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Potential new gene therapy option with sitimagene ceradenovec for newly diagnosed patients with glioblastoma multiforme

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Glioblastoma multiforme (GBM) is associated with a poor prognosis with a 5-year survival rate of less than 5%, making GBM one of the most aggressive neoplastic malignancies. However significant strides have been made over the past few years with respect to understanding the pathophysiology as well as treatment modalities. The use of local therapies, particularly gene therapy, has been evaluated, but have yet to make a major clinical impact on treatment of GBM. In a study published by Westphal and colleagues in *The Lancet Oncology*, the use of sitimagene ceradenovec, a first generation replication-deficient adenovirus containing a prodrug converting enzyme, herpes-simplex virus thymidine kinase, followed by intravenous ganciclovir administration and standard therapy was evaluated compared with standard therapy alone. Patients who received sitimagene ceradenovec had improved time to death or re-intervention, but did not show improvement in overall survival. Patients receiving sitimagene ceradenovec experienced more adverse effects related to treatment, including seizures and hyponatremia. While further studies need to be conducted to determine clinical significance, gene therapy appears to be a viable approach for patients who may be resistant to chemotherapy.

Keywords: glioblastoma multiforme, gene therapy, sitimagene ceradenovec, astrocytoma, local therapy

Abbreviations: GBM, glioblastoma multiforme; CNS, central nervous system; HSV-tk, herpes-simplex-virus thymidine kinase; MGMT, O^6-alkylguanine DNA alkyltransferase

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Glioblastoma multiforme (GBM) is one of the most commonly diagnosed central nervous system (CNS) malignancies in adults.[1] With an incidence of 3.2 per 100 000 person-years, it is one of the less frequently diagnosed cancers in adults. However, GBM has a very dismal prognosis with a 5-y survival rate of less than 5%, making GBM one of the most aggressive neoplastic malignancies faced by patients, clinicians, and researchers alike. One of the hallmark features of GBM that makes it so difficult to treat is the tumor often has significant heterogeneity within the same foci that results in different morphological features and therefore different intratumoral behavior.[2]

Current standard of care for GBM includes surgery, radiation therapy, and chemotherapy, with the most effective treatment currently being surgical resection.[3] However, resection is not often possible due to inoperable tumor location within the brain due to existing comorbidities or poor performance status. Following surgery, adjuvant therapy varies, taking into account tumor pathology, genetics, and patient performance status. Unfortunately, as GBM is likely to reoccur following surgical resection, the uses of peritumoral treatment options have been used. Currently, carmustine wafers are the only Food and Drug Administration (FDA)-approved therapy for intracranial use following tumor resection.[4]

One local therapy which had showed promising results in preclinical trials, was the use of gene therapy utilizing a retrovirus containing a prodrug converting enzyme, herpes-simplex-virus thymidine kinase (HSV-tk).[5] Following surgery, mice were injected intratumorally with a reproduction incompetent retroviral vector in vector-producing cells (VPC) containing the gene for HSV-tk. Following HSV-tk transduction into the tumor cells, ganciclovir was administered intravenously. Thymidine kinase phosphorylates

ganciclovir into its active metabolite, ganciclovir triphosphate. Once activated, ganciclovir triphoshate exhibits its cytotoxic effect by selectively targeting actively dividing cells by incorporating into DNA and inducing apoptosis. Not only was this effective against transduced tumor cells expressing thymidine kinase, but also against nearby tumor cells which did not express thymidine kinase, thus exhibiting a "bystander effect".[6] Toxicity from use of ganciclovir is minimized as it does not affect normal neuronal cells as they do not proliferate and thus not targeted by ganciclovir. In rodent studies, HSV-tk therapy showed promising results as it greatly caused regression of GBM or in some cases eradication.[5] A phase-3 trial conducted by GL1328 international study group, utilized VPCs to determine if gene therapy with ganciclovir would be effective in newly diagnosed GBM patients compared with standard treatment. For this study, standard of care was defined as surgery and radiation therapy (50–60 Gy in 2-Gy fractions days 14–21 × 6 wk), The study did not show improvement in survival (gene therapy median time to death: 365 d, 95% confidence interval [CI], 334–416 d; standard therapy median time to death: 354 d, 95% CI 327–382 d). The study group hypothesized while it is not necessary for all tumor cells to be transduced with HSV-tk, there does appear to be a clear threshold necessary for an effective tumor killing effect.

Recently in August 2013, a phase 3 trial published in *The Lancet Oncology* by Westphal and colleagues[7] described a new approach to gene therapy using sitimagene ceradenovec, a first generation replication-deficient adenovirus containing cDNA for HSV-tk (ASPECT trial). The study was designed as a randomized, open-label, parallel group, multicenter trial to evaluate the efficacy of intraoperative sitimagene ceradenovec followed by ganciclovir in addition to standard therapy or resection compared with standard therapy alone for treatment of newly diagnosed GBM. The study screened 256 patients, of which 124 patients were randomized into the experimental group and 126 into the control group. A composite primary endpoint of time to death or re-intervention was evaluated, with secondary endpoint of time to all-cause mortality.

Patients randomized in the trial had similar baseline characteristics except for a slight imbalance in Karnofsky score in favor of the standard of care group (15% of patients enrolled in experimental group had a Karnosky score of 70, while only 9% of patients in the standard of care group had a Karnofsky score of 70). Patients randomized to the experimental group, received a one-time treatment of sitimagene ceradenovec that was administered as a series of injections (30–70 injections) into the resected tumor cavity at the end of the completed resection. Five days following resection (to allow for transduction), intravenous ganciclovir 5 mg/kg twice daily was initiated and continued for 2 wk. Standard of care was defined as surgery and radiation therapy (60 Gy in 30 fractions to the tumor volume) but the Stupp protocol for radiochemotherapy was allowed in institutions that had temozolomide access.[8] More patients in the standard care group received temozolomide (65% vs 49%, respectively).

Findings from the first full analysis (August 2008) and from a subsequent analysis (October 2009) showed that sitimagene ceradenovec improved time to death or re-intervention, irrespective of temozolomide use (hazard ratio 1.53, 95% CI 1.13–2.07; P = 0.0057).[7] However, no statistically significant difference in overall survival was reported (sitimagene ceradenovec, 497 d vs standard care 452 d; hazard ratio [HR] 1.18, 95% CI 0.86–1.61; P = 0.31).

O^6-alkylguanine DNA alkyltransferase (MGMT) status in patients with glioblastoma increasingly is becoming of greater clinical significance as it can help guide chemotherapeutic regimens.[9] If the promoter region of MGMT is not methylated, then MGMT will be expressed and will reverse damage caused by alkylating agents. This is particularly important as MGMT expression can usually render patients resistant to temozolomide treatment.[10] In a subgroup analysis of patients that expressed MGMT in tumors within the experimental group, sitimagene ceravendoc was found to have a greater effect on the primary endpoint of the study compared with the whole cohort (HR 1.72, 95% CI 1.15–2.56, P = 0.008).[7]

The study also evaluated the effect of anti-adenoviral neutralizing antibodies as this could compromise efficacy of sitimagene ceradenovec. At baseline screening of patients, 46 patients in the experimental group and 37 patients in the control group had quantifiable anti-adenoviral antibody levels. By day 19, the number of patients with quantifiable antibody levels in the experimental group increased to 84 patients (and 14 patients with detectable, but nonquantifiable antibody levels). Patients in the control group did not have any change in patients with quantifiable antibody levels. The study reported that patients who had higher anti-adenoviral antibody titers (>100) actually experienced greater efficacy from sitimagene ceradenovec (primary endpoint HR 2.17 [95% CI, 1.01–4.64], P = 0.047).

Incidence of patients reporting adverse effects was similar among the two treatment groups. The study defined adverse effects occurring before day 19 as treatment related, while adverse effects reported after day 19 were considered likely related to other treatment modalities (i.e., temozolomide or radiation therapy). During days 0–19, patients in the experimental group had a higher incidence of cerebral edema, transient focal neurological deficits, and hyponatremia, with no reported life-threatening (grade 4) or fatal (grade 5) adverse reaction. The authors hypothesized that this increase in adverse effects was due to the multiple injections into the resected tumor cavity as well as ganciclovir administration, which is known to cause seizures. Use of other intracranial therapy (e.g., carmustine wafers) is also associated with increased adverse effects, such as seizures and cerebral edema).[5]

The investigators attempted to minimize the limitations to the open-label study. One of these included use of a re-intervention committee, which was masked to treatment, to minimize bias.[7] The committee found that when re-intervention was called for, no bias was detected among the whole cohort. The authors also disclosed conflict of interests, including 4 of the 9 authors had a vested interest in sitimagene ceradenovec, either

through direct employment for Ark therapeutics or as shareholders in the company. Ark therapeutics also funded the study adding further conflict of interest.

While the study was randomized, the study population consisted of primarily Caucasians. Although little evidence exists suggesting pathogenic GBM differences among ethnic groups, it is unknown whether MGMT status varies among ethnic groups. As MGMT status becomes more well understood, it may be important to evaluate results based on baseline status and also to determine if MGMT status varies among ethnic groups.

Another limitation is the use of a composite endpoint (i.e., time to death or re-intervention). The authors did not report the findings of each individual outcome, making it unclear whether one outcome had better findings than the other (e.g., more improvement in time to death vs time to re-intervention). In 2010, a commentary was published in the *Journal of the American Medical Association* discussing the use of composite end-points in studies.[11] It is interesting to note that death is often included in the composite endpoint as it is the most important, however, often statistically insignificant. Based on this assumption, when interpreting results from the ASPECT trial it seems patients are most likely to benefit from time to re-intervention outcome rather than time to death; however, this information was not reported.[7,11]

Gene therapy remains a promising new treatment modality for patients with resectable tumors and good performance status. While this study did not show improvement in overall survival, it did demonstrate moderate improvement in time to death or re-intervention, with similar adverse side effects when compared with standard treatments. Of particular interest, the study also showed improvement in patients with MGMT positive tumors. Unfortunately, the study was not designed or powered to evaluate MGMT in this subset of patients. Future studies in glioblastoma should be designed to evaluate MGMT status and its effect on outcome. Currently, several other ongoing trials in the United States examining the use of gene therapy in patients with GBM, however none are evaluating MGMT status. Overall, gene therapy has made great advancements and is a welcome addition to the future possibilities in the treatment of GBM.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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