TITLE PAGE

Title: Phase II Investigation of TVB-2640 (denifanstat) with Bevacizumab in Patients with First Relapse High-Grade Astrocytoma

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Translational Relevance: There remains a dire need for effective therapeutics for the treatment of recurrent glioblastoma. In this phase II study, modulation of fatty acid synthase by the small molecule inhibitor, TVB-2640 (now known as denifanstat), is investigated. Originally developed for the treatment of nonalcoholic steatohepatitis, the drug is a potent inhibitor of the ketoacylreductase activity of FASN and has been verified in both murine and phase I study. Current ongoing clinical trials are examining this strategy in NSCLC, breast, ovarian, prostate, colon and pancreatic cancer. Here, TVB-2640 is shown to have favorable safety profile in patients with recurrent glioblastoma and response signals supporting further study in a randomized phase 3 study.

ABSTRACT

Purpose: Glioblastoma (GBM) represents the most common primary brain tumor. Although anti-angiogenics are employed in the recurrent setting, they do not prolong survival. GBM is known to upregulate fatty acid synthase (FASN) to facilitate lipid biosynthesis. TVB-2640, a FASN inhibitor, impairs this activity.

Methods: We conducted a prospective, single-center, open-label, unblinded, phase II study of TVB-2640 plus bevacizumab in patients with recurrent high-grade astrocytoma. Patients were randomized to TVB-2640 (100mg/m2 oral daily) plus bevacizumab (10mg/kg IV, D1 and D15) or bevacizumab monotherapy for cycle 1 only (28 days) for biomarker analysis. Thereafter, all patients received TVB-2640 plus bevacizumab until treatment-related toxicity or progressive disease. The primary endpoint was progression-free survival.

Results: A total of 25 patients were enrolled. The most frequently reported AEs were palmar-plantar erythrodysesthesia, hypertension, mucositis, dry eye, fatigue and skin infection. Most were Grade 1 or 2 in intensity. The ORR for TVB-2640 plus bevacizumab was 56% (CR 17%, PR 39%). PFS6 for TVB-2640 plus bevacizumab was 31.4%. This represented a statistically significant improvement in PFS6 over historical bevacizumab monotherapy (BELOB 16%, p=0.008) and met the primary study endpoint. The observed OS6 was 68%, with survival not reaching significance by log rank test (p=0.56).

Conclusion: In this phase II study of relapsed high-grade astrocytoma, TVB-2640 was found to be a well-tolerated oral drug that could be safely combined with bevacizumab. The favorable safety profile and response signals support the initiation of a larger multicenter trial of TVB-2640 plus bevacizumab in astrocytoma.

INTRODUCTION:

Background: Glioblastoma (GBM, Grade 4 astrocytoma) is the most common and most aggressive primary brain malignancy in adults. The mainstay of treatment for newly diagnosed GBM consists of maximal surgical resection of the primary tumor, followed by radiation therapy (RT) with concurrent temozolomide (TMZ) daily for 6 weeks followed by maintenance TMZ for 6 months [1 2]. This combination of TMZ plus RT improves median and 2-year survival by 2.5 months (from 12.1 to 14.6 months), relative to RT alone. While initial treatment can prolong survival, disease progression is almost inevitable, and once a patient progresses through standard front-line therapy, prognosis is very poor. For patients with recurrent disease, the median survival is 5.0 months (95% CI, 4.6–5.4 months) [3]. Currently, there exists no standard of care for recurrent GBM, although recent survival data from autologous tumor lysate-loaded dendritic cell vaccination is promising [4]. In the era of targeted therapies, clinical studies have demonstrated anti-tumor activity in patients treated with anti-angiogenic agents such as with bevacizumab, a recombinant human monoclonal antibody against vascular endothelial growth factor A (VEGF-A). However, responses with bevacizumab are short, with no notable prolongation in survival [5].

Increasing evidence points to hypoxia as the root cause of angiogenesis and a driving force for resistance to anti-angiogenics. In brief, hypoxia-induced factor 1 α subunit (HIF-1α), normally degradable, is stabilized in hypoxic conditions such that it can dimerize with HIF-1β and regulate transcription a number of hypoxia-related genes including VEGF [6].
Common alterations involved in the tumorigenesis of GBM, notably EGFR, p53 and PTEN, alter HIF-1α signaling [7]. This gives rise to microvascular hyperplasia, a hallmark of GBM diagnosis. However, unlike normal neovascularizing processes, those involved in GBM are chaotic and do

not supply oxygen well [6]. Unsurprisingly HIF-1α expression correlates with both glioma grade and vessel density. Hypoxia in GBM has been shown to promote, rather than impede mitosis, migration and mutational accumulation [8].

Bevacizumab, through the inhibition of VEGF-A, further exacerbates the hypoxic environment of GBM [9]. In order to better characterize the metabolic changes associated with bevacizumab resistance and other antiangiogenics, we performed metabolomic profiling of tumors and sera from patients with progressive GBM using previously described methods [10 11] . Interestingly, the most significant change that correlated with degree of hypoxia was an increase in the presence of long-chain fatty acids. This finding was not entirely unexpected, as GBM is known to be a tumor type with large inclusions of fatty acids (i.e., lipid droplets) [12 13]. It has been proposed that these lipid droplets could represent a temporary storage compartment of fatty acids in the form of triglycerides that could in turn serve as reservoir for the tumor cells during times of metabolic stress [14]. Recent data using fluorescent labeling of lipid droplets in astrocytes shows restricted mobility in the presence of metabolic stress and inhibition of lipid droplet biogenesis in turn reduces astrocyte abundance [15]. It is hypothesized that native astrocytes shuttle and store free fatty acids as a way of decreasing lipotoxicity to neurons [16]. During times of limited glucose availability, GBM cells hydrolyze lipid droplets in lysosomes with resultant transfer to mitochondria for β-oxidation[13].

The changes observed in fatty acid content during hypoxia and bevacizumab resistance suggest a potentially targetable mechanism. Fatty acid synthase (FASN) is a homodimeric and multi-functional enzyme which catalyzes the biosynthesis of palmitate in a NADPH-dependent fashion [17]. Normal cells in adult tissue ubiquitously express low to moderate levels of FASN, however, these cells, which primarily import lipids from the extracellular milieu, do not have a

strict requirement for FASN activity [18]. In contrast, tumor cells have an increased requirement for lipids to facilitate membrane biosynthesis, protein modification, and signaling molecules. Consequently, tumor cells are more dependent on *de novo* palmitate synthesis catalyzed by FASN than normal cells [19]. Additionally, inhibition of FASN increases polyunsaturated fatty acids (PUFA), the accumulation of which may lead to iron-dependent cell death [20]. Many solid and hematopoietic tumors overexpress FASN, including non-small cell lung, breast, ovarian, prostate, colon, pancreatic cancers, non-Hodgkin lymphoma as well as GBM [21 22 23 24 25 26 27 28 29]. Moreover, FASN tumor expression has been found to be increased in a stage-dependent manner and correlate with diminished survival [30 19].

TVB-2640 (denifanstat) is a potent and reversible inhibitor of the FASN enzyme that has been validated in multiple tumor cell lines, as well as in clinical studies [31]. TVB-2640 inhibits the ketoacylreductase (KR) enzymatic activity of the FASN enzyme. TVB-2640 has been tested in murine models and a phase I, international, first-in-human, dose-escalation study confirmed predicable pharmacokinetic and tolerable side effects as well as establishing the recommended dose for phase II study [32]. There are several ongoing studies of TVB-2640 in KRAS-mutant non-small cell lung cancer, colon cancer and HER2-positive advanced breast cancer[33]. Preliminary data from breast cancer patients suggests prolonged disease control when given with cytotoxic chemotherapy[34].

Study Rationale: Given the findings of a) increased hypoxia with bevacizumab, b) increased long-chain fatty acid presence in the tumors of patients who have failed bevacizumab, and c) evidence suggesting that fatty acid metabolism could play a role in survival under hypoxia, we hypothesized that the addition of TVB- 2640 could overcome acquired resistance to bevacizumab. Since progression-free survival (PFS) with bevacizumab is short, we conducted a

phase II study in patients with high-grade astrocytoma to evaluate the potential effectiveness and safety of TVB-2640 in combination with bevacizumab.

MATERIALS AND METHODS:

Study Design: We conducted a prospective, single-center, open label phase II study of TVB-2640 plus bevacizumab in patients with high-grade astrocytoma in first relapse who were naïve to bevacizumab. A total of 24 patients were planned to be enrolled. For Cycle [C] 1, patients were assigned on a 1:1 ratio to either Arm 1 (bevacizumab + TVB-2640 [BEV/TVB]) or Arm 2 (bevacizumab [BEV] monotherapy). The purpose of C1 randomization was for exploratory analysis only (see Exosome and Proteomic Analysis). Starting on C2D1, all patients (Arms 1 and 2) received BEV/TVB until development of significant treatment-related toxicity or progressive disease (PD). The local institutional review board approved the study, and all patients provided informed written consent before performance of any study-related procedures. The study was conducted in accordance with recognized ethical guidelines (e.g., Declaration of Helsinki, CIOMS, Belmont Report, U.S. Common Rule).

Eligibility: Adults aged 18 years or older with histologically confirmed high-grade astrocytoma who experienced progression after standard combined modality treatment with RT plus TMZ and who had recovered from reversible toxicities of prior therapy were eligible. Of note, this study was designed and initiated prior to WHO Classification of CNS Tumors, 5th edition, which reclassified GBM, IDH mutant (secondary glioblastoma) as Astrocyte, IDH mutant, CNS WHO grade 4. Both IDH wild-type and IDH-mutant grade 4 astrocytoma were eligible for this study. Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status score of 0 to 2 and adequate renal, hepatic, and bone marrow function. Patients were

excluded if they were receiving warfarin, enzyme-inducing anti-epileptics, or had a history of non-standard RT, recent investigational agents, biologics, or other cytotoxic agents. Patients with a history of serious intercurrent illness, intracranial hemorrhage, coagulopathy, HIV or hepatitis were also excluded.

Treatment: In C1, all patients received bevacizumab 10 mg/kg intravenously (IV) on D1 and D15. Patients in Arm 1 also received TVB-2640 100 mg/m^2 orally (PO) once daily (QD) in C1. After completion of C1, all patients received bevacizumab and TVB-2640 in combination at these same dose regimens. The initial bevacizumab dose was infused over 90 minutes. If the initial infusion was tolerated, the second infusion could be shortened to 60 minutes, and the third and subsequent infusions could have been accordingly shortened to 30 minutes. Dose delays were instituted per standard of care. TVB-2640 was administered by the patient at the same time each day under fasted conditions (i.e., at least 2 hours after last food consumption and at least 1 hour before next food consumption), with each dose separated by 24 (±4) hours. If a TVB-2640 dose was missed by more than 8 hours, it was not made up.

Safety: Safety assessments included documentation of adverse events (AEs) and serious adverse events (SAEs), clinical laboratory tests (hematology, clinical chemistry, and urinalysis), physical examinations, ophthalmologic examinations, vital signs, ECOG performance status, and 12-lead electrocardiograms (ECGs). Toxicities were graded by the investigator using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 4.03.

Efficacy: Brain magnetic resonance imaging (MRI) was performed after every even cycle (e.g., C2, C4) during treatment, with tumor response assessed by the investigator for complete response (CR), partial response (PR) and PD according to the Response Assessment in Neuro-oncology (RANO) criteria. The same imaging technique was to be used throughout the study.

After completion of treatment, patients were followed for survival every 3 months through 1 year after the first dose. Any subsequent anti-tumor therapy (description and dates) was also documented.

Exosome and Proteomic Analysis: Before starting and after completion of C1, serum samples were collected for proteomic and lipidomic analyses using standard centrifugation techniques as previously described [35]. Relative protein levels in each sample were quantified by normalizing to total peptide-to-spectrum matches (PSMs). This was used to represent the percentage of total protein expression for each protein in each sample. After ultracentrifugation, proteomic analysis was conducted at the MD Anderson Proteomic and Metabolomics Facility. Additionally, serum was sent for exosome characterization through System Biosciences (Palo Alto, CA) using defibrination reagent (Cat#TMEXO-1) and ExoQuick (Cat 3EXOQ5A-1) for exosome isolation. Exosome Total RNA isolation was achieved using a SeraMir Exosome RNA Purification Column kit (Cat#RA808A-1) according to manufacturer's instruction. Final RNA concentration was determined by Agilent Bioanalyzer Small RNA Assay and using Bioanalyzer 2100 Expert instrumentation (Agilent Technologies, Santa Clara, CA). Concentration was analyzed in log units base 10. For exosome analysis, exosomes were defined according to 50-120nm size parameters. For exosome profiling, these samples were processed by System Biosciences using a standard protocol that consisted of Qiagen small RNA library prep, gel purification and sequencing on Illumina NextSeq SE75. For differential RNA analysis, a mean norm read counts cutoff of 10 was established. A log-fold cutoff was also used to identify targets greater than or equal to +1 and less than or equal to -1 (2-fold change). Accordingly, targets were ranked according to the number of patients whose samples met this cutoff after filtering (p value of 0.01) for significance, along with false-discovery rate (FDR) controlled for multiple-tests.

Statistical Considerations: The primary endpoint was PFS, defined as the time from study enrollment to the first occurrence of relapse, death from any cause, or, in the event of no progression event, until last contact before loss to follow up. Due to the lack of any systemic therapy with proven survival benefit in the recurrent setting, and the feasibility of this phase II study, a historical control was chosen over contemporaneous arms. Historical data comparisons were made with reference to the phase II BELOB bevacizumab monotherapy (BEV HIS) [36]. It was hypothesized that the combination of BEV/TVB would prolong PFS by 4 months as compared with BEV historical data. The primary efficacy analyses for estimation of PFS rates was to be performed using a one-sample one-sided log-rank test for an overall type-I error at 0.1 and a power of >90% against BEV HIS (BELOB), assuming a median PFS of 7 months with BEV/TBV and referencing the published BEV HIS median PFS of 3 months and an exponential model. Unless otherwise mentioned, efficacy and safety data for BEV/TVB refers to the aggregate of Arms 1 and 2, since all patients were treated with BEV/TVB from C2 on. Contrasts of the BEV/TVB study cohort with historical controls on overall survival were made with one-sample 1-sided log rank tests. All other statistical testing was two-sided with a significance level of 5%.

Statistical analyses of safety events were descriptive in nature. Continuous variables were summarized using descriptive statistics (n, mean, standard deviation, median, minimum, and maximum). Categorical variables were summarized showing the number and percentage (n, %) of patients within each classification. Statistical testing, estimates, and confidence intervals were computed with SAS Version 9.4 for Windows (SAS Institute, Cary, NC).

Data Availability: The data generated in this study are available within the article and its supplementary data files. Raw data for this study were generated at MD Anderson Proteomic and

Metabolomics Facility (Houston, TX), and System Biosciences (Palo Alto, CA). Derived data supporting the findings of this study are available from the corresponding author upon reasonable request. Data types for which community-recognized, structured repository does not exist, or where information could compromise patient privacy or consent, are available upon reasonable request from the corresponding author.

RESULTS:

Demographics: 28 patients were screened with 3 screen failures for histology, thrombocytopenia and intracranial bleeding respectively. A total of 25 patients were enrolled and treated with at least 1 dose of study drug. For the C1 treatment there were 13 patients randomized to Arm 1 (bevacizumab plus TVB-2640) and 12 to Arm 2 (bevacizumab monotherapy). Demographics (Table 1, Supplemental Table S1) and participant flow (Supplemental Figure S1) are summarized. The patient population was well-balanced with regard to sex (52% male). Most (24 patients; 96%) patients were white, with the remaining patient being Asian. Overall, the mean age of patients was 59 years. No significant differences were seen between C1 arms with regard to demographics. All patients had grade 4 astrocytoma. Of the 21 patients with evaluable IDH mutational status, 2 were IDH mutant (10%). Of the 18 patients with MGMT promoter methylation status, 6 were methylated (33%).

Safety: TVB-2640 plus bevacizumab was well tolerated with most AEs Grade 1 or 2 in intensity (Table 2), including palmar-plantar erythrodysesthesia (PPE), hypertension (HTN), mucositis, dry eye, fatigue, and skin infection. Treatment-related Grade 3 AEs included 4 events of PPE and single occurrences of alanine aminotransferase (ALT) elevation, deep venous thrombosis (DVT), HTN, mucositis, optic neuritis, perirectal abscess, and vomiting. Three of these events (DVT,

perirectal abscess, and vomiting) were serious. The only other treatment-related SAE was a single case of aphasia. Two Grade 4 AEs, fatigue and hemiplegia, and two Grade 5 AEs, intracranial hemorrhage and sepsis, were reported, all of which were deemed to be unrelated to the study drug.

Efficacy: The PFS6 observed for TVB/BEV was 31.4% and represented a statistically significant improvement in PFS6 over the BEV HIS (16%, p=0.008). 23 patients were evaluable by MRI with two patients being not assessable due to death prior to comparison scans and study withdrawal for patient preference, respectively. The BEV/TVB median PFS (Figure 1) was 4.6 months (95% CI 4.0 to 6.3) and the median OS (Figure 2) was 8.9 months (95% CI 5.2 to 13.6). A one-sided one-sample log rank test comparing BEV/TVB with BEV HIS regarding PFS, computed assuming exponential survival, gave p=0.008 (median 4.5 months, PFS6=31.4%), reflecting the larger median PFS in BEV/TVB relative to BEV HIS (3 months). The corresponding test for OS (BEV HIS median OS = 8 months) gave p=0.56 (median 8.9 months, OS6=68%); based on one-sample log rank testing when compared to BEV HIS (62%). The overall response rate (ORR) for BEV/TVB was 56% (CR 17%, PR 39%).

Exosome and Proteomic Analysis: One patient had no exosome data. Exosome characterization was conducted on serum samples, obtained prior to and following a 28-day cycle of treatment, comparing patients receiving BEV/TVB with BEV monotherapy (Supplementary Data File 1, Supplementary Data File 2). Minimal serum protein contamination was detected in samples collected for proteomic analysis. While serum albumin was present in most samples, it accounted for only a small proportion of the total protein content (<1.3%). In contrast, albumin constituted ~50% of plasma proteins, indicating that the proportion of proteins that are serum-derived rather than exosome-derived was relatively small[37]. All samples

expressed high levels of alpha-2-macroglobulin (11.4-69.4%). Galectins are non-glycosylated lectins which regulate formation of vesicles, and their identification is used to confirm exosome presence[38]. Galectin-3-binding was also present in all but one sample and accounted for 0.3-3.7% of all proteins. Taken together, these results suggest exosome purity.

The median size at C1D1 for BEV/TVB and BEV arms was 112.4nm (95% CI 97.6, 119) and 107nm (95% CI 87.2, 115.6), respectively (Supplemental Table S2). The median size at C2D1 for BEV/TVB and BEV arms was 108.8nm (95% CI 88.1, 120.1) and 107.55nm (95% CI 97.9, 123.9), respectively. The median concentration at C1D1 for BEV/TVB and BEV arms was 12.66 particles*10^8/ml (95% CI 12.38, 13.1) and 12.65 particles*10^8/ml (95% CI 12, 13.1), respectively. The median concentration at C2D1 for BEV/TVB and BEV arms was 12.64 particles*10^8/ml (95% CI 11.78, 13.2) and 12.87 particles*10^8/ml (95% CI 12.28, 13.3), respectively. While no statistically significant difference was observed for either size or concentration (Supplemental Figure S2, Supplemental Figure S3), a decrease in the median concentration of exosomes between C1D1 and C2D1 correlated with increased PFS when both groups were combined based on a proportional hazards model (LogHR 2.2±0.91, HR 9.1, (95% CI 1.5, 54.4), p=0.016, Supplemental Table S3).

Reactome pathway analysis (reactome.org) was conducted to quantify changes in the relative level of individual proteins between C1 and C2. In patients treated with bevacizumab plus TVB-2640 (C1), decreased expression of Annexin A3 (-0.01% of total protein, p=0.044) and A7 (-0.07% of total protein, p=0.047), as compared to bevacizumab monotherapy, was observed.

RNA analysis of exosome contents was also performed. For miRNA analysis of BEV pairwise samples, the upregulated targets of interest (equal or more than 5 samples) were has-

miR-338-3hashsa-miR-338, hsa-miR-4521, hsa-miR-146hasp, hsa-mhas6503, hsa-has-23b-5p, hshasiR-4111-5p, hsa-miR-411. For BEV patients, the downregulated targets of interest (equal or more thahas sampleshasere hsa-has-548q, hsa-has-548q, hsa-miR-6877-5p, hsa-miR-6877. For miRNA analysis of TVB/BEV pairwise samples, the upregulated targets of interest (equalhas more than 5 samples) were hsa-miR-582. For TVB/BEV patients, there were no downregulated targets of interest (equal or more than 5 samples) which met the predetermined levels of statistical significance.

DISCUSSION:

In this phase II study, administration of TVB-2640 plus bevacizumab was found to be well-tolerated with most AEs being of lower grade, and expected. In particular, palmar-plantar erythrodysesthesia was commonly observed. Previous research of TVB-2640 in cancer patients has shown that these symptoms were reversible. Although the pathophysiology of PPE remains poorly understood, eccrine accumulation of chemotherapeutic drugs and epithelial necrosis of eccrine ducts has been implicated but there is no data specific to TVB-2640 on this[39]. Interestingly, it has been demonstrated that TVB-2640 results in significant reductions in the saturated and monounsaturated triglyceride content of sebum [34]. This fatty acid reduction in sebaceous gland product is likely contributory to PPE.

As there is no systemic therapy with proven survival benefit in the treatment of recurrent GBM, practitioners are faced with the daunting task of extrapolating from what studies have shown the most benefit. Evidence for the utility of bevacizumab comes from two phase II studies which showed PFS6 of 29-42% [40]. The phase II BELOB study was chosen as the comparative arm in this study due to the apparent survival advantage of lomustine plus bevacizumab (PFS6

41%) over bevacizumab monotherapy (16%) observed in that study. However, the subsequent phase III study, EORTC 26101, was unable to confirm the superiority of lomustine plus bevacizumab over lomustine monotherapy (median PFS 4.2 versus 1.5 months, respectively) [41]. With this context in mind, the PFS6 observed for TVB-2640 plus bevacizumab (31.4%) in this study compares favorably with the initial phase II studies of bevacizumab as well the bevacizumab monotherapy arm of BELOB. While not adequately powered for comparisons of OS, the OS6 observed for TVB-2640 plus bevacizumab compares favorably with those seen in BELOB. Furthermore, the high ORR of TVB-2640 plus bevacizumab (56%) including 17% of patients experiencing a CR, suggests that the PFS advantage is not merely due to decreased contrast extravasation from bevacizumab's anti-angiogenic effects. Evidence supporting this inference comes from the observation that the ORR for TVB-2640 plus bevacizumab is superior to that seen in the bevacizumab monotherapy arm of BELOB (38%). In aggregate, these results suggest that TVB-2640 plus bevacizumab has activity in GBM which exceeds that of bevacizumab monotherapy. Accordingly, further validation with a larger randomized trial is required. Of note, there is an ongoing trial in China (NCT05118776) investigating TVB-2640 (under the identifier ASC40) in combination with bevacizumab in recurrent glioblastoma.

It is also worth mentioning that, in BELOB, there was suggestion that MGMT status could predict improved PFS and OS for those receiving bevacizumab alone, but not from lomustine or the combination of lomustine plus bevacizumab (table 5 of that paper) [36]. MGMT promoter methylation is of known prognostic value and predictive of benefit from temozolomide [42]. While our sample size precluded a dedicated subgroup analysis, we would recommend including methylation, as well as IDH, status as a design variable in any subsequent studies.

A cycle 1 randomization to either TVB-2640 plus bevacizumab or bevacizumab monotherapy was conducted in this trial for biomarker analysis only and was not intended to be comparative for either safety or efficacy. Exosome analysis showed adequate purification as assessed by the proportion of serum to exosome-derived proteins. Neither the size nor concentration of exosomes was found to be statistically different between the arms, suggesting inhibition of FASN does not alter exosome production. However, a statistically superior PFS was observed with decreased exosome concentrations between C1 and C2 when the arms were viewed in aggregate suggesting exosome concentration may be a prognostic biomarker for progression in GBM. This would require larger analysis in conjunction with a phase 3 study but establishing a correlation between a decrease in exosome concentration before starting therapy and at a later time period could potentially offer prognostic data.

Reactome pathway analysis showed that patients treated with BEV/TVB for C1 had very marginal decreases in expression of, annexin A3 and A7. Annexin A3 in particular is involved in VEGF signaling[43] and has been previously identified in ovarian cancer exosomes and associated with treatment resistance, but has a mixed role in tumor progression[44 45]. Annexin A7 appears to be a tumor suppressor in GBM, but has also been shown to promote tumor progression in other cancers [46 47]. All considered, these findings suggest a possible interplay between TVB-2640, VEGF signaling and treatment resistance. The RNA analysis of exosomes also highlighted has-miR-582 as being upregulated in patients receiving BEV/TVB. MicroRNA are single strands of non-coding RNA which regulate gene expression. The microRNA has-mir-582 has previously been shown to be upregulated in glioblastoma stem cells and promote survival by inhibiting Caspace 3 and 9 [48]. Although small RNA prep was employed, there was some expression of mRNA fragments (using NCBI RefSeq gene annotation) seen as have previously been described

in exosomes. The differential expression analysis of RefSeq results should be interpreted with caution due to the minimal alignment that was observed.

In conclusion, in this phase II study of relapsed high-grade astrocytoma, TVB-2640 was found to be a well-tolerated oral drug that could be safely combined with bevacizumab. The favorable safety profile and efficacy signals support the initiation of larger multicenter trial of TVB-2640 plus bevacizumab in this population.

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CAPTIONS:

FIGURE 1: KAPLAN-MEIER ESTIMATE OF PROGRESSION (ALL PATIENTS, N=25), MEDIAN=4.5, 95% CI 4.0, 6.3

FIGURE 2: Kaplan-Meier Estimate of Overall Survival (All patients, N=25) Median=8.9 95% CI 5.2, 13.6

Table 1: Patient Demographics

Characteristics	**No. (% evaluable)**
Mean Age (Range)	59 (39-87)
Sex/Gender (male)	13 (52)
Baseline ECOG	
0	11 (44)
1	8 (32)
2	6 (24)
Ethnicity/Race	
White Non-Hispanic	11 (44)
White Hispanic/Spanish/Latino	13 (52)
Asian	1 (4)
Histological Features	
IDH wild-type	19 (90)
MGMT promoter methylation	6 (33)

Table 2: Adverse Events

Adverse Event	TVB-2640 plus Bevacizumab (Any Grade)	TVB-2640 plus Bevacizumab (Grade 3-5)
PPE	18	3
Mucositis	15	1
HTN	13	1
Dry eye	12	0
Fatigue	7	2
Muscle Weakness	6	2
Alopecia	5	0
Skin Infection	5	0
Arthralgia	4	0
Myalgia	4	0
Elevated ALT	3	1
Allergic Rhinitis	3	0
Elevated AST	3	0
Constipation	3	0
Depression	3	0
Dysphasia	3	0
Headache	3	1
Hoarseness	3	0
Paresthesia	3	0
Urinary Tract Infection	3	1
Back Pain	2	0
Cognitive Disturbance	2	1
Conjunctivitis	2	0
Diarrhea	2	0
Dry Mouth	2	0
Dry Skin	2	0
Limb Edema	2	0
Hypokalemia	2	1
Pruritus	2	0
Seizure	2	1
Upper Respiratory Infection	2	0
Acute Kidney Injury	1	0
Anorexia	1	0
Anxiety	1	0
Aphasia	1	0
Bronchitis	1	0
Catheter-related Infection	1	1
Confusion	1	0
Increased Creatinine	1	0
Dysethesia	1	0
Encephalitis	1	1
Conjunctival Hemorrhage	1	0
Blepharitis	1	0

Fall	1	0
Flatulence	1	0
Gait Disturbance	1	0
Gastritis	1	1
GERD	1	0
Hallucination	1	1
Hyperglycemia	1	1
Hypermagnesemia	1	0
Hypomagnesemia	1	0
Intracranial Hemorrhage	1	1
Cerebrovascular Ischemia	1	0
Optic Neuritis	1	1
Thrombocytopenia	1	0
Proteinuria	1	0
Mood Lability	1	0
Rash	1	0
Rectal Hemorrhage	1	0
Perirectal Pain	1	0
Scalp Tenderness	1	0
Sepsis	1	1
Sinusitis	1	0
Candidasis	1	0
Syncope	1	1
DVT	1	1
Urinary Frequency	1	0
Urinary Incontinence	1	0
Vomiting	1	1
Watering Eyes	1	0
Weight Loss	1	0
Perirectal Abcess	1	1

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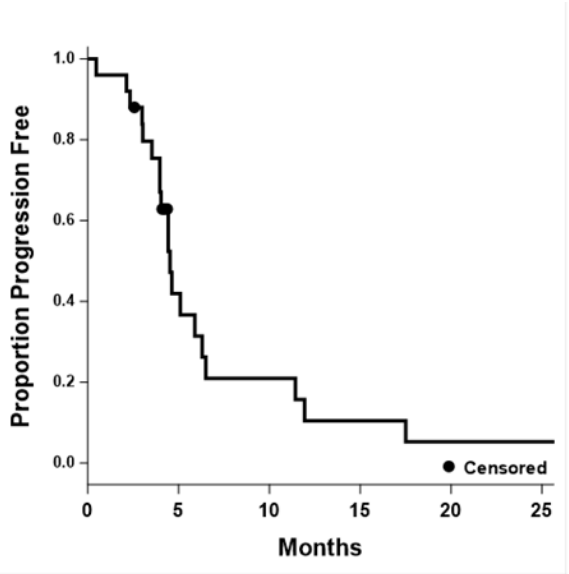
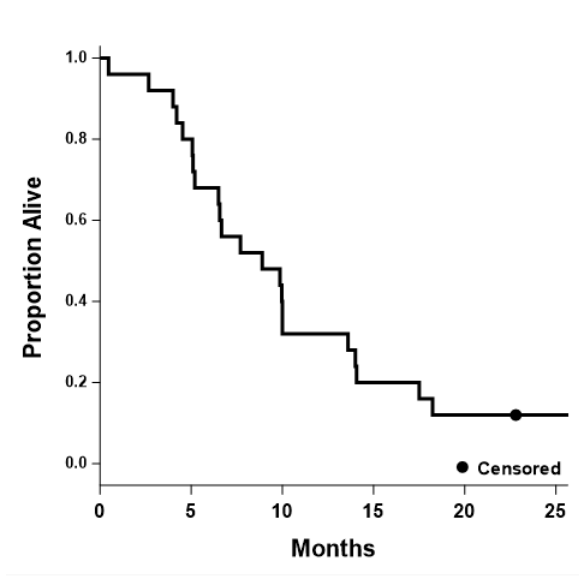
Figure 1



Kaplan-Meier Estimate of Progression (All patients, N=25), median=4.5, 95% CI 4.0, 6.3

Figure 2



Kaplan-Meier Estimate of Overall Survival (All patients, N=25) Median=8.9 95% CI 5.2, 13.6