IS ONCOLYTIC VIROTHERAPY A VIABLE PLATFORM FOR GLIOMA TREATMENT?

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ABSTRACT

Gliomas are devastating cancers of the nervous system with poor prognosis. Their aggressiveness produces a mortality rate rarely seen with other malignant tumours and the lack of effective treatment has left very few options. Oncolytic viruses, with their long history of experimentation, have been deemed to be a key player in the future treatment of gliomas. This review will focus on the two main contenders, adenovirus and herpes simplex virus, for glioma treatment and discuss how far the field has come since its conception. The concept of each vector and the rationales behind their use will be contrasted before discussing the future of the field. Data was located by accessing the MEDLINE database using the PubMed search system. Data was selected on the basis of the insight its information provided as well as on the dependability of the experimental method used.

Key Words: Glioma, Glioblastoma, Oncolytic virus, Herpes simplex virus, Adenovirus

INTRODUCTION & DISCUSSION

A glioma is any tumour that has its origins from a glial cell. These are supporting cells that nourish the neurones within the nervous system[1]. Patients experience a decline in cognitive function and score lower on quality of life scale than any other cancer [2]. Gliomas are also accountable for the greatest number of life years lost (an average of eight years) for any given cancer patient [3]. Furthermore, the 5-year survival rate is dismal with less than 10% surviving in patients with glioblastoma multiforme – the highest grade of glioma (Fig.1)[4].

Gliomas account for almost 90% of brain tumours, with the highest incidence in the Southeast of England [3]. Current treatments involve radiotherapy followed by Temozolomide chemotherapy and surgical resection[5]. However, efficacy is limited by the fact that alternative oncogenic pathways can mutate, offering resistance. Compounding this is the problem of targeting tumour elements within the central nervous system which requires drugs to pass the blood-brain-barrier as well as the resulting haematological toxicity in patients[6,7]. Financially, this therapy proves to be expensive especially as an adjuvant[8]. Looking at all these issues, the justification for novel approaches to glioma treatment becomes apparent.

The use of oncolytic viruses to treat cancer dates back as far as the 19th century although unbeknownst to doctors at the time. Multiple observations of remission in cancer patients with concurrent viral infections paved the way for research into the field[9]. We now know that the oncolytic effect of viruses is due to replication-mediated cell lysis[10].

This review will explore the current state of oncolytic virotherapy in treating glioma. It will focus on adenovirus and herpes simplex virus due to their effectiveness in clinical trials[11]. First, the rationales behind using each virus will be discussed before comparing the various mechanisms employed. Finally, the future of the field will be questioned to see if there is potential in oncolytic virotherapy.

Adenovirus (Ad) – Concept & Rationale

Adenoviruses are a good choice for gene therapy as a result of the ease with which their genome can be hijacked to carry transgenes. They also have the ability to grow exponentially in a short space of time thus boosting their oncolytic potential[12].

The replication cycle follows the mechanism of many viruses in that it begins with the infection of a cell and ends with the release of many virus particles. First, adenovirus interacts with the coxsackie adenovirus receptor (CAR) expressed by host cell membranes[13]. Internalisation is then promoted through an adenoviral penton base which recognises integrins on the cell surface and results in the formation of endosomes[14]. This allows adenoviral particles to relocate to the nuclear membrane where viral DNA can enter the nucleus[15]. Lastly, genomic transcription of viral DNA results in the organised production of early, immediate and late genes which trans-

locate to the cytoplasm to produce new viral particles. These are eventually released by cell lysis and are thus free to infect more cells[13].

Adenovirus – Rb Pathway

Retinoblastoma protein (pRb) is implicated in a variety of cancers including almost a third of malignant gliomas[16]. This protein works as a tumour suppressor gene where it binds to E2F transcription factors thereby preventing the cell from moving into S-phase. A mutation in pRb thus leads to unregulated cell proliferation[17]. One of the earlier experiments was to use Ad5CMV-Rb, which carried the Rb gene, in an effort to replace the aberrant protein expressed in cancer cells. The virus was made replication-deficient in order to limit gene expression in healthy tissue but this also reduced the capacity for gene transfer to a small number of cells[18]. As a result, it was observed that there was growth arrest in gliomas, however there was no actual remission i.e. the virus caused a cytostatic effect but was not cytopathic[16].

Studies like these led to the development of conditionally replicative adenoviruses (CRAds), which are unable to replicate in normal host cells but selectively target tumour cells[19]. In order to protect against infection, host cells will undergo cell cycle regulation and apoptosis[20]. To counter this, the adenovirus E1A gene codes for a protein which displaces E2F from pRb thus allowing cell transition into S-phase which favours viral DNA synthesis[21-23].

Ad5-Delta24 is a genetically modified adenovirus which has a 24-bp deletion in the region of its genome coding for E1A[24]. As a result, it can no longer prevent the pRb checkpoint in healthy cells and thus cannot divide. However, as pRb is already aberrant in tumour cells[25], there is nothing to stop progression into S-phase. This mechanism allows adenovirus to be conditionally-replicative in tumour cells whilst retaining its oncolytic potential. In vitro studies have shown Ad5-Delta24 to be a potent oncolytic virus in glioma cell cultures. By transferring pRb to pRb-null cells i.e. cancer cells, it was confirmed that the conditionally-replicative mechanism was indeed dependent on retinoblastoma protein. It was important to confirm this finding in order to be certain that there would be no bystander damage to brain tissue when running trials on humans. Multiplicity of infection ratios (MOI), the ratio between virus particles and tumour cells, as low as five caused noticeable cytopathic effects and within seven days some cell lines were in complete remission. With MOI ratios of ten, cell lines showed complete cytolysis within 14 days, however it must be noted that results differed between the cell lines used. In vivo studies were less marked with around 40% of live subjects showing tumour regression but multiple injections had to be used. Moreover, despite many different animals being used, to date there have been no clinical trials to authenticate the efficacy of the virus in humans[24].

Although the results of Ad5-Delta24 seemed promising, there was a stark difference between cell lines in terms of cytolytic effects. Bergelson et al. found that, despite adenovirus anchorage to tumour cells being related to CAR, internalisation of the virus relied on a secondary mechanism that was integrin dependent[26-28]. Also, Asaoka et al. found that the expression of CAR on glioma cells was quite variable and thus not a stable cellular identifier (Fig.2)[29-31]. Conclusively, Ad5-Delta24 affected cell lines differently due to their variability in CAR expression. Ad5-Delta24RGD was produced to include an RGD motif (arginine-glycine-aspartic acid), which binds strongly to integrins. Such a modification meant the virus would not have to rely on CAR but was instead integrin-dependent, resulting in a higher infectivity[32,33]. To simulate clinical conditions, cells were grown in spheroids which replicated a tumour mass that may actually be encountered instead of monolayers of glioma cells. Whilst replicative-deficient viruses barely broke the border of the mass, with Ad5-Delta24RGD the viability of cells was significantly reduced. Concordantly, when tested in nude mice with xenografts of low CAR-expressing human glioma, it was found that there was complete remission in 90% of mice and they survived free of any cancer for four months[31].

Unlike the variants previously mentioned, a phase I/II trial has recently been completed to assess the safety profile of Ad5-Delta24RGD. For the first time, it has been shown that this virus can cause complete remission in patients with no evidence of relapse more than three years after the disease. However, one must note that complete oncolysis was only seen in three patients and as the trial has been published quite recently, there has been little time to evidence any side effects or relapses[34].

Adenovirus – P53 pathway

P53 is a tumour suppressor gene that acts to inhibit cell cycle progression and cause apoptosis in order to prevent tumour formation[35]. Not only is p53 mutated in a vast array of cancers but also in more than a third of astrocytomas[36,37]. By upregulating p21, p53 inhibits cyclin-dependent kinases to retard progression of the cell cycle. A secondary effect includes the transcriptional activation of Bax which leads to apoptosis[38]. Thus, it makes sense that a transfer of p53 to p53-null cells results in apoptosis and indeed this has been shown[39].

Adenoviruses express the E1B-55K protein which binds to p53 in an attempt to stop apoptosis and allow the production of viral progeny[40]. Also they produce the E1B-19K protein to regulate free Bak and Bax proteins in a further attempt to reduce mitochondrial-dependent apoptosis[41].

ONYX-015 is the earliest example of a genetically engineered adenovirus and has a deletion in the E1B-55K gene. As a result, in normal cells ONYX-015 is unable to replicate but in cancerous cells where p53 is already mutated, the adenovirus is able to produce progeny. In this way, ONYX-015 is a conditionally replicative adenovirus[42-44]. However, this has been contested with recent studies that suggest ONYX-015 works by defecting the export of mRNA from the nucleus[45,46]. Phase I trials have shown that this virus has a good safety profile when injected into resected tumours[47]. A team in China have also completed a phase III trial of the virus with enough success to warrant FDA approval for its use in patients with head and neck cancer albeit in combination with chemotherapy[48]. Despite this, there has been much criticism of ONYX-015. Studies have found that many gliomas express functional p53 and may contain a small population of p21-expressing tumour cells rendering the virus ineffective[38]. Moreover, as E1B-55K is also involved in the translocation of nuclear viral mRNA to ribosomes and ONYX-015 is E1B-55K-null, the replicative potential is attenuated and indeed viral transduction in glioma models has not shown any substantial amount of oncolytic activity[47,49]. Likewise, progression was shown no more than two months after treatment on average in addition to the fact that only a third of the patients treated with the maximum dose were alive after 19 months. Thus, although ONYX-015 is safe, its therapeutic efficacy is questionable[47]. As such, one of the biggest hurdles in virotherapy is maintaining a fine balance between oncolytic potential and tumour selectivity.

Herpes Simplex Virus (HSV-1) – Concept & Rationale

HSV-1 is a neurotropic virus i.e. it preferentially infects the nervous system hence why it is favoured by current research for glioma (Fig.3)[50-52]. It has a relatively large genome which does not integrate with that of the hosts[53-56]. As a result, it can be modified to carry a large number of transgenes and exhibit latency without causing any insertional mutagenesis that may affect the cell unpredictably. Likewise, the genes associated with its neurovirulence are nonessential and can thus be modified without affecting the virus' survival[57]. The virus is inherently cytolytic and in case therapy goes awry, antiherpetic drugs such as gancyclovir can be used as a failsafe[58]. One caveat is that in the general population, a high rate of immunity already exists which may cause difficulties with regards to viral proliferation, although its habitation of a nuclear episomal state may avoid provoking an immune response altogether[59,60].

The first modified replicative-competent herpes virus was a mutant with a deletion in the tk gene and was called dlsptk. Briefly, for herpes virus to replicate, it requires the presence of thymidine kinase. This allows the phosphorylation of deoxythymidine – a precursor for deoxythymidine triphosphate which is used in DNA synthesis. In normal cells there are two types, TK1 and TK2, with the former only being present during cellular division (as more substrate is needed)[61]. HSV-1 codes for its own thymidine kinase allowing it to replicate in both dividing and non-dividing cells, however dlsptk is tk-null. As a result, it must rely on the cells inherent thymidine kinase activity, and as only replicating cells have both TK1 and TK2, only replicating cells are sufficiently suitable for HSV-1 replication. As a result, HSV-1 cells only replicate in dividing cells such as tumour cells in the nervous system[62]. In vivo studies proved that dlsptk was a potent oncolytic vector, however the deletion in the tk gene also meant the mutant was resistant to antiviral treatment. This, in combination with noxious effects at high titres eventually led to the dismissal of dlsptk[62,63]. Nevertheless, this example proved as a proof of concept that HSV-1 could indeed affect gliomas vigorously.

Herpes Simplex Virus 1 – PKR Pathway

Unlike adenovirus, a single gene deletion in HSV-1 does not render the virus innocuous to non-dividing cells[64,65]. Thus, to prevent herpes relapsing to its wild-type form, HSV-1 is often modified to have a double knockout. Viruses with such mutations are often referred to as 2nd generation oncolytic viruses with the first variant termed G207[60,66]. G207 has paired deletions in both of its $_{\gamma 1}$34.5 genes which regulate the neurovirulence of HSV-1 and overcome host cell defence. Usually when HSV-1 infects a cell, RNA-dependent protein kinase R (PKR) leads to an antiviral response that induces protein synthesis shutoff. This is carried out by the phosphorylation of eukaryotic initiating factor 2-alpha (eIF-2α) by PKR and culminates in the cessation of virus replication. The $_{\gamma 1}$34.5 genes code for infected cell protein 34.5 (ICP34.5) which blocks PKR mediated protein synthesis shutoff and therefore allows HSV-1 to continue replicating[67]. Genetic engineering of HSV-1 with its $_{\gamma 1}$34.5 gene deletions means that the virus can no longer overcome host defences in normal cells. However, in cancer cells with an oncogenic Ras system, PKR is already in a repressed state and so G207 no longer has to rely on its $_{\gamma 1}$34.5 genes[53,68]. Moreover, the U_L39 region of the HSV-1 genome codes for a subunit of ribonucleotide reductase called ICP6. This enzyme is needed for the synthesis of nucleotides post-infection and just as with tk deletions, can be provided by cells undergoing active cell division but not quiescent cells. G207 has a LacZ reporter gene (from bacteria) inserted into the U_L39 region to act as a disruption and allow detection by histochemical methods[69]. Overall, these two modifications result in a doubly attenuated virus to decrease the chance of wild-type reversion whilst also increasing its the sensitivity to acyclovir thus ensuring a high safety margin[70,71].

Preclinical studies in glioma cell lines showed that an MOI as low as 0.1 managed to completely lyse the entire cell population within two days[60].

However, as HSV-1 is one of the commonest causes of viral encephalitis, it is important to test the safety of the virus. When the highest dose possible was given to mice either intracerebrally or intraventricularly, there were no symptoms for over five months. Even with the most susceptible mouse strain, there was only slight non-fatal symptoms in a quarter of the population[72]. Additionally, mice that survived a previous infection with wild-type HSV were given a G207 inoculation. Despite both infections localising to the same area, there was no reactivation of latent HSV-1[73]. To further commend the safety of G207, trials were carried out in New World owl monkeys, primates with a propensity for HSV infection, but they were also asymptomatic[74]. Clinical trials in human patients did not elicit any adverse reactions nor could a maximum tolerated dose be identified. 20% of patients had reduced tumour volume and eight patients survived more than nine months with one example remaining alive even after five years. In a trial of 21 patients, only three patients suffered from side effects such as seizures or brain oedema[75]. Although the safety of G207 is almost unquestionable, almost all test subjects showed progression after ten months and even with immunosuppressive steroid therapy, most had seroconverted against the virus. One point to note is that G207 seems to hinder growth not only by replicative oncolysis, but also through an inflation of cytotoxic T-cell activity[76]. One could reason that immunosuppressive drugs to prevent seroconversion against HSV-1 could also decrease antitumor efficacy via the T-cell mechanism.

Figure 1: Kaplan-Meier plot showing how survival rates change depending on the type of treatment for patients between 20-70 years of age.
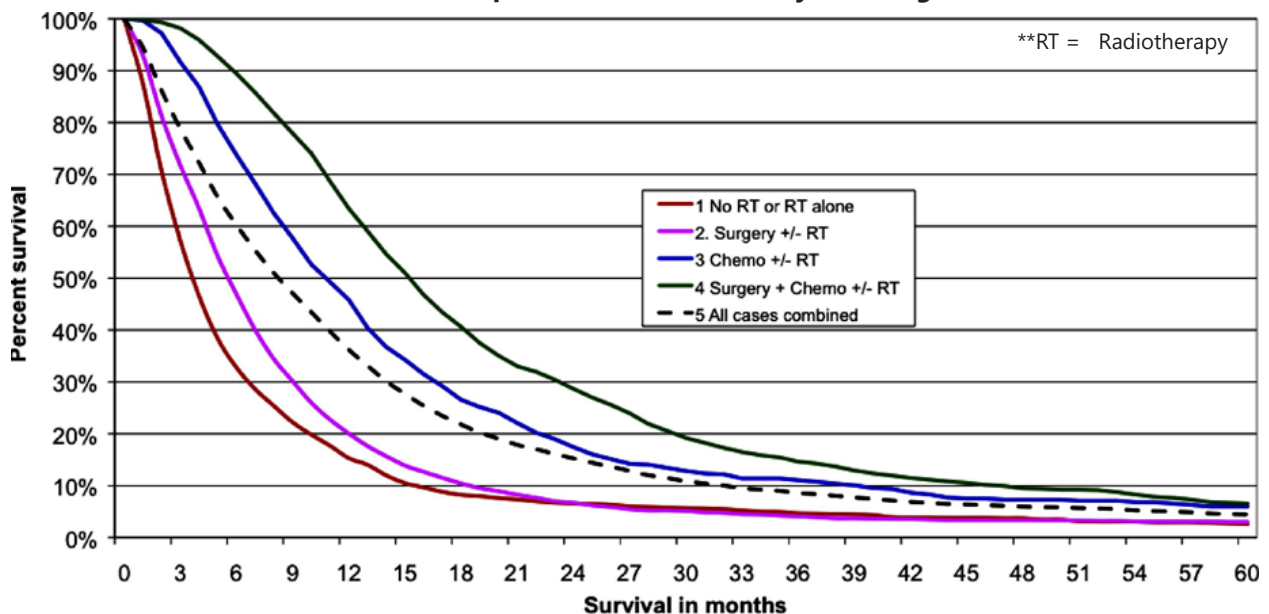
CONCLUSION

With the current state of treatment offering little improvement in prognosis, oncolytic virotherapy has shown promise as a main player in future glioma therapy. Not only have there been effective responses in preclinical studies but virotherapy has also proven to be extremely safe in humans despite using some of the most lethal viruses in existence.

In spite of this, more work still needs to be done to advance the field. Initial hypotheses of oncolytic mechanisms have had to be rethought several times illustrating just how complex the biology is. One area that still lacks significant knowledge is how the immune system interacts with both viral and tumour mechanisms, further highlighting that more research needs to be done to truly see how all innate processes interplay with each other.

Future approaches may seek to combine virotherapy with other novel approaches currently being developed such as virus-directed enzyme prodrug therapy (VDEPT) as well as cancer immunology. These collaborated efforts could potentially allow a deeper understanding of the field whilst also providing a substantial therapy for glioma.

Figure 2: Correlation between different CAR expression on different glioma cell lines and viability after infection with different adenovirus mutants
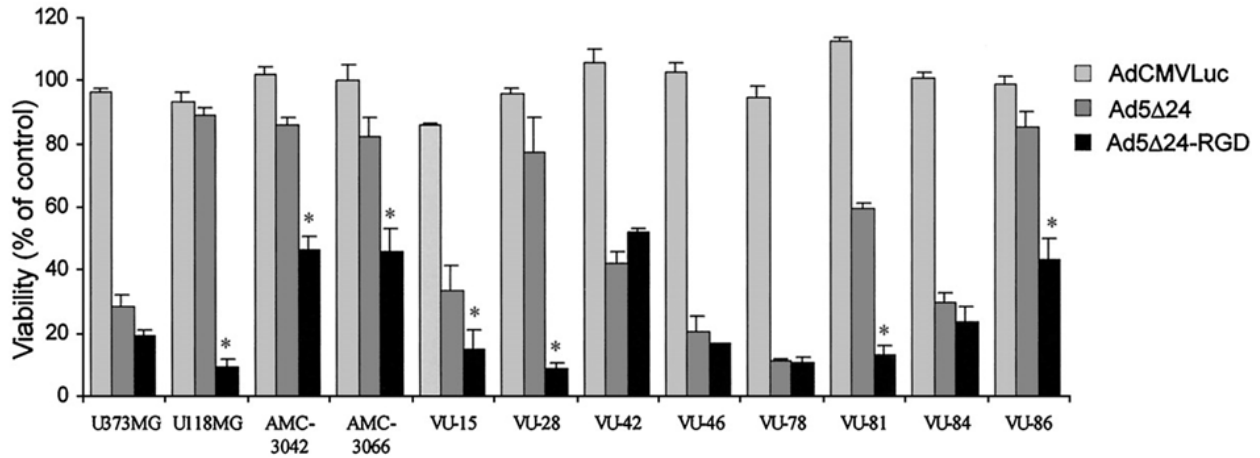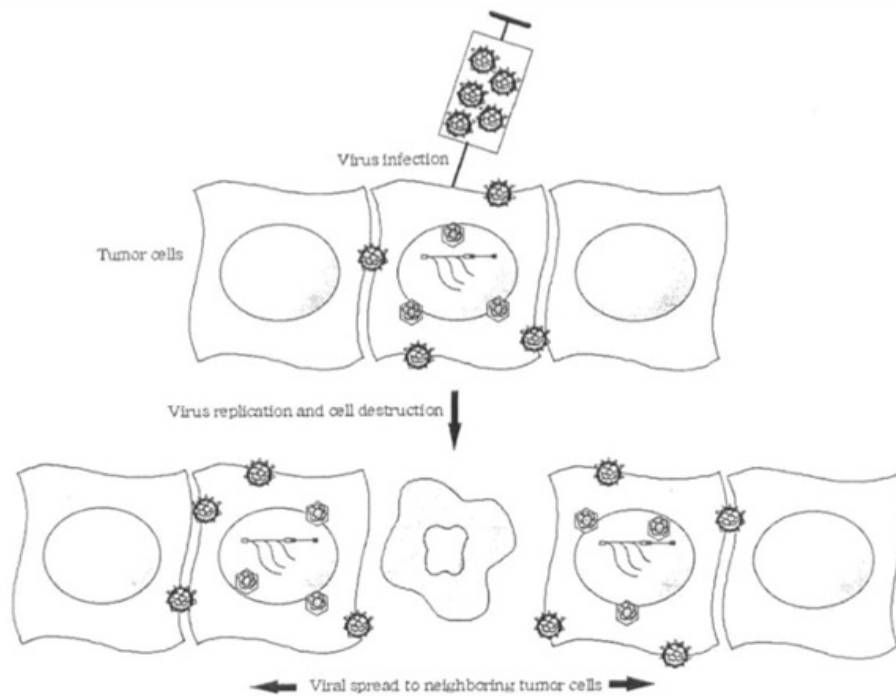

Figure 3: Mechanism of replicative-competent HSV-1

REFERENCES

1. Kleihues P, Cavenee WK. World Health Organization: classification of Tumours. Pathology and genetics of tumours of the nervous system. Int Agen Res Cancer Press: Lyon; 2000.

2. Halkett GK, Lobb EA, Rogers MM, Shaw T, Long AP, Wheeler HR, et al. Predictors of distress and poorer quality of life in High Grade Glioma patients. Patient Educ Couns 2015; 98:525-32.

3. Ratneswaren T, Jack RM, Tataru D, Davies EA. The survival of patients with high grade glioma from different ethnic groups in South East England. J Neurooncol 2014; 120:531-6.

4. Brodbelt A, Greenberg D, Winters T, Williams M, Vernon S, Collins VP, et al. Glioblastoma in England: 2007-2011. Eur J Cancer 2015; 51:533-42.

5. Minniti G, Muni R, Lanzetta G, Marchetti P, Enrici RM. Chemotherapy for glioblastoma: current treatment and

future perspectives for cytotoxic and targeted agents. Anticancer Res 2009; 29:5171-84.

6. Messaoudi K, Clavreul A, Lagarce F. Toward an effective strategy in glioblastoma treatment. Part I: resistance mechanisms and strategies to overcome resistance of glioblastoma to temozolomide. Drug Discov Today 2015; 20:899-905.

7. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJB, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005; 352:987-96.

8. Garside R, Pitt M, Anderson R, Rogers G, Dyer M, Mealing S, et al. The effectiveness and cost-effectiveness of carmustine implants and temozolomide for the treatment of newly diagnosed high-grade glioma: a systematic review and economic evaluation. Health Technol Assess 2007; 11:iii-iv, ix-221.

9. Dey M, Auffinger B, Lesniak MS, Ahmed AU. Antiglioma oncolytic virotherapy: unattainable goal or a success story in the making? Future Virol 2013; 8:675-93.

10. Dey M, Ulasov IV, Lesniak MS. Virotherapy against malignant glioma stem cells. Cancer Lett 2010; 289:1-10.

11. Murphy AM, Rabkin SD. Current status of gene therapy for brain tumors. Transl Res 2013; 161:339-54.

12. Sonabend AM, Ulasov IV, Han Y, Lesniak MS. Oncolytic adenoviral therapy for glioblastoma multiforme. Neurosurg Focus 2006; 20:E19.

13. McConnell MJ, Imperiale MJ. Biology of adenovirus and its use as a vector for gene therapy. Hum Gene Ther 2004; 15:1022-33.

14. Meier O, Boucke K, Hammer SV, Keller S, Stidwill RP, Hemmi S, et al. Adenovirus triggers macropinocytosis and endosomal leakage together with its clathrin-mediated uptake. J Cell Biol 2002; 158:1119-31.

15. Strunze S, Trotman LC, Boucke K, Greber UF. Nuclear targeting of adenovirus type 2 requires CRM1-mediated nuclear export. Mol Biol Cell 2005; 16:2999-3009.

16. Fueyo J, Gomez-Manzano C, Yung WK, Kyritsis AP. The functional role of tumor suppressor genes in gliomas: clues for future therapeutic strategies. Neurology 1998; 51:1250-5.

17. Riley DJ, Lee EY, Lee WH. The retinoblastoma protein: more than a tumor suppressor. Annu Rev Cell Biol 1994; 10:1-29.

18. Gomez-Manzano C, Yung WK, Alemany R, Fueyo J. Genetically modified adenoviruses against gliomas: from bench to bedside. Neurology 2004; 63:418-26.

19. Lin E, Nemunaitis J. Oncolytic viral therapies. Cancer Gene Ther 2004; 11:643-64.

20. Sonabend AM, Ulasov IV, Lesniak MS. Conditionally replicative adenoviral vectors for malignant glioma. Rev Med Virol 2006; 16:99-115.

21. Ulasov IV, Borovjagin AV, Schroeder BA, Baryshnikov AY. Oncolytic adenoviruses: A thorny path to glioma cure. Genes Dis 2014; 1:214-26.

22. Ramachandra M, Rahman A, Zou A, Vaillancourt M, Howe JA, Antelman D, et al. Re-engineering adenovirus regulatory pathways to enhance oncolytic specificity and efficacy. Nat Biotechnol 2001; 19:1035-41.

23. Dyson N, Harlow E. Adenovirus E1A targets key regulators of cell proliferation. Cancer Surv 1992; 12:161-95.

24. Fueyo J, Gomez-Manzano C, Alemany R, Lee PS, McDonnell TJ, Mitlianga P, et al. A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect in vivo. Oncogene 2000; 19:2-12.

25. Ueki K, Ono Y, Henson JW, Efird JT, von Deimling A, Louis DN. CDKN2/p16 or RB alterations occur in the majority of glioblastomas and are inversely correlated. Cancer Res 1996; 56:150-3.

26. Bergelson JM, Cunningham JA, Droguett G, Kurt-Jones EA, Krithivas A, Hong JS, et al. Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. Science 1997; 275:1320-3.

27. Wickham TJ, Mathias P, Cheresh DA, Nemerow GR. Integrins α v β 3 and α v β 5 promote adenovirus internalization but not virus attachment. Cell 1993; 73:309-19.

28. Honda T, Saitoh H, Masuko M, Katagiri-Abe T, Tominaga K, Kozakai I, et al. The coxsackievirus-adenovirus receptor protein as a cell adhesion molecule in the developing mouse brain. Mol Brain Res 2000; 77:19-28.

29. Asaoka K, Tada M, Sawamura Y, Ikeda J, Abe H. Dependence of efficient adenoviral gene delivery in malignant glioma cells on the expression levels of the Coxsackievirus and adenovirus receptor. J Neurosurg 2000; 92:1002-8.

30. Miller CR, Buchsbaum DJ, Reynolds PN, Douglas JT, Gillespie GY, Mayo MS, et al. Differential susceptibility of primary and established human glioma cells to adenovirus infection: targeting via the epidermal growth factor receptor achieves fiber receptor-independent gene transfer. Cancer Res 1998; 58:5738-48.

31. Lamfers ML, Grill J, Dirven CM, Van Beusechem VW, Geoerger B, Van Den Berg J, et al. Potential of the conditionally replicative adenovirus Ad5-Delta24RGD in the treatment of malignant gliomas and its enhanced effect with radiotherapy. Cancer Res 2002; 62:5736-42.

32. Suzuki K, Fueyo J, Krasnykh V, Reynolds PN, Curiel DT, Alemany R. A conditionally replicative adenovirus with enhanced infectivity shows improved oncolytic potency. Clin Cancer Res 2001; 7:120-6.

33. Fueyo J, Alemany R, Gomez-Manzano C, Fuller GN, Khan A, Conrad CA, et al. Preclinical characterization of the antiglioma activity of a tropism-enhanced adenovirus targeted to the retinoblastoma pathway. J Natl Cancer Inst 2003; 95:652-60.

34. Lang FF, Conrad C, Gomez-Manzano C, Tufaro F, Sawaya R, Weinberg J, et al. NT-18phase I clinical trial of oncolytic virus delta-24-rgd (dnx-2401) with biological endpoints: implications for viro-immunotherapy. Neuro-oncology 2014; 16:162.

35. Nigro JM, Baker SJ, Preisinger AC, Jessup JM, Hosteller R, Cleary K, et al. Mutations in the p53 gene occur in diverse human tumour types. Nature 1989; 342:705-8.

36. Sidransky D, Mikkelsen T, Schwechheimer K, Rosenblum ML, Vogelstein B. Clonal expansion of p53 mutant cells is associated with brain tumour progression. Nature 1992; 355: 846-7.

37. Levine A, Hu W, Feng Z. The P53 pathway: what questions remain to be explored? Cell Death & Differ 2006; 13:1027-36.

38. Gomez-Manzano C, Fueyo J, Kyritsis AP, McDonnell TJ, Steck PA, Levin VA, et al. Characterization of p53 and p21 functional interactions in glioma cells en route to apoptosis. J Natl Cancer Inst 1997; 89:1036-44.

39. Gomez-Manzano C, Fueyo J, Kyritsis AP, Steck PA, Roth JA, McDonnell TJ, et al. Adenovirus-mediated transfer of the p53 gene produces rapid and generalized death of human glioma cells via apoptosis. Cancer Res 1996; 56:694-9.

40. Jiang H, McCormick F, Lang FF, Gomez-Manzano C, Fueyo J. Oncolytic adenoviruses as antiglioma agents. Expert Rev Anticancer Ther 2006; 6:697-708.

41. Sundararajan R, Cuconati A, Nelson D, White E. Tumor necrosis factor-alpha induces Bax-Bak interaction and apoptosis, which is inhibited by adenovirus E1B 19K. J Biol Chem 2001; 276:45120-7.

42. Bischoff JR, Kirn DH, Williams A, Heise C, Horn S, Muna M, et al. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. Science 1996; 274:373-6.

43. Heise C, Sampson-Johannes A, Williams A, Mccormick F, Von Hoff DD, Kirn DH. ONYX-015, an E1B gene-attenuated adenovirus, causes tumor-specific cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. Nat Med 1997; 3:639-45.

44. Hall AR, Dix BR, O'Carroll SJ, Braithwaite AW. p53-dependent cell death/apoptosis is required for a productive adenovirus infection. Nat Med 1998; 4:1068-72.

45. O'Shea CC, Johnson L, Bagus B, Choi S, Nicholas C, Shen A, et al. Late viral RNA export, rather than p53 inactiva-

tion, determines ONYX-015 tumor selectivity. Cancer cell 2004; 6:611-23.

46. Parato KA, Senger D, Forsyth PA, Bell JC. Recent progress in the battle between oncolytic viruses and tumours. Nat Rev Cancer 2005; 5:965-76.

47. Chiocca EA, Abbed KM, Tatter S, Louis DN, Hochberg FH, Barker F, et al. A phase I open-label, dose-escalation, multi-institutional trial of injection with an E1B-Attenuated adenovirus, ONYX-015, into the peritumoral region of recurrent malignant gliomas, in the adjuvant setting. Mol Ther 2004; 10:958-66.

48. Xia ZJ, Chang JH, Zhang L, Jiang WQ, Guan ZZ, Liu JW, et al. Phase III randomized clinical trial of intratumoral injection of E1B gene-deleted adenovirus (H101) combined with cisplatin-based chemotherapy in treating squamous cell cancer of head and neck or esophagus. Ai Zheng 2004; 23:1666-70.

49. Kratzer F, Rosorius O, Heger P, Hirschmann N, Dobner T, Hauber J, et al. The adenovirus type 5 E1B-55K oncoprotein is a highly active shuttle protein and shuttling is independent of E4orf6, p53 and Mdm2. Oncogene 2000; 19:850-7.

50. Todo T. Oncolytic virus therapy using genetically engineered herpes simplex viruses. Front Biosci 2008; 13:2060-4.

51. Roizman B, Pellett P. The family Herpesviridae: A brief introduction. In: Knipe DM, Hawley PM, editors. Fields Virology. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001:2381-97.

52. Braun E, Zimmerman T, Hur TB, Reinhartz E, Fellig Y, Panet A, et al. Neurotropism of herpes simplex virus type 1 in brain organ cultures. J Gen Virol 2006; 87:2827-37.

53. Chou J, Kern ER, Whitley RJ, Roizman B. Mapping of herpes simplex virus-1 neurovirulence to gamma 134.5, a gene nonessential for growth in culture. Science 1990; 250:1262-6.

54. Roizman B. The function of herpes simplex virus genes: a primer for genetic engineering of novel vectors. Proc Natl Acad Sci USA 1996; 93:11307-12.

55. Terada K, Wakimoto H, Tyminski E, Chiocca E, Saeki Y. Development of a rapid method to generate multiple oncolytic HSV vectors and their in vivo evaluation using syngeneic mouse tumor models. Gene Ther 2006; 13:705-14.

56. Mellerick D. Physical state of the latent herpes simplex virus genome in a mouse model system: evidence suggesting an episomal state. Virology 1987; 158:265-75.

57. Nishiyama Y. Herpesvirus genes: molecular basis of viral replication and pathogenicity. Nagoya J Med Sci 1996;59:107-120.

58. Balfour HH Jr. Antiviral drugs. N Engl J Med 1999;

340:1255-1268.

59. Fink DJ, Glorioso JC. Engineering herpes simplex virus vectors for gene transfer to neurons. Nat Med 1997; 3:357-9.

60. Mineta T, Rabkin SD, Yazaki T, Hunter WD, Martuza RL. Attenuated multi-mutated herpes simplex virus-1 for the treatment of malignant gliomas. Nat Med 1995; 1:938-43.

61. Wintersberger E. Regulation and biological function of thymidine kinase. Biochem Soc Trans 1997; 25:303-8.

62. Martuza RL, Malick A, Markert JM, Ruffner KL, Coen DM. Experimental therapy of human glioma by means of a genetically engineered virus mutant. Science 1991; 252:854-6.

63. Herrlinger U, Jacobs A, Aghi M, Schuback DE, Breakefield XO. HSV-1 Vectors for Gene Therapy of Experimental CNS Tumors. Methods Mol Med 2000; 35:287-312.

64. McMenamin MM, Byrnes AP, Pike FG, Charlton HM, Coffin RS, Latchman DS, et al. Potential and limitations of a gamma 34.5 mutant of herpes simplex 1 as a gene therapy vector in the CNS. Gene Ther 1998; 5:594-604.

65. Lasner TM, Tal-Singer R, Kesari S, Lee VM, Trojanowski JQ, Fraser NW. Toxicity and neuronal infection of a HSV-1 ICP34. 5 mutant in nude mice. J Neurovirol 1998; 4:100-5.

66. Shen Y, Nemunaitis J. Herpes simplex virus 1 (HSV-1) for cancer treatment. Cancer Gene Ther 2006; 13:975-92.

67. He B, Gross M, Roizman B. The gamma(1) 34.5 protein of herpes simplex virus 1 complexes with protein phosphatase 1 alpha to dephosphorylate the alpha subunit of the eukaryotic translation initiation factor 2 and preclude the shutoff of protein synthesis by double-stranded RNA-activated protein kinase. Proc Natl Acad Sci USA 1997; 94:843-8.

68. Farassati F, Yang A, Lee PW. Oncogenes in Ras signalling pathway dictate host-cell permissiveness to herpes simplex virus 1. Nat Cell Biol 2001; 3:745-50.

69. Kramm C, Chase M, Herrlinger U, Jacobs A, Pechan P, Rainov N, et al. Therapeutic efficiency and safety of a second-generation replication-conditional HSV1 vector for brain tumor gene therapy. Hum Gene Ther 1997; 8:2057-68.

70. Goldstein DJ, Weller SK. Factor (s) present in herpes simplex virus type 1-infected cells can compensate for the loss of the large subunit of the viral ribonucleotide reductase: characterization of an ICP6 deletion mutant. Virology 1988; 166:41-51.

71. Coen DM, Goldstein DJ, Weller SK. Herpes simplex virus ribonucleotide reductase mutants are hypersensitive to acyclovir. Antimicrob Agents Chemother 1989; 33:1395-9.

72. Todo T, Martuza RL, Rabkin SD, Johnson PA. Oncolytic herpes simplex virus vector with enhanced MHC class I presentation and tumor cell killing. Proc Natl Acad Sci USA 2001; 98:6396-401.

73. Sundaresan P, Hunter WD, Martuza RL, Rabkin SD. Attenuated, replication-competent herpes simplex virus type 1 mutant G207: safety evaluation in mice. J Virol 2000; 74:3832-41.

74. Hunter WD, Martuza RL, Feigenbaum F, Todo T, Mineta T, Yazaki T, et al. Attenuated, replication-competent herpes simplex virus type 1 mutant G207: safety evaluation of intracerebral injection in nonhuman primates. J Virol 1999; 73:6319-26.

75. Markert JM, Medlock MD, Rabkin SD, Gillespie GY, Todo T, Hunter WD, et al. Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. Gene Ther 2000; 7:867-74.

76. Todo T, Rabkin SD, Sundaresan P, Wu A, Meehan KR, Herscowitz HB, et al. Systemic antitumor immunity in experimental brain tumor therapy using a multimutated, replication-competent herpes simplex virus. Hum Gene Ther 1999; 10:2741-55.