Stem Cells Loaded With Multimechanistic Oncolytic Herpes Simplex Virus Variants for Brain Tumor Therapy

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Background The current treatment regimen for malignant glioblastoma multiforme (GBM) is tumor resection followed by chemotherapy and radiation therapy. Despite the proven safety of oncolytic herpes simplex virus (oHSV) in clinical trials for GBMs, its efficacy is suboptimal mainly because of insufficient viral spread after tumor resection.

Methods Human mesenchymal stem cells (MSC) were loaded with oHSV (MSC-oHSV), and their fate was explored by real-time imaging in vitro and in vivo. Using novel diagnostic and armed oHSV mutants and real-time multimodality imaging, the efficacy of MSC-oHSV and its proapoptotic variant, oHSV-TRAIL encapsulated in biocompatible synthetic extracellular matrix (sECM), was tested in different mouse GBM models, which more accurately reflect the current clinical settings of malignant, resistant, and resected tumors. All statistical tests were two-sided.

Results MSC-oHSVs effectively produce oHSV progeny, which results in killing of GBMs in vitro and in vivo mediated by a dynamic process of oHSV infection and tumor destruction. sECM-encapsulated MSC-oHSVs result in statistically significant increased anti-GBM efficacy compared with direct injection of purified oHSV in a preclinical model of GBM resection, resulting in prolonged median survival in mice (P < .001 with Gehan–Breslow–Wilcoxon test). To supersede resistant tumors, MSC loaded with oHSV-TRAIL effectively induce apoptosis-mediated killing and prolonged median survival in mice bearing oHSV- and TRAIL-resistant GBM in vivo (P < .001 with $\chi^2$ contingency test).

Conclusions Human MSC loaded with different oHSV variants provide a platform to translate oncolytic virus therapies to clinics in a broad spectrum of GBMs after resection and could also have direct implications in different cancer types.


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Methods

Parental and Engineered Cell Lines
Human bone marrow–derived MSC (kindly provided by David Prockop, Tulane University, New Orleans, LA) were grown as previously described (26). Gli36vIII (Gli36 cells expressing EGFRvIII, a constitutively active variant of EGFR), U87, U251, LN319, U138, U251, and LN229 GBM cell lines were obtained from the American Type Culture Collection (Manassas, VA) and grown as described previously (27). MSC and GBM cell lines (LN229 and Gli36vIII cells) were transduced with lentivirus (LV) bearing green fluorescent protein (GFP or GF) fused to firefly luciferase (Fl): LV-GFl or LV-GFP at multiplicity of infection of 2 in medium containing protamine sulfate (2 µg/ mL). All cells were visualized by fluorescence microscopy for GFP expression 36 hours after transduction. Lentiviral packaging was performed by transfection of 293T cells as previously described (28).

Recombinant Oncolytic Herpes Simplex Viruses and Viral Growth Assay
G47Δ-TRAIL carries S-TRAIL cDNA driven by the IE4/5 immediate early promoter of HSV and G47Δ-Fluc carries firefly luciferase cDNA driven by cytomegalovirus immediate early promoter. oHSV-mCherry (oHSV-mCh) was generated by cloning mCherry cDNA under the IE4/5 immediate early promoter of HSV using the same BAC technique and the shuttle plasmid as with G47Δ-TRAIL (29). All of the recombinant oHSVs express Escherichia coli lacZ driven by endogenous ICP6 promoter.

In Vivo Mouse Experiments
Female SCID mice (aged 6–8 weeks) obtained from Charles River laboratories (Wilmington, MA) were used in three different in vivo experiments. All of the animal care procedures were approved by the Subcommittee on Research Animal Care at MGH.

To assess cell viability of MSC-oHSV, MSC-GFl (mice; n = 3), or MSC-GFl infected with oHSV-mCh (n = 5), MSC were stereotactically implanted into the brains of mice, and bioluminescence imaging was performed as described in the Supplementary Methods (available online).

To assess the therapeutic effects of MSC-oHSV, Gli36vIII-GFl cells were stereotactically implanted into the brains of SCID mice (n = 12), and tumor-bearing mice were injected with MSC (n = 3), oHSV-mCh (n = 3), or MSC-oHSV-mCh (n = 6) intratumorally at the same coordinate as the tumor cell implantation. Mice were followed for changes in tumor volumes by Fluc bioluminescence imaging.

To assess the efficacy of MSC-oHSV or MSC-oHSV-TRAIL in a mouse model of tumor resection, a cranial window was created over the original implantation site for tumor debulking. One week later, Gli36vIII-GFl or LN229-GFl cells were stereotactically implanted into the brains of 10 mice, and tumor debulking was performed 7 days (Gli36vIII-GFl) or 21 days (LN229-GFl) after implantation as described in the Supplementary Materials (available online). sECM-encapsulated MSC or naked/purified oHSV were injected into the resection cavity (mice: n = 5). Mice were serially imaged for Fluc activity as described in the Supplementary Methods (available online).

For survival studies, mice bearing Gli36vIII-GFl GBM tumors (n = 20) underwent tumor debulking and were treated with sECM-encapsulated MSC (n = 5), purified oHSV-mCh (n = 5) or sECM-encapsulated MSC-oHSV-mCh (n = 10). Mice were imaged for Fluc activity an followed for survival and killed when neurological symptoms became apparent. For oHSV-TRAIL in vivo studies, LN229-GFl GBM cells were implanted (n = 12), and 21 days later tumor debulking was performed followed by injection of sECM-encapsulated MSC-oHSV-mCh (n = 6) or sECM-encapsulated MSC-oHSV-TRAIL (n = 6), and mice were followed for survival.

Statistical Analysis
Data were analyzed by Student t test when comparing two groups. Data were expressed as mean ± standard deviations. Differences were considered statistically significant at P less than .05. Kaplan–Meier analysis was used for mouse survival studies, and the groups were compared using Gehan–Breslow–Wilcoxon test or the χ2 contingency test. All statistical tests were two-sided.

Further methodological details are described in the Supplementary Methods (available online).

Results

MSC as a Cellular Delivery Vehicle for oHSV
To assess whether MSC are capable of serving as a cellular delivery vehicle for oHSV, we first studied oHSV replication and release of infectious viral particles in human MSC in vitro. Human MSC were infected with a G47Δ-based recombinant oHSV in which cDNA encoding the mCherry fluorescent protein is placed under the IE4/5 immediate-early promoter of HSV (oHSV-mCh) (29). Infection of MSC with oHSV-mCh resulted in exponential amplification of virus during the first 24 hours (Figure 1A), which was associated with marker protein mCherry expression and resulted in decreasing MSC survival in vitro (Figure 1B–E). To assess the survival of oHSV-mCh–loaded MSC (MSC-oHSV-mCh) in vivo, MSC were first engineered to express a bimodal fluorescent and bioluminescent fused protein, GFP. A statistically significant decrease in cell viability was seen in MSC-GFl–loaded oHSV-mCh implanted into the brains of mice as compared with the controls (P = .006) (Figure 1F). Brain sections of mice at different time points after oHSV-mCh injection showed cytopathic effect of virus-loaded, mCh-positive MSC as virus amplification and mCh expression intensified, eventually resulting in cell lysis with mCh-positive cell debris (Figure 1G–I).

To assess the oncolytic activity of MSC-oHSV-mCh in GBM cells, we used human GBM cell lines Gli36vIII (highly proliferating) and U87 (intermediately proliferating) engineered to express the diagnostic marker GFl, Gli36vIII-GFl, and U87-GFl. A direct association between GBM-GFl cell numbers, Fluc signal intensity, and GFP-positive cells was seen in vitro within the ranges tested (Supplementary Figure 1, available online). Release of oHSV-mCh from MSC resulted in the infection of engineered GBM cells and spread of oHSV-mCh among GBM cells, leading to extensive oncolysis (Figure 2, A–F). In vitro, coculture of these GBM lines with MSC-oHSV-mCh resulted in drastic cell killing of both highly proliferating Gli36vIII-GFl (Figure 2, A–C and G) and intermediately proliferating U87-GFl (Figure 2, D–G) GBM cells. Similarly,
MSC-oHSV-mCh cocultured with GBMs resulted in increased killing of oHSV-sensitive (U87, U251, U373, and Gli36vIII) compared to the oHSV-resistant (LN229, U138, and LN319) GBM cells (Supplementary Figure 2, available online). Despite the aggressiveness of Gli36vIII-GFl GBM in vitro as well as in established GBMs in vivo.

MSC-oHSV-mCh expressed a fusion of firefly luciferase-mCherry (Fluc-mCh). Fluc bioluminescence imaging revealed that intra-venously injected MSC did not home to the tumors in the brain and got trapped in the lungs (Supplementary Figure 4, available online). These results reveal that oHSV-mCh–loaded MSC effectively produce oHSV progeny, which results in effective killing of GBM cells in vitro as well as in established GBMs in vivo.

Dynamics of oHSV Infection and Oncolysis In Vivo

To investigate the dynamics of oHSV spread and GBM cell killing mediated by oHSV-loaded MSC, mice bearing highly proliferating Gli36vIII-GFl tumors were treated with MSC-oHSV-mCh. Multicolor fluorescence imaging of serial brain sections showed rapid spread of oHSV-mCh emanating from MSC-oHSV-mCh implantation site with concomitant shrinkage of Gli36vIII-GFl tumor area within 96 hours (Figure 3, A–D). At 24 hours, a higher number of yellow cells (GFP-positive, mCherry-positive) emerged around the MSC implantation site, confirming infection of these cells. Bioluminescence firefly luciferase (Fluc) signal intensity as a measure of tumor volume changes in mice bearing intracerebral Gli36vIII-GFl tumors treated with MSC, oHSV-mCh, or MSC-oHSV-mCh. Bars: + standard deviation. Sizing of scale bars: 200 μm. In all panels, *P < .05 vs controls (two-sided t test).
of tumor cells (Figure 3, E and F, white arrowheads). oHSV amplification and spread penetrating into tumor tissue was seen at 48 hours, and rounded tumor cells showing weakened GFP expression were observed, indicating widespread cytopathic effect (Figure 3G, black arrowheads). After 72 hours, the forefront of mCherry-positive area extended to near the tumor periphery, leaving vast areas of mCherry-positive cell debris behind. Viral production in human MSC and infection of Gli36vIII-GFl resulted in almost entire destruction of original tumor mass (white dotted line). E and F) Large amounts of yellow cells (EGFP+, mCherry+; arrowheads) at the tumor areas surrounding the MSC implantation site 24 hours after MSC implantation were observed. G and H) Virus penetrating into the tumor at 48 hours with widespread cytopathic effect (black arrowheads) and ongoing rounds of tumor infection (white arrowheads). I and J) Forefront of mCherry-positive area extended to near tumor periphery at 72 hours leaving mCherry-positive cell debris behind. K) High magnification photomicrograph showing infected GBM cells at varying stages of infection. L) Hematoxylin & Eosin (H&E) and X-gal staining of a brain section adjacent to the one shown in (E) to confirm that fluorescence imaging results were associated exactly with histological analysis. M) Plot showing quantification of uninfected Gli36vIII tumor cells (GFP+), oHSV-mCh infected MSC (mCherry+), and oHSV-mCh infected Gli36vIII (GFP+ and mCherry+) at different times after treatment. Cells were counted and plotted relative to total cell number. Bars: + standard deviation. Scaling of scale bars: 200 µm in (A–D); 100 µm in (E, G, I, and L); 50 µm in (F, H, and J); 20 µm in (L).

**Figure 3.** Fluorescent imaging to follow progression of viral oncolysis by mesenchymal stem cell (MSC) loaded with oncolytic herpes simplex virus (oHSV)-mCherry (mCh). A–D) Gli36vIII-GFl (green fluorescent protein-firefly luciferase) tumor-bearing mice were treated with MSC-oHSV-mCh. Production of oHSV-mCh (mCh) in MSC and infection of Gli36vIII-GFl cells (GFP+mCh) were monitored over time. Low magnification sections showing viral infection (red) of tumor cells (green) over 4 days after MSC-oHSV-mCh implantation. Arrowheads show MSC-oHSV-mCh implantation site. Viral production in human MSC and infection of Gli36vIII-GFl resulted in almost entire destruction of original tumor mass (white dotted line). E and F) Large amounts of yellow cells (EGFP+, mCherry+; arrowheads) at the tumor areas surrounding the MSC implantation site 24 hours after MSC implantation were observed. G and H) Virus penetrating into the tumor at 48 hours with widespread cytopathic effect (black arrowheads) and ongoing rounds of tumor infection (white arrowheads). I and J) Forefront of mCherry-positive area extended to near tumor periphery at 72 hours leaving mCherry-positive cell debris behind. K) High magnification photomicrograph showing infected GBM cells at varying stages of infection. L) Hematoxylin & Eosin (H&E) and X-gal staining of a brain section adjacent to the one shown in (E) to confirm that fluorescence imaging results were associated exactly with histological analysis. M) Plot showing quantification of uninfected Gli36vIII tumor cells (GFP+), oHSV-mCh infected MSC (mCherry+), and oHSV-mCh infected Gli36vIII (GFP+ and mCherry+) at different times after treatment. Cells were counted and plotted relative to total cell number. Bars: + standard deviation. Sizing of scale bars: 200 µm in (A–D); 100 µm in (E, G, I, and L); 50 µm in (F, H, and J); 20 µm in (L).

**MSC-Mediated Delivery of oHSV in a Mouse Model of GBM Resection**

We have recently developed a clinically relevant mouse model of GBM resection and shown the ECM encapsulation of therapeutic MSC allows retention of a higher number of MSC in the GBM tumor resection cavity, resulting in higher therapeutic efficacy (30). Based on these studies, we first assessed the production and release of oHSV-mCh from sECM-encapsulated MSC. In vitro sECM-MSC-oHSV-mCh produced oHSV-mCh during the first 24 hours and reached plateau 36 hours after infection (Supplementary Figure 5A, available online). Further, coculture of sECM-encapsulated MSC-oHSV-mCh with U87-GFl GBM cells statistically significantly reduced GBM cell viability over time compared with sECM-encapsulated oHSV-mCh (P = .004) and sECM-MSC (P = .002) (Supplementary Figure 5, B and C, and sECM-MSC (P = .002) (Supplementary Figure 5, B and C, and sECM-MSC (P = .002) (Supplementary Figure 5, B and C, and sECM-MSC (P = .002) (Supplementary Figure 5, B and C, and sECM-MSC (P = .002) (Supplementary Figure 5, B and C, and sECM-MSC (P = .002) (Supplementary Figure 5, B and C,
We then sought to determine whether oHSV delivery by sECM-encapsulated MSC increases oHSV persistence and oncolytic activity in a clinically relevant model of GBM resection when compared with direct injection of concentrated oHSV. We used an in vivo imageable version of G47Δ recombinant oHSV in which cDNA encoding Fluc is placed under the cytomegalovirus immediate early promoter (oHSV-Fluc) (31). sECM-encapsulated MSC loaded with oHSV-Fluc (MSC-oHSV-Fluc, 3 × 10^6 PFU when loading) led to statistically significantly increased expression of Fluc when compared with conventional direct injection of purified oHSV-Fluc (1 × 10^6 PFU) in the tumor resection cavity before (C) and after (D) resection. E) Fluorescent image showing sECM-encapsulated MSC-oHSV-mCherry (MSC-oHSV-mCh) (red) implanted at close proximity to the residual GFP+ tumor cells. F) Plot showing Fluc signal as a measure of Gli36vIII-GFl (GFP-firefly luciferase) tumor burden followed over time after intracavitary injections of purified oHSV-mCh or sECM-encapsulated MSC-oHSV-mCh. G) Established intracranial Gli36vIII-GFl tumors were resected and treated with intracavitary injections of phosphate-buffered saline (PBS), purified oHSV-mCh, or sECM-encapsulated MSC-oHSV-mCh. Kaplan–Meier survival curves of treated mice. P < 0.001 (Wilcoxon test), sECM-MSC-oHSV-mCh vs oHSV-mCh and vs PBS. The number of mice at risk is shown below the graph. Bars: + standard deviation. *P < .05 vs controls (two-sided t test). Sizing of scale bars: 400 µm.

Figure 4. Fate and therapeutic efficacy of synthetic extracellular matrix (sECM)–encapsulated mesenchymal stem cells (MSC) loaded with oncolytic herpes simplex virus (oHSV) in a clinically relevant glioblastoma multiforme (GBM) resection model. Mice bearing established intracranial Gli36vIII-GFP (green fluorescent protein) tumors were resected and treated with intracavitary injections of purified oHSV–firefly luciferase (Fluc) or sECM-encapsulated MSC-oHSV-Fluc. A) Plot showing changes in Fluc activity as a measure of virally infected cells monitored over time. A representative bioluminescence image from a mouse of each group and time is shown (13.2 ± 1.82 times higher Fluc expression in the MSC-oHSV-Fluc group in comparison with the purified oHSV-Fluc group). B) Light microscopic image of the brain after craniotomy. The white dotted line shows the drilled rim around a cranial window. C and D) Fluorescent microscopic images showing GFP+ intracerebral tumor visualized through the cranial window before (C) and after (D) resection.
(Figure 4E) or purified oHSV-mCh. A statistically significant suppression of tumor growth (oHSV-mCh vs sECM-MSC-oHSV-mCh: \( P = 0.004 \)) and increased median survival time was seen in mice treated with sECM-MSC-oHSV-mCh as compared with the mice treated with purified oHSV-mCh (MSC-oHSV-mCh group: 32 days; PBS group: 19 days; purified oHSV-mCh group: 20 days; \( P < .001 \) with Gehan–Breslow–Wilcoxon test) (Figure 4, F and G).

We also assessed the viral yield of intratumorally transplanted MSC-oHSV-mCh by X-gal staining of serially collected brain sections. oHSV-mCh initially produced by MSC (day 1) infected neighboring GBM tumor cells, which was followed by further oHSV-mCh propagation in tumor cells (day 3) (Supplementary Figure 6, A and B, available online). Furthermore, brain sections from mice treated with MSC-oHSV-mCh did not show evidence of oHSV infection in peritumoral normal brain (neurons and astrocytes) 12 days after treatment (Supplementary Figure 6C, available online), confirming the safety of this approach. These results demonstrate that encapsulated MSC-oHSV results in an increased anti-GBM efficacy compared with direct injection of purified oHSV in a preclinical model of GBM resection possibly because of long-lasting production of oHSV in the vicinity of GBM deposits.

**MSC-Mediated Delivery of an Armed oHSV Mutant**

We recently created an armed version of G47Δ recombinant oHSV in which cDNA encoding secretable TRAIL is placed under the IE4/5 immediate-early promoter of HSV (oHSV-TRAIL) and showed that it targets a broad spectrum of GBM lines, including oHSV-resistant and TRAIL-resistant lines (31). To develop MSC loaded with oHSV therapies for a broad spectrum of GBMs, we next investigated whether MSC loaded with oHSV-TRAIL could target both oHSV- and TRAIL-resistant GBM lines. oHSV-TRAIL released from MSC loaded with oHSV-TRAIL (MSC-oHSV-TRAIL) exponentially amplified during the first 36 hours and reached a plateau 48 hours after infection (Figure 5A). Similar to MSC-oHSV-mCh, a time-dependent decrease in MSC viability was seen in MSC-oHSV-TRAIL over 120 hours (Figure 5B).

Time-course enzyme-linked immunosorbent assay on MSC-oHSV-TRAIL confirmed the release of S-TRAIL into the culture media over time (Figure 5C). As compared with MSC-oHSV-mCh or MSC-TRAIL treatment, MSC-oHSV-TRAIL treatment resulted in greater cell killing when cocultured with different engineered GBM lines that are either fully or semi-resistant to TRAIL and have low susceptibility to oHSV-mediated oncolysis (31) (Figure 5D; Supplementary Figures 7, A–C, and 8A, available online). GBM cell killing by MSC-oHSV-TRAIL was mediated by activated caspase-3/7 (Figure 5E; Supplementary Figure 8B, available online). Western blotting analysis of LN229 GBM cell lysates obtained from transwell inserts culture showed a greater increase in cleaved caspase-8, cleaved caspase-9, and cleaved PARP in the MSC-oHSV-TRAIL treatment group as compared with controls (Figure 5F). These results show that MSC loaded with the armed oHSV mutant encoding secretable TRAIL effectively produce oHSV-TRAIL progeny and induce apoptosis-mediated killing of both oHSV- and TRAIL-resistant GBM.

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**Figure 5.** Therapeutic efficacy of mesenchymal stem cells (MSC) loaded with oncolytic herpes simplex virus (oHSV)–TRAIL in oHSV- and TRAIL-resistant glioblastoma multiforme (GBMs) in vitro. MSC were infected with oHSV-TRAIL and followed over time for virus yield and MSC viability. A) Plot showing viral yield of MSC-oHSV-TRAIL in vitro over time. B) Plot showing survival of MSC-oHSV-TRAIL in vitro over time. C) Enzyme-linked immunosorbent assay showing secretion of S-TRAIL from MSC-oHSV-TRAIL over time. D and E) Coculture assay of MSC, MSC-oHSV-mCherry (MSC-oHSV-mCh), or MSC-OHSV-TRAIL with different fully or semi-TRAIL-resistant GBM lines (LN229, LN319, U138, U251) engineered to express GFP–firefly luciferase (Fluc). Plots representing tumor cell viability at day 3 (D) and caspase-3/7 activation in GBM lines at day 2 (E) show increased tumor cell killing and caspase activation by MSC-oHSV-TRAIL. F) Western blotting analysis of TRAIL-resistant LN229 cell lysate collected after 20 and 40 hours of incubation with MSC, MSC-oHSV-mCh, or MSC-OHSV-TRAIL. Bars: + standard deviation. \( *P < .05 \) vs controls (two-sided t test).
To test in vivo efficacy of MSC-oHSV-TRAIL in a clinically relevant GBM mouse model, mice bearing established LN229-GFl tumors underwent GBM resection followed by injection of sECM-encapsulated MSC, MSC-oHSV-mCh, or MSC-oHSV-TRAIL. A suppression in the relapse of LN229-GFl tumors was seen in the sECM-MSC-oHSV-TRAIL–treated group as compared with the controls (Figure 6A). T2-weighted magnetic resonance imaging showed a localized high-signal-intensity area at the site of sECM-MSC-oHSV-TRAIL injection on day 1 after resection, which persisted for 2 weeks (Figure 6B). T1-weighted magnetic resonance imaging with contrast confirmed the efficacy by sECM-encapsulated MSC-oHSV-TRAIL because it revealed sustained tumor regression after treatment as compared with the controls (Figure 6C). This anti-GBM activity by sECM-MSC-oHSV-TRAIL resulted in statistically significant prolongation of median survival time of mice (41 days) as compared with the sECM-MSC-oHSV-mCh–treated group (20 days; \( P < .001 \) with \( \chi^2 \) contingency test) (Figure 6D). These results demonstrate that MSC can serve as a robust cellular delivery vehicle for oHSV armed with a proapoptotic molecule, and when applied within sECM to a clinically relevant mouse model of GBM resection, this treatment modality targets resistant GBM, resulting in a statistically significant survival benefit (\( P = 0.04 \)).

**Discussion**

In this study, we showed the dynamics of diagnostic oHSV mutants, oHSV-mCh, and oHSV-Fluc delivered by MSC (MSC-oHSV) in real time in vitro and in vivo in mouse models of GBMs. We also showed the efficacy of sECM-encapsulated MSC-oHSV and its proapoptotic variant MSC-oHSV-TRAIL in clinically applicable mouse models that represent clinical scenarios of tumor resection and resistance.

In an effort to circumvent the issues dampening the current oHSV trials in GBM, we sought to develop a cell-based strategy to deliver oHSV that takes into account the challenges found in a clinical scenario of GBM resection. We have previously shown that both human neural stem cells (NSC) and MSC can home to tumors in the brain and can effectively deliver therapeutic proteins on site, resulting in a substantial therapeutic efficacy (26,32,33). The use of MSC as delivery vehicles opposed to NSC has major advantages in that they can be easily isolated from patients and grown in culture and have high metabolic activity (26,34). Using oHSV mutants showing tumor regression after sECM-MSC-oHSV-TRAIL treatment (1st and 3rd row) and tumor relapse after sECM-MSC-oHSV-mCh treatment (2nd and 4th row) in T2-weighted sequences (B) and T1-weighted sequences with contrast agent (C). White arrowheads in (B) point out edema caused by tumor growth, which persists in the treatment group. Despite no obvious tumor regrowth on day 15 (C), an area with high T2 signal persisted after sECM-MSC-oHSV-TRAIL treatment (white arrowheads in B). D) Kaplan–Meier survival curves of mice treated with sECM-encapsulated MSC-oHSV-mCh or MSC-oHSV-TRAIL (n = 6 mice per group). \( P < .001 \) (\( \chi^2 \) contingency test), sECM-MSC-oHSV-TRAIL vs sECM-MSC-oHSV-mCh. The number of mice at risk is shown below the graph.

**Figure 6.** Therapeutic efficacy of synthetic extracellular matrix (sECM) mesenchymal stem cells (MSC) loaded with oncolytic herpes simplex virus (oHSV)–S-TRAIL in oHSV- and TRAIL-resistant glioblastoma multiforme (GBMs) in vivo. A–D) Mice bearing established LN229-GFl (green fluorescent protein-firefly luciferase) intracranial tumors were resected and treated with sECM-encapsulated MSC, MSC-oHSV-mCherry (MSC-oHSV-mCh), or MSC-oHSV-TRAIL. A) Plot showing bioluminescence signal intensity over time with increased tumor suppression in the sECM-MSC-oHSV-TRAIL group against sECM-MSC and sECM-MSC-oHSV-mCh groups. A representative bioluminescence image from a mouse of each group at day 9 is shown. Bars: + standard deviation. B and C) Representative serial magnetic resonance images

![Figure 6](https://academic.oup.com/jnci/article-abstract/106/6/dju090/948257)
bearing diagnostic proteins and combining bioluminescence imaging, our results revealed that the robust changes in virus spread and oncolysis occurred during the initial 48 hours after MSC-oHSV implantation, which may be crucial for overall therapeutic success. Comparison of therapeutic activity between MSC-oHSV and naked oHSV revealed that both potently induced tumor volume reduction. However, MSC-oHSV treatment resulted in superior efficacy that may be associated with the different dynamics of virus production in situ, spread, and clearance after injection of MSC-oHSV and purified oHSV.

We have previously shown that encapsulation of stem cells in biodegradable sECM is a promising approach toward successful stem cell–based therapy after GBM resection (30). Most of the experiments in this study were performed on Gli36vIII-GFl, which is an extremely proliferative GBM line and supports poorer oHSV replication rates than other GBM lines such as U87-GFl (31). We showed that Gli36vIII-GFl can be successfully targeted with MSC-oHSV encapsulated in sECM in vivo despite its aggressive and difficult-to-treat nature. Using a diagnostic oHSV variant, oHSV-Fluc, and real-time Fluc bioluminescence imaging, we showed that sECM-encapsulated MSC loaded with oHSV when transplanted in the tumor resection cavity released oHSV for a longer period in the brain when compared with conventional direct injection of purified oHSV. This persistence of oHSV when delivered by sECM-encapsulated MSC results in suppression of tumor growth and a substantially increased survival of treated animals as compared with oHSV alone.

We previously showed that oHSV susceptibility varies among GBM lines, and some lines are resistant to oHSV-mediated oncolysis (31). This implies that patient GBM tumors have heterogenous responsiveness to oHSV and suggests the need to develop oHSV strategies that target a broad spectrum of GBM tumors. In our previously published study, we engineered an armed oHSV mutant encoding secretable TRAIL and showed its ability to successfully target GBM lines that are both less permissive to oHSV-mediated oncolysis and also resistant to TRAIL (31). In this study, we assessed the feasibility of using oHSV-TRAIL–loaded MSC and showed that MSC are capable of amplifying oHSV-TRAIL, producing secretable TRAIL and inducing caspase-mediated apoptosis in GBM lines nonpermissive to oHSV and resistant to TRAIL.

Our study is not without limitations. We only used the preclinical GBM models that are based on conventional GBM cell lines, which may not represent the phenotypic and genotypic hallmarks of GBM (35). Future work should address whether MSC loaded with oHSV retain their efficacy in mouse models generated with patient–derived tumor-initiating cells that mirror the human disease and provide the challenge of tumor invasion (36). Although MSC are known to be nonimmunogenic after transplantation (37), it would be ideal to use a patient’s own MSC or reprogrammed induced pluripotent cells loaded with oHSV and its variants (38). We envisage that, after the neurosurgical removal of the main tumor mass, the patient’s own reprogrammed cells or MSC loaded with different variants of oHSV tailored to the molecular profile of the tumor will be encapsulated in sECM and used in patients after GBM resection.

In summary, our findings demonstrate the feasibility and impact of MSC delivery of oncolytic virus in clinical scenarios of GBM resection, underlining the translatability of this approach. Stem cell–based delivery of oHSV can overcome the problems associated with the current clinical practice involving direct oncolytic virus injection into resection cavities, which has produced minimal therapeutic effect. Thus our results have direct implications for designing future clinical trials using oncolytic viruses for GBM therapy. Because different oHSV mutants have been widely used for the treatment of different cancer types (18,39,40), this study will have an impact on the development of viral delivery systems in other solid tumors, such as liver, prostate, ovarian, breast, and lung cancer.

References


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M. Duebgen was responsible for conception and design, collection and assembly of data, data analysis and interpretation, and manuscript writing. J. Martinez-Quintanilla was responsible for collection and assembly of data, data analysis and interpretation, and manuscript writing. K. Tamura was responsible for collection and assembly of data. S. Hingtgen was responsible for collection and assembly of data. N. Redjali was responsible for collection and assembly of data. H. Wakimoto was responsible for collection and assembly of data, data analysis and interpretation, and manuscript writing. K. Shah was responsible for conception and design, data analysis and interpretation, financial support, and manuscript writing.

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