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Phase 1 Safety, Pharmacokinetics, and Fluorescence Imaging Study of Tozuleristide (BLZ-100) in Adults With Newly Diagnosed or Recurrent Gliomas

BACKGROUND: Fluorescence-guided surgery (FGS) can improve extent of resection in gliomas. Tozuleristide (BLZ-100), a near-infrared imaging agent composed of the peptide chlorotoxin and a near-infrared fluorophore indocyanine green, is a candidate molecule for FGS of glioma and other tumor types.

OBJECTIVE: To perform a phase 1 dose-escalation study to characterize the safety, pharmacokinetics, and fluorescence imaging of tozuleristide in adults with suspected glioma.

METHODS: Patients received a single intravenous dose of tozuleristide 3 to 29 h before surgery. Fluorescence images of tumor and cavity in Situ before and after resection and of excised tissue ex Vivo were acquired, along with safety and pharmacokinetic measures.

RESULTS: A total of 17 subjects received doses between 3 and 30 mg. No dose-limiting toxicity was observed, and no reported adverse events were considered related to tozuleristide. At doses of 9 mg and above, the terminal serum half-life for tozuleristide was approximately 30 min. Fluorescence signal was detected in both high- and low-grade glial tumors, with high-grade tumors generally showing greater fluorescence intensity compared to lower grade tumors. In high-grade tumors, signal intensity increased with increased dose levels of tozuleristide, regardless of the time of dosing relative to surgery.

CONCLUSION: These results support the safety of tozuleristide at doses up to 30 mg and suggest that tozuleristide imaging may be useful for FGS of gliomas.

KEY WORDS: Brain neoplasms, Glioma, Craniotomy, Cystine-knot miniproteins, Fluorescent dyes

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For patients with malignant brain tumors, surgery is a primary treatment modality with maximal safe tumor resection, providing the greatest chance of long-term and progression-free survival.[1-3] Aggressive tumor resection must be balanced with preservation of normal brain structures to avoid neurological impairment. However, it is often difficult for surgeons to distinguish tumor from normal brain tissue, particularly at tumor margins, because of the infiltrative nature of the tumors and their similar appearance.[4] Development of intraoperative imaging technologies that can aid surgeons in identifying tumor margins in real time should lead to completer and more precise resection. This remains a significant unmet clinical need.

Current imaging technologies such as magnetic resonance imaging (MRI) and computed tomography are important for surgical planning and registration of frameless stereotactic surgical navigation systems.[5] The accuracy of these navigation methods is reduced by tissues shifting during surgery,[6] making them less reliable as resection progresses. Although MRI and ultrasound can be used intraoperatively, issues such as poor resolution, prolonged operative times, high cost, and disruption of surgical workflow have limited their use.[5]

ABBREVIATIONS: AE, adverse event; **CTX,** chlorotoxin; **CTCAE,** Common Terminology Criteria for Adverse Events; **DLT,** dose limiting toxicity; **EPR,** enhanced permeability and retention; **FGS,** fluorescence-guided surgery; **ICG,** indocyanine green; **MRI,** magnetic resonance imaging; **MTD,** maximum tolerated dose; **NCI,** National Cancer Institute; **NIR,** near-infrared; **SAE,** serious adverse event; **SIRIS,** Synchronized Infrared Imaging System; **TEAE,** treatment-emergent adverse event; **WHO,** World Health Organization

Supplemental digital content is available for this article at www.neurosurgery-online.com.

Fluorescence-guided surgery (FGS) is an emerging technology that pairs fluorescent contrast agents with detection devices.[7,8] Tozuleristide is a peptide/fluorophore conjugate in development for FGS. It selectively binds to neoplastic tissue and fluoresces in the near-infrared (NIR) range. The peptide component is a 36 amino acid synthetic peptide derived from chlorotoxin (CTX).[9] CTX binds to many different types of human tumor in Vitro, including high- and low-grade glial tumors such as glioblastoma, astrocytoma, oligodendroglioma, and ependymoma.[9,10] A radiolabeled conjugate of CTX tested in phase 1 and 2 therapeutic clinical trials selectively bound to solid tumors, including glioma.[11-13] Fluorescent CTX conjugates have demonstrated tumor-selective uptake in mouse models of brain cancer and other cancers.[14-16] The fluorophore component of tozuleristide is an indocyanine green (ICG). It emits NIR light, which is minimally absorbed by water or hemoglobin, making it well suited for intraoperative imaging.[17] ICG is frequently employed in vascular and ophthalmic surgeries and sentinel lymph node mapping.[18]

Tozuleristide was well tolerated in preclinical toxicology studies.[19] In preclinical imaging studies, it bounds selectively to human and animal tumors with good tumor-to-background signal, including rodent head and neck carcinoma,[20] naturally occurring tumors in dogs,[21] and a mouse model of glioblastoma.[22] Tozuleristide may improve extent of resection in a variety of tumors by enabling surgeons to visualize tumor margins and foci of cancer cells intraoperatively.

Here, we describe a phase 1 dose-escalation study evaluating the safety, tolerability, pharmacokinetics, and preliminary fluorescence imaging of tozuleristide in adult subjects with gliomas.

METHODS

Subject Eligibility

Subjects were recruited at 2 clinical sites. Eligible subjects were 18 to 75 yr of age and had grade 1 to 4 glioma, for which surgical resection was indicated. Subjects with recurrent disease were eligible if the previous brain surgery was ≥3 mo prior to study surgery. Complete eligibility criteria are listed in **Text**, **Supplemental Digital Content 1**.

Study Design and Safety Oversight

The study was conducted in full conformance with the protocol, the Declaration of Helsinki prior to 2000 and its amendments, the national drug and data protection laws of Australia and the United States, and the principles of Good Clinical Practice as outlined in regulation 12AB(2)(a) of the Australian Therapeutic Goods Regulations and the National Statement on Ethical Conduct in Research Involving Humans and International Conference on Harmonisation guidelines. Written informed consent was obtained from each subject prior to participation.

The primary objective of this nonrandomized, single-dose, open-label, dose-escalation study was to evaluate the safety and tolerability of tozuleristide in adult subjects with glioma undergoing surgery. A 3 + 3 dose-escalation design was used to identify a maximum tolerated dose (MTD) or until the highest prespecified dose level was tested. Secondary objectives were an evaluation of pharmacokinetics parameters, quantitation of fluorescence in excised tumors, and determination of a dose for phase 2 studies in subjects with brain tumors. Exploratory objectives included evaluation of fluorescence in Situ.

Tozuleristide was administered as a slow intravenous bolus injection over 1 to 5 min, 3 to 29 h before surgery. Each vial contained 5 mg of sterile liquid drug product at 2 mg/mL in 10 mM Tris, 5% mannitol, pH 6.8. Dosing was to be stopped or modified if suspected adverse drug reactions posed a significant health risk. Supportive care was allowed as deemed appropriate by the investigator. Safety monitoring and PK sample collection took place during the 2 h after injection before proceeding with surgery. Thus, 3 h represented the realistically earliest time point for intraoperative imaging. The protocol allowed the flexibility for surgery to take place the day following tozuleristide administration based on preclinical data showing durable fluorescence in tumors.[21]

Safety Evaluation

Subjects were monitored for at least 7 and 30 d after surgery for primary safety monitoring and serious adverse event (SAE) reporting, respectively. Laboratory monitoring was conducted at screening, presurgery, 24 h postsurgery, and day 7 postdose. All clinically significant abnormal measurements of vital signs and laboratory parameters were reported as adverse events (AEs). A dose limiting toxicity (DLT) was defined as any study product-related (as determined by the investigator) AE of greater or equal to grade 3 according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 occurring through day 7 postdosing despite optimal supportive care. A Protocol Steering Committee reviewed safety data for each cohort before escalation.

Pharmacokinetics

Serum samples for PK analyses were collected before dosing at 1, 5, 15, 30, 60, and 120 min postdose, presurgery, and 24 h after surgery. Samples were analyzed with a validated LC-MS/MS method (TetraQ, Brisbane, Australia). Lower limit of quantitation was 10 ng/mL. Tozuleristide serum concentration vs time profiles were evaluated by noncompartmental analysis using WinNonlin Phoenix version 6.3 software using actual sample collection times.

Fluorescence Imaging

Fluorescence signal in tumor and nontumoral tissues was measured in Situ (surgical cavity) and ex Vivo (tissue specimens removed at the time of surgery). The FLUOBEAM® 800[23] (Fluoptics, Grenoble, France) and/or the Synchronized Infrared Imaging System (SIRIS)[22,24] (Teal Light Surgical, Inc, Seattle, Washington) was used for intraoperative imaging. Images were acquired according to manufacturers' instructions. Ambient lighting was subdued by turning off overhead lights and redirecting surgical lighting. Windows, when present, were covered to the extent feasible. In Situ imaging was not allowed to be used to guide surgical decisions. (See **Text**, **Supplemental Digital Content 1** for detailed description of imaging systems, imaging rationale, and data acquisition.)

Surgical Technique

Patients underwent standard craniotomy based on clinical criteria and surgeon-preferred method. With the tumor exposed, the surgeon held the imaging device directly over the tumor, and images were acquired. This required moving the surgical microscope out of the field to bring the imaging system over the patient. Once images were acquired, the

TABLE 1. Demographics and Disease Characteristics

	Cohort 1 3 mg tozuleristide	Cohort 2 9 mg tozuleristide	Cohort 3 18 mg tozuleristide	Cohort 4 24 mg tozuleristide	Cohort 5 30 mg tozuleristide	All subjects
All subjects	3	4	4	3	3	17
Mean age (yr)[a,b]	58.0 ± 16.4	48.5 ± 6.6	49.3 ± 12.1	39.3 ± 9.5	38.3 ± 9.5	46.9 ± 11.9
Gender, *n* (%)						
Male	3 (100.0%)	3 (75.0%)	3 (75.0%)	2 (66.7%)	2 (66.7%)	13 (76.5%)
Female	0 (.0%)	1 (25.0%)	1 (25.0%)	1 (33.3%)	1 (33.3%)	4 (23.5%)
Mean time since suspected cancer diagnosis (d)[b,c]	11.3 ± 10.4	48.0 ± 61.6	6.3 ± 5.1	7.7 ± 6.5	60.0 ± 22.6	24.6 ± 36.2
Recurrent disease at screening, *n* (%)						
Yes	2 (66.7%)	3 (75.0%)	2 (50.0%)	1 (33.3%)	1 (33.3%)	9 (52.9%)
No	1 (33.3%)	1 (25.0%)	2 (50.0%)	2 (66.7%)	2 (66.7%)	8 (47.1%)
Mean time since last brain surgery[b,d] (mo)	18.00 (5.66)	37.33 (28.02)	58.00 (14.14)	43.00 (-)	27.00 (-)	37.11 (21.04)
Karnofsky performance status score, *n* (%)						
100	1 (33.3%)	1 (25.0%)	2 (50.0%)	2 (66.7%)	1 (33.3%)	7 (41.2%)
90	1 (33.3%)	2 (50.0%)	1 (25.0%)	1 (33.3%)	2 (66.7%)	7 (41.2%)
80	1 (33.3%)	1 (25.0%)	1 (25.0%)	0 (.0%)	0 (.0%)	3 (17.6%)
Tumor type, *n* (%)						
Grade 4 glioma[e]	2 (66.7%)	1 (25.0%)	2 (50.0%)	1 (33.3%)	0 (.0%)	6 (35.3%)
Grade 3 astrocytoma[f]	1 (33.3%)	1 (25.0%)	0 (.0%)	0 (.0%)	0 (.0%)	2 (11.8%)
Grade 1 pilocytic astrocytoma	0 (.0%)	0 (.0%)	0 (.0%)	1 (33.3%)	0 (.0%)	1 (5.9%)
Grade 2/3 oligodendroglioma[f]	0 (.0%)	2 (50.0%)	2 (50.0%)	1 (33.3%)	2 (66.7%)	7 (41.2%)
Grade 2 oligoastrocytoma	0 (.0%)	0 (.0%)	0 (.0%)	0 (.0%)	1 (33.3%)	1 (5.9%)

[a] Age (yr) = integer value ((date of informed consent – date of birth + 1)/365.25).
[b] Mean values are expressed ± SD.
[c] Data available for 2 of 3 subjects in the 30 mg cohort and 16 of 17 subjects overall.
[d] Percentages are based on the number of subjects with recurrent disease at screening.
[e] Includes glioblastoma and gliomatosis cerebri.
[f] Includes cases described as anaplastic.

surgeon biopsied an area-representing tumor based on visual impression. The surgeon then proceeded with resection based on standard clinical methods. At any point, the surgeon could choose to reimage the surgical cavity with the NIR imaging device and/or biopsy areas of suspected tumor. At completion of resection, the surgeon imaged the tumor cavity. Any tissue with residual fluorescence could be biopsied at the surgeon's discretion. However, the surgeon could not use the presence or absence of fluorescence to guide surgical decision making or extent of resection.

Image Analysis

Three independent reviewers scored images for each case for in Situ contrast and overall signal ex Vivo. In Situ contrast was scored as none, weak (some contrast apparent, but not well defined), or strong (well-defined contrast that was visually obvious). Ex Vivo signal was scored as none, weak (exposure > 33 ms), or strong (exposure < 33 ms). The 33-ms cutoff was selected to accommodate video frame rates of 30 frames per second.

Tumor tissue samples were either fixed in formalin for standard of care clinical pathology or frozen for quantitative fluorescence imaging. Tissues to be frozen were trimmed, embedded in O.C.T. Compound (Tissue-Tek), frozen, and stored at –70°C. Cryosectioning and analysis were performed at PhenoPath (Seattle, Washington). Sections were imaged using the Odyssey CLx NIR scanner (LI-COR Biosciences, Lincoln, Nebraska). Serial sections were stained with hematoxylin and eosin (H&E), and areas of tumor, necrosis, infiltration, nontumor abnormal, or normal tissue were marked by a clinical pathologist. Fluorescence intensity within each region was analyzed using ImageJ v1.48 with Bio_Format plugin (NIH).

Statistical Considerations

There was no formal hypothesis testing planned. All analyses were descriptive and conducted by the authors.

Treatment-emergent adverse events (TEAEs) were summarized by the Medical Dictionary for Regulatory Activities-coded preferred term and system organ class for each dose cohort and graded for severity using CTCAE Version 4.0 (NCI). Laboratory parameters, ECGs, and vital signs were summarized with descriptive statistics of the change from baseline value by time point.

Assuming true incidence rates of DLT of 10% or 50%, the probability of escalation to the next dose cohort was 91% or 17%, respectively.

RESULTS

Subject Characteristics and Safety Summary

Seventeen subjects (Table 1) were accrued and completed the study from November 2014 to January 2016. Tozuleristide was well tolerated. No DLTs were reported, and the MTD was not

TABLE 2. Tozuleristide Pharmacokinetic Parameters

Parameter, units	Cohort 1 3 mg ($n = 3$)			Cohort 2 9 mg ($n = 4$)			Cohort 3 18 mg ($n = 4$)			Cohort 4 24 mg ($n = 3$)			Cohort 5 30 mg ($n = 3$)		
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
T_{max} (h)[a]	3	0.083	0.029-0.133	4	0.117	0.050-0.150	4	0.092	0.083-0.117	3	0.100	0.100-0.300	3	0.083	0.050-0.100
C_{max} (ng/mL)	3	350	115	4	2260	321	4	3980	212	3	5130	1310	3	7120	1310
$AUC_{0\text{-}t}$, (h × ng/mL)	3	134	36.9	4	1150	125	4	2370	363	3	4320	1320	3	4630	789
$AUC_{0\text{-}inf}$, (h × ng/mL)	3	139	36.2	4	1170	127	3	2600	255	3	4690	1450	3	4960	835
$t_{1/2}$ (h)	3	0.242	0.0466	4	0.326	0.0131	3	0.392	0.0838	3	0.521	0.0764	3	0.485	0.0532
CL (mL/h)	3	22 800	6480	4	7790	876	3	6980	726	3	5420	1460	3	6160	949
Vss (mL)	3	5870	2460	4	3390	223	3	3570	385	3	3940	889	3	3880	850

[a]Median values and ranges are shown for T_{max}.

$AUC_{0\text{-}inf}$ = area under the concentration vs time curve from time 0 extrapolated to time infinity; $AUC_{0\text{-}t}$ = area under the serum concentration vs time curve from time 0 to the timepoint with the last measurable concentration; CL = clearance; C_{max} = maximum observed serum concentration; SD = standard deviation; $t_{1/2}$ = half-life; T_{max} = time of maximum observed serum concentration; Vss = steady-state volume of distribution.

identified. Dose escalation continued to the highest prespecified dose level (30 mg). Nine of seventeen subjects reported TEAEs, none of which were considered related to tozuleristide. No laboratory abnormalities or vital sign changes were considered related to tozuleristide.

Three SAEs were reported for three subjects. One subject in the 3-mg cohort and 1 subject in the 18-mg cohort each experienced postprocedural infection and a different subject in the 18-mg cohort experienced pulmonary embolism. Both infections occurred in patients undergoing reoperation for high-grade glioma. Each event was grade 3 in severity. None of the SAEs were considered by the investigator to be related to treatment with tozuleristide.

Pharmacokinetics

Mean serum concentrations were measurable out to 1 h postdose at the 3-mg dose level, and out to 2 h postdose at the higher doses. Exposure increased in a greater than dose-proportional manner between the 3 and 9 mg dose levels (Table 2). Exposure based on mean C_{max} appeared dose proportional for 9 to 30 mg doses. Mean AUC values increased in a slightly higher than dose-proportional manner for the 9 to 24 mg dose levels, but not for the 30-mg dose level.

The $t_{1/2}$ increased with dose across the 3 to 24 mg dose levels, but not for the 30 mg dose level. The changes in $t_{1/2}$ appeared to be due to a decrease in clearance with increasing dose. These data indicate that tozuleristide is rapidly cleared from blood by 1 to 2 h after injection, 30-mg doses do not appear to result in substantially higher exposure than 24-mg doses, and doses between 9 and 24 mg provide increasing amount of circulating tozuleristide available for uptake in tumor.

Imaging

Intraoperative imaging was conducted as an exploratory objective. Two intraoperative imaging devices were used, the FLUOBEAM® 800 and the SIRIS. In Situ imaging was done with FLUOBEAM in 9 cases, SIRIS in 6 cases, and with both instruments in 2 cases (Table 3).

In Situ images of the tumor and/or tumor bed were collected during pauses in surgery to avoid interference between the imaging equipment and the surgical microscope. The degree to which tumors were exposed for these images varied and depended on surgical approach and the point during the procedure the surgeon could pause for imaging.

Fresh surgical specimens and biopsied areas of interest were also imaged ex Vivo, with gross tumor fully exposed, thus providing a better estimation of the fluorescence. Sample in Situ and ex Vivo images are shown in Figure 1. Imaging results for all subjects are summarized in Table 3. See **Text**, **Supplemental Digital Content 1** for further discussion of intraoperative imaging.

Overall, 12 of 17 tumors demonstrated positive fluorescence on ex Vivo imaging. At doses of 9 mg or higher, 6 of 6 high-grade tumors showed positive fluorescence ex Vivo. Importantly, at these doses, 4 of 8 (50%) of low-grade tumors also showed positive fluorescence ex Vivo (Table 3).

Because SIRIS imaging is semiquantitative and subject to confounding factors, such as variation in ambient light, tissue thickness, and orientation, the Odyssey NIR scanner was used to quantify fluorescence intensities in frozen tissues (a secondary endpoint). The Odyssey is a flat-bed scanner, which controls for variables such as camera distance and ambient light. Tissues were sectioned to control for tissue thickness.

Odyssey fluorescence data correlated with ex Vivo imaging results obtained with freshly excised tissues and with tumor histopathology (Figure 2). Data from representative excised tumor samples (Table 3) were analyzed with respect to variables such as dose, time between dose and surgery, and tumor type. The high-grade glioma cases showed a clear positive association between tozuleristide dose and fluorescence intensity. Fluorescence in low-grade tumors was less consistent, and a relationship

TABLE 3. Summary of Imaging Results

Subject ID	Diagnosis	WHO grade	Dose (mg)	Time dose to surgery (h)	In Situ device(s)	In Situ score	Odyssey score[a]	Ex Vivo device(s)	Ex Vivo score
T101	Astrocytoma	3	3	29	FB	Negative	409	FB	Negative
T102	Glioblastoma	4	3	21	FB	Negative	1890	FB/SIRIS	Weak
T103	Glioblastoma	4	3	26	FB	Negative	556	FB/SIRIS	Weak
T201	Oligodendroglioma	2	9	27	FB	Weak	1336	FB/SIRIS	Weak
T202	Anaplastic astrocytoma	3	9	22	FB	Weak	6204	FB/SIRIS	Weak
T203	Glioblastoma	4	9	23	FB	Negative	4560	FB/SIRIS	Weak
T204	Oligodendroglioma	2	9	28	FB	Negative	389	FB	Negative
T301	Oligodendroglioma	2	18	21	FB/SIRIS	Negative	191	SIRIS	Negative
T302	Glioblastoma	4	18	21	FB/SIRIS	Weak	32 141	SIRIS	Strong
T303	Glioblastoma	4	18	4	SIRIS	Strong	5729	SIRIS	Strong
T304	Oligodendroglioma	2	18	16	FB	Weak	17 033	FB/SIRIS	Strong
T401	Anaplastic oligodendroglioma	3	24	5	FB	Negative	1821	FB	Weak
T402	Gliomatosis cerebri	4	24	7	SIRIS	Strong	36 109	SIRIS	Strong
T403	Pilocytic astrocytoma	1	24	3	SIRIS	Negative	4543	SIRIS	Strong
T501	Oligodendroglioma	2	30	4	SIRIS	Negative	644	SIRIS	Weak
T502	Oligodendroglioma	2	30	17	SIRIS	Negative	480	SIRIS	Negative
T503	Oligoastrocytoma	2	30	18	SIRIS	Negative	971	SIRIS	Negative

[a]Representative tumor section Odyssey score (MFI/mm^2).
FB = FLUOBEAM® 800; NA = not applicable; ND = not done; SIRIS = Synchronized Infrared Imaging System.

between dose and signal was not apparent; however, positive imaging results were obtained in some of these cases, and, in one case (T304), were similar to the results seen in glioblastoma within the same cohort.

Odyssey scan data were available for sections scored as normal brain parenchyma from 4 cases (T304, T401, T402, and T403), and fluorescence intensities of 491, 331, 892, and 401 MFI/mm^2 were recorded (mean 529 ± 251 MFI/mm^2). Tumor sections considered positive for fluorescence at these dose levels ranged from 1821 to 36 109 MFI/mm^2 (3.44-68.3 times the mean normal brain MFI/mm^2).

DISCUSSION

This study showed that tozuleristide can be safely administered to subjects undergoing surgical resection of gliomas. There were no DLTs in this study, and none of the TEAEs were considered related to tozuleristide. Two unrelated postprocedural infections were noted; however, both infections occurred in patients with recurrent tumors undergoing repeat craniotomies, which have a substantially higher infection rate.[25] Furthermore, imaging was conducted with devices not optimized for neurosurgery, which required interruptions in surgical flow, which likely increased the risk of infection. Future studies will use an optimized imaging system that is integrated into the surgical microscope, which will preserve surgical flow. These studies will better assess if tozuleristide use increases infection risk. Strong fluorescence signal in tumors was achieved starting at the 18-mg dose level. Quantitative fluorescence intensity data show that increasing dose positively influenced fluorescence intensity for high-grade gliomas; however, variation in the time after administration when imaging occurred did not. These findings are consistent with other studies using tozuleristide[21,26] and support the view that imaging can be reliably carried out the day of or the day following injection. Administering tozuleristide a few hours before the start of surgery allows for sufficient tumor uptake and clearance of unbound drug from the blood for optimal imaging, consistent with the observed half-life in blood of ~30 min.

The entry criteria allowed for a relatively broad range of primary brain tumors. There were fluorescence-positive examples of World Health Organization (WHO) grade 1 to 4 tumors in this study, suggesting tumor grade per se does not influence uptake of tozuleristide. A greater proportion of the grade 3 and 4 tumors were positive for fluorescence and had some of the highest intensities; yet, 2 of the low-grade cases treated at 18 or 24 mg were strongly fluorescent ex Vivo. A few of the negative low-grade cases occurred at the 30-mg dose level, suggesting that the lack of tumor fluorescence is not due to inadequate exposure to tozuleristide, but more likely to lack of uptake by the tumor. This could be due to low expression of cell-surface tozuleristide receptors or other biological features. This study was conducted before the 2016 WHO guidelines were issued, and the complete set of recommended molecular pathology tests for IDH and 1p/19q testing are not available for these cases. A trial using an optimal tozuleristide dose and including detailed molecular analysis could address which tumor types demonstrate consistent fluorescence. The cell-surface target of CTX has been debated in the literature, with chloride channels,[27] matrix metalloproteinase 2,[28]

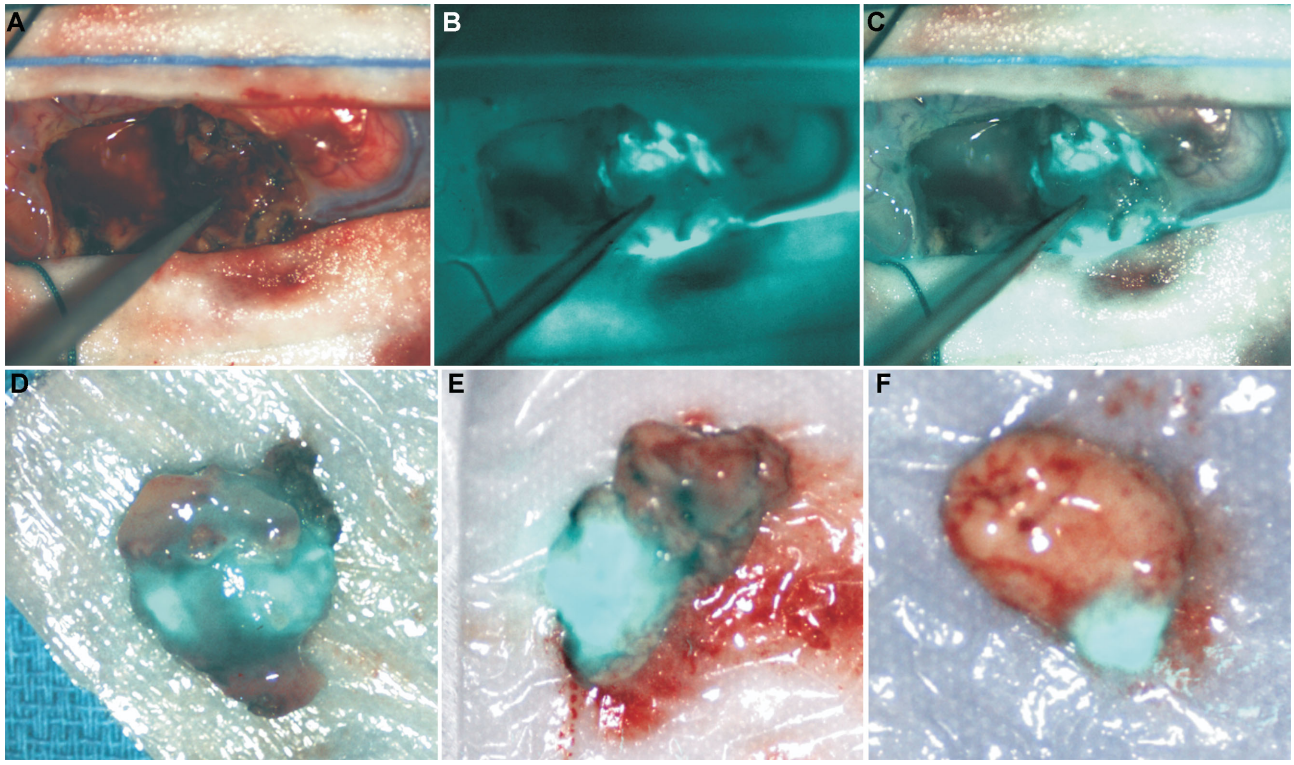

FIGURE 1. *In Situ and ex Vivo imaging. Examples of in Situ SIRIS images from subject T303 (glioblastoma) are shown as visible only* **A**, *NIR only* **B**, *and merge* **C**. *In Situ imaging was performed partway through tumor resection. Forceps indicate the area of exposed tumor. Fluorescence is visible within the area of tumor (135-ms NIR exposure). Ex Vivo SIRIS images are shown as visible NIR merge only for subjects T303* **D**, *T403* **E**, *and T402* **F**. *One hundred thirty-five millisecond NIR exposures. Images have been brightened and sharpened.*

and annexin A2 (ANXA2)[29] being the best supported proposed targets. Given the clear demarcation of positive and negative cases in this study, future trials also could explore cell-surface targets and/or markers of tozuleristide uptake.

Normal brain parenchyma was generally not fluorescent, which contributed to good fluorescence contrast between normal and tumor tissue. However, because of limitations of the imaging devices used in this study, it was not possible to precisely observe specimens near the tumor edge or border to ascertain how clearly tozuleristide fluorescence could demarcate between tumor and normal brain tissue.

Although the imaging results in this phase 1 study must be interpreted with caution, the overall pattern of tumor fluorescence ex Vivo was favorable. Perhaps most encouraging was the fluorescence in half of the nonenhancing low-grade gliomas. The ability to identify low-grade glioma and infiltrating margins of low-grade tumor remains one of the great promises for FGS. Neither fluorescein nor ICG demonstrates meaningful fluorescence for these tumors, and 5-ALA has also proven disappointing with less than 20% of low-grade tumors showing uptake. Considering that future trials of tozuleristide will include more uniform dosing regimens and incorporate imaging technology optimized for neurosurgery, greater detection of LGG seems realistic. Preliminary data from a trial of tozuleristide in pediatric brain tumors[26] support this conclusion.

Alternate Approaches for FGS

The promise of FGS to improve extent of resection and minimize injury to normal brain structures has been promoted for several decades. To date, clinical applications in neurosurgery have been limited to dyes such as fluorescein and ICG, which nonspecifically accumulate in high-grade tumors because of the enhanced permeability and retention (EPR) effect observed in many high-grade neoplasms.[30] Second-window ICG imaging,[31] in which fluorescence imaging is employed 24 h after ICG administration, takes advantage of EPR and washout of intravascular ICG from the circulation to improve contrast. Unfortunately, these methods have limited value as they do not differentiate tumor cells from normal and are ineffective in lower-grade tumors. The amino acid 5-ALA, which drives the conversion of protoporphyrin IX in mitochondria, has been demonstrated to improve extent of resection in high-grade gliomas.[32] It is

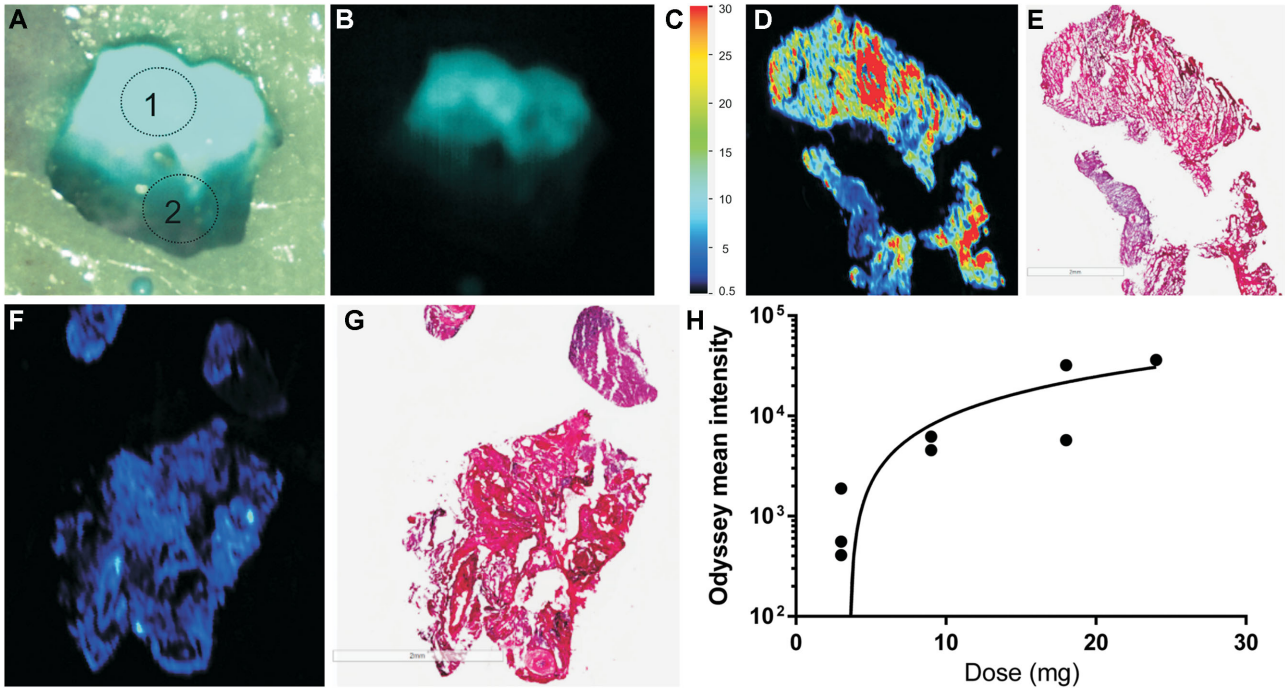

FIGURE 2. *Odyssey analysis of excised samples.* **A**, *Visible + NIR overlay and* **B**, *NIR (3-ms exposure) ex Vivo SIRIS images of tumor sample from subject T302. Overlay image is deliberately overexposed to demonstrate lack of signal from dim area. Samples of tissue from areas of bright (1) and dim (2) fluorescence were frozen for further analysis. Scale bar for the Odyssey heat map is shown in* **C**. *Cryosection analysis of samples from region 1 is shown in* **D** *and* **E**, *and from region 2 is shown in* **F** *and* **G**. *Odyssey scan* **D** *and* **F**, *and H&E stain* **E** *and* **G** *were performed on serial sections from each sample. Region 1 was viable tumor, and region 2 was necrosis (independent pathology assessment). Scale bar = 2 mm.* **H**, *Graph of Odyssey mean fluorescence intensity (MFI/mm^2) by dose for high-grade glioma samples.*

FDA approved for FGS, and is widely used in Europe. However, 5-ALA fluorescence is not observed in the majority of LGG. Autofluorescence in the blue light range interferes with 5-ALA imaging. Finally, 5-ALA may induce toxicities and photosensitivity days after administration, thus requiring special precautions for routine use.

Tozuleristide represents the first tumor-specific, NIR-targeting agent for fluorescence-guided neurosurgery that has advanced into human clinical trials. Its tumor cell selectivity is a distinct advantage compared with naked fluorescein or ICG. Its NIR fluorescence is an advantage compared with 5-ALA because of lower autofluorescence and better tissue penetration in this range.[33]

Limitations

This study has several limitations. There were multiple tumor types, multiple dose levels, and a range of times between dose and imaging. The imaging devices used to collect in Situ and immediate ex Vivo imaging data changed during the study and were not optimized for neurosurgical applications. Ambient light in the operating room differed between the sites, potentially affecting image acquisition and contrast. Variations in surgeon use of NIR imaging devices and number of biopsies acquired per case resulted in less than optimal comparisons between histology and fluorescence. All these limitations will be addressed in future studies, including a recently opened phase 2 study in pediatric CNS tumors (clinicaltrials.gov: NCT03579602).

CONCLUSION

This study showed that tozuleristide is well tolerated at doses up to 30 mg in patients undergoing neurosurgery. The preliminary imaging data show that contrast can be achieved within a few hours of dosing; however, larger clinical trials at a single-dose level are needed to better characterize true clinical utility. Coupled with an adequately sensitive NIR imaging device that can be continually used during neurosurgery, fluorescence guidance with tozuleristide has the potential to highlight residual cancer and increase the extent of resection while minimizing damage to normal brain. Further clinical trials in neurosurgery are justified for tozuleristide.

Disclosures

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Byrnes-Blake, and Mamelak are consultants for Blaze Bioscience, Inc. Drs Butte, Black, and Mamelak are shareholders of Blaze Bioscience, Inc. Teal Light Surgical, Inc is a wholly owned subsidiary of Blaze Bioscience, Inc.

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Supplemental digital content *is available for this article at http://www.neurosurgeryonline.com.*

Supplemental Digital Content 1. Text. Supplemental methods and discussion. This document contains complete eligibility criteria for the study and an expanded discussion of imaging device considerations and approach.

COMMENTS

Extent of resection is known to have impact on survival outcome in glioma surgery. Intraoperative imaging and neuronavigation are tools that are being used for this purpose. However, there are limitations associated with them, like prolonged operative times, high cost, and disruption of surgical workflow. Fluorescence-guided surgery (FGS) is a cheaper technique to improve extent of resection and is proven to be effective, at least for centers that intraoperative magnetic resonance imaging was deemed not to be cost effective. Current commercially available agents include indocyanine green (ICG) and 5-aminolevulinic acid (5-ALA).

This is a phase 1 study regarding a new, near-infrared agent (tozuleristide). This agent is a peptide that selectively binds to neoplastic tissue. A potential advantage of tozuleristide to ICG is its selectivity to tumor, and to 5-ALA is the near-infrared fluorescence that has better penetration to the tissue. The study design is a nonrandomized, single-dose, open-label, dose-escalation study to evaluate safety and tolerability of this agent. Patients were followed up to 1 mo for possible adverse events. Two types of fluorescence were measured: at surgical cavity (in Situ) and ex Vivo. Per study design, in Situ imaging was not allowed to be used as guide of surgery. However, they obtained multiple biopsies from surgical cavity based on fluorescence imaging. Seventeen

patients enrolled, and tozuleristide was well tolerated even in the highest prespecified dose. Three patients had an adverse event: 2 infections in recurrent high-grade glioma and one pulmonary embolism. Twelve patients had positive ex Vivo results, and in higher doses of the drug, all of high-grade glioma and half of low-grade glioma patients were positive in ex Vivo study.

Although this is a safety study and proved that tozuleristide is safe in all administered doses as well as had acceptable results in ex Vivo imaging, however, in Situ imaging didn't have promising results, which needs to be investigated and fixed for phase 2-3. Especially if this agent is going to be used for FGS like 5-ALA and ICG, its major use will be intraoperative and not ex Vivo. Overall, this is a strong first step and we will stay tuned for phase 2-3 results.

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In glioma surgery, maximal safe resection is well established to improve overall survival while preserving quality of life. Given the infiltrative nature of gliomas, tumor margin is often ill defined and difficult to discern with standard white light microscopy. A number of modalities have been developed to aid the surgeon in obtaining more complete resection, including stereotactic navigation, intraoperative imaging, FGS, etc. A number of fluorescent agents have also been developed in glioma surgery, with 5-ALA and fluorescein having the broadest clinical application to date.[1,2] All of them have a number of advantages and disadvantages. The limitations of the currently available fluorophores have led to additional fluorescent agents being sought for glioma excision.

This study is an initial phase 1 dose-escalation trial for the use of tozuleristide in human glioma surgery after previously demonstrating this compound has specificity for solid tumors, including gliomas in cited preclinical research. At first glance, tozuleristide has a number of advantages over 5-ALA, as mentioned by the authors. It is administered intravenously, operating can continue under the near infrared used to see the coupled ICG fluorophore, and it has a reasonable safety profile in this dose-escalation trial. Although it is unclear if tozuleristide will find useful application in glioma surgery, this is an important first step in its potential clinical translation. Additional phase 2-3 studies will be important to further understand risks and benefits of this novel agent and its potential application in neurosurgery.

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1. Stummer W, Pichlmeier U, Meinel T, et al. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: A randomized controlled multicenter phase III trial. *Lancet Oncol*. 2006;7(5):392-401.
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This phase 1 trial using tozuleristide for intraoperative fluorescence shows a good safety profile and supports the potential use of tozuleristide in surgical resection of primary brain tumors. This study used a heterogeneous group of gliomas, noting better fluorescence is seen ex Vivo compared to in Vivo. The authors have also observed that the system for visualizing fluorescence while operating has not been streamlined. Thus, the potential uses for tozuleristide as presented in this study for FGS is limited because of these factors. After the work flow has improved, further studies on both high-grade and low-grade gliomas will help determine the usefulness of this drug for FGS. With that accomplished, a meaningful comparison with currently available fluorescent agents, such as 5-ALA and fluorescein, can be undertaken.

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