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Phase Ib Trial of Oncolytic Herpes Virus G207 Shows Safety of Multiple Injections and Documents Viral Replication

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doi:10.1038/mt.2008.275

The prognosis for patients with glioblastoma remains poor despite numerous phase I–II clinical trials on systemic chemotherapies such as the vascular endothelial growth factor–neutralizing antibody bevacizumab.[1] Indeed, only two chemotherapies—implantable carmustine-containing Gliadel wafers and temozolomide[2]—have been approved for glioblastoma by the Food and Drug Administration, because these two agents showed benefit in phase III clinical trials by prolonging median survival by approximately 8 weeks. Therefore, there is a continued need to explore new treatments or to further refine and optimize older treatments for this type of brain cancer.

Oncolytic viruses (OVs), which can be implanted into a glioblastoma resection cavity after surgery, are promising agents for the treatment of glioblastoma because (i) glioblastoma infiltration into surrounding white matter leads to eventual recurrence after surgical removal, and OVs could potentially replicate in and lyse infiltrating tumor cells at the margin of the resection cavity, and (ii) OVs can be engineered to selectively replicate in tumor cells targeting oncogenic or tumor suppressor pathways that have been disrupted.[3,4] So far, there have been six published reports of administration of OVs into glioblastoma multiforme (GBM), all at the level of phase I–II clinical trials. Injected OVs have included recombinant or mutant strains of herpes simplex virus type 1 (HSV1), adenovirus, reovirus, and Newcastle disease virus. Safety of intracerebral OV administration has been confirmed in a total of 92 patients, alleviating concerns that these agents could cause encephalitis when injected into the brain. However, only 5 of the 92 GBM patients (5%) studied in OV clinical trials to date appeared to show some evidence of a radiographic response.[5–10] This survey of the published literature thus calls into question whether the OVs used thus far in the clinics are effective in their anticancer effects.

Because the initial mechanism of OV antitumor action consists of intratumoral replication, it must be determined whether inoculated OVs are truly replicating. In this issue of *Molecular Therapy*, Markert *et al.*[11] provide evidence for *in vivo* viral replication, which has not yet been performed in OV glioblastoma clinical trials. The virus that they used, G207, is a recombinant HSV1 that possesses deletions of both copies of the viral genes encoding ICP34.5 and a *LacZ* gene insertion into the viral gene encoding ICP6. This particular mutant has previously been shown to be safe in a clinical trial,[8] and the lack of ICP34.5 is thought to be a safety feature to ensure lack of neurovirulence and may also allow for replicative targeting of tumor cells,[4] whereas the ICP6 defect is an additional targeting mechanism for tumor cells with p16 tumor suppressor defects.[3]

Glioblastoma oncolytic viral therapy faces a challenge different from that in other cancer types, because GBM tumors cannot be easily accessed without costly procedures associated with some morbidity. A two-stage scheme, composed of stereotactic viral inoculation followed by repeat inoculation during a craniotomy, can provide tissue that allows study of the antiviral immune response and confirmation of whether viral replication occurred. Although this surgical strategy seems very logical and has been used in the past for gene therapy trials,[12,13] GBM patients typically require just one procedure, usually a craniotomy, meaning that a stereotactic needle–guided procedure may not be of clinical necessity and its cost may not be covered by routine insurance. For example, the hospital costs for a craniotomy can be US$50,000 to $100,000, and the risk of perioperative complications and neurologic complications can be high.[14] Therefore, trials that make use of a stereotactic viral inoculation followed by a craniotomy, although providing valuable scientific information about viral replication and the immune response, are performed only if the company sponsoring the trial or another funding agency can cover the costs of the second procedure and if the trial can justify the need for acquisition of tissue.

The study by Markert *et al.* enrolled six individuals with recurrent glioblastoma, each of whom received two doses of the OV G207 totaling 1.15×10^9 plaque-forming units; 13% of the dose was injected stereotactically via a catheter and the remainder was injected into a resection cavity after tumor removal *en bloc* 2–5 days later. In using this two-stage schema for their trial, Markert *et al.* were able to use reverse transcriptase–polymerase chain reaction (RT-PCR) to detect viral RNA encoding HSV DNA polymerase (*pol*) within the lysates of glioblastoma tissue inoculated with G207 during the first procedure and resected during the second procedure. The detection of *pol* mRNA in excised tumor tissue indicates viral replication but does not provide quantitative information about the extent and amount of such replication. The worst-case scenario would be some minimal replication in a few tumor cells (easily detected by RT-PCR) with lack of extensive distribution and high levels of progeny virus in tumor.

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Additional studies—involving, for example, recovery of virus from inoculated tissue for titration or immunohistochemistry to visualize distribution throughout tumor tissue—would determine the efficacy of the OV inoculation.

An additional challenge faced by this trial and other OV trials is that the preclinical production, toxicology, and process development can be limiting. Usually the amount of OV produced is sufficient to cover only a small number of affected individuals, and limitations on the maximal dose that can be studied complicate data interpretation. Illustrative of this problem, the patient in this trial with the longest survival had the highest detectable amount of HSV *pol* RNA and the smallest amount of lymphocyte infiltration. This may suggest that viral replication correlates directly with survival and that an antiviral immune response, as assessed by lymphocyte infiltration, negatively influences survival, supporting the findings in animal models in which a cellular antiviral immune response inhibited viral replication.[15–17] However, larger numbers of subjects are needed to prove this, and this would also allow determination of whether there are tumor-specific mutations that enhance[3,4] or inhibit viral replication, allowing a better understanding of which patients might most benefit from these therapies.

Although overcoming an inhibitory antiviral immune response might require immunomodulation,[15] there are other potential limitations (intracellular antiviral responses and extracellular matrix and microenvironmental barriers) to the success of OV clinical trials for GBM.[18] Future trials should also investigate (i) whether standard glioblastoma chemotherapy or radiation therapy enhances oncolytic HSV replication, as has been shown in preclinical murine models for G207 [19,20], the virus studied by Markert *et al.*,and (ii) whether oncolytic HSVs expressing multiple prodrug-activating enzymes can provide an enhanced anticancer effect through multimodal cell killing.[21,22] The challenges in fulfilling the potential of OVs in the treatment of individuals with cancer, particularly those with glioblastoma, are numerous, but the work of Markert *et al.* is an important step in the right direction.

REFERENCES

1. Vredenburgh, JJ, Desjardins, A, Herndon, JE, Marcello, J, Reardon, DA, Quinn, JA *et al.* (2007). Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. *J Clin Oncol* **25**: 4722–4729.
2. Stupp, R, van den Bent, MJ and Hegi, ME (2005). Optimal role of temozolomide in the treatment of malignant gliomas. *Curr Neurol Neurosci Rep* **5**: 198–206.
3. Aghi, M, Visted, T, Depinho, RA and Chiocca, EA (2008). Oncolytic herpes virus with defective ICP6 specifically replicates in quiescent cells with homozygous genetic mutations in p16. *Oncogene* **27**: 4249–4254.
4. Smith, KD, Mezhir, JJ, Bickenbach, K, Veerapong, J, Charron, J, Posner, MC *et al.* (2006). Activated MEK suppresses activation of PKR and enables efficient replication and *in vivo* oncolysis by Deltagamma(1)34.5 mutants of herpes simplex virus 1. *J Virol* **80**: 1110–1120.
5. Chiocca, EA, Abbed, KM, Tatter, S, Louis, DN, Hochberg, FH, Barker, F *et al.* (2004). 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* **10**: 958–966.
6. Forsyth, P, Roldan, G, George, D, Wallace, C, Palmer, CA, Morris, D *et al.* (2008). A phase I trial of intratumoral administration of reovirus in patients with histologically confirmed recurrent malignant gliomas. *Mol Ther* **16**: 627–632.
7. Harrow, S, Papanastassiou, V, Harland, J, Mabbs, R, Petty, R, Fraser, M *et al.* (2004). HSV1716 injection into the brain adjacent to tumour following surgical resection of high-grade glioma: safety data and long-term survival. *Gene Ther* **11**: 1648–1658.
8. Markert, JM, Medlock, MD, Rabkin, SD, Gillespie, GY, Todo, T, Hunter, WD *et al.* (2000). Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. *Gene Ther* **7**: 867–874.
9. Papanastassiou, V, Rampling, R, Fraser, M, Petty, R, Hadley, D, Nicoll, J *et al.* (2002). The potential for efficacy of the modified (ICP 34.5$^-$) herpes simplex virus HSV1716 following intratumoural injection into human malignant glioma: a proof of principle study. *Gene Ther* **9**: 398–406.
10. Rampling, R, Cruickshank, G, Papanastassiou, V, Nicoll, J, Hadley, D, Brennan, D *et al.* (2000). Toxicity evaluation of replication-competent herpes simplex virus (ICP 34.5 null mutant 1716) in patients with recurrent malignant glioma. *Gene Ther* **7**: 859–866.
11. Markert, JM, Liechty, PG, Wang, W, Gaston, S, Braz, E, Karrasch, M *et al.* (2009). Phase Ib trial of mutant herpes simplex virus G207 inoculated pre- and post-tumor resection for recurrent GBM. *Mol Ther* **17**: 199–207.
12. Ram, Z, Culver, KW, Oshiro, EM, Viola, JJ, DeVroom, HL, Otto, E *et al.* (1997). Therapy of malignant brain tumors by intratumoral implantation of retroviral vector-producing cells. *Nat Med* **3**: 1354–1361.
13. Harsh, GR, Deisboeck, TS, Louis, DN, Hilton, J, Colvin, M, Silver, JS *et al.* (2000). Thymidine kinase activation of ganciclovir in recurrent malignant gliomas: a gene-marking and neuropathological study. *J Neurosurg* **92**: 804–811.
14. Chang, SM, Parney, IF, McDermott, M, Barker, FG 2nd, Schmidt, MH, Huang, W *et al.* (2003). Perioperative complications and neurological outcomes of first and second craniotomies among patients enrolled in the Glioma Outcome Project. *J Neurosurg* **98**: 1175–1181.
15. Fulci, G, Breymann, L, Gianni, D, Kurozomi, K, Rhee, SS, Yu, J *et al.* (2006). Cyclophosphamide enhances glioma virotherapy by inhibiting innate immune responses. *Proc Natl Acad Sci USA* **103**: 12873–12878.
16. Fulci, G, Dmitrieva, N, Gianni, D, Fontana, EJ, Pan, X, Lu, Y *et al.* (2007). Depletion of peripheral macrophages and brain microglia increases brain tumor titers of oncolytic viruses. *Cancer Res* **67**: 9398–9406.
17. Ikeda, K, Ichikawa, T, Wakimoto, H, Silver, JS, Deisboeck, TS, Finkelstein, D *et al.* (1999). Oncolytic virus therapy of multiple tumors in the brain requires suppression of innate and elicited antiviral responses. *Nat Med* **5**: 881–887.
18. Chiocca, EA (2008). The host response to cancer virotherapy. *Curr Opin Mol Ther* **10**: 38–45.
19. Aghi, M, Rabkin, S and Martuza, RL (2006). Effect of chemotherapy-induced DNA repair on oncolytic herpes simplex viral replication. *J Natl Cancer Inst* **98**: 38–50.
20. Stanziale, SF, Petrowsky, H, Joe, JK, Roberts, GD, Zager, JS, Gusani, NJ *et al.* (2002). Ionizing radiation potentiates the antitumor efficacy of oncolytic herpes simplex virus G207 by upregulating ribonucleotide reductase. *Surgery* **132**: 353–359.
21. Aghi, M, Chou, TC, Suling, K, Breakefield, XO and Chiocca, EA (1999). Multimodal cancer treatment mediated by a replicating oncolytic virus that delivers the oxazaphosphorine/rat cytochrome P450 2B1 and ganciclovir/herpes simplex virus thymidine kinase gene therapies. *Cancer Res* **59**: 3861–3865.
22. Tyminski, E, Leroy, S, Terada, K, Finkelstein, DM, Hyatt, JL, Danks, MK *et al.* (2005). Brain tumor oncolysis with replication-conditional herpes simplex virus type 1 expressing the prodrug-activating genes, *CYP2B1* and secreted human intestinal carboxylesterase, in combination with cyclophosphamide and irinotecan. *Cancer Res* **65**: 6850–6857.