumor tissues and selected internal organs were analysed by PCR and immunohistochemistry for the presence of HSV1790. Immunohistochemical staining with an anti-HSV antibody demonstrated the presence of the virus within the xenograft tumors (Figure 4A-F). Figures 4 A (×5 magnification) and Figure 4B (×20), and Figure 4C (×40) are tumour 1 day post *i.v.* viral injection and show small areas of cells positive for HSV. Figure 4C shows an area with a typical HSV infection – giant multi-nucleated cells have formed holes, where cells have been killed. Figures 4D, E and F are tumour at day 7 post *i.v.* injection. The areas positive for HSV are much larger than at Day 1. Necrosis was also widespread in the areas of positive staining, with cell debris visible (Figure 4 F). There was no indication of positive staining in any other organ (Figure 4G-L) with the exception of skin, in which some positive staining was visible in cells below the dermal and epidermal layer (Figure 4K).

DNA and RNA from tumor tissues and from selected internal organs were also analysed by PCR to determine the presence of virus and the replication of the virus within these tissues (Figure 4M). Both viral DNA and RNA were detected at low levels in the tumor tissue at day 1 following *i.v.* administration of HSV1790. By day 7 following *i.v.* injection of HSV1790, the PCR band intensity had increased demonstrating that HSV was replicating within the tumor tissue. Furthermore *ntr* DNA and RNA were detectable in the tumor demonstrating that HSV1790 was replicating within the tumor and was also producing the NTR protein. A high level of viral DNA was seen in the spleen at day 1 following *i.v.* administration of HSV1790. However, there was no replicating virus detectable by RT-PCR. This suggests that the virus was not actively replicating and the positive PCR result was likely due to splenic clearance of the virus from the circulating blood.

Discussion

The safety and potential efficacy of HSV1716, a selectively replication competent ICP34.5 null mutant of HSV-1 have been previously described, both in *in vivo* laboratory experiments (5-13) and in Phase I clinical studies in humans (14-17). A second-generation virus, HSV1790, which contains the *E. coli* nitroreductase (*ntr*) gene inserted into the RL1 locus of the HSV1716 genome was generated and characrterized. In this study it has been demonstrated that the NTR protein was expressed following infection of cells *in vitro* with HSV1790, and that the subsequent addition of the prodrug CB1954 to 3T6 cells infected with HSV1790 resulted in a significantly enhanced cell kill compared to infection with HSV1790 alone. *In vivo*, administration of the prodrug CB1954 to athymic mice bearing either A431 or A2780 tumor xenografts, 48 hours after intra-tumoral injection of HSV1790, resulted in a marked reduction in tumor volumes compared to administration of HSV1790 alone. Furthermore, administration of CB1954 48 hours after intra-tumoral injection of HSV1790 resulted in a significantly improved survival for mice bearing A431 tumors compared to these with administration of HSV1790 alone.

In contrast, administration of CB1954 following administration of HSV1790 to mice bearing CP70 tumor xenografts had no additional antitumor effect compared to administration of HSV1790 alone. CP70 is a derivative of the ovarian carcinoma cell line A2780, and has a drug-resistant phenotype due to the loss of MLH1, a key protein involved in mismatch repair (25, 26). These cells are resistant to a variety of DNA damaging agents and alkylating agents, and the IC_{50} values *in vitro* for A2780 and CP70 are 29 μM and 60 μM respectively (26). Expression of NTR in these cells results in similar-fold sensitization to the prodrug, with the IC_{50} value for A2780 remaining approximately half of that for CP70 cells. As the active form of CB1954 is an alkylating agent, it is possible that the intra-tumoral activation of CB1954 to its active form by NTR expression, following HSV1790 administration, is insufficient to overcome the drug resistant phenotype of these CP70 cells.

The concept of using viral vectors to deliver therapeutic genes to tumors is well established. In this study, it has been demonstrated that an additional antitumor effect, above that of the oncolytic effect of the virus alone, can be obtained by engineering the oncolytic virus to express a therapeutic gene to activate the prodrug CB1954. A number of other studies have evaluated the antitumor activity of oncolytic viruses engineered to express either immuno-stimulatory genes or therapeutic genes including those that can activate prodrugs (27, 28). One of the most reported enzyme-prodrug approaches is the use of the HSV thymidine kinase (*tk*) gene to convert ganciclovir into toxic metabolites (29). However, combining this system with an oncolytic virus has the drawback that ganciclovir inhibits viral spread.

Other prodrug activating systems that have been studied include the rat cytochrome P450 (CYP2B1) which converts cyclophosphamide into the alkylating toxin phosphoramide mustard. This results in enhanced efficacy in suppressing tumor growth (30), although expression of CYP2B1 in normal liver cells could have toxic side-effects and may limit its use when given by systemic administration. Viral vectors in combination with cytosine deaminase (CD), which converts the non toxic 5-flurocytosine into 5-fluorouracil, a highly toxic chemotherapeutic agent, has also shown considerable promise in the treatment of cancer. The replication-selective HSV vector engineered to express CD (M012) has recently shown increased tumoricidal effects both *in vitro* and *in vivo* with no toxicity observed (19).

Efficacy of a gene directed enzyme prodrug therapy system using both replication defective (18) and selectively replicative adenoviruses (31) has also been demonstrated *in vivo*. However, there is a need to improve adenoviral vectors to overcome unresolved issues including the adenovirus-triggered immune response which may compromise its efficacy (32, 33). Futhermore, HSV1790 has three potential advantages compared with ntr-expressing adenoviruses: (a) HSV1790 can replicate in most tumor cells and does not rely solely on CB1954 activation for cell killing; (b) As HSV1790 is replication competent, a major benefit is the amplification of the input dose within the tumor; (c) HSV1790 also expresses the HSV *tk* gene and this could be exploited in two ways. Administration of ganciclovir following injection of virus could be an important safety procedure should treatment require to be terminated. Alternatively, if HSV1790 is allowed to replicate extensively within the tumor first, addition of ganciclovir may further enhance tumor cell killing from tk activation of ganciclovir.

Most human clinical trials of genetic therapies for cancer, including those using oncolytic viruses, continue to use direct intra-tumoral injection as the route of administration of the therapeutic virus. However, systemic administration of oncolytic viruses by the intravenous route remains the ultimate goal as most patients have metastatic, rather than loco-regional, disease that cannot be accessed by direct injection. Intravenous administration of PV701, an oncolytic strain of Newcastle disease virus (34) and Onyx 015 (35) have been well tolerated in initial clinical studies with minimal toxicity.

These preliminary results demonstrate replication of HSV1790 and expression of the *ntr* transgene within tumor tissue following intravenous administration, but with no evidence of viral replication in normal organ tissues. However, as athymic nude mice have a compromised immune system, the effect of a normal immune system on viral toxicity and efficacy is unknown. Viral infection of tumors can attribute an antitumor immune response which is beneficial (7, 36, 37). In contrast, the host's immune system has been shown to neutralise virus and inhibit oncolytic

activity (38). Further studies of systemic administration of varying doses of HSV1790 in seronegative and seropositive immunocompetent models are required.

In conclusion, it has been demonstrated that the combination of an oncolytic herpes simplex virus with a "suicide gene" therapy approach can enhance antitumor efficacy in comparison with the effects of the oncolytic virus alone. It has also been demonstrated with encouraging initial data that intravenous administration of HSV1790 results in expression of the *ntr* transgene in tumor tissues, but not in normal organs, suggesting that it may be feasible to administer this virus by the intravenous route.

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