Postmortem study of organ-specific toxicity in glioblastoma patients treated with a combination of temozolomide, irinotecan and bevacizumab

Guangrong Lu1 · Ping Zhu1,7 · Mayank Rao1 · Nadine Linendoll2 · L. Maximilian Buja3 · Meenakshi B. Bhattacharjee3 · Robert E. Brown3 · Leomar Y. Ballester1,3 · Xuejun Tian4,8 · Monika Pilichowska4 · Julian K. Wu5 · Georgene W. Hergenroeder1 · Williams F. Glass3 · Lei Chen3 · Rongzhen Zhang3 · Anil K. Pillai6 · Robert L. Hunter3 · Jay-Jiguang Zhu1

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Abstract

Purpose Systemic chemotherapy including monotherapy with temozolomide (TMZ) or bevacizumab (BEV); two-drug combinations, such as irinotecan (IRI) and BEV, TMZ and BEV and a three-drug combination with TMZ, IRI and BEV (TIB) have been used in treating patients with progressive high-grade gliomas including glioblastoma (GBM). Most patients tolerated these regimens well with known side effects of hypertension, proteinuria, and reversible clinical myelosuppression (CM). However, organ- or system- specific toxicities from chemotherapy agents have never been examined by postmortem study. This is the largest cohort used to address this issue in glioma patients.

Methods Postmortem tissues (from all major systems and organs) were prospectively collected and examined by standard institution autopsy and neuropathological procedures from 76 subjects, including gliomas (N = 68, 44/M, and 24/F) and brain metastases (N = 8, 5/M, and 3/F) between 2009 and 2019. Standard hematoxylin and eosin (H&E) were performed on all major organs including brain specimens. Electronic microscopic (EM) study was carried out on 14 selected subject’s kidney samples per standard EM protocol. Medical records were reviewed with adverse events (AEs) analyzed and graded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 4.03. A swimmer plot was utilized to visualize the timelines of patient history by treatment group. The binary logistic regression models were performed to explore any associations between treatment strategies and incident myelosuppression.

Results Twenty-four glioma subjects were treated with TIB [median: 5.5 (range: 1–25) cycles] at tumor recurrence. Exposure to IRI significantly increased the frequency of CM (p = 0.05). No unexpected adverse events clinically, or permanent end-organ damage during postmortem examination was identified in glioma subjects who had received standard or prolonged duration of BEV, TMZ or TIB regimen-based chemotherapies except rare events of bone marrow suppression. The most common causes of death (COD) were tumor progression (63.2%, N = 43) followed by aspiration pneumonia (48.5%, N = 33) in glioma subjects. No COD was attributed to acute toxicity from TIB. The study also demonstrated that postmortem kidney specimen is unsuitable for studying renal ultrastructural pathological changes due to autolysis.

Conclusion There is no organ or system toxicity by postmortem examinations among glioma subjects who received BEV, TMZ or TIB regimen-based chemotherapies regardless of durations except for occasional bone marrow suppression and reversible myelosuppression clinically. IRI, but not the extended use of TMZ, significantly increased CM in recurrent glioma patients. COD most commonly resulted from glioma tumor progression with infiltration to brain stem and aspiration pneumonia.

Keywords Autopsy · Postmortem · End-organ toxicity · Chemotherapy · Temozolomide · Irinotecan · Bevacizumab · Glioblastoma (GBM) · Glioma · Myelosuppression · Brain metastasis

Jay-Jiguang Zhu
jay.jiguang.zhu@uth.tmc.edu

Extended author information available on the last page of the article
Glioblastoma (GBM) is the most common primary malignant brain tumor with an extremely poor prognosis. The median overall survivals (mOS) ranges from 14.6 [1] to 23.1 months [2] based on phase III clinical trial results and 11 months in the real-world GBM population [3]. The disease usually recurs within 3.3–8.2 months while on treatments [4]. There is no uniform standard of care (SOC) for recurrent GBM (rGBM). For example, bevacizumab (BEV) is commonly used in the USA for rGBM, but it was not approved in the European Union [5]. Unfortunately, no new therapeutic drug was demonstrated improving the mOS of GBM patients in phase III clinical studies in the last decade compared to established therapies [6, 7].

Two-drug combinations, temozolomide (TMZ)/BEV, irinotecan (IRI)/BEV and TMZ/IRI, have been studied in adult rGBM. However, none of these combinations were further evaluated in phase III studies [5, 8, 9]. A phase III clinical trial using BEV with lomustine (CCNU) (N = 288) for rGBM failed to demonstrate an overall survival advantage over CCNU monotherapy (N = 149) [10]. In the pediatric oncology community, the TMZ/IRI combination is adopted as an effective “backbone” regimen [11]. Combination therapy with three drugs TMZ/BEV/IRI (TIB) was studied in clinical trials for treating pediatric diseases [12, 13] and gliomas: (1) a phase I study of unresectable GBM (N = 41); [14] (2) a phase II study of newly diagnosed GBM (N = 75); [15] and (3) a phase I study of high-grade glioma (N = 12) [16]. Our retrospective study demonstrated an improved mOS among rGBM patients receiving TIB plus Tumor Treating Fields (TTFields) therapy (N = 18) compared to those receiving non-TIB BEV-based chemotherapies with TTFields (N = 30) without unexpected side effects [17]. All these studies concluded that the TIB regimens were well-tolerated without unforeseen toxicities [12–18]. The safety profiles support the TIB regimen administration in rGBM patients with normal bone marrow function since rGBM patients have limited treatment options.

BEV is a recombinant humanized monoclonal antibody against vascular endothelial growth factor A. The most common and predictable side effects of its long-term use are hypertension (HTN) and proteinuria. It is postulated that kidney injury from long-term use of BEV is related to the development of HTN and proteinuria, but few renal biopsies in patients who developed BEV-related HTN and proteinuria have been carried out [19].

The standard duration of TMZ chemotherapy for GBM is 6 weeks of daily therapy with concurrent conformal external beam fractionated radiation followed by 6 cycles of adjuvant treatment per the Stupp protocol [1]. It is not uncommon for GBM patients to take up to 12 cycles of TMZ (prolonged duration) or even longer. However, there is concern that prolonged use of TMZ alone or with other chemotherapy drugs may produce more harm than benefit, including alkylating agent-related leukemia [20, 21].

At Tufts Medical Center and Memorial Hermann Hospital (MHH)-Texas Medical Center (TMC)/UTHHealth, the TIB regimen was offered to rGBM patients with normal bone marrow function. Treatment decisions were based upon the physician’s best knowledge and judgment plus tumor board recommendations while monitoring for side effects closely, balancing the needs of controlling the aggressive and resistant rGBM and minimizing side effects to patients. Clinical side effects from individual chemotherapy drug, including TMZ, BEV, IRI, and a combination of TMZ/IRI, have been well-documented by the Cancer Therapy Evaluation Program (CTEP). However, there are few reports on the side effects from long-term use of TIB, BEV/TMZ, or TMZ monotherapy beyond 12 months. Autopsy studies of 117 GBM [22] and 40 cases of brain stem gliomas [23] did not report chemotherapy-related end-organ toxicity. This study aimed to determine any significant end-organ or system damage from TIB therapy, prolonged use of TMZ monotherapy or TMZ based two-drug therapy. We also attempted to identify pathological changes of kidney post BEV therapy by standard postmortem examination plus electron microscopic (EM) study of selected kidney specimens prospectively.

From 2009 to 2019, 76 deceased subjects were examined in this study, including 24 patients treated with TIB and 14 subjects’ kidney specimens were evaluated by EM.

Subjects and methods

Regulatory approval and consent

The study protocol and consent received approvals from the Health Sciences Institutional Review Board at Tufts Medical Center and the Committee for the Protection of Human Subjects (CPHS) at Memorial Hermann Hospital (MHH) at Texas Medical Center (TMC) and UTHHealth (HSC-MS-11-0133), respectively. Consents for autopsy were obtained from patients antemortem or next of kin postmortem prior to autopsy.

Autopsy, organ sample collection, neuropathological and EM examinations

Twelve subjects from Tufts Medical Center and 64 subjects from the MHH-TMC/UTHHealth were in the study between 2009 and 2019 prospectively (see Supplemental Table 2 for detail of individual subject). Autopsies and neuropathological exam were done at subjects’ respective institution.
per the institution’s standard of practice (SOP) including unrestricted autopsies (N = 69), restricted autopsies to brain (N = 6), and to brain and kidney (N = 1). Eight brain metastasis (BM) subjects were included. Most of the subjects’ bodies were transported to hospital morgue within two to four hours. Bodies were stored at the morgue (+4 °C) until autopsy. Postmortem organ studies included gross organ examinations and microscopic tissue evaluation with hematoxylin and eosin (H&E) staining of all systems and organs except reproductive organs per SOP. Kidney tissues from 14 subjects, including ultrasound-guided biopsies of four subjects before the full autopsy, were analyzed by EM. The tissues destined for EM examination were collected in formaldehyde fixative solution and then prepared per EM examination SOP. Cadaver kidney biopsies were performed with ultrasound guidance by an interventional radiologist (AKP) prior to autopsy to shorten the delay of kidney sample collection. EM slides were reviewed by EM trained neuropathologists (WFG and MP). Three subjects who never received BEV (#31, #49, and #60) were included as controls. The brain was harvested at the time of autopsy and placed in the fixative solution until brain cutting which ranged from 3 to 6 weeks. Neuropathological exam was performed per SOP and included gross inspection and microscopic examination with H&E stain. Fresh samples from each major organ (lung, heart, kidney, spleen, liver, pancreas, small and large intestines, bone marrow, adrenal gland, thyroid, muscle and skin except reproductive organ) and selected brain tissues were also collected at the time of autopsy. The samples were placed in paraffin cassettes for microscopic examination per SOP. Duplicate specimens were snap-frozen in liquid nitrogen and stored at −80 °C for other use. Brain specimens from selected anatomic locations including brainstem were collected subsequently after brain fixation was complete during brain cutting sessions by a staff neuropathologist and then analyzed by gross and microscopic examinations per institutional SOP.

Medical records review and side effects grading

Medical records were reviewed, including operative reports, lab results, MRI reports, pathology and autopsy reports of body and brain, chemotherapy received and documented adverse events. Clinical notes were reviewed to extract age, gender, body weight, diagnosis, overall survival, and adverse events (AEs). The Common Terminology Criteria for Adverse Events (CTCAE), version 4.03, was used for AE grading.

Chemotherapy regimens

Each TIB cycle consists of one 5-day period of TMZ (150 mg/M², PO, QD during days 1–5 every 28 days), two BEV (10 mg/kg), and two IRI (125 mg/M²) infusions every 14 ± 2 days in a 28–32 days period with permission for a delayed start of next treatment of either TMZ or IRI or both based on lab results. No patient was on p450 enzyme-inducing anti-seizure medication. TIB was offered to rGBM patients for up to 12 cycles, depending on the patient’s tolerance or resume at subsequent GBM progressions. Four patients received more than 12 cycles either continuously or cumulatively including resuming TIB therapy after GBM further progressions. When TMZ was used for rGBM, but not as a component of the TIB regimen, it was first administered at 200 mg/m², PO, QD on days 1–5 of a 28-day cycle, and then the dose was reduced to 150 mg/m² if grade III or higher CM was observed. Detailed chemo/anti-angiogenesis therapy records for each patient are provided in Supplemental Table 1.

Molecular test of isocitrate dehydrogenase (IDH) in glioma subjects

For subjects who did not have IDH status determined at the time of pathological diagnosis either by immunohistochemistry (IHC) with IDH R132H antibody or by next generation sequencing (NGS) (FoundationOne, Foundation Medicine, Cambridge, MA), we did it retrospectively in subjects with available original tumor tissue by IDH R132H antibody IHC per SOP in UTHealth pathology lab which is a CLIA approved laboratory.

Glioma patients grouping based on therapies received

Glioma patients were separated into four groups: Group 1: no chemotherapy beyond concurrent chemoradiation (CCRT), or NCBC (N = 7), including three subjects never received chemotherapy; Group 2: received 1–5 cycles of TIB (N = 12); Group 3: received 6–25 cycles of TIB (N = 12); Group 4: SOC group (N = 37), including 8 patients on maintenance TMZ only while the other 29 patients received BEV alone or BEV/IRI doublet therapies, with 3 patients discontinued TMZ during CCRT. The total cycles of chemotherapies received in each group are summarized in Table 1 and details of drugs administered for individual subject are provided in Supplemental Table 2.

Clinical myelosuppression score (CMS) and subgrouping based on cycle number of chemotherapy received

Clinical myelosuppression (CM) is define as severe bone marrow suppression measured by complete blood count (CBC) as grade III or higher based on CTCAE, version 4.03. Clinical myelosuppression score (CMS), the sum
of the highest grade of AEs based on the worst results of white blood cell, hemoglobin or platelet, respectively, for each subject. Grade 1 and 2 AEs do not receive score while grade III, IV or V AE is assigned with 3, 4 or 5, respectively. For example, subject #36 (Supplemental Table 1) experienced grade IV leukocytopenia and thrombocytopenia, but with grade II anemia. So her CMS was $4 + 4 + 0 = 8$. CMS was analyzed based on the number of TMZ cycles received: reference group (1–6 cycles) and four subgroups (7–12, 13–18, 19–24, and 25–47 cycles). In addition, CMS was compared in patients who received

| Groups | BM (N = 8) | Recurrent gliomas, treatment based (N = 68) |
|--------|-----------|------------------------------------------|
|        | NCBC (Group 1) | 1–5 TIB (Group 2) | 6–25 TIB (Group 3) | SOC (Group 4) |
|        | (N = 7) | (N = 12) | (N = 12) | (N = 37) |
| Groups |          |          |          |          |
| Treatments |          |          |          |          |
| TMZ/BEV/IRI | NA | 0 | 12 | 12 | 0 |
| TMZ first, then BEV | NA | 0 | 18 |
| TMZ | 1¢ | 0 | 6 |
| BEV | NA | 0 | 1 |
| BEV/IRI | NA | 0 | 5 |
| NCBC | NA | 7 |
| SOC chemo for primary disease | 8 | NA | NA | NA |
| Median TMZ cycle, cycles (range) | NA | 12.5 (2–47) | 20 (7–40) | 3 (0–30) |
| Median BEV, infusions (range) | NA | 10 (2–13) | 30 (12–59) | 8 (0–49) |
| Median IRI, infusions (range) | NA | 4 (1–11) | 23 (12–52) | 0 (0–19) |
| Adverse Events |          |          |          |          |
| Body weight change (%) | $-3.9 \pm 7.7$ | $-2.0 \pm 7.7$ | $-8.6 \pm 4.1$ | $-3.3 \pm 5.1$ | $-19.6 \pm 2.4$ |
| Grade III weight loss N, (%) | 1 (12.5%) | 1 (8.3%) | 1 (8.3%) | 11 (29.7%) |
| Grade III weight gain N, (%) | 1 (12.5%) | 1 (14.3%) | 2 (16.7%) | 1 (2.7%) |
| Seizure, grade III | 1¢ (14.3%) | 1 (8.3%) | 4 (10.8%) |
| Grade III, proteinuria | 5 (41.7%) | 5 (41.7%) | 12 (37.5%) |
| Grade III, anemia | 1 (12.5%) | 1 (14.3%) | 1 (8.3%) | 4 (10.8%) |
| Grade III, thrombocytopenia | 1 (14.3%) | 2 (16.7%) | 2 (16.7%) | 2 (5.4%) |
| Grade III, neutropenia | 3 (25%) | 2 (5.4%) |
| Grade IV, hypertension | 1 (12.5%) | 1 (14.3%) | 5 (13.5%) |
| Grade IV, thrombocytopenia | 1 (12.5%) | 1 (14.3%) | 3 (8.1%) |
| Grade IV, neutropenia | 1 (12.5%) | 2 (16.7%) | 1 (8.3%) | 9 (24.3%) |
| Grade V, perforated diverticulitis | 1 (12.5%) | 1 (14.3%) | 1 (2.7%) |
| Grade III, kidney failure | 1 (14.3%) | 1 (2.7%) |
| Grade IV, kidney failure | 1 (14.3%) | 1 (2.7%) |
| Abnormal liver enzymes, grade IV | 1 (14.3%) | 1 (8.3%) | 1 (2.7%) |
| CK and LDH elevation, grade III | 1 (14.3%) | 1 (8.3%) | 1 (2.7%) |
| Abnormal electrolytes, grade III | 1 (14.3%) | 1 (8.3%) | 2 (5.4%) |
| Meningitis, grade III | 1 (14.3%) | 1 (8.3%) |
| PCP, grade IV | 1 (14.3%) | 1 (2.7%) |
| CMV pneumonitis, grade III | 1 (12.5%) | 1 (2.7%) |
| Shingles, grade III | 1 (14.3%) | 1 (2.7%) |
| Cellulitis, grade III | 1 (14.3%) | 1 (2.7%) |

*Pseudo-seizure, chorea with diagnosis of Huntington’s disease
¢Subject participated in clinical trial and the study drug is known to cause elevation of creatine kinase (CK) and lactate dehydrogenase (LDH)
£Patient #72 received low dose TMZ for 42 days consecutively.
PCP: Pneumocystis carinii pneumonia

Table 1: Summaries of chemotherapies received and adverse events (grade III and above)
TIB (cycles: 1–5, 6–25, and 1–25) vs. Group 4, which included various numbers of IRI infusions (0, 1–6, 7–52).

**Statistical analysis**

Univariable binary logistic regression models were performed to explore the associations between different treatment strategies and incident myelosuppression. Odds ratios (OR) and 95% confidence intervals (95% CIs) are presented. A swimmer plot was utilized to illustrate and compare the timelines of patient history by treatment group. Statistical analyses were performed using Stata IC 15.1 (StataCorp, College Station, TX). P values are two-sided and are considered statistically significant when \( P < 0.05 \).

**Results**

**Clinical and pathological characteristics of patients**

A total of 76 subjects (49 males, ages ranges 23–80 years-old at initial diagnosis) were enrolled prospectively with initial diagnoses as GBM (N = 53), grade III glioma (N = 8), brain metastasis (BM, N = 8), grade II glioma (N = 5), and spinal glioma (N = 2 (Supplemental Table 2). At autopsy, 4 grade II and 5 grade III gliomas had progressed to secondary GBMs (Table 2). Except 9 cases without IDH test result, 4 glioma subjects harbor IDH mutant (#20 and #62 based on NGS, #3 and #4 by IHC staining with IDH R132H antibody) and the rests of the 55 glioma patients were IDH wild type (Supplemental Table 2). The swimmer plot is presented in Fig. 1. The average interval from death to postmortem examination at MHH-TMC/UTHealth was 35.3 ± 3.7 h (range: 7–100 h, calculated from 38 cases with documentation of timing of death).

**Table 2** Summary of patient characteristics

| Characteristics                   | BM   | DMG | Grade II Glioma | Grade III Glioma | GBM  |
|-----------------------------------|------|-----|-----------------|-----------------|------|
| Number of patients at autopsy     | 8    | 3   | 1               | 2               | 62   |
| Number of patients at initial diagnosis | 8    | 2 (spine) | 5               | 8               | 53   |
| Age at diagnosis (median, range)  | 60 (39–75) | 28 (23–33) | 48 (31–56) | 38.5 (27–76) | 61 (25–80) |
| Gender (Male/Female)              | 5/3  | 0/2 | 4/1             | 5/3             | 35/18|
| Race:                             |      |     |                 |                 |      |
| Caucasian                         | 7    | 1   | 3               | 8               | 44   |
| African American                  | 0    | 0   | 0               | 0               | 4    |
| Latino                            | 1    | 0   | 1               | 0               | 0    |
| Asian                             | 0    | 1   | 0               | 0               | 2    |
| Unspecified                       | 0    | 0   | 1               | 0               | 3    |

*One anaplastic glioma (grade III) and 2 spine glioma cases were confirmed to be diffuse midline gliomas (DMG) at autopsy based on anatomic location and histone H3F3A K27M mutation.

**Nine cases were confirmed of progression to secondary GBMs at autopsy**
Gross and microscopic examinations of all organs except reproductive organ

Post-mortem external and internal as well as microscopic examinations per SOP at Tufts Medical Center and UTHealth/MHH-TMC were carried out by pathology residents and attendings at respective institutions including all systems (cardiovascular, respiratory, gastrointestinal, hepatobiliary, genitourinary, female/male genitalia, endocrine, reticuloendothelial system, bone, marrow and joints and central nervous system) and all major organs (lung, heart, kidney, spleen, liver, pancreas, small and large intestines, bone marrow, adrenal gland, thyroid, muscle and skin) except reproductive system and organ.

Besides the known antemortem myelosuppression as summarized using CMS (see later), there is no end-organ or system damage identified during postmortem examination except for bone marrow and kidney changes reported below.

Kidney ultrastructure examination by EM

EM studies were performed in 14 subjects to understand the ultrastructural pathological changes in those who had HTN and proteinuria while on prolonged BEV therapy except 3 of them never received BEV (serving as control). The interval from death to tissue collection was 19.8 ± 7.5 h from 9 glioma subjects at MHH-TMC/UTHealth while such information was not available from 5 subjects at Tufts Medical Center. To differentiate true pathological microscopic findings from possible artifacts from autolysis, interventional radiologist (AKP) biopsied cadaver kidneys under ultrasound guidance on four subjects #22 (3 h), #20 (6 h), #17 (7 h), and #49 (9 h). The EM results of podocyte effacement with basement membrane detachment were observed in subjects exposed to BEV and naïve to BEV (Supplemental Fig. 1), making the observation of podocyte effacement with basement membrane detachment in relation to BEV therapy inconclusive due to the inevitable presence of autolysis artifact.

Bone marrow examinations

Among 39 subjects who had bone marrow examination, 13 subjects showed evidence of bone marrow suppression with 4 subjects (Subjects # 9, 46, 65, 69) displayed mild hypocellular bone marrow and 9 cases with severe hypocellularity: 2 from Group 2 (Subjects # 17 and 23), 3 from Group 4 (Subjects # 29, 52 and 59), and 4 from the brain metastasis (BM) group (Subjects # 71, 73, 74 and 76). Three subjects with severe hypocellular bone marrow finding (Subjects # 17, 23, and 52) had matched antemortem CM history. On the contrary, subject #36’s (Group 4) bone marrow, who had a history of leukocytopenia and thrombocytopenia, was hypercellular. Subject #9 (Group 3) who was diagnosed of spine glioma, grade II, C7 through T4, and had received spinal radiation (50.4 Gy in 28 fractions) and had hypocellular bone marrow at autopsy (Fig. 2D) which is expected. Otherwise, there was no evidence of postmortem bone marrow abnormality among subjects in Groups 1 and 3.

Five subjects (#29, 32, 38, 62, and 63) discontinued TMZ due to severe CM during CCRT. But CM in glioma patients did not commonly develop permanent bone marrow damage as often comparing to subjects with brain metastasis treated with varies chemotherapy (Supplemental data for chemotherapy received). Patients received prolonged TMZ (Fig. 2C) demonstrated normal bone marrow findings. Most patients received TIB regimen therapies have normal bone marrow exam or mild bone marrow dysfunction on post-mortem exam.

Clinical myelosuppression rates on active therapies

Analysis showed that 41.7% (5/12), 16.7% (2/12), and 20% (10/37) of subjects experienced grade III or worse CM during active therapies in Groups 2, 3, and 4, respectively. Compared to Group 4, ORs were 1.93 (95% CI: 0.50–7.50, \( P = 0.343 \)) and 0.54 (95% CI: 0.10–2.91, \( P = 0.473 \)) for Groups 2 and 3, respectively (Table 3). The CM rates were 57.1% and 47.6% in IRI infusion subgroups who received 1–6 cycles (N = 14) and 1–12 cycles (N = 21), respectively. The ORs in these two groups were 1.71–34.33, \( P = 0.008 \)) and 5.23 (95% CI: 1.34–20.45, \( P = 0.018 \)) (Table 3). By contrast, the likelihoods of CM for patients treated with 7–12, 13–18, 19–24, and 25–47 cycles of TMZ were not significantly increased compared to those receiving 1–6 cycles (all \( P > 0.05 \)) (Table 3).

Other clinical adverse events

The three most common AEs in Group 4 were grade III HTN (37.5%), weight loss (29.7%), and venous thromboembolism (VTE, 24.3%). Grade III HTN (41.7%) was also the most common AE among patients who received TIB (Groups 2 and 3 combined). The grade III CM was 33.3% and 21.6% in TIB (Groups 2 and 3 combined) and Group 4, respectively. However, no grade IV thrombocytopenia was observed in the two TIB groups, while the rate was 13.5% in Group 4. All grade III or above AEs are listed in Table 1.

Cause of death (COD) for recurrent/progressive glioma patients including GBM patients

Among the 68 glioma subjects, disease progression was the most common COD (N = 43, 63.2%) while the second most common COD was aspiration pneumonia (N = 33, 48.5%).
Herniation with brain swelling was detected in 12 (17.6%) subjects, while cerebral or intraventricular hemorrhages were documented in 7 cases. Rare CODs include subject #67, who died at home from sudden death with autopsy findings of cardiomyopathy; subjects #26 and #76 expired from cerebral herniation soon after decompression surgeries. Three subjects (#52, 57, and 60) passed away in hospital due to severe adverse events while on active anti-cancer treatments.

Both treatment effects and active tumor cells were detected in majority of brain autopsy specimens. Excluding 22 cases without microscopic exam reports, 26 subjects (56.5% out of 46 subjects) had microscopic evidence of GBM infiltration including brain stem. In contrast, 7 subjects showed the presence of treatment effects without evidence of active tumor or with minimal active tumors in and around surgical cavities by gross and microscopic examinations of brain specimens (H&E stain only).

Fig. 2 Bone marrow pathological changes in different diseases and treatment groups. A Patient #72 in BM group passed away from a stage IV parotid gland carcinosarcoma. Tumor infiltrate (insert: H&E 200X) completely destroyed normal bone marrow cells (H&E 40X). B Patient #65 (Group 1: NCBC) did not receive chemotherapy. Examination of her bone marrow revealed as hypocellular for age (30–40%) with trilineage hematopoiesis. C Patient #61 (Group 4, SOC) was diagnosed of grade III glioma. He was treated with 9 cycles TMZ before disease progression to GBM. He received experimental Toca 511 virus injection into resection cavity wall and took 1 cycle of Toca FC before withdrew further treatments. His bone marrow showed as normocellular for age (40–50%, H&E 40X) with trilineage hematopoiesis. D Patient #9 (27 years old woman received spinal radiation for her spinal glioma, grade II from C7 to T4, Group 3, cycle 6–25 TIB) was treated with 7 TIB cycles, 21X BEV and 10X IRI infusions. Her bone marrow contained 40% cellularity as hypocellularity for her age (H&E 100X)
Discussion

Postmortem examination is the gold standard to identify end-organ toxicities from chemotherapies [24]. In this study, we documented clinical AEs and autopsy findings from 76 subjects prospectively collected, including 68 glioma patients with the majority of them as de novo GBM, IDH wild type (Tables 1 and 3 and Supplemental Table 2). Sequela from TIB chemotherapy-related end-organ toxicity including kidney injury from Bev-based therapy were investigated. There is no evidence of system or organ injury by TIB or SOC chemotherapy including prolonged use of TMZ except for infrequent damage to the bone marrow (Fig. 2).

Hypertension (41.7%) and combined CM (33.3%) were the two most common AEs among patients who received TIB treatment. In contrast, hypertension (32.1%), weight loss (29.7%), and VTE (24.3%) were the most common AEs among patients treated with SOC. We found that IRI, but not the extended TMZ administration, significantly

| Predictors | Myelosuppression rate |
|------------|-----------------------|
|            | Total # | Myelo-Suppression score (CMS) | OR | 95%CI | P |
| Standard care vs. TIB, N=61 | | | | | |
| Standard care | 37 | 10 | 1.00 | – | – |
| TIB | 24 | 7 | 1.11 | 0.36–3.48 | 0.856 |
| Standard care vs. TIB (cutoff: 6), N=61 | | | | | |
| Standard care | 37 | 10 | 1.00 | – | – |
| TIB: ≤ 6 | 12 | 5 | 1.93 | 0.50–7.50 | 0.343 |
| TIB: ≥ 6 | 12 | 2 | 0.54 | 0.10–2.91 | 0.473 |
| TMZ cycles: continuous, N=58 | | | | | |
| TMZ cycles | 58 | 15 | 0.97 | 0.91–1.03 | 0.312 |
| TMZ cycles: cutoff—6, 12, 18, 24; N=58 | | | | | |
| 1–6 | 28 | 8 | 1.00 | – | – |
| 7–12 | 10 | 4 | 1.67 | 0.37–7.53 | 0.507 |
| 13–18 | 8 | 1 | 0.36 | 0.04–3.39 | 0.370 |
| 19–24 | 4 | 1 | 0.83 | 0.08–9.25 | 0.882 |
| 25–47 | 8 | 1 | 0.36 | 0.04–3.39 | 0.370 |
| TMZ cycles: cutoff, 12; N=58 | | | | | |
| < 12 | 35 | 11 | 1.00 | – | – |
| ≥ 12 | 23 | 4 | 0.46 | 0.13–1.67 | 0.238 |
| TIB cycles: cutoff, 6; N=24 | | | | | |
| < 6 | 12 | 5 | 1.00 | – | – |
| ≥ 6 | 12 | 2 | 0.28 | 0.04–1.88 | 0.190 |
| IRI cycles: cutoff, 0; N=61 | | | | | |
| 0 | 27 | 4 | 1.00 | – | – |
| > 0 | 34 | 13 | 3.56 | 1.00–12.64 | 0.050 |
| IRI cycles: cutoff, 0, 6; N=61 | | | | | |
| 0 | 27 | 4 | 1.00 | – | – |
| ≤ 6 | 14 | 8 | 7.67 | 1.71–34.33 | 0.008 |
| > 6 | 20 | 5 | 1.92 | 0.44–8.31 | 0.385 |
| IRI cycles: cutoff, 0, 12; N=61 | | | | | |
| 0 | 27 | 4 | 1.00 | – | – |
| ≤ 12 | 21 | 10 | 5.23 | 1.34–20.45 | 0.018 |
| > 12 | 13 | 3 | 1.72 | 0.32–9.17 | 0.523 |

OR odds ratio; 95%CI, 95% confidence interval; TIB Temozolomide + Irinotecan + Bevacizumab; TMZ Temozolomide; IRI Irinotecan

*Univariable binary logistic regression models

Bold numbers in the last column are used to highlight that they are statistically significant.
increased the likelihood of CM among rGBM patients. Long-term use of TIB (up to 25 cycles) did not cause unexpected toxicities or end-organ injuries among rGBM patients. For patient who are considered for IRI therapy, we recommend screening patients for the polymorphism of the UDP-glucuronosyl-transferase 1A1 (UGT1A1) locus [15] and closely monitoring bone marrow function.

TMZ-induced myelosuppression is a known side effect and was reported predominantly in female patients [25]. Five (7.7%) subjects had to discontinue TMZ permanently after brief exposure to low daily doses during the CCRT period because of severe myelosuppression. Most brain tumor treating physicians discontinue TMZ for rGBM patients based on the assumption that TMZ will no longer be effective and could generate more toxicity than benefit. TMZ with BEV and IRI for rGBM have not been studied in phase III clinical trials. Given the fact that GBM tumor cells grow while on BEV alone or BEV-based doublet chemotherapies are resistant to all subsequent therapies, physicians (J-J Z) used TIB regimen in selected rGBM patients with normal bone marrow functions and had seen clinical benefit with manageable myelosuppression episodes [17]. In addition, 101 [26] and 108 [27] cycles of TMZ had been used to treat recurrent glioma patients without reports of severe myelosuppression. In the current study, administration of up to 47 cycles of TMZ or 25 cycles of TIB were not associated with an increased risk of developing severe CM or any end-organ toxicity among recurrent glioma patients.

Bone marrow from 3 (#17, 23, and 52) glioma subjects demonstrated chemotherapy-related hypocellularity with documented clinical histories of CM. Subject #9’s (Group 3) bone marrow study also showed evidence of hypocellularity (Fig. 2D), but without clinical myelosuppression. She had grade II spinal glioma and had received spinal radiation (C7—T4). So, the finding in her bone marrow is not representative for all subjects from Group 3.

The analysis revealed that infusion with 1–6 (N = 14) and 1–12 (N = 21) cycles of IRI increased the risk of CM by 7.67 (P = 0.008) and 5.23 (P = 0.018) times. CTEP-issued side effects report from the TMZ/IRI combination include a 4–20% chance of developing irreversible bone marrow damage with the observation that most of the CM events occurred soon after a new treatment drug started. Genetic variants of the UGT1A1 gene and NADPH dehydrogenase quinone 1 might predispose susceptible patients to develop CM from IRI [28–30]. These genetic factors may explain why some patients developed CM soon after IRI was given, while other patients rarely experienced CM, even with prolonged usage. It is also likely that previous TMZ treatment as adjuvant therapy predisposes patients to an elevated risk for CM when IRI is added to salvage treatment. To reduce overlapping side effects on myelosuppression, TMZ dosage in the TIB regimen was set at 150 mg/M² and it was subjected to further reduction if a given patient could not tolerate the dosage.

According to forensic studies, fast morphological changes (autolysis, degenerative) at the microscopic and ultrastructural level have been reported in human [31] and animal [32] kidneys within hours of death. Variable degrees of autolysis in kidney specimens (Supplemental Fig. 1) prevented us from studying glomerular ultrastructural changes using postmortem specimens. Meanwhile, ultrastructural changes such as unique hyaline occlusive glomerular microangiopathy were demonstrated by renal biopsy from patients actively treated with BEV [33]. There was no evidence of fibrin polymers or extensive loss of podocyte foot processes [33] which is different from acute or chronic cases due to thrombotic microangiopathy.

This study demonstrated that rGBM patients predominantly died directly from tumor progression and aspiration pneumonia. It is likely that respiratory dysfunction is due to incapacitation (dysphagia and weakened diaphragm function) from tumor progression including brain stem infiltration (Supplemental Table 2). This study revealed microscopic evidence of GBM brain stem infiltration among 56.5% of subjects (22/46), which is consistent with a finding reported by Drumm et al. [34] Reasonably attributes to treatment advances such as repeated debulking surgery, ventriculoperitoneal shunt placement, BEV, and steroid use, death from brain herniation is not as common today as was reported in the 1990’s [25].

This retrospective study has its limitations: (1) Toxicity to male and female reproductive organs was not evaluated; (2) Participation from brain metastasis subjects is low; (3) Medical records review and AE assessment are retrospective. There is selection bias since subjects received the TIB regimen dictated by normal or near-normal bone marrow function before TIB therapy; (4) Targeted drug treatments in addition to TMZ, BEV, and/or IRI are potential confounding factors; (5) There were variable intervals from death to tissue collection; (6) Testing O6-methylguanine methyltransferase (MGMT) promoter methylation and UGT1A1 polymorphism were not a routine practice several years ago and we do not have fund currently to perform MGMT analysis retrospectively.

In summary, the TIB regimen might have caused a higher rate of manageable CM than patients on SOC therapy. This difference is likely due to IRI involvement rather than the extended use of TMZ or BEV. There were no unexpected clinical adverse effects or organ-specific toxicities by postmortem examinations, even in patients who received long-term uses of TIB. We demonstrated that GBM disease progression and aspiration pneumonia are the most common CODs among glioma subjects. These results will allow physicians to plan the best treatment regimens for rGBM.
and design clinical trials with BEV and BEV-based therapies with less fear of end-organ damage besides side effects clinically known.

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**Author contributions** All authors contributed to the study. J-JZ initiated the project and mobilized resources for the study at Tufts Medical Center first, then at University of Texas Health Science Center at Houston (UTHealth®) McGovern Medical School and Memorial Hermann at Texas Medical Center. JK. Wu and J-JZ were investigators at Tuft Medical Center and University of Texas Health Science Center at Houston (UTHealth®) McGovern Medical School and Memorial Hermann at Texas Medical Center; respectively. Autopsies, tissue collections, storage and retrieval, gross anatomy and microscopic analysis of tissues were performed at the Department of Pathology, supervised by MB, MBB, LYB, REB, XT, MP, GWH, RZ, and RLH. Ultrasound guided kidney biopsy in cadaver was performed by AKP. Slides review and pathology reports were issued by MB, MBB, LYB, XT, MP, RLH. WFG and LC. Clinical data collection and analysis were performed by GL, MR, NL, and J-JZ. Statistical analysis was performed by PZ. The first draft of the manuscript was written by GL. J-JZ, GL, and PZ had worked on all versions of the manuscript. All authors reviewed and approved the final manuscript.

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**Data availability** This manuscript has processed data included as electronic supplementary material. The datasets (raw data) generated during and/or analyzed during the current study are available from the corresponding author upon request.

**Declarations**

**Conflicts of interest** The authors have no relevant financial or non-financial interests to disclose.

**Research involving human participants** This study used postmortem human specimens and had reviewed medical records from deceased subjects. The study was performed in line with the principles of the Declaration of Helsinki. It is approved by the Health Sciences Institutional Review Board (IRB) at Tufts Medical Center. The same study protocol was also approved by the Committee for the Protection of Human Subjects (CPHS) at the University of Texas Health Science Center at Houston (UTHealth) (HSC-MS-11–0133).

**Informed consent** Consents for autopsy examination were obtained from all participants, either from patients when patients were alive or from the next of kin after subjects had deceased before autopsy [full autopsies (N = 69), restricted autopsies to the brain (N = 6) and restricted to the brain and kidney (N = 1)].

**References**

1. Stupp R, Mason WP, van den Bent MJ et al (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352:987–996
2. Liu LM, Ashkan K, Tran DD et al (2018) First results on survival from a large Phase 3 clinical trial of an autologous dendritic cell vaccine in newly diagnosed glioblastoma. J Transl Med 16:142
3. Zhu P, Du XL, Lu G et al (2017) Survival benefit of glioblastoma patients after FDA approval of temozolomide concomitant with radiation and bevacizumab: a population-based study. Oncotarget 8:44015–44031
4. Stupp R, Taillibert S, Kanner A et al (2017) Effect of tumor-treating fields plus maintenance temozolomide vs maintenance temozolomide alone on survival in patients with glioblastoma: a randomized clinical trial. JAMA 318:2306–2316
5. Seystahl K, Wick W, Weller M (2016) Therapeutic options in recurrent glioblastoma–An update. Crit Rev Oncol Hematol 99:389–408
6. Stupp R, Wong ET, Kanner AA et al (2012) NovoTTF-100A versus physician’s choice chemotherapy in recurrent glioblastoma: a randomised phase III trial of a novel treatment modal. Eur J Cancer 48:2192–2202
7. Roth P, Gorlia T, Rejnveielcd JC et al (2021) EORTC 1709/CCTG CE.8: A phase III trial of marizomib in combination with temozolomide-based radiochemotherapy versus temozolomide-based radiochemotherapy alone in patients with newly diagnosed glioblastoma. JCO 39:2004–2004
8. Weller M, Cloughesy T, Perry JR et al (2013) Standards of care for treatment of recurrent glioblastoma–are we there yet? Neuro Oncol 15:4–27
9. Gilbert MR, Pugh SL, Aldape K et al (2017) NRG oncology RTOG 0625: a randomized phase II trial of bevacizumab with either irinotecan or dose-dense temozolomide in recurrent glioblastoma. J Neurooncol 131:193–199
10. Wick W, Gorlia T, Bendszus M et al (2017) Lomustine and bevacizumab in progressive glioblastoma. N Engl J Med 377:1954–1963
11. Bagatell R, London WB, Wagner LM et al (2011) Phase II study of irinotecan and temozolomide in children with relapsed or refractory neuroblastoma: a Children’s Oncology Group study. J Clin Oncol 29:208–213
12. Levy A, Krailo M, Chi S et al (2017) PDCT-09. Temozolomide with irinotecan versus temozolomide, irinotecan plus bevacinumab for recurrent medulloblastoma/cns pnet of childhood: report of a cog randomized phase ii screening trial. Neuro Oncol 19:v186
13. Modak S, Kushner BH, Basu E et al (2017) Combination of bevacizumab, irinotecan, and temozolomide for refractory or relapsed neuroblastoma: results of a phase II study. Pediatr Blood Cancer. https://doi.org/10.1002/hpc.26448
14. Peters KB, Lou E, Desjardins A et al (2015) Phase II trial of upfront bevacizumab, irinotecan, and temozolomide for unresectable glioblastoma. Oncologist 20:727–728
15. Vredenburgh JJ, Desjardins A, Reardon DA et al (2011) The addition of bevacizumab to standard radiation therapy and temozolomide followed by bevacizumab, temozolomide, and irinotecan for newly diagnosed glioblastoma. Clin Cancer Res 17:4119–4124
16. Hummel TR, Sallom R, Drissi R et al (2016) A pilot study of bevacizumab-based therapy in patients with newly diagnosed high-grade gliomas and diffuse intrinsic pontine gliomas. J Neurooncol 127:53–61
17. Lu G, Rao M, Zhu P et al (2019) Triple-drug therapy with bevacizumab, irinotecan, and temozolomide plus tumor treating fields for recurrent glioblastoma: a retrospective study. Front Neurol 10:42
18. Aguilera D, Mazewski C, Fangusaro J et al (2013) Response to bevacizumab, irinotecan, and temozolomide in children with relapsed medulloblastoma: a multi-institutional experience. Childs Nerv Syst 29:589–596
19. Shye M, Hanna RM, Patel SS et al (2020) Worsening proteinuria and renal function after intravitreal vascular endothelial growth factor blockade for diabetic proliferative retinopathy. Clin Kidney J 13:969–980
20. Perry JR, Brown MT, Gockerman JP (1998) Acute leukemia following treatment of malignant glioma. J Neurooncol 40:39–46
21. Liu P, Li P, Lei T et al (2018) Acute lymphoblastic leukemia following temozolomide treatment in a patient with glioblastoma: a case report and review of the literature. Oncol Lett 15:8663–8668
22. Silbergeld DL, Rostomily RC, Alvord EC (1991) The cause of death in patients with glioblastoma is multifactorial: clinical factors and autopsy findings in 117 cases of supratentorial glioblastoma in adults. J Neurooncol 10:179–185
23. Yoshimura J, Onda K, Tanaka R et al (2003) Clinicopathological study of diffuse type brainstem gliomas: analysis of 40 autopsy cases. Neurol Med Chir (Tokyo) 43:375–382
24. Penninckx B, Van de Voorde WM, Casado A et al (2012) A systemic review of toxic death in clinical oncology trials: an Achilles’ heel in safety reporting revisited. Br J Cancer 107:1–6
25. Kourelis TV, Buckner JC, Gangat N et al (2015) Temozolomide induced bone marrow suppression–A single institution outcome analysis and review of the literature. Am J Hematol 90:E183–184
26. Barbagallo GMV, Paratore S, Caltabiano R et al (2014) Long-term therapy with temozolomide is a feasible option for newly diagnosed glioblastoma: a single-institution experience with as many as 101 temozolomide cycles. Neurosurg Focus 37:E4
27. Hirano H, Kawahara T, Niño M et al (2017) Anaplastic astrocytoma cells not detectable on autopsy following long-term temozolomide treatment: a case report. Mol Clin Oncol 6:321–326
28. Tsunedomi R, Hazama S, Fujita Y et al (2014) A novel system for predicting the toxicity of irinotecan based on statistical pattern recognition with UGT1A genotypes. Int J Oncol 45:1381–1390
29. Innocenti F, Undevia SD, Iyer L et al (2004) Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. J Clin Oncol 22:1382–1388
30. Liu D, Li J, Gao J et al (2017) Examination of multiple UGT1A and DPYD polymorphisms has limited ability to predict the toxicity and efficacy of metastatic colorectal cancer treated with irinotecan-based chemotherapy: a retrospective analysis. BMC Cancer 17:437
31. Kushwaha V, Yadav M, Srivastava A et al (2010) Time since death from degenerative changes in the Kidney. J Indian Acad Forensic Med 32:37–41
32. Tomita Y, Nihira M, Ohno Y et al (2004) Ultrastructural changes during in situ early postmortem autolysis in kidney, pancreas, liver and skeletal muscle of rats. Leg Med 6:25–31
33. Person F, Rinschen MM, Brix SR et al (2019) Bevacizumab-associated glomerular microangiopathy. Mod Pathol 32:684–700
34. Drum M, Dixit KS, Grimm S et al (2020) Extensive brainstem infiltration, not mass effect, is a common feature of end-stage cerebral glioblastomas. Neuro Oncol 22:470–479

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Authors and Affiliations

Guangrong Lu1 · Ping Zhu1,7 · Mayank Rao1 · Nadine Linendoll2 · L. Maximilian Buja3 · Meenakshi B. Bhattacharjee3 · Robert E. Brown3 · Leomar Y. Ballester1,3 · Xuejun Tian4,8 · Monika Pilichowska4 · Julian K. Wu5 · Georgene W. Hergenroeder1 · Williams F. Glass5 · Lei Chen3 · Rongzhen Zhang3 · Anil K. Pillai6 · Robert L. Hunter3 · Jay-Jiguang Zhu1

1 The Vivian L. Smith Department of Neurosurgery, The University of Texas Health Science Center at Houston (UTHealth®) McGovern Medical School and Memorial Hermann Hospital at Texas Medical Center, 6400 Fannin Street, Suite 2800, Houston, TX 77030, USA
2 Department of Hematology-Oncology, Tufts Medical Center, Boston, MA 02111, USA
3 Department of Pathology and Laboratory Medicine, UTHealth® McGovern Medical School, Houston, TX 77030, USA
4 Department of Pathology and Laboratory Medicine, Tufts Medical Center, Boston, USA
5 Department of Neurosurgery, Tufts Medical Center, Boston, MA 02111, USA
6 Diagnostic & Interventional Imaging, UTHealth® McGovern Medical School, Houston, TX 77030, USA
7 Department of Neurosurgery, University of Minnesota, Minneapolis, MN 55455, USA
8 Department of Pathology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY 10467, USA