Chronic convection-enhanced delivery of topotecan for patients with recurrent glioblastoma: a first-in-patient, single-centre, single-arm, phase 1b trial

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Summary

Background Topotecan is cytotoxic to glioma cells but is clinically ineffective because of drug delivery limitations. Systemic delivery is limited by toxicity and insufficient brain penetrance, and, to date, convection-enhanced delivery (CED) has been restricted to a single treatment of restricted duration. To address this problem, we engineered a subcutaneously implanted catheter-pump system capable of repeated, chronic (prolonged, pulsatile) CED of topotecan into the brain and tested its safety and biological effects in patients with recurrent glioblastoma.

Methods We did a single-centre, open-label, single-arm, phase 1b clinical trial at Columbia University Irving Medical Center (New York, NY, USA). Eligible patients were at least 18 years of age with solitary, histologically confirmed recurrent glioblastoma showing radiographic progression after surgery, radiotherapy, and chemotherapy, and a Karnofsky Performance Status of at least 70. Five patients had catheters stereotactically implanted into the glioma-infiltrated peritumoural brain and connected to subcutaneously implanted pumps that infused 146 µM topotecan 200 µL/h for 48 h, followed by a 5–7-day washout period before the next infusion, with four total infusions. After the fourth infusion, the pump was removed and the tumour was resected. The primary endpoint of the study was safety of the treatment regimen as defined by presence of serious adverse events. Analyses were done in all treated patients. The trial is closed, and is registered with ClinicalTrials.gov, NCT03154996.

Findings Between Jan 22, 2018, and July 8, 2019, chronic CED of topotecan was successfully completed safely in all five patients, and was well tolerated without substantial complications. The only grade 3 adverse event related to treatment was intraoperative supplemental motor area syndrome (one [20%] of five patients in the treatment group), and there were no grade 4 adverse events. Other serious adverse events were related to surgical resection and not the study treatment. Median follow-up was 12 months (IQR 10–17) from pump explant. Post-treatment tissue analysis showed that topotecan significantly reduced proliferating tumour cells in all five patients.

Interpretation In this small patient cohort, we showed that chronic CED of topotecan is a potentially safe and active therapy for recurrent glioblastoma. Our analysis provided a unique tissue-based assessment of treatment response without the need for large patient numbers. This novel delivery of topotecan overcomes limitations in delivery and treatment response assessment for patients with glioblastoma and could be applicable for other anti-glioma drugs or other CNS diseases. Further studies are warranted to determine the effect of this drug delivery approach on clinical outcomes.

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Introduction

Glioblastoma, the most common primary brain malignancy, is locally invasive and universally recurrent, and thus, the prognosis is poor despite conventional surgery, radiotherapy, and chemotherapy, underscoring the need for more effective treatments.[1] Promising antiglioma drugs have not succeeded clinically because of limitations in drug delivery.[2,3] Topotecan is a topoisomerase inhibitor that effectively kills proliferating glioma cells, but is clinically impractical as a systemically delivered chemotherapeutic because of systemic toxic effects and insufficient brain penetrance.[4,5] We previously showed the safety and feasibility of short-term, single-dose convection-enhanced delivery (CED) in a clinical trial with toptecan for patients with refractory malignant gliomas.[6] CED is a method of locoregional drug infusion that delivers high

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See **Comment** page 1347

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Research in context

Evidence before this study

We searched PubMed for preclinical animal studies and human clinical trials using topotecan or convection-enhanced delivery for gliomas published between Jan 1, 1996, and Dec 31, 2018, using the search term "((glioma[Title/Abstract]) OR (glioblastoma[Title/Abstract])) AND ((topotecan[Title/Abstract]) OR (convection-enhanced delivery[Title/Abstract]))". The search returned 372 articles; studies that included human or animal studies were included and review papers and non-English-language publications were excluded. Topotecan is cytotoxic to glioma cells but was ineffective with systemic delivery in a phase 2 clinical trial. Although topotecan with convection-enhanced delivery led to tumour regression in a phase 1 clinical trial of malignant glioma, the reliance of this technique on external pumps and catheters limited treatment to a single infusion over a short period of time to minimise infection risk. Evidence from preclinical animal studies showed that chronic infusion strategies that prolong the duration of infusion provide survival benefits for topotecan and could be effective in humans if technological obstacles can be overcome.

Added value of this study

To achieve intratumoural delivery of topotecan at high concentrations and for unlimited duration, we developed a subcutaneously implanted catheter-pump system and tested this prospectively for the first time, to our knowledge, in human patients with glioma. By delivering topotecan, an antiproliferative chemotherapeutic drug, for multiple cycles over a 4-week period, we were able to show the clinical safety and therapeutic efficacy of direct interstitial topotecan delivery into the tumour and peritumoural brain tissue. The treatment protocol incorporated a unique method for collecting tissue specimens immediately before and after treatment to provide an opportunity to directly analyse treatment effects in tissue for the first time, to our knowledge, in a human glioma trial. The clinical utility of this treatment was further enhanced by MRI of co-infused gadolinium, which showed broad drug distribution into the brain where unresectable invaded tumour cells reside and can cause recurrence. Tissue analysis supported topotecan treatment effectiveness by showing significant reductions of proliferating tumour cells without toxic effects in neurons.

Implications of all the available evidence

Despite conventional therapy, glioblastoma is a rapidly fatal disease and new treatment strategies are needed. Since treatment failure with chemotherapeutics such as topotecan is primarily due to limitations in drug delivery, our ability to use an implantable pump-catheter to deliver drugs at high concentrations and for extended periods of time has the potential to transform approaches to brain tumour therapy. Although we showed success in patients with glioma using topotecan, this system could potentially have broad clinical applicability, as it is adaptable for multi-drug regimens and new classes of drugs, including high-molecular-weight compounds, proteins, viruses, liposomes, nanoparticles, and other biologicals that would not be feasible with systemic delivery. This treatment strategy could also be applied to other CNS diseases in which therapeutic effectiveness is constrained by drug delivery. Additionally, as used in this study to show the effectiveness of our treatment strategy, our unique approach of collecting and analysing pre-treatment and post-treatment tissue provides insight into effects for individual patients and a new methodology for overall response assessment in glioma clinical trials.

concentrations of therapeutic compounds directly into the brain through a surgically implanted thin cannula attached to a microinfusion pump. Because glioblastomas are locally invasive, rarely metastasise, and usually recur within 2 cm of the original resection margin,[7] local drug delivery strategies have the potential to impact patient survival and provide insight into the direct effects of chemotherapy on the tumour microenvironment.[8,9] Drugs are infused at flow rates that generate a positive hydrostatic pressure to distribute the infusate by bulk flow through the interstitial space. As this strategy circumvents blood–brain barrier limitations and avoids systemic toxic effects, CED provides a pharmacokinetic advantage that is several orders of magnitude greater for maximising drug concentrations in a targeted region of the brain compared with conventional diffusion-driven systemic methods, such as oral or intravenous delivery.[9–12] However, to the extent that CED relies on an external catheter and bedside pump, the inherent infection risks impose a restriction that limits treatment to only a single infusion of insufficient duration to achieve meaningful clinical results. Sustained chronic (repeated, prolonged) delivery with multiple treatment cycles is important therapeutically because topoisomerase poisons such as topotecan, like most chemotherapies, are cytotoxic to cycling cells in the S-phase, where only a small percentage of glioma cells reside at any given time.[13,14] Preclinical studies in a rat glioma model with locally delivered topotecan showed improved survival when the infusion duration was extended to allow more tumour cells to cycle through the vulnerable S-phase.[15] Therefore, a chronic local topotecan delivery technique for gliomas that circumvents blood–brain barrier limitations, avoids systemic toxic effects, and provides unlimited drug regimens to achieve sustained effective intratumoural drug concentrations is needed.

To achieve this goal, we engineered a subcutaneously implanted catheter-pump system similar to one effectively used in patients with Parkinson's disease.[16] After successful preclinical testing in a large animal model,[17,18] we designed a prospective clinical trial for patients with refractory glioblastoma using chronic prolonged pulsatile CED with a refillable pump subcutaneously implanted in the abdomen for long-term and repeated intracerebral infusions of high-dose topotecan chemotherapy. We

aimed to test the clinical utility and safety of topotecan delivery by chronic CED in patients with glioblastoma using a subcutaneous pump-catheter construct. Given the unique nature of this clinical trial, which incorporated both a novel device and off-label use of a drug, the US Food and Drug Administration (FDA) provided approval for a small number of patients, which precluded us from measuring treatment efficacy with conventional response parameters such as survival or tumour progression. Therefore, we devised a novel trial protocol design that included the procurement of tissue both immediately before and after treatment to facilitate a direct tissue-based assessment of treatment response. This analysis approach enabled us to show antitumour treatment effects within our small number of patients and overcome challenges in conventional response assessment in glioma clinical trials, which currently rely on ambiguous radiographic and clinical endpoints or analysis of post-mortem tissue that is confounded by the effects of recurrent disease.[19,20]

Methods

Study design and participants

This study was an investigator-initiated, single-centre, single-arm, phase 1b clinical trial at New York-Presbyterian-Columbia University Irving Medical Center (New York, NY, USA). Patients were recruited from neuro-oncology practices at Columbia University Irving Medical Center and approved at the weekly Brain Tumor Board. The estimated life expectancy for enrolled patients with recurrent glioblastoma was not prespecified by the protocol but was expected to be 3–7 months based on the available literature.[21] Eligible patients were at least 18 years of age with previously histologically confirmed malignant glioma (WHO grade III–IV) treated with surgical resection, temozolomide chemotherapy, and external beam radiotherapy, who showed clinical and radiographic evidence of recurrent glioblastoma and who were willing and medically able to undergo surgery. Additional eligibility criteria were Karnofsky performance status (KPS) of 70 or greater and a solitary stereotactically accessible supratentorial contrast-enhancing tumour localised to a region less than 32 cm^3 in volume on pre-enrolment MRI, with histopathological confirmation of recurrent glioblastoma at the time of catheter placement. Basic laboratory tests, including but not limited to serum chemistry, complete blood count (to ensure adequate bone marrow function), coagulation studies, and pregnancy test (if applicable), were required before enrolment. Men and women of child-beraing age must have been using contraception. Exclusion criteria included patients who were previously treated systemically with topotecan, cerebellar, multi-focal, or ventricular disease, as well as patients with known HIV, hepatitis B, hepatitis C, or other known active infection or intercurrent uncontrolled illness. Patients also must not have been on any medications solely metabolised by the CYP450 enzyme system and have a narrow therapeutic index (see appendix pp 22 for medications concominantly taken by each enrolled patient). Patients had to be able to receive MRI and PET scans. Further information can be found in the published study protocol (appendix).

All patients gave written, informed consent to the protocol approved by the Columbia University Irving Medical Center Institutional Review Board (protocol #AAAQ9520) and the FDA. The principal investigator (JNB) was the sponsor of the investigational new drug approval from the FDA. Ethics approval was obtained from the Columbia University Irving Medical Center Institutional Review Board and the FDA.

Procedures

Treatment comprised four 48-h infusions of topotecan through a surgically placed intracerebral catheter connected to a subcutaneously implanted pump, followed by radical resection of the tumour and removal of the pump-catheter 4 weeks later (appendix pp 5–6). A preoperative MRI was done to optimise selection of localised tumour biopsies and catheter trajectory. Multiple stereotactic biopsies of the tumour and infiltrated brain tissue were collected through small twist drill holes for comprehensive immunohistopathological and molecular analyses, as well as for intraoperative histopathological assessment to verify the diagnosis of recurrent glioblastoma. A 1·5-mm outer diameter silastic Spetzler lumbar shunt catheter (Integra; Plainsboro, NJ, USA) was stereotactically positioned between the contrast-enhancing tumour and the margin of the planned surgical resection to be done at the end of the 4-week treatment (appendix pp 5–6). This catheter placement strategy was designed to maximise drug delivery into the peritumoural brain tissue. The catheter was connected to silastic tubing that was subcutaneously tunnelled and connected to a SynchroMed II infusion pump (Medtronic; Minneapolis, MN, USA) implanted in the abdomen. The infusate was prepared by the research pharmacy and consisted of 1:100 gadolinium Gadavist (GE Healthcare; Marlborough, MA, USA) plus 146 µM topotecan (Hycamtin; GlaxoSmithKline; Research Triangle Park, NC, USA). Topotecan is stable for at least 10 days at 37°C.[18]

Four treatment pulses were given, comprising 146 µM of topotecan infused over 48 h at 200 µL/h, followed by a 5–7-day washout period before the next infusion. The topotecan dose was derived from our previous phase 1 clinical trial.[6] The rationale for 48-h pulsatile infusion was derived from preclinical porcine studies showing that the largest relative gains in the volume of distribution of infusate occur within the first 24–48 h, before they decline as infusions reach a steady state.[17] Gadolinium was co-infused during the first and fourth pulse and T1-weighted MRI was used to monitor the volume of distribution and backflow (appendix pp 5–6).

Basic laboratory data, including serum chemistries (including liver enzymes), complete blood counts, and coagulation studies were taken at relative intervals.

See Online for appendix

Adverse events were defined as in the protocol (appendix). Adverse events were to be recorded irrespective of causality, and each event was described by its severity (mild, moderate, severe, or life-threatening). Safety and adverse events were assessed through daily neurological examinations during the treatment period, and at continued periodic timepoints after treatment, as follows: 1–2 weeks, 4–6 weeks, and then every 1–3 months. If at any time the principal investigator determined that the dose must be modified for patient safety, they could make the following modifications: decrease active pulse rate, decrease active pulse duration, or decrease or increase resting duration between active pulses to less than 5 or more than 7 days. Criteria for removal of patients from the study are found in the protocol (appendix), and included intercurrent illness, unacceptable adverse events (per the discretion of the principal investigator), patient withdrawal, or no recurrent tumour present histologically on pre-CED biopsy.

Immediately after completion of the fourth treatment pulse, a final MRI was done, after which the patient underwent multiple radiographically localised stereotactic-guided biopsies of the tumour, tumour margin, and surrounding invaded brain tissue, followed by surgical tumour resection along with removal of the pump and catheter system (appendix pp 5–6). A mean of seven biopsies were taken during catheter-pump implantation; a mean of 11 biopsies were taken during pump removal and surgical resection. Tissue analysis included immunohistochemistry and RNA sequencing to analyse treatment effects and matrix-assisted laser desorption/ionisation mass spectrometry for drug-concentration analysis. Tumour burden was assessed by staining for SOX2, a highly pervasive glioma cell marker that is expressed by most tumour cells in glioblastoma,[22] as well as Ki67, a marker for proliferation. Immunohistochemical staining was also done to assess changes to the glioma microenvironment, using CD68 (marker of inflammatory microglia and macrophages) and NeuN (a marker of neurons). We extracted RNA and sequenced MRI localised biopsies taken pre-CED and post-CED using the Illumina TruSeq-NovaSeq pipeline, and aligned, mapped, and quantified using the Kallisto pipeline (appendix p 2).

3T MRI was done pre-operatively and on post-operative day 0 or 1 after catheter implantation to confirm catheter placement before infusion initiation. For the first infusion, MRIs were done approximately 8 h, 14 h, 24 h, and 48 h after the start of infusion, and approximately 8 h, 14 h, and 24 h after completion. For the remaining infusions, MRIs were done immediately before and approximately 48 h after the start of each infusion. After the first two patients were treated, the protocol was amended on April 4, 2019, and [^{18}F]fluorodeoxyglucose ([^{18}F]FDG)-PET imaging was done on patients 3, 4 and 5 pre-operatively, approximately 48 h after the start of pulse 1, and approximately 24 h after start of pulse 4.

Outcomes

The primary endpoint of this study was the safety of chronic CED of topotecan in patients with recurrent glioblastoma, as defined by the occurence of serious adverse events.

Secondary endpoints per the study protocol were measurement of the steady-state volume of drug distribution as measured on volumetric MRI, correlation of intraparenchymal topotecan concentration with contrast enhancement intensity on MRI, time to tumour progression or recurrence, and time to death. We report survival (ie, time to death) from CED, as well as overall survival from diagnosis. Tumour progression is typically determined by the Response Assessment in Neuro-Oncology (RANO) criteria, which use a combination of radiographic and clinical parameters to determine tumour progression.[19,20] However, radiographic changes related to local treatment and surgical resection precluded use of these criteria for assessing tumour progression. Additionally, prespecified tissue-based analyses were done on pre-treatment and post-treatment MRI-localised biopsies to characterise the biological effects of treatment.

Quality-of-life and neurocognitive testing using the Cognitive Stability Index, Functional Assessment of Chronic Illness Therapy, FACT-Brain, Karnofsky Performance Scale, and Patient-Reported Outcomes Measurement Information System were done immediately before and after each treatment pulse for all patients.[23,24]

Statistical analysis

Clinical toxicity (defined as a grade 2 or worse serious adverse event) was projected to be 5% or less at 30 days. A clinical toxicity rate that exceeded 20% was considered unacceptable for the study procedure, which was the rationale for assessment of a minimum of five patients in the present study. The trial was ended after five patients completed the study, since there were no serious adverse events. A truncated sequential probability ratio test was used to determine whether the rate of toxic effects exceeded our target. We used correlation analysis to determine the relationship between gadolinium signal intensity and distribution on MRI and the direct measurement of topotecan concentrations. The power calculations were framed to assess our ability to detect these correlations. All statistical analysis was done at a significance level of $p<0·05$.

Quality-of-life survey data were analysed using descriptive statistics and were scored as follows: KPS scored from 0–100 (a higher score indicates better performance); neurocognitive assessments scored by percentile 0–100 (a higher percentile indicates better performance); Funcational Assessment of Cancer Therapy-Brain scored from 0–200 (a higher value indicates worse performance); Functional Assessment of Chronic Illness Therapy-Fatigue scored from 0–52 (a higher value indicates worse fatigue); and Patient-Reported Outcome Measurement Information System

	Sex	Age at enrolment, years	Race	Initial diagnosis	Tumour location	Tumour volume, mL	*IDH1* status	*MGMT* promoter methylation	Survival from enrolment, months
Patient 1	Male	34	White	Anaplastic astrocytoma grade III*	Right, frontal	1·9	Wild-type	Unmethylated	12
Patient 2	Female	58	White	GBM, *IDH* wild-type	Right, temporal	12·9	Wild-type	Unmethylated	17
Patient 3	Male	61	White	GBM, *IDH* wild-type	Left, frontal	5·1	Wild-type	Methylated	10
Patient 4	Male	56	White	GBM, *IDH* wild-type	Right, temporal	18·0	Wild-type	Unmethylated	5
Patient 5	Female	51	White	GBM, *IDH* wild-type	Right, frontal	4·1	Wild-type	Methylated	25

See appendix (pp 12–13) for more information. *At recurrence, this patient's tumour showed histopathological and molecular features diagnostic for GBM, *IDH* wildtype. GBM=glioblastoma multiforme.

Table 1: **Baseline clinical and pathological data from the five patients who completed the study**

global health measure scored by T-score (a higher score indicates better performance). A worsening of 20% in any of the metrics after a treatment pulse that was not attributed to surgical resection or non-CED-related events was deemed a meaningful change.

All bioinformatic tissue analysis, including of RNA sequencing data, was done in programming language R (version 4.1.2). RNA count data from all biopsies were processed and normalised using the R package DESeq2, and pre-treatment and post-treatment biopsies were compared across all patients via differential gene expression analysis using DESeq2.[25] Gene Set Enrichment Analysis (implemented via the GSEA desktop version 4.1.0) was done using the 50 Cancer Hallmarks gene sets patient-by-patient comparing RNA sequencing data from pre-treatment and post-treatment MRI-localised biopsies.[26] Fisher p-integration was done on FDR-adjusted p-values across all patients for each pathway, and significant pathways ($p<0·05$) were visualised.

All immunohistochemical stains were quantified using a labelling index (count of positive cells for a stain divided by the count of all cells), and *t* tests were done between pre-CED and post-CED conditions for significance testing (appendix p 2).

Single-sample gene set variation analysis was done on all MRI-localised biopsies using gene signatures for six distinct glioma cell states derived from single-nuclei and single-cell RNA sequencing signatures (appendix p 3). Each biopsy was annotated by the glioma cell state it was most enriched in (appendix p 67). Biopsies were stratified into whether they were taken pre-CED or post-CED, and if they were taken post-CED, they were further separated into whether or not the biopsy was within the radiographically determined maximal volume of distribution of treatment. Pearson's χ^2 test was then done to statistically quantify change in phenotype between conditions.

All analyses were done on all treated patients, unless otherwise stated.

The data safety monitoring committee of the Herbert Irving Comprehensive Cancer Center of Columbia University provided oversight and review of the primary endpoints. Independent monitoring was also provided by the Herbert Irving Comprehensive Cancer Center Clinical Protocol and Data Management Compliance Core. The trial is registered with ClinicalTrials.gov, NCT03154996.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Jan 22, 2018, and July 8, 2019, six patients were enrolled in the study. One patient was excluded because histopathological analysis of their pre-treatment biopsy showed no recurrent tumour. Basic demographic and clinical data of enrolled patients are summarised in table 1. Three male patients and two female patients (median age 56 years [IQR 48–57]) were treated for a median of 11 months (IQR 7–16) from the time of initial diagnosis. All patients had biopsy-proven *IDH1* wild-type glioblastoma according to WHO criteria. Detailed pathology descriptions and treatment history are provided in the appendix (pp 14–18).

The median survival duration for all five patients was 12 months (IQR 10–17) from the time of enrolment and the median overall survival from the time of initial diagnosis was 23 months (21–28). The median follow-up duration was 12 months (10–17) from pump explant. All five (100%) patients eventually died due to tumour progression, and there were no known treatment-related deaths. Radiographic changes related to CED and surgical resection precluded a reliable determination of time to tumour progression or recurrence based on RANO criteria.[19,20]

Overall, topotecan delivered by chronic CED was generally well tolerated and complications were uncommon and transient. No significant systemic complications were reported (appendix p 8). Regarding the most common treatment complaints, five (100%) of five patients had pain at the incision site, three (60%) of five patients had fatigue, and two (40%) of five patients had headache, all of which were grade 1 or 2 in severity (table 2). One patient had worsening of a baseline

	Patients (n=5)
Grade 1–2	
Pain at incision site	5 (100%)
Fatigue	3 (60%)
Headache	2 (40%)
Stroke*	1 (20%)
Grade 3	
Intraoperative stroke†	1 (20%)
Supplementary Motor Area (SMA) syndrome‡	1 (20%)

Data are n (%). No grade 4 or 5 adverse events were reported. A full list of pre-trial and post-trial symptoms for each patient can be found in the appendix (pp 19–21). *This adverse reaction was not directly related to the treatment delivery, but instead the surgical resection; however, this event affected the patient's postoperative quality of life and performance outcomes (appendix pp 24–26). †This adverse reaction was not directly related to the treatment delivery; the patient had arm and face weakness 5 days after completion of treatment due to a middle cerebral artery stroke that was attributed to a radiographically confirmed stenosis of the M2 branch from previous radiotherapy (appendix p 23). ‡Led to temporary dose reduction for this patient.

Table 2: **Selected treatment-related and non-treatment-related adverse events**

supplementary motor area syndrome during the initial infusion pulse, which improved over the ensuing treatment holiday, and the remaining infusions were given at a 50% reduced infusion rate without further incident. Because this patient also had a seizure between the second and third infusion pulse, the third infusion was reduced to 24 h as a precaution, given the patient's pre-treatment baseline seizure disorder. This patient also developed transient syndrome of inappropriate anti-diuretic hormone secretion, which resolved with fluid restriction. Furthermore, the same patient developed a lower extremity deep venous thrombosis during the treatment period, which was attributed to glioma associated coagulopathy (appendix p 19).[27] An inferior vena cava filter was placed with no further complications. The four (80%) other patients completed the treatment protocol as intended.

All patients were ambulatory and maintained their baseline KPS throughout treatment (appendix p 7). One patient had a transient decrease in KPS to as low as 70 because of transient supplementary motor area syndrome; however, their KPS returned to the baseline value of 90 at the end of pulse 4. Quality of life assessments showed no meaningful changes (appendix p 7). Serious adverse events in the follow-up period related to surgical resection or underlying disease, but unrelated to topotecan treatment, are reported in the appendix (p 3). The trial was ended as expected after completion by a minimum of five eligible patients.

Using the MRI signal of co-infused gadolinium, chronic CED resulted in a large and stable volume of distribution for all patients (mean maximal volume of distribution 20·4 mL [SD 10·5]; figure 1A) with mean time to peak volume after start of infusion of 43·1 h (11·5; figure 1B). At the time of maximal volume of distribution, the mean ratio

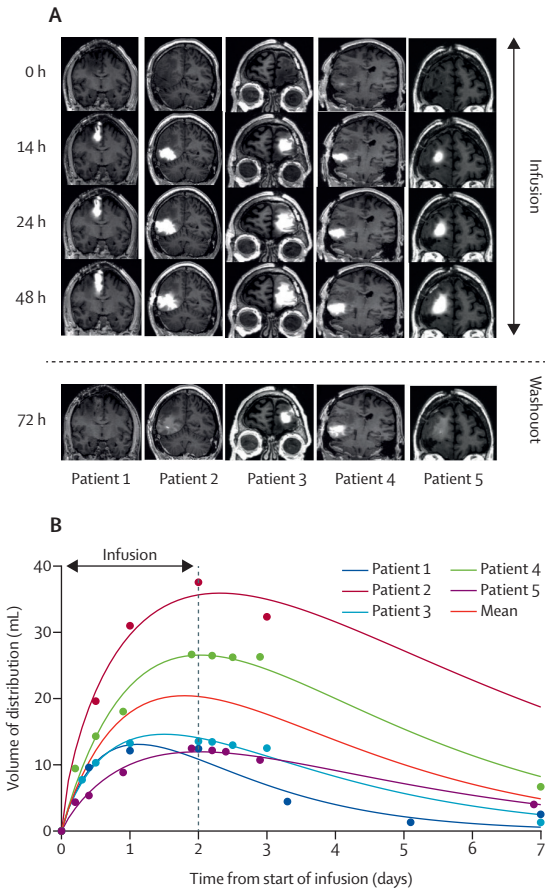

Figure 1: **Chronic CED of topotecan achieves large and stable volumes of distribution**

(A) Serial coronal T1-post contrast MRIs for each patient at selected timepoints during and immediately after first infusion. Contrast-enhancing regions (hyper-intense or bright on MRI) show the volume of distribution of treatment using co-delivered gadolinium as a proxy. Timepoints were 0 h (start of infusion), 14 h, 24 h, 48 h (end of infusion), and 72 h (as gadolinium begins to wash out). (B) Estimated volume of distribution of the infused contrast plotted as a function of time and fit to a γ function for each patient. The dashed vertical line indicates when the infusion took place. CED=convection-enhanced delivery.

of the volume of distribution to volume of infusion was 2·37 (SD 0·75; appendix p 27). Backflow was a small fraction of the total volume of distribution (mean maximum backflow volume 1·8 mL [SD 2·1; 8·8%; 1·8 mL [mean maximum backflow volume] divided by 20·5 mL [mean maximum volume of distribution]; appendix p 8).

11 (92%) of 12 biopsies analysed with mass spectrometric imaging had detectable levels of topotecan, with maximum pixel values above the limit of detection (3·2 μM; appendix p 9) and topotecan concentrations ranging from 1·1 μM to 30 μM (mean 8·9 μM [SD 2·3]). Micromolar concentrations of topotecan were present up to 6·5 cm (range 1·1–6·5) from the catheter tip and in all biopsies taken within the maximum volume of gadolinium distribution (appendix p 9).

Tissue collected from patients pre-treatment and post-treatment was analysed by immunohistochemistry to

determine the effects of topotecan on tumour cells (figure 2A, B). The SOX2 labelling index was significantly decreased in post-CED biopsies compared with pre-CED biopsies (18·5% *vs* 25·8%; p=0·031). Additionally, the Ki67 labelling index was significantly lower in post-CED biopsies compared with pre-CED biopsies (1·4% *vs* 3·9%; p=0·0090).

To further characterise the functional effect of chronic infusion of topotecan, [^{18}F]FDG-PET was done in three patients and glucose metabolic activity was assessed in the treated regions, as defined by the maximal volume of the infused gadolinium (figure 2C). CED treatment resulted in a significant reduction in glucose uptake relative to before treatment (patient 3 –17·2%; patient 4 –12·7%; patient 5 –6·2%; figure 2D). A comparison of post-CED biopsies showed an inverse exponential correlation between the Ki67 proliferation labelling index and [^{18}F]FDG-PET uptake (p=0·0020; figure 2E).

To further understand the effects of topotecan, RNA sequencing and differential gene-expression analyses were done on all MRI-localised biopsies with sufficient quality RNA (RNA integrity number >7), which included pre-CED at the time of catheter placement (n=35) and after treatment at the time of tumour resection (n=51; appendix pp 28–64). Gene set enrichment analysis showed substantial heterogeneity across patients before and after treatment but showed clear patterns of tissue response, with increases in gene signatures for DNA damage, apoptosis, and generation of reactive oxygen species, as well as upregulation of several metabolic programmes, including oxidative phosphorylation (appendix pp 10–11, 65–66). Mitotic spindle and other proliferation-associated gene ontologies were decreased across all patients, further showing the impact of chronic topotecan infusion on the proliferating tumour cell population. Single-sample gene set variation analysis revealed a significant shift in gene signatures for glioma cell states,[28] with post-CED samples within the volume of distribution of treatment showing a significant decrease (p=0·031) in the samples showing highest enrichment for the proliferative or proneural signatures and a significant increase (p=0·0006) in the samples showing highest enrichment for mesenchymal signatures (appendix pp 4, 10–11, 67). We also analysed the effects of chronic CED with topotecan on the tumour microenvironment. Expression of CD68 on MRI-localised biopsies was significantly increased in post-CED biopsies as assessed by immunohistochemistry (17·4% *vs* 9·6%; p=0·020; appendix pp 10–11). Post-CED biopsies were also significantly positively enriched in pro-inflammatory transcriptional programmes (appendix pp 10–11, 65–66). Differential gene-expression analysis was done by pooling biopsies across all patients, and pre-CED versus post-CED comparison showed a significant increase in several pro-inflammatory cytokines and other immunoactive markers (appendix pp 10–11, 28–64). Immunostaining with the neuronal marker NeuN showed no significant difference in post-treatment biopsies compared with pre-treatment biopsies, providing supportive evidence that topotecan was not substantially toxic to neurons in the peritumoural invaded brain (post-CED 5·4% *vs* pre-CED 4·7%; p=0·77; appendix pp 10–11).

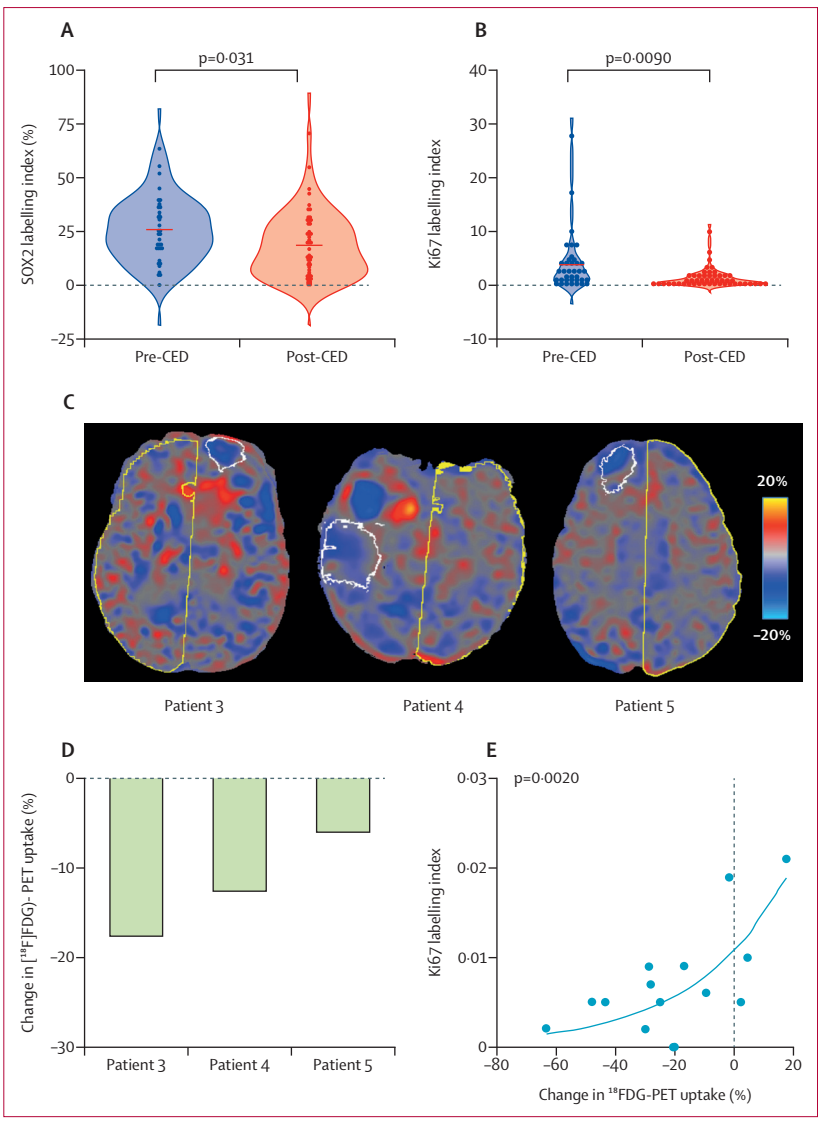

Figure 2: **Effects of chronic CED of topotecan on proliferating tumour populations and tumour phenotype**
Violin plots displaying quantification of SOX2 (A) and Ki67 (B) by labelling index across all MRI-localised biopsies from all patients, comparing biopsies taken pre-CED and post-CED using Student's *t* test (n=86). (C) PET scans were only done on the last three patients in this series. The difference between post-infusion and pre-infusion PET images were computed and converted to percentage signal change. The white outline represents the maximum infused volume; the yellow outline represents the control hemisphere. Blue voxels represent decreases in metabolism, red voxels represent increases in metabolism, and grey voxels represent no change after treatment. (D) Change in metabolism within the infused volume mask. (E) Change in Ki67 labelling and [^{18}F]FDG metabolism (n=14) after topotecan treatment. CED=convection-enhanced delivery. [^{18}F]FDG=[^{18}F]fluorodeoxyglucose.

Discussion

In this study, we showed that a subcutaneously implanted pump can feasibly and safely provide repeated prolonged infusion of topotecan in patients with glioma, overcoming

traditional shortcomings in the treatment of gliomas. Previous CED trials for glioblastoma used externalised hardware, which restricts treatment to a single infusion of restricted duration. We used this system to significantly reduce proliferating tumour cells in patients with refractory glioblastoma, delivering multiple cycles of topotecan at high concentrations, directly into the tumour and surrounding brain over 4 weeks, without serious neurological or neurobehavioural events, thereby circumventing the limitations associated with traditional systemic delivery and with stable quality of life. Using MRI to non-invasively monitor the distribution of co-infused gadolinium as a surrogate for topotecan distribution, we found large and stable volumes of drug distribution effectively targeting peritumuoral brain tissue where unresectable invasive tumour cells reside. Comparison of pre-treatment and post-treatment tissue showed consistent responses to topotecan after treatment, including a significant reduction in proliferating tumour cells and an increase in mesenchymal and inflammatory gene signatures. These changes were only seen in biopsies taken within the CED treatment volume, providing further evidence that they represent a response to topotecan. Larger studies will be needed to determine if chronic CED with topotecan will significantly improve survival or delay recurrence in patients with glioblastoma multiforme.

An effective treatment response with topotecan was possible by modifying previous CED constructs to facilitate chronic delivery, with potential unlimited regimens modelled after a similar protocol in patients with Parkinson's disease.[16] The advantage of an implantable CED pump allows for percutaneous refilling and the capacity for sequential or simultaneous treatment algorithms using one or more drugs, while maximising distribution volume. The pump–tubing–catheter construct that we used was improvised from various commercial sources and was designed to be used with a skill set common to neurosurgeons. The tested system has numerous features designed to ease its clinical use, as follows: self-contained subcutaneously implanted hardware to avoid infection and facilitate chronic use; an implanted microinfusion pump with a reservoir containing the treatment drug, which can be percutaneously refilled or emptied with a needle and syringe; wireless programming pump technology to modify flow rate; a simple flexible thin-bore catheter, which can be implanted precisely with stereotactic guidance and can accommodate flow rates within the therapeutic range without substantial backflow; incorporation of a technique for co-infusing gadolinium to monitor the volume of delivery in real time using a safe and reliable non-invasive methodology; and a safety profile suitable for use in an outpatient setting. Improvements in the CED construct are a source of ongoing study by our laboratory and others, including catheter design, use of multiple catheters, and improvements in pump technology, monitoring software, and stereotactic technologies. Future studies are needed to optimise infusion variables and treatment indications, including potentially eliminating the need for tumour resection.

Although our study showed the safety and feasibility of chronic CED, a major limitation was that the study was underpowered and did not have a comparison group for determination of definitive survival benefit. A further limitation was our inability to assess disease progression and treatment response radiographically via the RANO criteria due to effects of local drug infusion and surgical resection. However, our treatment protocol provides a novel broad clinical framework to study the effects of locally delivered therapy at the tissue-based level, using patients as their own controls. Performing a series of pre-therapy and post-therapy MRIs and PET scans, as well as taking dozens of MRI-localised biopsies upon implantation and removal of the CED pump-catheter system, enabled patient-specific measurements of drug delivery and treatment response. MRI-localised biopsies taken before treatment at pump implantation could allow tailoring of treatment regimens to each patient's tumour, and could facilitate combination therapies to target distinct glioma subpopulations. Biopsies taken immediately after therapy could provide an view into mechanisms of tumour resistance and recurrence, from which new therapeutic approaches could be designed.

Additionally, because the blood–brain barrier is bypassed by our system, new classes of drugs and targeted compounds could potentially be used, including high-molecular-weight compounds, proteins, viruses, liposomes, nanoparticles, and other biologicals that would not be feasible with systemic delivery because of toxic effects or metabolic breakdown. This is a useful and strategic approach to study the tissue-specific effects of novel therapies to the brain in a direct clinical setting with ever greater utility, not only for gliomas but other non-neoplastic CNS diseases.[16,29,30]

In conclusion, we present the results of a phase 1b clinical trial in which we show that chronic convection-enhanced delivery of topotecan is a safe and feasible therapeutic approach for patients with recurrent glioblastoma. Analysis of MRI-localised biopsies collected before and after treatment provided a tissue-based assessment of treatment response without the need for large numbers of patients. This novel drug delivery strategy and innovative clinical trial framework overcomes limitations in delivery and treatment response assessment in patients with glioma, and larger studies are warranted to determine the effect of this drug delivery approach on clinical outcomes.

Contributors

EFS, MGA, PSU, and MAB equally share first authorship. JNB, PC, RSD, and JG equally share senior authorship. EFS, MGA, PSU, MAB, JNB, PC, and JG wrote the original manuscript. JNB and PC originally conceived and designed the study and have independently accessed and verified all data presented. EFS, MAB, JAN, DMOH, RSD, and JNB were involved in intraoperative tissue acquisition. EFS, MAB, JAN, DMOH, RSD, JNB,

FMI, ABL, AL, MB, LG, and MRW were involved in patient care. EFS, MGA, PSU, MAB, AVS, DMB, TM, JLF, TDS, PP, BP, AMi, and NH were involved in acquisition and processing of samples. EFS, PSU, BP, MGA, and DMB did the histological analysis. MGA, MAB, EFS, WZ, OA-D, PAS, and PC did the RNA sequencing analysis. SAS, MSR, VC, and NYRA did drug level analysis. PBW, JG, SJ, AMi, and AL analysed and interpreted radiographic data. All authors had access to all the data reported in the study, were involved in drafting the Article or revising it critically for intellectual content and approved the final version for publication. The corresponding author had full access to all of the data and the final responsibility to submit for publication. All authors had final responsibility for the decision to submit for publication.

Declaration of interests

JNB has a consulting agreement with Theracle and held the sponsor-investigator Investigational New Drug Application from the US Food and Drug Administration for this study. NYRA receives support from EMD Serono and Bruker Daltonics. FMI has obtained grants or contracts through Columbia from Merck, Bristol Myers Squibb, Roche, Sapience, Novocure, Celldex, Tocagen, Forma, Celldex, and Northwest Biotherapeutics; is in consulting agreements with Novocure, Regeneron, Tocagen, Alexion Pharmaceuticals, Abbvie, Guidepoint Global, Merck, Kiyatec, PPD, Massive Bio, Medtronic, MimiVax, Gennao Bio, and Xcures; has two US provisional patent applications (62/739,617 and 63/062,805) through Columbia University; received support for meetings and travel from Roche and Oncoceutics; and participates on advisory boards of Mimivax and Northwest Biotherapeutics. AMi is in consulting agreements and on the advisory board of Regeneron. PAS receives consulting fees from Wilson Sonsini and EpiCypher, received payment from AstraZeneca for an honorarium for a seminar, and royalties from Guardant Health through Harvard University. SZ is the paediatric oncology lead at Bristol Myers Squibb. ABL receives consulting fees or personal financial support for honoraria or meetings from Affinia, Bioclinica, Elsevier, Fondazion AIRC, National Cancer Institute, Novocure, Sapience, Leal, Abbott, AbbVie, Clinical Education Alliance, MJH Healthcare, Novartis, Northwest Biotherapeutics, Oligonation, Pfizer, Radiation Therapy Oncology Group Foundation, American Society of Clinical Oncology, Bayer, US Food and Drug Administration, Forma, Karyopharm, QED, Global Coalition for Adaptive Research, Matheson Foundation, NHS Blood and Transplant, SNO, and VBI Vaccines, and is on advisory boards of Abbvie, Bayer, Chimerix, Forma, Karyopharm, Novocure, Orbus, QED, and Vivacitas. No authors are employees of WHO, International Agency for Research on Cancer, or Pan American Health Organization. All other authors declare no competing interests.

Data sharing

All individual participant data that underlie the results reported in this Article, after de-identification (text, tables, figures, and appendices) will be available immediately after publication (no end date) upon reasonable request to the corresponding author.

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