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Journal

Science translational medicine, 8(341)

ISSN

1946-6234

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Publication Date

2016-06-01

DOI

10.1126/scitranslmed.aad9784

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Science Translational Medicine

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Phase 1 trial of vocimagene amiretrorepvec and 5-fluorocytosine for recurrent high-grade glioma

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Toca 511 (vocimagene amiretrorepvec) is an investigational nonlytic, retroviral replicating vector (RRV) that delivers a yeast cytosine deaminase, which converts subsequently administered courses of the investigational prodrug Toca FC (extended-release 5-fluorocytosine) into the antimetabolite 5-fluorouracil. Forty-five subjects with recurrent or progressive high-grade glioma were treated. The end points of this phase 1, open-label, ascending dose, multicenter trial included safety, efficacy, and molecular profiling; survival was compared to a matching subgroup from an external control. Overall survival for recurrent high-grade glioma was 13.6 months (95% confidence interval, 10.8 to 20.0) and was statistically improved relative to an external control (hazard ratio, 0.45; $P = 0.003$). Tumor samples from subjects surviving more than 52 weeks after Toca 511 delivery disproportionately displayed a survival-related mRNA expression signature, identifying a potential molecular signature that may correlate with treatment-related survival rather than being prognostic. Toca 511 and Toca FC show excellent tolerability, with RRV persisting in the tumor and RRV control systemically. The favorable assessment of Toca 511 and Toca FC supports confirmation in a randomized phase 2/3 trial (NCT02414165).

INTRODUCTION

High-grade gliomas (HGGs), including glioblastomas and anaplastic astrocytomas, are the most aggressive malignant brain tumors (*1*). Treatments for recurrent glioblastoma are associated with an overall survival (OS) of 7.1 to 9.8 months (*2*–*6*). Patients generally suffer considerable morbidity from the underlying disease, including seizures, peritumoral edema, venous thromboembolism, fatigue, cognitive dysfunction, and depression. There are few therapeutic options for recurrent HGG, and the improvement in care over the last several decades has lagged behind nearly all other malignant tumors.

Toca 511 (vocimagene amiretrorepvec) is an investigational nonlytic, retroviral replicating vector (RRV) (*7*). As an RRV based on a gammaretrovirus with an amphotropic envelope, Toca 511 infects human cells with selectivity for cancer cells because genome integration is dependent on cell division and viral replication is normally restricted by innate and adaptive immune responses that are defective in malignant tissues (*8*–*10*). Toca 511 spreads through cancer cells and stably delivers a codon-optimized yeast cytosine deaminase (CD) gene whose protein product converts courses of the prodrug Toca FC [an investigational extended-release version of 5-fluorocytosine (5-FC)] into 5-fluorouracil (5-FU). 5-FU is a canonical chemotherapeutic that has a poor therapeutic index for the treatment of brain tumors, unlike 5-FC, which is commonly used to treat fungal infections of the brain and more efficiently crosses the blood brain barrier (*7*, *11*, *12*). In addition to direct killing of cells by production of intracellular 5-FU, Toca 511 and Toca FC therapy also operates through metabolic cooperation, in which noninfected but dividing neighboring cells are killed through the transfer of the antimetabolite 5-FU from nearby CD-expressing cells. This type of bystander effect has been associated with CD and 5-FC because 5-FU is a small molecule capable of diffusion through cellular membranes (*13*, *14*). Furthermore, 5-FU has direct cytotoxic effects on myeloid-derived suppressor cells located in glioblastoma tumors (*15*). Because 5-FU is generated directly within infected tumors and its half-life is short, adverse effects of systemic chemotherapy, such as myelotoxicity, are avoided. This also enables the immune system to remain intact, preserving the capacity to develop antitumor immune responses. In vivo studies demonstrated that Toca 511 and 5-FC treatment stimulates a local and systemic immune response against the tumor (*8*, *16*).

This phase 1 trial (NCT01470794) includes subjects with HGG that have recurred after treatment with at least subtotal resection, postoperative radiation, and temozolomide. This trial evaluated combination therapy, consisting of surgical resection followed by increasing doses of Toca 511 administered under direct visualization by multiple injections using a blunt-tipped needle into the walls of the resection cavity. This method of administration has been used in previous gene transfer studies and has generally been well tolerated

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(*17*). About 6 weeks after Toca 511 injection and consequent viral replication, just before Toca FC administration, subjects underwent evaluation, including radiological assessment by magnetic resonance imaging (MRI) and neurological examination. Subjects were subsequently treated with Toca FC administered for 7 days every 4 to 8 weeks in repeat cycles until radiological tumor progression or clinical progression. Some subjects received Toca FC beyond tumor progression. Here, we report the results of the phase 1 study including safety, OS, objective response rate by independent radiology review, progression-free survival, and putative biomarker identification. To provide context to the results observed, the OS and safety profile of subjects with glioblastoma in first or second recurrence, who were treated with Toca 511 and Toca FC, were compared to an external control of matched subjects who received standard therapy with lomustine (*6*).

RESULTS

Subjects

Between February 2012 and May 2015 across seven medical centers, 45 subjects were enrolled and treated with Toca 511. As of 18 September 2015, 43 subjects, considered the efficacy evaluable population, received at least one planned course of Toca FC (fig. S1 and table S1). Most of the subjects were diagnosed with glioblastoma (82.2%). Their median age was 56 years, with first (51.1%), second (22.3%), or >2 (26.6%) recurrences and a Karnofsky performance status (KPS) of 70 to 80 (24.5%) or 90 to 100 (75.5%). All subjects had previously received radiotherapy and chemotherapy with temozolomide (table S2). Most of these recurrent HGGs were located in the cerebral hemispheres, including the frontal, temporal, parietal, or occipital lobes. Although it was not confirmed by a comparison of the preoperative and immediate postoperative MRI scans, the estimated percentage of tumor resection in most of the cases was 80 to 100%. Subjects were treated with Toca 511 from 1.4×10^7 to 4.8×10^9 transducing units and with Toca FC from 130 to 220 mg/kg per day (table S3), generating concentrations of 5-FU known to be effective in cultured cells and in vivo (*8*, *18*).

Efficacy

Treatment with Toca 511 resulted in viral transduction of tumor cells, as demonstrated by detection of Toca 511 DNA and expression of Toca 511 RNA and CD protein in resected recurred tumor samples after Toca 511 delivery and several cycles of Toca FC (table S4 and fig. S2). In the efficacy evaluable population, OS for recurrent HGG subjects (n = 43) was 13.6 months [95% confidence interval (CI), 10.8 to 20.0], and that for HGG subjects at first or second recurrence was 14.4 months (95% CI, 11.3 to 32.3) (Table 1). OS for glioblastoma subjects was 11.6 months (95% CI, 9.2 to 14.6), and that for glioblastoma subjects at first or second recurrence was 13.6 months (95% CI, 11.1 to 20.0). In the efficacy evaluable population, landmark survival data included OS at 6 months (OS6) (87.9%), OS9 (72.4%), OS12 (52.5%), and OS24 (29.1%). In an analysis comparing the higher-dose cohorts (cohorts 4 to 7a) with the lower-dose cohorts (cohorts 1 to 3), there was a trend for dose response in survival [hazard ratio (HR), 0.56; 95% CI, 0.26 to 1.20] for the higher-dose cohort with median survival of 14.4 months (95% CI, 10.8 to not reached) versus the lower-dose cohort with median survival of 11.8 months (95% CI, 6.7 to 16.8) (Fig. 1A). Progression-free survival was 3.2 months (95% CI, 3.0 to 3.4), and progression-free survival at 6 months was 16.3%. Evidence of potential pseudoprogression in resected tumors after Toca 511 and Toca FC treatment was observed (fig. S3).

Evaluating tumor response in the postoperative setting requires careful analysis because subjects may have evaluable but not measurable disease. On the basis of an independent radiology review, best overall response included complete response (4.7%, in two subjects with anaplastic astrocytoma), partial response (4.7%, in two subjects with glioblastoma), an objective response rate (9.3%), stable disease rate (18.6%), and a clinical benefit rate (27.9%) (table S5). The objective response rate (13.3%), stable disease rate (23.3%), and clinical benefit rate (36.7%) were seen in the higher-dose cohorts. Two subjects with anaplastic astrocytoma were reported by an independent radiology review to have a complete response with a duration of response of more than 1 year. Concerted efforts were made to approach six sponsors of randomized phase 2 and 3 trials, including trials with surgical resection of recurrent glioblastoma. One sponsor of a phase 3 trial agreed to share data from subjects in the control arm, who were treated with lomustine. Efficacy evaluable subjects with glioblastoma at first and second recurrence were compared to the external control lomustine-treated subjects who met eligibility criteria of a phase 3 trial, potentially identifying a less ill population than typical historical controls (Table 2). The study subjects and the external control lomustine studies were nearly contemporaneous (study subjects, 2012–2015; lomustine, 2006–2010).

Table 1. Toca 511 and Toca FC OS. K-M, Kaplan-Meier.

Population	*n*	Median survival, 95% CI (months)
Efficacy evaluable—HGG	43	13.6 (10.8–20.0)
Efficacy evaluable—HGG, first and second recurrence	32	14.4 (11.3–32.3)
Glioblastoma efficacy evaluable	35	11.6 (9.2–14.6)
Glioblastoma efficacy evaluable—first and second recurrence	27	13.6 (11.1–20.0)
Landmark OS for efficacy evaluable—HGG	43	K-M survival rate
OS6		87.9%
OS9		72.4%
OS12		52.5%
OS24		31.6%

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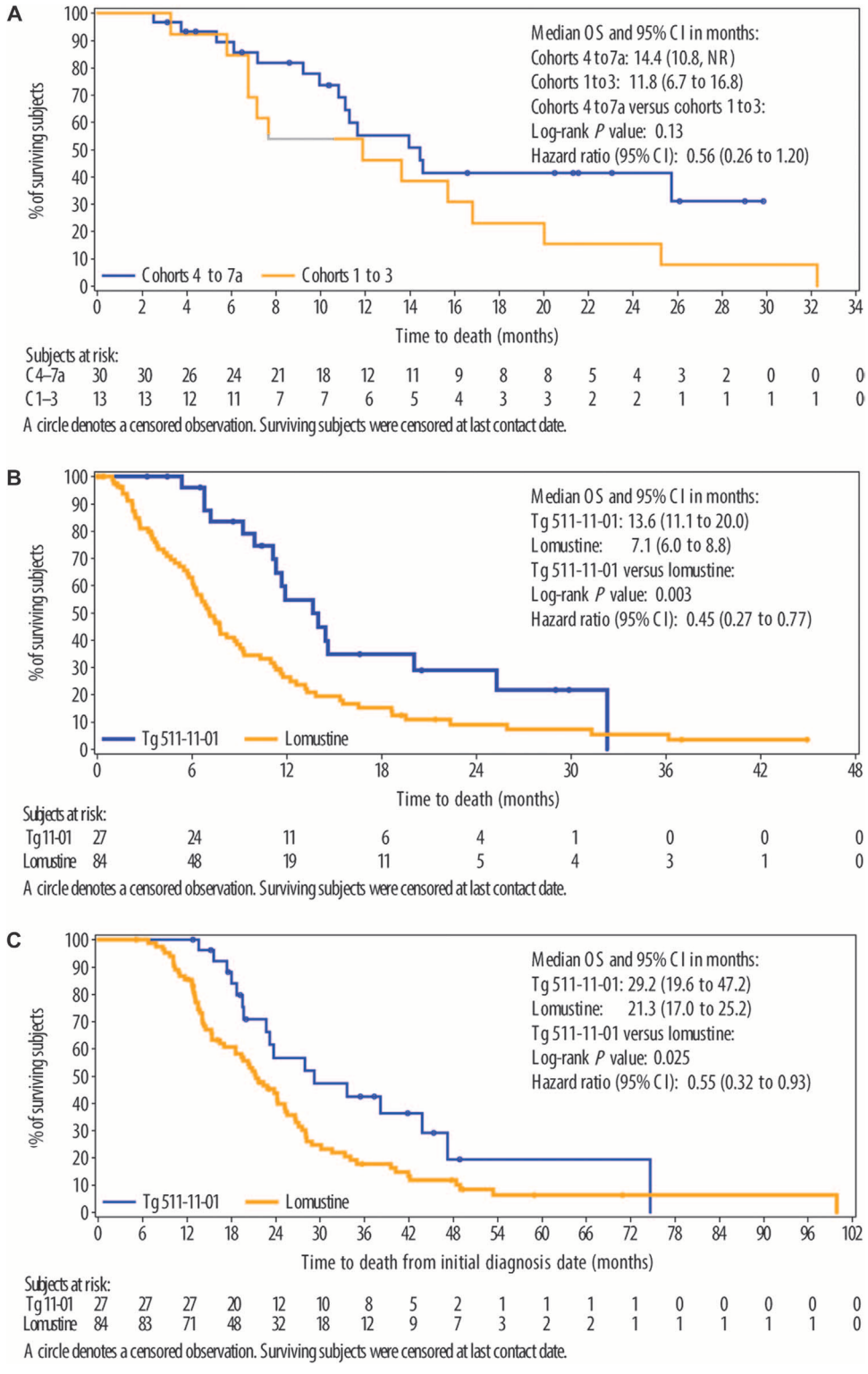

Fig. 1. OS and comparison of Toca 511 and Toca FC to lomustine. (**A**) The OS Kaplan-Meier plot of subjects who have received higher [cohorts 4 to 7a (C4–7a)] versus lower doses [cohorts 1 to 3 (C1–3)] of Toca 511 and Toca FC. NR, not reached. (**B**) The OS Kaplan-Meier plot of subjects with glioblastoma at first or second recurrence who received Toca 511 and Toca FC versus the lomustine external control. (**C**) The OS Kaplan-Meier plot from initial diagnosis of subjects with glioblastoma at first or second recurrence treated with Toca 511 and Toca FC versus lomustine external control. Tg 511-11-01 and Tg 11-01 are abbreviations for Toca 511 + Toca FC treatment.

The demographics of the study subjects with glioblastoma at first and second recurrence and lomustine control were comparable, although data were not available to match the subjects for enhancing tumor volume at the start of treatment with lomustine. The lomustine control had a slightly younger population, more subjects in first recurrence and fewer subjects requiring corticosteroids at baseline, whereas the Toca 511 and Toca FC trial had a higher percentage of subjects with a KPS of 90 to 100. Median OS for study subjects was 13.6 months (95% CI, 11.1 to 20) compared to 7.1 months (95% CI, 6.01 to 8.80) for the lomustine control. The OS HR was 0.45 (95% CI, 0.27 to 0.77; $P = 0.003$). The separation of the curves occurred initially with OS6 of 96.0% versus 61.8% ($P < 0.001$) and continued through 30 months (Fig. 1B and Table 3). Analysis of survival comparing time from initial diagnosis shows a median OS at 29.2 months for study subjects (95% CI, 19.6 to 47.2) compared to 21.3 months for lomustine control (95% CI, 17.0 to 25.2). The OS HR was 0.55 (95% CI, 0.32 to 0.93; $P = 0.025$) (Fig. 1C). A forest plot for subgroups showed improved survival for study subjects in all subgroups (fig. S4).

During the trial, increases were observed in total circulating CD4-positive T cell counts over the course of treatment (fig. S5). The increase in CD4-positive T cells more than 21 weeks after delivery of Toca 511 was significant ($P = 0.019$) compared to the start of therapy using Wilcoxon signed-rank test.

Adverse events

The median duration of treatment with Toca FC was two cycles (range, one to seven cycles). About 35% of the subjects on this trial received Toca FC on a continuation protocol of cycles of 7 days every 6 weeks (range, 1 to 14 cycles). Toca FC serum concentrations increased in a dose-dependent fashion (Fig. 2). Toca 511 and Toca FC treatment produced few related adverse events and serious adverse events (table S6). Two dose-limiting toxicities (DLTs) were observed:

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Table 2. Demographics of glioblastoma at first or second recurrence: Toca 511 and Toca FC versus lomustine.

Treatment group characteristics	Toca 511 and Toca FC (*n* = 27) *n* (%)	Lomustine (*n* = 84) *n* (%)
Sex		
Female	4 (14.8)	33 (39.3)
Male	23 (85.2)	51 (60.7)
Age, years		
Median	61	54
Range	41–71	18–75
Age, years		
<50	5 (18.5)	27 (32.1)
≥50	22 (81.5)	57 (67.9)
KPS		
70–80	7 (25.9)	41 (48.8)
90–100	20 (74.1)	43 (51.2)
Time from diagnosis (months)		
Median	11.6	11.5
Range	5.1–49.4	4.4–92.1
Recurrence		
First	19 (70.4)	65 (77.4)
Second	8 (29.6)	19 (22.6)
Baseline steroid administration		
Yes	23 (85.2)	46 (54.8)
No	4 (14.8)	38 (45.2)

Table 3. Landmark OS in glioblastoma at first or second recurrence with Toca 511 and Toca FC versus lomustine.

Treatment group	Toca 511 and Toca FC (*n* = 27)	Lomustine (*n* = 84)	*P* value
Landmark OS rate			
OS6	96.0%	61.8%	<0.001
OS9	83.5%	38.4%	<0.001
OS12	54.8%	26.4%	0.017
OS24	29.1%	9.1%	0.065

one in cohort 3 of grade 3 asthenia, which was possibly related to Toca 511, and one in cohort 5a of grade 3 normal pressure hydrocephalus, which was considered unrelated. A maximum tolerated dose was not reached. There were no treatment-related deaths. Additionally, safety analyses were conducted comparing adverse event profiles in glioblastoma study subjects in the first and second recurrence subgroup to the lomustine external control. Subjects treated with Toca 511 and Toca FC had fewer related treatment-emergent grade ≥3 adverse events (3.7% versus 36.9%) (Table 4). There were far fewer grade ≥3 adverse events and a virtual absence of hematologic toxicity for study subjects relative to the lomustine control, where grade ≥3 thrombocytopenia occurred in 23.8% of lomustine patients (table S7). Given that Toca 511 is surgically delivered, the treatment-emergent adverse events regardless of attribution were also reported, including grade 2 incision site pain (25.9%) and procedural pain (18.5%) (table S8). These results demonstrate a more favorable safety profile with fewer severe toxicities for Toca 511 and Toca FC compared to lomustine. To date, there has been no persistent viremia observed; Toca 511 virus in blood appears to be well controlled and cleared quickly (fig. S6).

mRNA expression profiles associated with survival after Toca 511 and Toca FC therapy

HGG is molecularly heterogeneous, with several subtypes classically defined by histology and more recently through mRNA expression and genomic mutation profiles (*19*). Variability in the underlying tumor molecular features resulting in differences in virus-host interactions, 5-FU–induced cell death, and immune activation are expected to contribute to the differential benefit derived from Toca 511 and Toca FC, as measured by OS. Therefore, we systematically profiled tumor RNA expression from frozen tissue biopsies, taken at the time of tumor resection and immediately before Toca 511 administration, by next-generation sequencing. Available RNA from two or three spatially distinct pieces was isolated from biopsies to gauge intratumor heterogeneity. A total of 64 available tumor samples were tested from 26 efficacy evaluable subjects. To gauge variation in mRNA expression across tumors, the 4314 mRNAs with the greatest variation in expression (SD, >0.8) were identified, and their relative expression was used as a metric of similarity for unsupervised hierarchical clustering (Fig. 3). In this analysis, subject samples clustered into four groups (color-coded) based on expression of functionally related sets of mRNAs (sets 1 to 5). As expected, most of the grade 3 samples clustered together (gray). In the purple-coded group, 76% of tumor samples (13 of 17) came from five of eight glioblastoma subjects who lived more than 1 year after Toca 511 and Toca FC therapy. Tumor samples preferentially expressed mRNAs encoding proteins involved in neuronal functions, which we termed survival-related neuronal subtype (SRNS) and is represented in the purple branch in Fig. 3.

Previous studies by The Cancer Genome Atlas (TCGA) consortium have shown that HGG RNA expression profiles from newly diagnosed tumors reproducibly segregate samples into four molecular classes, coinciding with specific genomic alterations and cell types of origin: "classical" astrocytes, "mesenchymal" microglia, "proneural" oligodendrocytes, and "neural" neuron-like cells (*19*, *20*). To determine whether this classification was applicable to recurrent HGG, for which there is a paucity of published genomic data sets, we hierarchically clustered study samples using a molecular classification gene set provided by TCGA consortium for newly diagnosed HGG (fig. S7). Samples were clustered into four groups, coinciding with the defined molecular classes: neural (n = 19), mesenchymal (n = 14), proneural (n = 14), and classical (n = 4) (Fig. 4A). Most grade 3 samples were proneural (n = 10/14), consistent with published results on newly diagnosed tumors (Fig. 4B) (*21*). Samples showed differing ranges of intratumor heterogeneity (fig. S8) (*22*), which was not attributable to RNA quality or sequencing coverage (fig. S9). In 23 of 26 efficacy evaluable subjects, most of the samples corresponded to a specific subtype, but 8 of these 23 subjects displayed multiple subtypes within their tumor (Fig. 4C), underscoring the heterogeneity maintained in recurrent HGG. A multivariate Cox regression analysis showed that the HR

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of death of the neural signature is significantly reduced compared to other subtypes (HR, 0.11; $P = 0.01$) after controlling for the number of recurrences and HGG grade (table S9). However, the TCGA neural signature is not a prognostic marker for survival in newly diagnosed HGG, where typically, only the G-CIMP phenotype confers a survival advantage (fig. S10). G-CIMP refers to glioblastoma tumors that are proneural and also have glioma-CpG island methylator phenotype (*23*).

The SRNS identified in recurrent glioblastoma tumors via unsupervised hierarchical clustering (Fig. 3) bears functional similarities to the TCGA neural subtype identified in newly diagnosed HGG tumors. To more precisely classify subjects via the SRNS and its relationship to the neural subtype signature, we hierarchically clustered samples on the basis of the expression of the 890 mRNAs in cluster 5 from Fig. 3 (fig. S11A). Tumors from subjects who survived at least 1 year after Toca 511 treatment disproportionately displayed the SRNS (19 of 21) (fig. S11B). Using the first bifurcation of the dendrogram to separate SRNS from non-SRNS, 28 of 50 samples from efficacy evaluable glioblastoma subjects were SRNS (fig. S11A), including 18 of 19 tumors defined as neural and 4 of 4 tumors defined as classical (fig. S11C). The HR of death of those with SRNS was significantly reduced compared to non-SRNS (HR, 0.11, $P = 0.003$) after controlling for the number of recurrences and HGG grade using a multivariate Cox regression analysis (table S10). Like the TCGA neural signature, the SRNS signature is not associated with survival in newly diagnosed glioblastoma (fig. S12).

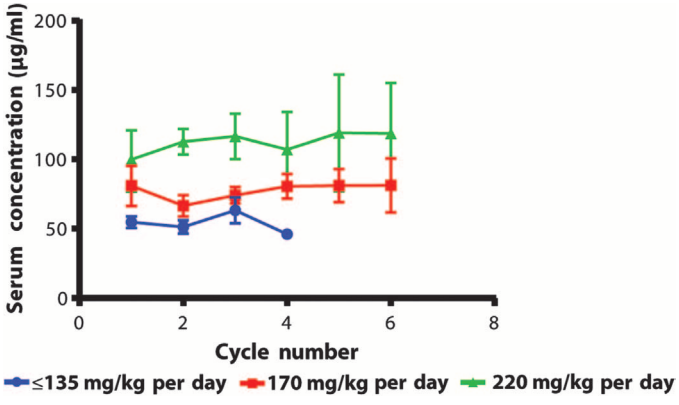

Fig. 2. Toca FC concentrations. The Toca FC serum concentrations (microgram/milliliter) over time at increasing Toca FC doses (means ± SE). Cycles are about 1 week long and 4 to 8 weeks apart; measurements were normally on day 4 or 5 of the cycle.

A recent study compared expression profiles of glioblastoma tumors from the contrast-enhancing tumor core (CE) with the leading edge nonenhancing (NE) margins of the tumors (*24*). Samples from CE resembled classical, mesenchymal, and proneural subtypes, whereas samples from NE largely resembled the neural type. The raw sequencing data from this study were retrieved and processed identically to the samples from the Toca 511 and Toca FC study and then combined. Neural tumors were segregated with NE samples and normal brain, whereas classical, mesenchymal, and proneural samples were intermixed with CE samples (fig. S13A). Neural tumor samples were distinct from normal brain, including alterations in mRNA expression of genes involved in proliferation (fig. S13B).

Separate from the SRNS signature, there were numerous mRNAs whose expression correlated with survival time. For instance, expression of *SPOC1/PHF13* negatively correlates (Pearson correlation, −0.71) with survival after Toca 511 therapy in glioblastoma patients (fig. S14). SPOC1 (survival-time associated PHD protein in ovarian cancer 1) modulates chromatin structure, acts as an adenovirus host restriction factor, and therefore has the potential to interfere with the retroviral life cycle (*25*, *26*).

O-6-methylguanine-DNA methyltransferase (MGMT) encodes a DNA repair protein that repairs alkylation at the O^6 position of guanine (*27*). MGMT promoter hypermethylation reduces its expression and impairs the cells' ability to repair damage induced by chemotherapeutic agents and radiation (*28*, *29*). Accordingly, MGMT promoter hypermethylation is associated with improved outcome in newly diagnosed HGG (*30*). MGMT promoter methylation was measured with a bisulfite-free DNA methylation detection assay (*31*). Most study samples were MGMT methylation–negative, but there were three instances where MGMT methylation status differed between two distinct samples from the same tumor (fig. S15A). Unlike SRNS, MGMT methylation status did not correlate with survival (fig. S15B).

DISCUSSION

Recurrent HGG is associated with dismal clinical outcomes, and patients are in need of safe and more efficacious therapy. The nonlytic RRV Toca 511 and an extended-release 5-FC have the potential to fill this medical need. Detection of viral transduction in resected tumors after Toca 511 treatment, despite it being cleared from blood, suggests

Table 4. Summary of adverse and serious adverse events.

	All grades		Grade ≥3	
	Toca 511 and Toca FC *n* = 27 *n* (%)	Lomustine *n* = 84 *n* (%)	Toca 511 and Toca FC *n* = 27 *n* (%)	Lomustine *n* = 84 *n* (%)
Any treatment-emergent adverse event	27 (100.0)	70 (83.3)	17 (63.0)	45 (53.6)
Related treatment-emergent adverse event	11 (40.7)	52 (61.9)	1 (3.7)	31 (36.9)
Leading to study discontinuation treatment-emergent adverse event	0	4 (4.8)	0	4 (4.8)
Treatment-emergent serious adverse event	9 (33.3)	24 (28.6)	7 (25.9)	21 (25.0)
Related treatment-emergent serious adverse event	2 (7.4)	6 (7.1)	1 (3.7)	5 (6.0)

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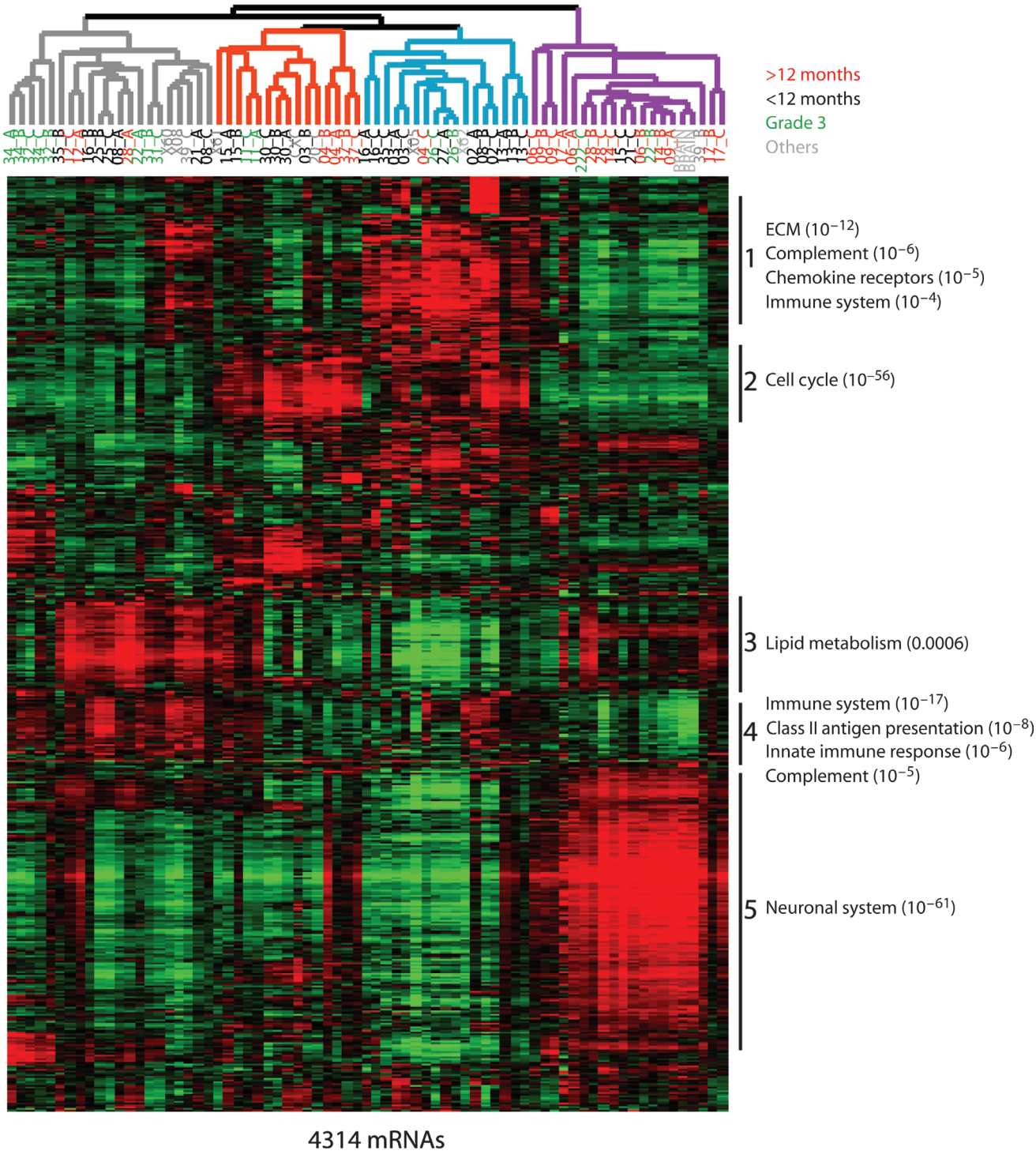

Fig. 3. Tumor mRNA expression profiles and correlation with survival. Unsupervised hierarchical cluster of mRNA expression across 64 efficacy evaluable study tumor samples from 26 biopsies. The heatmap includes the 4313 mRNAs with the greatest variation in expression (SD, >0.8), with red and green bars representing increased and decreased gene expression, respectively, compared to normal human brain. Samples were segregated into four main groups, which are color-coded in the dendrogram. Sample numbers are red, black, green, and gray from subjects with glioblastoma who survived more than 12 months after Toca 511 delivery, from subjects with glioblastoma that survived less than 12 months, from subjects with grade 3 tumors, and from other sample numbers, respectively. Many of these 4313 mRNAs fell into one of five distinct clusters, characterized by common functional themes. Functional themes associated with the proteins encoded by mRNAs in specific clusters were identified using Reactome gene sets obtained from the Molecular Signatures Database. Examples of gene sets whose members are overrepresented in specific clusters are listed to the right of the heatmap along with associated *P* values (hypergeometric distribution function; *P* values are represented in figure parentheses). ECM, extracellular matrix.

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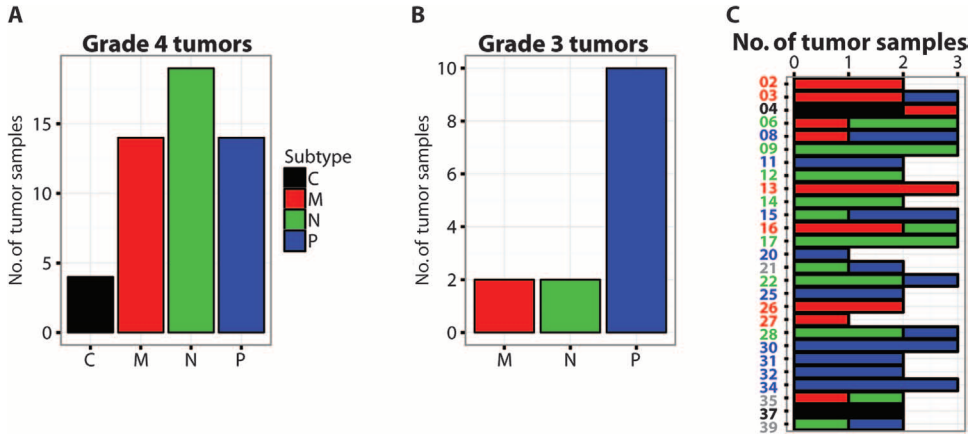

Fig. 4. Molecular classification of tumor samples from study subjects based on mRNA expression. (**A**) Bar plot representation of the number of glioblastoma (grade 4) samples in each molecular subtype: classical (C) (black), mesenchymal (M) (red), neural (N) (green), and proneural (P) (blue). (**B**) Same as in (A), except for grade 3 samples. (**C**) Bar plot representation of molecular subtype for each subject, with the predominant subtype indicated by the color of the subject identifier. Gray indicates that no consensus was reached, which means that the two samples profiled had different subtypes.

that a reservoir of Toca 511 may exist selectively in tumors to continually kill tumor cells and activate the immune system over multiple cycles of Toca FC (*8*). Cancer selectivity for Toca 511 is supported by the lack of virus staining in areas of normal brain tissue included in the tumor resection after Toca 511 delivery and has been well established in preclinical tumor models (*8*, *10*).

To provide context to the results observed, we compared our data to the only available contemporaneous randomized phase 2/3 external controls. Subjects with glioblastoma at first and second recurrence treated with Toca 511 and Toca FC were compared to those treated with lomustine. Although such a comparison does not overcome the lack of an internal control and randomized patient assignment, it is considered robust, reflecting a phase 3 clinical trial patient population and establishing comparability to the subjects receiving Toca 511 and Toca FC. Efficacy is strongly suggested in the Toca 511– and Toca FC–treated recurrent HGG population by almost twofold improvement in OS compared to this external control and by a median OS of 13.6 months that approaches the OS observed in newly diagnosed glioblastoma (*32*, *33*). Although Toca 511 and Toca FC are administered after tumor resection and lomustine is not, a similar effect was seen in another phase 1 Toca 511 trial, which did not require surgical resection (*34*). The improvement in OS is not reflected by an improvement in progression-free survival, which may be attributable to radiological changes of pseudoprogression as has been observed with immunotherapies for HGG (*35*). Although evaluating tumor response in the postoperative setting and in the context of a potentially immune-activating therapy presents challenges, longer follow-up of 30 evaluable subjects in the higher-dose cohorts demonstrated that 2 had complete responses, 2 had partial responses, and 8 had stable disease, which provides additional supportive evidence of efficacy. The safety profile appears manageable, but further attribution of the adverse events observed as complications of surgery, Toca 511, and/or Toca FC treatment will need to be assessed in a randomized trial. There were no reports of spontaneous cases of autoimmune disorders, such as vitiligo, thyroiditis, or hypophysitis, which have been observed with the immune checkpoint inhibitors anti–CTLA-4 (cytotoxic T lymphocyte-associated antigen 4) and anti–PD-1 (programmed cell death protein 1).

The improvement in OS and a favorable safety profile of Toca 511 and Toca FC support moving forward with this treatment regimen. In the external control comparison, there was a separation of the OS curves that occurred initially and continued through 30 months with an OS24 of 31.6%. This is consistent with an initial cytoreductive effect from intratumorally generated 5-FU, followed by a sustained effect from augmented antitumor immunity resulting from a combination of cancer and immunosuppressor cell killing by 5-FU in the tumor microenvironment (*8*, *36*). A maximum tolerated dose has not been established because DLTs were infrequent. A randomized trial (NCT02414165) is being conducted at a maximum feasible dose of 4 ml using 40 injections into the wall of the resection cavity. As evidenced by immunohistochemistry and analysis of viral RNA and DNA, Toca 511 successfully transduced recurrent HGG with selectivity for tumor cells and was readily cleared from the subjects' blood. The documentation of tumor virus persistence with systemic clearance supports the importance of further study of RRVs in humans for cancer treatment. Although persistence of virus was shown in tumors that were resected after Toca 511 treatment, it is not possible to estimate the percentage of tumor that was transduced before resection in these samples. These post–Toca 511–resected tumors received at least one cycle of Toca FC, which is expected to kill most of the transduced cells before resection occurred, so an accurate assessment of transduction frequency by Toca 511 was not possible in this setting.

Toca 511 and Toca FC therapy increased the number of $CD4^+$ T cells in the blood, suggesting that immunomodulatory activity may be part of this therapy. In addition to the adjuvant properties of a replicating virus (Toca 511) and cancer cell killing by 5-FU, myeloid-derived suppressor cells are known to be sensitive to 5-FU, and the changes in immune cell populations in the blood may result from the induction of an antitumor response generated by Toca 511 and Toca FC (*15*).

Our molecular analyses reinforce the heterogeneity of HGG tumors including MGMT methylation, a prognostic factor that may sensitize cells to 5-FU (*37*, *38*). These results indicate the importance of obtaining measurements of multiple spatially distinct tumor biopsy samples when prognostic determinations can potentially affect treatment decisions.

The underlying prognostic and predictive molecular features that contribute to survival in this study may identify subjects most likely to benefit from Toca 511 and Toca FC therapy and suggest pathways to further improve treatment. MGMT promoter methylation did not explain variation in survival among treated subjects with glioblastoma at first or second recurrence. Subjects whose tumors displayed the SRNS (and TCGA neural subtype) survived significantly longer than subjects whose tumors displayed other subtypes (SRNS, $P = 0.003$; TCGA, $P = 0.01$). In principle, subjects with SRNS could have a less aggressive, more differentiated tumor or a more successful surgery and thus live longer. However, neither the SRNS nor the related TCGA neural subtype correlates with survival in newly diagnosed glioblastoma, suggesting that SRNS tumor phenotype predicts response to Toca 511

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and Toca FC therapy (*21*, *23*). Our results are consistent with the SRNS signature being associated with the nonenhancing region of advancing edge of the tumor (*24*). This environment may be more favorable for Toca 511 persistence and spread because the advancing edges are associated with increased cell division, which is important for Toca 511 infection and 5-FU killing (*39*). A recent clinical trial reported encouraging survival in recurrent HGG transcranially injected with Toca 511 into the nonenhancing edge of a glioma using a biopsy needle compared to infusion into the center of the tumor (*34*). Expression of a known virus-host interaction viral restriction factor, SPOC1, has a strong negative correlation with survival after treatment with Toca 511 and Toca FC, supporting the importance of Toca 511 tumor spread for survival improvement.

Two meta-analyses have demonstrated that surgical resection of recurrent tumor does not affect OS (*40*, *41*). Adverse events will be understood more clearly from a randomized trial of Toca 511 and Toca FC in combination with surgical resection, which is currently ongoing (NCT02414165).

Use of Toca 511 and Toca FC after glioma resection may be an optimal setting for testing this immunotherapy treatment. Typically, neurosurgeons try to resect enhancing tumor, leaving behind nonenhancing tumor in the resection cavity wall. Toca 511 can then be injected into the nonenhancing region left behind in the advancing edge of the tumor where the SRNS cells likely reside.

A randomized phase 2/3 trial (NCT02414165) in subjects with recurrent glioblastoma and anaplastic astrocytoma is under way. This trial will compare the OS of subjects treated with Toca 511 combined with Toca FC to subjects treated according to standard of care after tumor resection. This design provides an opportunity to validate the promising OS, tolerability, immune cell changes, and mRNA expression signature observed in this phase 1 study and to quantify cytotoxic and immune-mediated effects in an effort to further confirm the mechanism of action of Toca 511 and Toca FC. It is conceivable that treatment with Toca 511 at the time of resection of newly diagnosed HGG may provide better efficiency of treatment, and this will further explored in another phase 1 trial (NCT02598011).

MATERIALS AND METHODS

Study design

Eligible patients had histologically proven HGG with recurrence or progression after initial definitive therapy or therapy for recurrence. Other key eligibility criteria included age between 18 and 80 years, Karnofsky performance score ≥70, single recurrent HGG tumor ≤6 cm, preoperative evaluation indicating that ≥80% of tumor was resectable, and adequate organ function.

The protocol was approved by the institutional review board at each site and complied with the International Ethical Guidelines for Biomedical Research Involving Human Subjects, good clinical practice guidelines, the Declaration of Helsinki, and local laws. All subjects provided written informed consent.

This phase 1, open-label, ascending dose, multicenter trial was designed to evaluate the safety and tolerability of Toca 511 and Toca FC. The primary end point was to identify the highest, safe, and well-tolerated dose of Toca 511. The secondary objective was to evaluate safety and tolerability of repeated treatment with Toca FC at various doses and schedules after administration of Toca 511. Efficacy was to be assessed by OS, OS6, OS9, OS12, and OS24. We used an independent radiology review (ICON Imaging) to determine objective response rate, progression-free survival, and progression-free survival at 6 months. Exploratory objectives included developing a predictive diagnostic factor based on RNA expression and assessing potential clinical utility. This was done in accordance with the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) 2013 Guidelines.

Subjects underwent gadolinium-enhanced MRI scan of the brain about every 8 weeks, with the baseline MRI scan obtained after surgical resection and just before Toca FC. Tumor response was assessed by an independent radiology review using the Macdonald criteria, which require measurable contrast-enhancing disease of at least 10 mm in both dimensions and confirmation at least 4 weeks after the initial response (*42*).

All adverse events were classified and graded using the Common Terminology Criteria for Adverse Events version 4.0 (*43*). If any subject experienced a DLT attributed to Toca 511, Toca FC, or the combination, then another three subjects would be studied at that dose level. If two of six subjects experienced a related DLT at any dose level, then no further dose escalation was planned. The highest dose at which six subjects were studied with <2 DLTs would have been considered the maximum tolerated dose. A data monitoring committee periodically reviewed the safety data. Subjects were assessed for adverse events from the time of Toca 511 administration and will be followed for up to 15 years through a continuation study, as stipulated by U.S. Food and Drug Administration (FDA) guidance (*44*).

Testing for the presence of viral RNA by quantitative reverse transcription polymerase chain reaction (PCR), viral DNA by quantitative PCR, antibodies to Toca 511, and immunologic parameters was performed in blood. Urine and saliva were monitored for viral shedding.

Molecular analyses

Genomic DNA and total RNA from spatially distinct bulk tumor pieces were isolated and analyzed further. Additional methodologic details are provided in the Supplementary Materials and Methods.

Study oversight

The study was designed jointly by the investigators and representatives of the sponsor, Tocagen. The sponsor collected and analyzed the data. All the authors were involved in the data analysis and manuscript preparation and vouch for the completeness and accuracy of the data and analyses and for the adherence of the study to the protocol. No one who is not listed as an author contributed to the writing of the manuscript.

External control

Tocagen approached six sponsors with recently published phase 2 or 3 data in recurrent HGG or glioblastoma, including a study in which subjects underwent resection of recurrent glioblastoma. One sponsor (Denovo Biopharma LLC) agreed to share lomustine data from a phase 3 randomized, open-label study evaluating enzastaurin in subjects with recurrent glioblastoma who did not undergo resection of recurrent tumor. The study enrolled subjects meeting the following criteria: ≥18 years of age; KPS ≥70; histologically confirmed glioblastoma at first or second recurrence; ≤2 previous chemotherapy regimens; and adequate organ function. Although 92 subjects were

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randomized to lomustine, 84 received lomustine treatment. The study was discontinued early for futility after an interim analysis of the primary end point of progression-free survival (*6*). A subset of subjects with recurrent glioblastoma at first or second recurrence treated with Toca 511 and Toca FC was compared to these lomustine-treated subjects.

Statistical analysis

The efficacy evaluable population included subjects who received at least one dose of Toca 511 and one planned course of Toca FC. The safety population included all subjects who received Toca 511. Continuous variables were summarized with means, SDs, medians, minimums, and maximums. Categorical variables were summarized by counts and by percentage of subjects in corresponding categories. OS and progression-free survival were analyzed using the Kaplan-Meier method, and plots were generated with the median OS and progression-free survival and 95% CI summarized. The log-rank test was used for between-group comparisons, and the HRs with 95% CI were presented. In addition, *Z* test was used for between-group comparison in landmark survival rate at 6 (OS6), 9 (OS9), 12 (OS12), and 24 months (OS24). All analyses were performed using SAS version 9.4 (SAS Institute Inc.). Molecular characteristics of neural subtypes versus other subtypes and SRNS versus other subtypes were investigated using a multivariate Cox regression model for prediction of survival. For this ongoing trial, these analyses were performed on the basis of a data transfer date of 18 September 2015. Additionally, an independent radiology review (ICON Imaging) occurred on the basis of a data transfer date of 22 January 2016.

SUPPLEMENTARY MATERIALS

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Materials and Methods
Fig. S1. Simplified schematic for the phase 1 trial (NCT01470794).
Fig. S2. Tumor-specific Toca 511 staining in re-resected tumors after multiple cycles of Toca FC.
Fig. S3. Evidence of potential pseudoprogression on resected tumor after Toca 511 and Toca FC treatment.
Fig. S4. Comparison of subjects with glioblastoma at first or second recurrence treated with Toca 511 and Toca FC to lomustine.
Fig. S5. Peripheral blood $CD4^+$ T cell modulation after Toca 511 and Toca FC dosing.
Fig. S6. Tracking of Toca 511 DNA and RNA signal in whole blood and plasma over time.
Fig. S7. Molecular classification of tumor samples from study subjects based on mRNA expression.
Fig. S8. Intratumor versus intertumor heterogeneity in RNA expression profiles.
Fig. S9. No relationship between variation in mRNA expression and confounding technical factors.
Fig. S10. No correlation between neural subtype and prognosis in newly diagnosed glioblastoma.
Fig. S11. Identification of an mRNA profile (SRNS) associated with longer survival after Toca 511.
Fig. S12. No correlation between SRNS and prognosis in newly diagnosed glioblastoma.
Fig. S13. Study survival–related neural subtype samples likely derive from nonenhancing regions of tumors.
Fig. S14. Negative expression between *SPOC1* expression and subject survival time with Toca 511 and Toca FC therapy.
Fig. S15. MGMT promoter methylation.
Table S1. Toca 511 and Toca FC: Baseline demographic and clinical characteristics.
Table S2. Additional baseline demographic and clinical characteristics.
Table S3. Dosing cohorts.
Table S4. Detection of Toca 511 in re-resected tumors.
Table S5. Best overall response in the efficacy evaluable population.
Table S6. Adverse events and serious adverse events related to Toca 511 and Toca FC.
Table S7. Related adverse events: Toca 511 and Toca FC compared to lomustine.
Table S8. Adverse events regardless of attribution: Toca 511 and Toca FC compared to lomustine.
Table S9. Summary of multivariate analysis for survival (TCGA neural signature).
Table S10. Summary of multivariate analysis for survival (SRNS signature).
Table S11. RNA sequencing normalized results (reads per kilobase of transcript per million mapped reads values).
Table S12. SRNS gene list.
Table S13. Subject summary.
References (*45*, *46*)

REFERENCES AND NOTES

1. M. C. Chamberlain, Treatment options for glioblastoma. *Neurosurg. Focus* **20**, E19 (2006).
2. W. Taal, H. M. Oosterkamp, A. M. E. Walenkamp, H. J. Dubbink, L. V. Beerepoot, M. C. J. Hanse, J. Buter, A. H. Honkoop, D. Boerman, F. Y. F. de Vos, W. N. M. Dinjens, R. H. Enting, M. J. B. Taphoorn, F. W. P. J. van den Berkmortel, R. L. H. Jansen, D. Brandsma, J. E. C. Bromberg, I. van Heuvel, R. M. Vernhout, B. van der Holt, M. J. van den Bent, Single-agent bevacizumab or lomustine versus a combination of bevacizumab plus lomustine in patients with recurrent glioblastoma (BELOB trial): A randomised controlled phase 2 trial. *Lancet Oncol.* **15**, 943–953 (2014).
3. H. S. Friedman, M. D. Prados, P. Y. Wen, T. Mikkelsen, D. Schiff, L. E. Abrey, W. K. A. Yung, N. Paleologos, M. K. Nicholas, R. Jensen, J. Vredenburgh, J. Huang, M. Zheng, T. Cloughesy, Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. *J. Clin. Oncol.* **27**, 4733–4740 (2009).
4. S. Kunwar, S. Chang, M. Westphal, M. Vogelbaum, J. Sampson, G. Barnett, M. Shaffrey, Z. Ram, J. Piepmeier, M. Prados, D. Croteau, C. Pedain, P. Leland, S. R. Husain, B. H. Joshi, R. K. Puri, P. S. Group, Phase III randomized trial of CED of IL13-PE38QQR vs Gliadel wafers for recurrent glioblastoma. *Neuro Oncol.* **12**, 871–881 (2010).
5. T. T. Batchelor, P. Mulholland, B. Neyns, L. B. Nabors, M. Campone, A. Wick, W. Mason, T. Mikkelsen, S. Phuphanich, L. S. Ashby, J. Degroot, R. Gattamaneni, L. Cher, M. Rosenthal, F. Payer, J. M. Jürgensmeier, R. K. Jain, A. G. Sorensen, J. Xu, Q. Liu, M. van den Bent, Phase III randomized trial comparing the efficacy of cediranib as monotherapy, and in combination with lomustine, versus lomustine alone in patients with recurrent glioblastoma. *J. Clin. Oncol.* **31**, 3212–3218 (2013).
6. W. Wick, V. K. Puduvalli, M. C. Chamberlain, M. J. van den Bent, A. F. Carpentier, L. M. Cher, W. Mason, M. Weller, S. Hong, L. Musib, A. M. Liepa, D. E. Thornton, H. A. Fine, Phase III study of enzastaurin compared with lomustine in the treatment of recurrent intracranial glioblastoma. *J. Clin. Oncol.* **28**, 1168–1174 (2010).
7. O. D. Perez, C. R. Logg, K. Hiraoka, O. Diago, R. Burnett, A. Inagaki, D. Jolson, K. Amundson, T. Buckley, D. Lohse, A. Lin, C. Burrascano, C. Ibanez, N. Kasahara, H. E. Gruber, D. J. Jolly, Design and selection of Toca 511 for clinical use: Modified retroviral replicating vector with improved stability and gene expression. *Mol. Ther.* **20**, 1689–1698 (2012).
8. D. Ostertag, K. K. Amundson, F. Lopez Espinoza, B. Martin, T. Buckley, A. P. Galvão da Silva, A. H. Lin, D. T. Valenta, O. D. Perez, C. E. Ibañez, C.-I. Chen, P. L. Pettersson, R. Burnett, V. Daublebsky, J. Hlavaty, W. Gunzburg, N. Kasahara, H. E. Gruber, D. J. Jolly, J. M. Robbins, Brain tumor eradication and prolonged survival from intratumoral conversion of 5-fluorocytosine to 5-fluorouracil using a nonlytic retroviral replicating vector. *Neuro Oncol.* **14**, 145–159 (2012).
9. C. Dalba, D. Klatzmann, C. R. Logg, N. Kasahara, Beyond oncolytic virotherapy: Replication-competent retrovirus vectors for selective and stable transduction of tumors. *Curr. Gene Ther.* **5**, 655–667 (2005).
10. K. Hiraoka, T. Kimura, C. R. Logg, N. Kasahara, Tumor-selective gene expression in a hepatic metastasis model after locoregional delivery of a replication-competent retrovirus vector. *Clin. Cancer Res.* **12**, 7108–7116 (2006).
11. C. O. Wilson, J. M. Beale, J. H. Block, *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry* (Lippincott Williams & Wilkins, Baltimore, MD, ed. 12, 2011).
12. V. Formica, A. Leary, D. Cunningham, Y. J. Chua, 5-Fluorouracil can cross brain–blood barrier and cause encephalopathy: Should we expect the same from capecitabine? A case report on capecitabine-induced central neurotoxicity progressing to coma. *Cancer Chemother. Pharmacol.* **58**, 276–278 (2006).
13. S. Kuriyama, K. Masui, T. Sakamoto, T. Nakatani, M. Kikukawa, H. Tsujinoue, A. Mitoro, M. Yamazaki, H. Yoshiji, H. Fukui, K. Ikenaka, C. A. Mullen, T. Tsujii, Bystander effect caused by cytosine deaminase gene and 5-fluorocytosine in vitro is substantially mediated by generated 5-fluorouracil. *Anticancer Res.* **18**, 3399–3406 (1998).
14. B. E. Huber, E. A. Austin, C. A. Richards, S. T. Davis, S. S. Good, Metabolism of 5-fluorocytosine to 5-fluorouracil in human colorectal tumor cells transduced with the cytosine deaminase gene: Significant antitumor effects when only a small percentage of tumor cells express cytosine deaminase. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 8302–8306 (1994).
15. J. Vincent, G. Mignot, F. Chalmin, S. Ladoire, M. Bruchard, A. Chevriaux, F. Martin, L. Apetoh, C. Rébé, F. Ghiringhelli, 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell–dependent antitumor immunity. *Cancer Res.* **70**, 3052–3061 (2010).

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16. T. T. Huang, S. Parab, R. Burnett, O. Diago, D. Ostertag, F. M. Hofman, F. L. Espinoza, B. Martin, C. E. Ibañez, N. Kasahara, H. E. Gruber, D. Pertschuk, D. J. Jolly, J. M. Robbins, Intravenous administration of retroviral replicating vector, Toca 511, demonstrates therapeutic efficacy in orthotopic immune-competent mouse glioma model. *Hum. Gene Ther.* **26**, 82–93 (2015).
17. N. G. Rainov, A phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme. *Hum. Gene Ther.* **11**, 2389–2401 (2000).
18. C. G. Twitty, O. R. Diago, D. J. Hogan, C. Burrascano, C. E. Ibanez, D. J. Jolly, D. Ostertag, Retroviral replicating vectors deliver cytosine deaminase leading to targeted 5-fluorouracil-mediated cytotoxicity in multiple human cancer yypes. *Hum. Gene Ther. Methods* **27**, 17–31 (2016).
19. R. G. W. Verhaak, K. A. Hoadley, E. Purdom, V. Wang, Y. Qi, M. D. Wilkerson, C. R. Miller, L. Ding, T. Golub, J. P. Mesirov, G. Alexe, M. Lawrence, M. O'Kelly, P. Tamayo, B. A. Weir, S. Gabriel, W. Winckler, S. Gupta, L. Jakkula, H. S. Feiler, J. G. Hodgson, C. D. James, J. N. Sarkaria, C. Brennan, A. Kahn, P. T. Spellman, R. K. Wilson, T. P. Speed, J. W. Gray, M. Meyerson, G. Getz, C. M. Perou, D. N. Hayes; Cancer Genome Atlas Research Network, Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in *PDGFRA*, *IDH1*, *EGFR*, and *NF1*. *Cancer Cell* **17**, 98–110 (2010).
20. C. W. Brennan, R. G. W. Verhaak, A. McKenna, B. Campos, H. Noushmehr, S. R. Salama, S. Zheng, D. Chakravarty, J. Z. Sanborn, S. H. Berman, R. Beroukhim, B. Bernard, C.-J. Wu, G. Genovese, I. Shmulevich, J. Barnholtz-Sloan, L. Zou, R. Vegesna, S. A. Shukla, G. Ciriello, W. K. Yung, W. Zhang, C. Sougnez, T. Mikkelsen, K. Aldape, D. D. Bigner, E. G. Van Meir, M. Prados, A. Sloan, K. L. Black, J. Eschbacher, G. Finocchiaro, W. Friedman, D. W. Andrews, A. Guha, M. Iacocca, B. P. O'Neill, G. Foltz, J. Myers, D. J. Weisenberger, R. Penny, R. Kucherlapati, C. M. Perou, D. N. Hayes, R. Gibbs, M. Marra, G. B. Mills, E. Lander, P. Spellman, R. Wilson, C. Sander, J. Weinstein, M. Meyerson, S. Gabriel, P. W. Laird, D. Haussler, G. Getz, L. Chin; TCGA Research Network, The somatic genomic landscape of glioblastoma. *Cell* **155**, 462–477 (2013).
21. L. A. D. Cooper, D. A. Gutman, Q. Long, B. A. Johnson, S. R. Cholleti, T. Kurc, J. H. Saltz, D. J. Brat, C. S. Moreno, The proneural molecular signature is enriched in oligodendrogliomas and predicts improved survival among diffuse gliomas. *PLOS One* **5**, e12548 (2010).
22. N. R. Parker, P. Khong, J. F. Parkinson, V. M. Howell, H. R. Wheeler, Molecular heterogeneity in glioblastoma: Potential clinical implications. *Front. Oncol.* **5**, 55 (2015).
23. X. Guan, J. Vengoechea, S. Zheng, A. E. Sloan, Y. Chen, D. J. Brat, B. P. O'Neill, J. de Groot, S. Yust-Katz, W.-K. Yung, M. L. Cohen, K. D. Aldape, S. Rosenfeld, R. G. W. Verhaak, J. S. Barnholtz-Sloan, Molecular subtypes of glioblastoma are relevant to lower grade glioma. *PLOS One* **9**, e91216 (2014).
24. B. J. Gill, D. J. Pisapia, H. R. Malone, H. Goldstein, L. Lei, A. Sonabend, J. Yun, J. Samanamud, J. S. Sims, M. Banu, A. Dovas, A. F. Teich, S. A. Sheth, G. M. McKhann, M. B. Sisti, J. N. Bruce, P. A. Sims, P. Canoll, MRI-localized biopsies reveal subtype-specific differences in molecular and cellular composition at the margins of glioblastoma. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 12550–12555 (2014).
25. S. Schreiner, S. Kinkley, C. Burck, A. Mund, P. Wimmer, T. Schubert, P. Groitl, H. Will, T. Dobner, SPOC1-mediated antiviral host cell response is antagonized early in human adenovirus type 5 infection. *PLOS Pathog.* **9**, e1003775 (2013).
26. S. Kinkley, H. Staege, G. Mohrmann, G. Rohaly, T. Schaub, E. Kremmer, A. Winterpacht, H. Will, SPOC1: A novel PHD-containing protein modulating chromatin structure and mitotic chromosome condensation. *J. Cell Sci.* **122**, 2946–2956 (2009).
27. A. E. Pegg, Mammalian O^6-alkylguanine-DNA alkyltransferase: Regulation and importance in response to alkylating carcinogenic and therapeutic agents. *Cancer Res.* **50**, 6119–6129 (1990).
28. M. E. Hegi, A.-C. Diserens, T. Gorlia, M.-F. Hamou, N. de Tribolet, M. Weller, J. M. Kros, J. A. Hainfellner, W. Mason, L. Mariani, J. E. C. Bromberg, P. Hau, R. O. Mirimanoff, J. G. Cairncross, R. C. Janzer, R. Stupp, *MGMT* gene silencing and benefit from temozolomide in glioblastoma. *N. Engl. J. Med.* **352**, 997–1003 (2005).
29. M. Esteller, J. Garcia-Foncillas, E. Andion, S. N. Goodman, O. F. Hidalgo, V. Vanaclocha, S. B. Baylin, J. G. Herman, Inactivation of the DNA-repair gene *MGMT* and the clinical response of gliomas to alkylating agents. *N. Engl. J. Med.* **343**, 1350–1354 (2000).
30. M. N. T. Thuy, J. K. T. Kam, G. C. Y. Lee, P. L. Tao, D. Q. Ling, M. Cheng, S. K. Goh, A. J. Papachristos, L. Shukla, K.-L. Wall, N. R. Smoll, J. J. Jones, N. Gikenye, B. Soh, B. Moffat, N. Johnson, K. J. Drummond, A novel literature-based approach to identify genetic and molecular predictors of survival in glioblastoma multiforme: Analysis of 14,678 patients using systematic review and meta-analytical tools. *J. Clin. Neurosci.* **22**, 785–799 (2015).
31. D. R. McCarthy, M. M. Hanna, P. D. Cotter, *MethylMeter: A Quantitative, Sensitive, and Bisulfite-Free Method for Analysis of DNA Methylation* (InTech Open Access Publisher, Croatia, 2012).
32. O. L. Chinot, W. Wick, W. Mason, R. Henriksson, F. Saran, R. Nishikawa, A. F. Carpentier, K. Hoang-Xuan, P. Kavan, D. Cernea, A. A. Brandes, M. Hilton, L. Abrey, T. Cloughesy, Bevacizumab plus radiotherapy–temozolomide for newly diagnosed glioblastoma. *N. Engl. J. Med.* **370**, 709–722 (2014).
33. R. Stupp, W. P. Mason, M. J. van den Bent, M. Weller, B. Fisher, M. J. B. Taphoorn, K. Belanger, A. A. Brandes, C. Marosi, U. Bogdahn, J. Curschmann, R. C. Janzer, S. K. Ludwin, T. Gorlia, A. Allgeier, D. Lacombe, J. G. Cairncross, E. Eisenhauer, R. O. Mirimanoff; European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups; National Cancer Institute of Canada Clinical Trials Group, Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Engl. J. Med.* **352**, 987–996 (2005).
34. J. Landolfi, T. Cloughesy, M. A. Vogelbaum, S. Kesari, S. Kalkanis, J. Portnow, T. Mikkelsen, J. B. Elder, D. Baskin, A. Chu, J. Skillings, H. Gruber, C. Cobb, G. Foltz, G. Kaptain, M. K. Aghi, in Society of Neurooncology 2015 Annual Conference, San Antonio, TX, 2015.
35. H. Okada, M. Weller, R. Huang, G. Finocchiaro, M. R. Gilbert, W. Wick, B. M. Ellingson, N. Hashimoto, I. F. Pollack, A. A. Brandes, E. Franceschi, C. Herold-Mende, L. Nayak, A. Panigrahy, W. B. Pope, R. Prins, J. H. Sampson, P. Y. Wen, D. A. Reardon, Immunotherapy response assessment in neuro-oncology: A report of the RANO working group. *Lancet Oncol.* **16**, e534–e542 (2015).
36. W. Wang, C. K. Tai, A. D. Kershaw, S. K. Solly, D. Klatzmann, N. Kasahara, T. C. Chen, Use of replication-competent retroviral vectors in an immunocompetent intracranial glioma model. *Neurosurg. Focus* **20**, E25 (2006).
37. J. Murakami, Y.-J. Lee, S. Kokeguchi, H. Tsujigiwa, J.-I. Asaumi, H. Nagatsuka, K. Fukui, M. Kuroda, N. Tanaka, N. Matsubara, Depletion of O6-methylguanine-DNA methyltransferase by O6-benzylguanine enhances 5-FU cytotoxicity in colon and oral cancer cell lines. *Oncol. Rep.* **17**, 1461–1467 (2007).
38. J. A. Oliver, R. Ortiz, C. Melguizo, P. J. Álvarez, J. Gómez-Millán, J. Prados, Prognostic impact of MGMT promoter methylation and MGMT and CD133 expression in colorectal adenocarcinoma. *BMC Cancer* **14**, 511 (2014).
39. T. Mazor, A. Pankov, B. E. Johnson, C. Hong, E. G. Hamilton, R. J. A. Bell, I. V. Smirnov, G. F. Reis, J. J. Phillips, M. J. Barnes, A. Idbaih, A. Alentorn, J. J. Kloezeman, M. L. M. Lamfers, A. W. Bollen, B. S. Taylor, A. M. Molinaro, A. B. Olshen, S. M. Chang, J. S. Song, J. F. Costello, DNA methylation and somatic mutations converge on the cell cycle and define similar evolutionary histories in brain tumors. *Cancer Cell* **28**, 307–317 (2015).
40. T. Gorlia, R. Stupp, A. A. Brandes, R. R. Rampling, P. Fumoleau, C. Dittrich, M. M. Campone, C. C. Twelves, E. Raymond, M. E. Hegi, D. Lacombe, M. J. van den Bent, New prognostic factors and calculators for outcome prediction in patients with recurrent glioblastoma: A pooled analysis of EORTC Brain Tumour Group phase I and II clinical trials. *Eur. J. Cancer* **48**, 1176–1184 (2012).
41. J. L. Clarke, M. M. Ennis, W. K. A. Yung, S. M. Chang, P. Y. Wen, T. F. Cloughesy, L. M. Deangelis, H. I. Robins, F. S. Lieberman, H. A. Fine, L. Abrey, M. R. Gilbert, M. Mehta, J. G. Kuhn, K. D. Aldape, K. R. Lamborn, M. D. Prados; North American Brain Tumor Consortium, Is surgery at progression a prognostic marker for improved 6-month progression-free survival or overall survival for patients with recurrent glioblastoma? *Neuro Oncol.* **13**, 1118–1124 (2011).
42. D. R. Macdonald, T. L. Cascino, S. C. Schold Jr., J. G. Cairncross, Response criteria for phase II studies of supratentorial malignant glioma. *J. Clin. Oncol.* **8**, 1277–1280 (1990).
43. Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 (28 May 2009); http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40
44. FDA Guidance for Industry Considerations for the Design of Early-Phase Clinical Trials of Cellular and Gene Therapy Products; http://www.fda.gov/downloads/Biologi.../UCM359073.pdf
45. D. Kim, G. Pertea, C. Trapnell, H. Pimentel, R. Kelley, S. L. Salzberg, TopHat2: Accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol.* **14**, R36 (2013).
46. R. Sandberg, J. R. Neilson, A. Sarma, P. A. Sharp, C. B. Burge, Proliferating cells express mRNAs with shortened 3′ untranslated regions and fewer microRNA target sites. *Science* **320**, 1643–1647 (2008).

Acknowledgments: We thank J. Skillings, N. Boyle, A. M. Richter, and J. Wood for critical readings of the manuscript. We also wish to thank R. Verhaak for providing updated glioma classification gene lists developed by the TCGA glioblastoma multiforme consortium and F. M. Hofman, professor of pathology, Keck School of Medicine of the University of Southern California, for analysis of the pseudoprogression slides. We also wish to thank Denovo Biopharma LLC for providing the lomustine external control data. Study sites, principal investigators, and primary study coordinators: University of California, Los Angeles: T.F.C., A.L., L.M.L., P.L.N., E. Filka, and S. Green; New Jersey Neuroscience Institute, John F. Kennedy (JFK) Medical Center, Edison, NJ: J.L., S.B., C. Porbeni, and L. Thomas; Moores Cancer Center, Department of Neurosciences, University of California, San Diego: D.P., S.K., B.C., C.C.C., L. Rose, and B. Brown; Ohio State University Wexner Medical Center, Columbus, OH: J.B.E., C. Rond; Henry Ford Hospital, Detroit, MI: T.W., T.M., S.NK., I.Y.L., J. Gaggin, and S. Marl; Cleveland Clinic Foundation, Cleveland, OH: M.A.V., C. Brewer, and J. Biscup; Swedish Neuroscience Institute, Seattle, WA: C. Cobbs and N. Hansen. **Funding:** The authors thank the Accelerate Brain Cancer Cure Foundation (Washington, DC), the National Brain Tumor Society (Watertown, MA), the American Brain Tumor Association (Chicago, IL), the Musella Foundation (Hewlett, NY), and Voices Against Brain Cancer (New York, NY) for their support and collaborations. N.K. was also supported in part by U01NS059821 from the National Institute of Neurological Disorders and Stroke. **Author contributions:** T.F.C., J.L., S.B., B.C., C.C.C., J.B.E., S.N.K., S.K., A.L., I.Y.L., L.M.L., T.M., P.L.N., D.P., T.W., and M.A.V. ran the clinical trial at their respective sites, provided clinical samples, and reviewed and provided insight to the manuscript. D.J.H., A.C., A.D., D.G., D.O., L.M., M.R.-A., O.R.D., J.M.R., and D.J.J. performed the experiments and analyzed the data. H.E.G., J.M.R., D.J.J, A.D., and D.O. designed the study and supervised the overall project. A.D., D.J.H.,

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and D.O. wrote the manuscript. N.K. developed the founding technology on which Toca 511 is based. All authors reviewed the final manuscript. **Competing interests:** D.J.H., A.C., A.D., D.G., H.E.G., D.J.J., D.O., L.M., M.R., O.R.D., and J.M.R. are employees and/or shareholders of Tocagen. N.K. is a consultant, has ownership interest in, and is the recipient of a research grant from Tocagen. T.W. and J.L. are advisors for Novocure. L.M.L. is an advisor for Genentech/Roche; M.A.V. is an advisor with Infuseon Therapeutics. Materials presented in this manuscript are covered by the following U.S. patents: 6,410,313; 6,899,871; 8,652,460; 8,722,867; 8,741,279; 8,829,173; 7,045,319; 7,226,738; 7,468,261; 7,470,511; 7,473,775; 7,541,165; 8,211,644; 8,242,243; and 8,263,339. **Data and materials availability:** All reasonable requests for nonclinical collaboration involving data generated in the manuscript, or for use of non–good manufacturing practice (GMP) material, from qualified groups or institutions will be fulfilled provided that a written agreement is executed in advance between Tocagen Inc. or Ribomed and the requestor (and his or her affiliated institution). Such requests should be directed to D.J.J. at Tocagen Inc. Clinical use of GMP-manufactured drugs is regulated by FDA in the United States, and Tocagen is responsible for the safe use of Tocagen-manufactured material. Clinical collaborations with use of GMP materials require a clinical trial agreement, drug availability, and a fit with Tocagen's plans for drug approval and use. Requests for clinical collaborations should be directed to A.D. at Tocagen Inc.

Submitted 4 December 2015
Accepted 2 May 2016
Published 1 June 2016
10.1126/scitranslmed.aad9784

Citation: T. F. Cloughesy, J. Landolfi, D. J. Hogan, S. Bloomfield, B. Carter, C. C. Chen, J. B. Elder, S. N. Kalkanis, S. Kesari, A. Lai, I. Y. Lee, L. M. Liau, T. Mikkelsen, P. L. Nghiemphu, D. Piccioni, T. Walbert, A. Chu, A. Das, O. R. Diago, D. Gammon, H. E. Gruber, M. Hanna, D. J. Jolly, N. Kasahara, D. McCarthy, L. Mitchell, D. Ostertag, J. M. Robbins, M. Rodriguez-Aguirre, M. A. Vogelbaum, Phase 1 trial of vocimagene amiretrorepvec and 5-fluorocytosine for recurrent high-grade glioma. *Sci. Transl. Med.* **8**, 341ra75 (2016).

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(June 1, 2016)
Science Translational Medicine **8** (341), 341ra75. [doi: 10.1126/scitranslmed.aad9784]

Editor's Summary

Tag-team attack on glioma

Toca FC (extended-release 5-fluorocytosine) and Toca 511 (vocimagene amiretrorepvec) are an investigational therapeutic combination for glioma, consisting of two parts: a prodrug that is inactive on its own and a modified virus that infects the tumor and delivers an enzyme, which then activates the drug and allows it to kill the glioma cells. Cloughesy *et al.* tested this therapy in 45 human patients with recurrent or progressive high-grade glioma and discovered that the treatment was well tolerated and improved survival compared to an external control group. In addition, the authors identified a gene signature that correlated with response to the treatment, which may help identify the patients most likely to benefit from this approach.

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