

# Oncolytic HSV-1 for the Treatment of Brain Tumours

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## KEY WORDS

■ HERPES SIMPLEX VIRUS ■ BRAIN TUMOURS ■ GENE THERAPY  
■ GLIOMA ■ CLINICAL TRIAL

## SUMMARY

The prognosis for patients diagnosed with malignant glioma, the most common primary tumour of the central nervous system, remains poor despite decades of research and advances in surgery, radiation and chemotherapy. The development of new approaches for the treatment of these tumours has led to the emergence of oncolytic viral therapy, with the use of conditionally replicating viruses, as a potential new intervention. Herpes simplex virus type 1 has emerged as the leading candidate oncolytic virus, with six different trials either completed or underway for patients with malignant glioma. In this review, the background of this approach will be discussed, followed by a discussion of the clinical trials, as well as potential directions for future trials.

## Introduction

THE DEVELOPMENT OF oncolytic herpes simplex virus type 1 (HSV-1) vectors for the treatment of malignant brain tumours has emerged in the past decade as a result of the dismal prognosis for people with these lesions. The onset of glioblastoma multiforme (GBM), the most common of these tumours, results in a median life expectancy of 12–15 months<sup>1</sup> despite decades of research in the traditional oncological approaches, namely surgery, radiation and chemotherapy. The tendency of these tumours to invade adjacent and distant brain, including eloquent functional areas, renders complete surgical resection impossible. Radiation therapy has been shown to increase the duration of patient survival, but 90% of patients still suffer recurrences within 2 cm of the original tumour. Recently, Stupp *et al.*<sup>1</sup> have shown an increase in survival in a subset of patients with GBM when the chemotherapeutic agent temozolomide is added to the radiation treatment regimen; however, the effect was modest, with overall survival still remaining under 15 months.

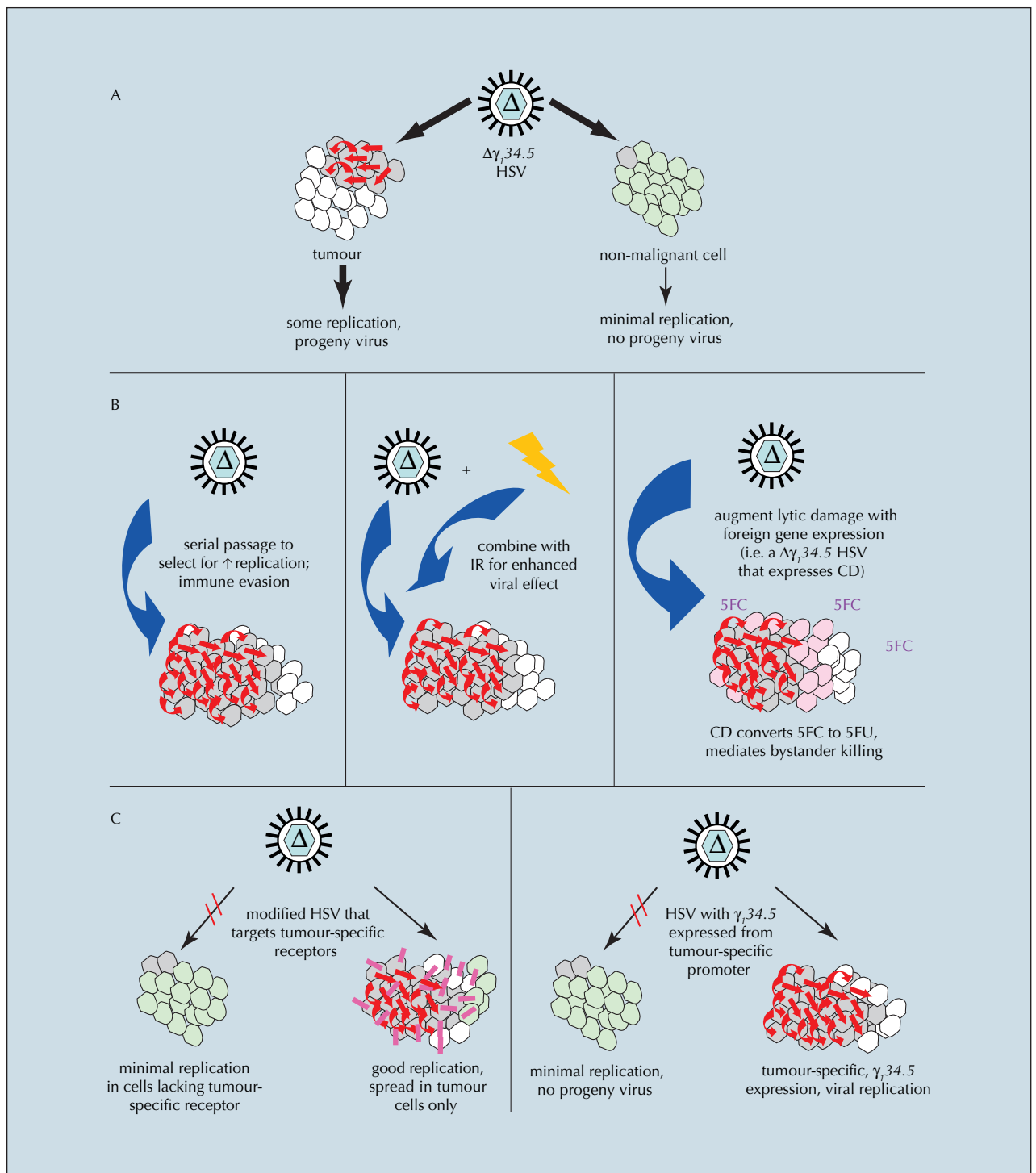
As a result of the generally poor outcomes for these patients, research efforts shifted to the evaluation of novel experimental therapies for GBM. In 1991, a genetically engineered HSV-1 that exhibited decreased neurovirulence, due to a mutation in the viral thymidine kinase (*tk*) gene, was shown to kill human glioma cells *in vitro*, inhibit tumour growth in *in vivo* models of human glioma, and to increase survival in orthotopic models of glioma.<sup>2</sup> To improve the potential application of oncolytic therapy within the clinical realm, the number of viral mutants studied was expanded. HSV-1-containing mutations within the  $\gamma_134.5$  gene retained the capacity to inhibit tumour growth and extend survival *in vivo*, but were also

sensitive to common antiherpetic agents in clinical use (e.g. aciclovir).<sup>3</sup> This ability potentially to interrupt the viral replication cycle, to prevent viral-induced toxicity or the development of encephalitis, was critical to the use of these viruses in clinical trials, particularly as the entire field of genetically engineered oncolytic viruses was in its infancy at that time. Thus, further studies with  $\gamma_134.5$ -deleted HSV-1 have taken advantage of their lack of neurovirulence and their sensitivity to aciclovir. This review examines the latest preclinical developments in oncolytic HSV-1 therapy (Figure 1), the current status of clinical trials, and future directions of both preclinical and clinical research. Basic details of preliminary work will be described here briefly for clarity; for more details, the reader is referred to an earlier review.<sup>4</sup>

## Initial Preclinical Research

The initial  $\gamma_134.5$  mutant HSV-1 studied, as described by Roizman and colleagues,<sup>5</sup> demonstrated a complete lack of neurovirulence in murine models. The  $\gamma_134.5$  gene is present in two copies in the HSV-1 genome, in the inverted repeat which flanks the Unique Long ( $U_L$ ) segment of the genome; its 263 amino acid protein product (ICP34.5) has been demonstrated to have at least two functions. The mechanism of the first function, neurovirulence, is not well described. More is understood about the second function of ICP34.5, which is subversion of the host-cell response to HSV-1 infection. In the non-malignant cell, protein kinase R (PKR) is activated following viral infection, shutting down translation in the infected cell as an antiviral protective mechanism by phosphorylating and inactivating eukaryotic initiation factor-2 $\alpha$  (eIF-2 $\alpha$ ). ICP34.5 recruits protein phosphatase-1 $\alpha$ , which in turn dephosphorylates eIF-2 $\alpha$ , restoring protein synthesis and, consequently, viral replication.<sup>6</sup> As such,  $\gamma_134.5$  mutants are unable to overcome the shut-off of host-protein synthesis in non-malignant cells. This effect, however, is absent or reduced in malignant cells, which permits for replication of such mutants. Alterations in expression of the protein Ras are present in many cancer cells, and these alterations have been suggested as the mechanism for this selectivity of replication in tumour cells, although further work suggests that the actual mechanism may be more complex than first appreciated.<sup>7</sup>

Research involving the  $\gamma_134.5$ -deleted HSV-1 mutant R3616 (Table 1) demonstrated that it did indeed exhibit antiglioma effects while retaining sensitivity to aciclovir.<sup>3</sup> At that time, however, there was still concern as to the possibility of recombination with wild-type HSV-1 occurring in the human patient population. To minimize this risk a novel mutant, G207, was



**Figure 1:** Strategies to enhance tumour-cell killing by oncolytic HSV-1. (A) Conditionally replication-competent HSVs (e.g.  $\Delta\gamma_{1,34.5}$ ) can infect and replicate in tumour cells (white), but not in non-malignant cells (light green). Grey, HSV-infected cells; red arrows, HSV replication, spread to other cells. Two strategies are being investigated to enhance tumour-cell killing by conditionally replication-competent,  $\gamma_{1,34.5}$ -deleted HSV (B) or by specific targeting of wild-type HSV replication in tumour cells (C). Tumour-cell killing by conditionally replication-competent viruses ( $\Delta\gamma_{1,34.5}$ ) can be enhanced either by serial passage (panel B, left), combination with low-dose IR (panel B, centre) or by introducing foreign gene inserts, including suicide genes (pink cells, tumour cells killed by bystander effect) or immune-modulating molecules which enhance the antitumour immune responses (panel B, right). Modified HSVs are being developed that specifically target tumour cells either through tumour cell-specific receptors (panel C, left), or through control of viral virulence genes by tumour-specific promoters (panel C, right). HSV, herpes simplex virus; IR, ionizing radiation; 5FC, 5-fluorocytosine; 5FU, 5-fluorouracil; CD, cytosine deaminase.

constructed which contained a mutation in the viral gene encoding the large subunit of the ribonucleotide reductase ( $U_L39$ ).<sup>8</sup> This mutation had been previously described as reducing neurovirulence in wild-type HSV-1.<sup>9</sup> Despite the presence of the additional mutation, G207 exhibited similar antiglioma properties to R3616 and maintained its safety. Further studies in the

hypersensitive simian primate *Aotus* confirmed that the virus was aneurovirulent.<sup>10</sup>

### Clinical Studies

Details of the G207 and HSV1716 clinical trials are summarized in Table 2.

**Table 1: Summary of the key oncolytic herpesviruses and genomic modifications**

Virus	Parent HSV-1 strain	Genomic changes
R3616	F	1 kbp deletion in $\gamma_134.5$ gene (both copies)
R3659	F	Insertion of two copies of <i>hsv-1</i> tk gene under alpha-27 promoter with deletions in $\gamma_134.5$ genes
G207	F	1 kbp deletion in $\gamma_134.5$ gene (both copies) <b>and</b> disabling <i>lacZ</i> insertion in $U_L39$
HSV1716	17	759 bp deletion in $\gamma_134.5$ gene (both copies)
M002	F	1 kbp deletion in $\gamma_134.5$ gene (both copies) <b>and</b> insertion at same locus of Egr-1 promoter expressing p40 and p35 subunits of murine IL-12 linked by an IRES sequence
M012	F	1 kbp deletion in $\gamma_134.5$ gene (both copies) <b>and</b> insertion at same locus of Egr-1 promoter expressing the bacterial cytosine deaminase gene

HSV, herpes simplex virus; IRES, internal ribosome entry site; IL-12, interleukin-12.

**G207 CLINICAL TRIALS**

Following its demonstrated safety in multiple preclinical animal studies, a Phase I study was initiated to determine if G207 could be safely administered, by direct intratumoural injection, in patients with recurrent or progressive malignant glioma who had failed standard therapies. The trial was a dose-escalation study, designed to determine the maximum tolerated dose of G207.<sup>11</sup> Twenty-one patients, all of whom had undergone prior external beam radiotherapy in addition to either craniotomy and surgical debulking (17 patients) or biopsy alone (four patients) were entered into the trial. Ten patients had received one or more chemotherapeutic agent. A Karnofsky Performance score of  $\geq 70$  was required for entry (Table 3). Primary tumour histologies included 15 glioblastomas, one gliosarcoma, four anaplastic astrocytomas and one anaplastic mixed glioma. Patients were allocated to cohorts by dose level (three patients per cohort) using a standard dose-escalation scheme of half-log increments. Initially, patients were given one intratumoural injection into the enhancing portion of the tumour (according to magnetic resonance imaging [MRI] or computed tomography scans). All patients treated at the highest dose level,  $3 \times 10^9$  plaque forming units (pfu), had their tumours stereotactically inoculated in five different areas of the enhancing tumour,

as demonstrated by pre-operative imaging studies.

The results of this study confirmed the safety of intratumoural G207 administration. A maximum tolerated dose was not established as, even with inoculation of  $3 \times 10^9$  pfu (the highest planned dose level), there were no definitive dose-limiting toxicities. Seven post-treatment biopsy specimens and five post-treatment autopsy specimens were available for histological review. No evidence of encephalitis or major inflammatory changes was observed.

Additional, non-safety related end-points were also examined. Five of 19 evaluable patients were confirmed to be HSV-1 seronegative prior to treatment with G207; HSV-1 titres were not confirmed preoperatively in two other patients, hence they were not included in this analysis. One of these five (from the  $3 \times 10^9$  pfu treatment cohort) seroconverted after inoculation, demonstrating that even in this extremely immunocompromised group of patients, an immune response against G207 could occur in a fraction of patients treated intracerebrally. While efficacy was not the primary end-point of this study, certain findings support the antiglioma effects of G207 in this trial. Eight out of the 20 patients examined with MRI had a decrease in the amount of enhancing tumour at 1 month after inoculation. Long-term survivors, including one GBM

**Table 2: Summary and evaluation of clinical trials for the oncolytic herpesviruses G207 and HSV1716**

Virus	Study type	Design	Study population	Patients enrolled	Viral dose	Reference
G207	Phase I	Stereotactic inoculation of virus into tumour; dose-escalation	RMG	21	Dose escalation from $1 \times 10^6$ pfu to $3 \times 10^9$ pfu	11
	Phase Ib	Stereotactic catheter inoculation followed 2–5 days later by resection and reinoculation into tumour bed	Recurrent GBM	6	$1.15 \times 10^9$ pfu (total, divided into two doses)	Manuscript in preparation
	Phase I	Stereotactic inoculation into tumour in five loci followed 24 h later by 5Gy IR or SRS	RMG	In progress	$1 \times 10^9$ pfu (dose de-escalate as necessary)	Under enrolment
HSV1716	Phase I	Stereotactic inoculation of virus into tumour; dose escalation	RMG	9	Dose escalation from $1 \times 10^3$ pfu to $1 \times 10^5$ pfu	12
	Phase Ib	Stereotactic inoculation followed 4–9 days later by resection	RMG (11); NDMG (1)	12	$1 \times 10^5$ pfu	13
	Phase I	Tumour resection followed by inoculation into tumour bed	RMG (6); NDMG (6)	12	$1 \times 10^5$ pfu	14

RMG, recurrent malignant glioma; pfu, plaque forming units; GBM, glioblastoma multiforme; IR, ionizing radiation; SRS, stereotactic radiosurgery; NDMG, newly diagnosed malignant glioma.

**Table 3: The Karnofsky Performance Scale<sup>15</sup>**

Description	(%)
Normal; no complaints; no evidence of disease	100
Able to carry on normal activity; minor signs and symptoms of disease	90
Normal activity with effort; some signs and symptoms of disease	80
Cares for self; unable to carry on normal activity or do work	70
Requires occasional assistance, but is able to care for most personal needs	60
Requires considerable assistance and frequent medical care	50
Disabled; requires special care and assistance	40
Severely disabled; hospitalization indicated although death not imminent	30
Very sick; hospitalization necessary; requires active support treatment	20
Moribund; fatal processes progressing rapidly	10
Dead	0

patient surviving >7 years after G207 therapy, were also seen in the treatment cohort.

Having demonstrated the safety of G207 in a one-time inoculation regimen, several questions remained to be answered. Thus, a Phase Ib study was designed to evaluate the potential toxicity of a two-dose regimen, the safety of inoculation of G207 into the brain surrounding the gross tumour, to obtain G207-treated tumour specimens for analysis of viral replication and spread, and to assess host immune responses elicited against the virus and/or against the tumour. Confirmation of studies conducted in the initial trial (including the degree of virus shedding, time to progression and overall survival) were other trial end-points. For this trial, the first dose was administered via a catheter placed stereotactically within the tumour, followed by tumour resection 2–5 days later and reinoculation of the tumour bed. A manuscript describing the results of this trial is currently in preparation.

#### HSV1716 CLINICAL TRIALS

G207 is not the only HSV-1 mutant to be examined in human clinical trials for the treatment of malignant glioma. HSV1716, a  $\gamma_134.5$ -deleted mutant constructed from wild-type strain 17 of HSV-1 (G207 was constructed from strain F), has been examined in early-phase clinical studies in the UK. HSV1716 (Table 1) contains only the  $\gamma_134.5$  mutation and no changes in the  $U_L39$  locus. Lower dosages were used in these trials, with the maximum dosage being  $10^5$  pfu. In the initial HSV1716 trial, similar results were seen to the Phase I G207 trial, with some long-term survivors and no dose-related toxicities produced by the virus.<sup>12</sup> A second ‘proof of principle’ study was undertaken, to demonstrate viral replication and the potential for efficacy of oncolytic HSV-1 therapy. In this study, the investigators could recover virus from treated tumours at higher titres than the input dose, indicating that viral replication was occurring. In addition, polymerase chain reaction (PCR) analysis of treated tumour

specimens indicated HSV-1 DNA in tumour sections distal to the injection site.<sup>13</sup> More recently these investigators injected HSV1716 directly into the brain, following surgical resection of tumour from patients newly diagnosed or with recurrent high-grade glioma.<sup>14</sup> As before, there was no evidence of clinical toxicity associated with virus administration. In addition, three patients were surviving between 15 and 22 months following HSV1716 injection. Efficacy trials are forthcoming as a result of these promising data.

### Future Directions for Oncolytic HSV-1 Glioma Therapy

#### ADJUNCTIVE THERAPY WITH ONCOLYTIC HSV-1

While promising results have been seen in certain patients treated with G207 in two trials, many patients progressed as expected, with no obvious benefit from viral treatment. A variety of studies in the preclinical arena have supported the concept that irradiation of  $\Delta\gamma_134.5$  HSV-1-treated tumour in the time-period directly after viral inoculation produces an increase in viral replication and a concomitant increase in antitumour effects (Figure 1B).<sup>16–19</sup> Doses of radiation that are considered subtherapeutic (e.g. a single fraction of 3.3 Gy; G Yancey Gillespie, personal communication) can produce this effect without an evident increase in toxicity. As a result of these data, a new study of G207 in patients with malignant brain tumours has recently begun enrolment at the University of Alabama at Birmingham, USA. The trial is divided into two patient groups. The first group will undergo inoculation of  $1 \times 10^9$  pfu of virus into five different loci within the enhancing portion of the tumour. The day following G207 inoculation, the patients undergo treatment with a single dose of 5 Gy of external beam-ionizing radiation (administered to the enhancing portion of the tumour on T1-weighted imaging as well as a portion of the abnormality present on T2-weighted imaging). If nine patients are enrolled under this dosing paradigm without dose-limiting toxicity, an additional nine patients will be treated with G207 in an identical fashion and then undergo treatment, as in the first group, with ionizing radiation on the day following viral treatment. This group, however, will be treated with stereotactic radiosurgery to the T1-enhancing portion of the tumour, based on the dosing levels suggested by Radiation Therapy Oncology Group Study 9005. Toxicities, as well as potential benefits in response, determined by overall survival and time to progression, will be evaluated between the two groups. These data will then be utilized to design a Phase II study to attempt to examine the efficacy of treatment.

#### DEVELOPMENT OF TARGETED ONCOLYTIC HSV-1 VECTORS

While these clinical studies have been underway, laboratory efforts have focused on the development of new-generation vectors that will retain the safety exhibited by these first-generation viruses. Development of targeted vectors specific for tumour cells has been notoriously difficult to accomplish with HSV-1 due to its complex binding and entry programme. Initial attempts resulted in endosomal degradation of the virus or cellular apoptosis. Recently, Roizman and colleagues<sup>20,21</sup> have successfully retargeted the virus to the interleukin 13 (IL-13) receptor, which is highly expressed in glioma cells<sup>22</sup> and not expressed in other cells of the central nervous system. The IL-13 receptor has been successfully used for targeting glioma cells in

other paradigms, and a Phase III clinical trial using IL-13 bound to *Pseudomonas* exotoxin has recently completed enrolment in an international trial of recurrent glioma patients, although the results have not yet been reported. More recently, Roizman's group<sup>23</sup> has retargeted the virus to another glioma cellular target, urokinase-type plasminogen activator. While initial viruses did not eliminate entry via the cell-to-cell adhesion molecule, nectin-1, or the herpesvirus entry mediator A, and consequently are not yet specific to tumour cells, this problem has been overcome in more recent work. This demonstrates that targeting of HSV-1 vectors to alternative receptors is possible, and that further tumour-specific vectors are forthcoming (Figure 1C).<sup>24</sup>

#### ONCOLYTIC HSV-1 FOR ANTITUMOUR GENE THERAPY

Another promising strategy has come from engineering  $\gamma_134.5$ -deleted viruses to express foreign genes (Figure 1B). Several successful approaches have been reported, including the use of suicide genes in combination with prodrugs, expression of antiangiogenic genes, and cell-surface antigens.<sup>4,25,26</sup> The most successful approach to date, however, may be viruses engineered to express cytokines. Such cytokines can potentially augment antitumour effects either directly or through stimulation of the antitumour response by immune effector cells. The authors' group has produced viruses expressing proteins from each of these groups, and by far the most

successful approach has been the use of cytokines. One such cytokine-expressing virus, M002, harbours a  $\gamma_134.5$  deletion and expresses murine IL-12 under a constitutively active early-growth response gene (*Egr-1*) promoter. This virus has maintained safety in a variety of murine models and has also demonstrated increased efficacy in syngeneic models of murine glioma<sup>26</sup> and a stringent, non-immunogenic murine neuroblastoma model.<sup>25</sup> Preparations are underway to create clinical Good Manufacturing Practice virus stock under a National Cancer Institute-funded 'Rapid Access to Intervention Development' application: a Phase I study using this virus would then be conducted.

Suicide-gene prodrug viruses are other viruses that have demonstrated an increase in efficacy over parental HSV-1 mutants not expressing foreign genes. Chiocca and colleagues<sup>27,28</sup> have demonstrated increased efficacy using a virus expressing cytochrome P450 in combination with cyclophosphamide. Parker and Markert have successfully utilized a mutant-expressing cytosine deaminase, in combination with 5-fluorocytosine, to inhibit tumour growth in the flanks of A/J mice bearing Neuro2A neuroblastoma (Parker JN, Markert JM, personal communication) (Figure 2).<sup>29</sup>

#### Conclusions

The future of HSV-1 oncolytic treatment for malignant glioma appears promising. Multiple doses of virus,

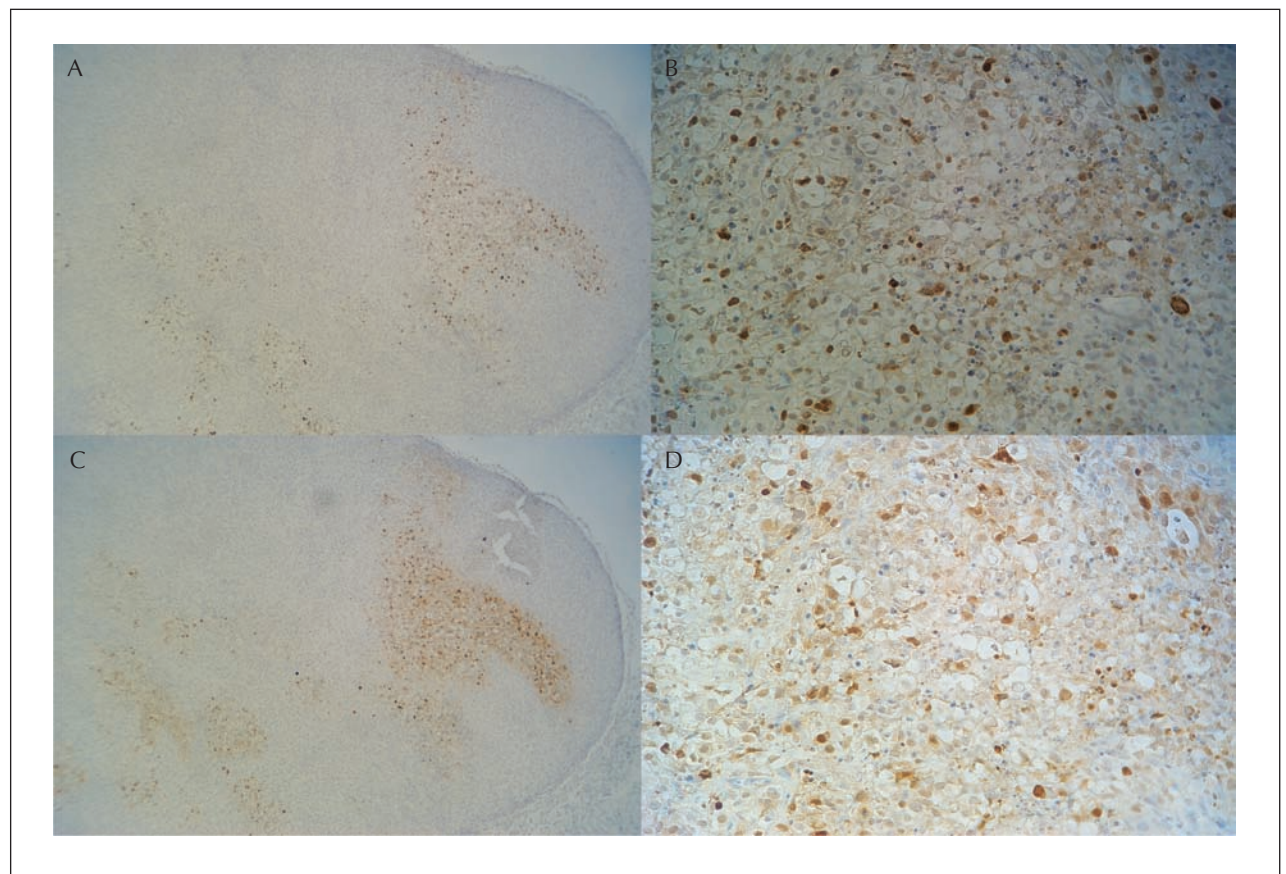


Figure 2: Immunohistochemistry staining of tumour sections for herpes simplex virus (HSV) and cytosine deaminase. U87 flank tumours were propagated in nude mice and inoculated with  $1 \times 10^7$  plaque-forming units of M012, a  $\gamma_134.5$ -deleted HSV-1 expressing the *Escherichia coli* cytosine deaminase gene under the *Egr-1* promoter. Four days later, mice were killed and tumours harvested. Shown are tumour sections stained for HSV (A and B) using a polyclonal antibody against HSV-1 and HSV-2, and sections stained for cytosine deaminase (C and D). Note the widespread tumour necrosis associated with the staining, as well as the concordance of staining for HSV and cytosine deaminase, indicating consistent expression of the foreign gene product. A and C, magnification  $\times 2.7$ ; B and D, magnification  $\times 13$ .

adjunctive agents such as radiation (that increase viral replication and spread), targeted viruses, and viruses that express foreign genes that augment antitumour responses are promising avenues of research with potential clinical impact. Future efforts will focus on increasing the efficacy of viral delivery and spread, as well as developing imaging approaches to allow rational administration of the virus, including determining the proper timing and geographic location for injection into the tumour.

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## Conflicts of Interest

No conflicts of interest were declared in relation to this article.

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