A Phase II trial of tandutinib (MLN 518) in combination with bevacizumab for patients with recurrent glioblastoma

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Practice points

- Surgical pathology is required for the definitive diagnosis of glioblastoma.
- After maximal safe resection or stereotactic biopsy, standard treatment for newly diagnosed glioblastoma includes involved field radiation with concurrent and adjuvant temozolomide.
- Tumor treating fields (NovoTTF) was approved in 2015 for use in combination with adjuvant temozolomide given improved overall survival compared with temozolomide alone in a pivotal Phase III randomized controlled trial. NovoTTF was previously approved as monotherapy for recurrent glioblastoma.
- Bevacizumab monotherapy was approved for recurrent glioblastoma, improved response and progression-free survival was mainly shown for combination with nitrosoureas. Most other combinations, such as tandutinib, have not shown improved response compared with bevacizumab monotherapy and often result in greater toxicity.
- Repeat surgery and radiotherapy are reasonably tolerated and effective in select patients with recurrent glioblastoma.
- Molecularly targeted agents and immunotherapy have shown promising results in early phase studies, but await randomized controlled studies to confirm efficacy.
- Trial enrollment for eligible patients with glioblastomas is strongly advised.

Aim: A Phase II trial of bevacizumab plus tandutinib. **Methods:** We enrolled 41 recurrent, bevacizumab-naïve glioblastoma patients for a trial of bevacizumab plus tandutinib. Median age was 55 and 71% were male. Treatment consisted of tandutinib 500 mg two-times a day (b.i.d.) and bevacizumab 10 mg/kg every 2 weeks starting day 15. Of 37 (90%) evaluable, nine (24%) had partial response. **Results & conclusion:** Median overall and progression-free survival was 11 and 4.1 months; progression-free survival at 6 months was 23%. All patients suffered treatment-related toxicities; common grade ≥ 3 toxicities were hypertension (17.1%), muscle weakness (17.1%), lymphopenia (14.6%) and hypophosphatemia (9.8%). Four of six with grade ≥ 3 tandutinib-related myasthenic-like muscle weakness had electromyography-proven neuromuscular junction pathology. Tandutinib with bevacizumab was as effective but more toxic than bevacizumab monotherapy.

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Background
Glioblastomas are the most frequent adult primary malignant brain tumors, with an incidence of 3.19 per 100,000 per year [1]. Glioblastomas are the second leading cause of cancer mortality in adults under 35 years of age [2]. Despite advances in imaging, anesthesia and surgical techniques, the prognosis of glioblastomas treated by surgical resection alone is dismal with a median survival of 4–6 months [3–5]. Radiotherapy extends median survival to 8–9 months [6–8], while temozolomide improves median survival to 15 months [9,10]. Cytotoxic agents are generally ineffective in recurrent disease [5].
Recent therapies target abnormal angiogenesis central to glioblastoma growth, as evidenced by vascular permeability on MRI and endothelial proliferation on pathology. Specifically, glioblastoma growth depends on angiogenesis induced by VEGF expression [11]. The VEGF ligand binds receptor tyrosine kinases VEGFR-1 (Flt-1) and VEGFR-2 (KDR), thus, promoting endothelial cell proliferation, survival and migration. Glioblastoma cells overexpress VEGF and tumor-associated endothelial cells express KDR [12]. Mutations of the *VEGF* receptor or antibodies to VEGF and Flt-1 can inhibit malignant glioma growth in mice [13,14]. The signal transduction pathways of the KDR/Flk-1 and Flt-1 receptors include tyrosine phosphorylation, activation of PLC- β , as well as PI3K/Akt signaling with downstream activation or nuclear translocation of PKC plus activation of the MAPK pathway [15–20].

Bevacizumab is a humanized IgG1 monoclonal antibody with high affinity to VEGF [21]. In 2009, bevacizumab received accelerated FDA approval for recurrent glioblastomas due to improved median progression-free survival of 4–6 months in Phase II trials [22,23]. Bevacizumab led to radiographic response rates of 71% of glioblastomas, durable response in above 30% with progression-free survival at 6 months of 29–46% [22,24]. The Phase III trial for newly diagnosed glioblastoma revealed no overall survival benefit when added to upfront radiation and temozolomide, though *post hoc* analysis suggest putative benefit in IDH1 wild-type proneural glioblastomas [25,26].
Tandutinib (MLN 518, Millennium Pharmaceuticals, MA, USA) is an orally active small molecule selective inhibitor of Type III receptor tyrosine kinases, specifically FLT3, c-Kit and PDGFR β [27]. It demonstrated anti-leukemic activity in patients with relapsed and refractory acute myelocytic leukemia (AML) whose blasts contain an activating internal tandem duplication (ITD) mutation of *FLT3*. Tandutinib was effective in chronic myelomonocytic leukemia mice models with chromosomal translocation between the *PDGFR β* gene and the gene encoding the ets-like transcription factor (*TEL*). The *TEL/PDGFR* gene causes constitutive receptor activation leading to a lethal proliferative disease.
The initial Phase I clinical trial of tandutinib was conducted in patients with relapsed and/or refractory AML or high-risk myelodysplastic

Table 1. Demographics and outcomes.	
Demographics and outcomes	Descriptive statistic
Enrolled (n)	41
Age (median; min–max)	55 (26–75) years
Gender (female; %)	12 (29)
KPS (%; min–max)	90 (70–100)
Prior therapies (median; min–max)	1 (1–5)
Evaluable for response (n; %)	37 (90)
Partial response (n; %)	9 (24)
Median PFS (months; range)	4.1 (0.4–26.2)
Median OS (months; range)	11.0 (0.4–26.2)
6-month PFS (%; 95% CI)	23 (12–37)
KPS: Karnofsky performance status; OS: Overall survival; PFS: Progression-free survival.	

syndrome [28]. The maximally tolerated dose of 525 mg twice daily, limited by severe but rapidly reversible muscle weakness. Two of the six patients treated at the maximally tolerated dose exhibited decreases in peripheral and bone marrow blasts, though no objective responses were noted in the Phase I study. Only AML patients

with ITD mutations of *FLT3* were enrolled in the Phase II trial of tandutinib. Of the 25 enrolled patients, 11 (44%) exhibited decreases in peripheral and/or bone marrow blasts, with only one (4%) in complete remission [29].

We combined tandutinib with bevacizumab primarily for its activity against the PDGFR

Figure 1. Progression and overall survival. Kaplan–Meier curves for (A) progression-free survival and (B) overall survival are depicted along with the corresponding median survival in months.

Table 2. Treatment-related toxicities.

CTCAE v3.0 Term (n = 41)	Grade (frequency, percent)					
	1–2	%	3–4	%	Any	%
Any toxicity					41	100.0
Diarrhea	31	75.6	1	2.4	32	78.0
Edema	24	58.5			24	58.5
– Edema: head and neck	17	41.5			17	41.5
– Edema: limb	9	22.0			9	22.0
Lymphopenia	21	51.2	6	14.6	23	56.1
Nausea	22	53.7	2	4.9	23	56.1
Fatigue/malaise	21	51.2	2	4.9	21	51.2
Leukopenia (WBC)	21	51.2	2	4.9	21	51.2
Prolonged QTc interval	20	48.8	4	9.8	21	51.2
Proteinuria	18	43.9	1	2.4	19	46.3
Hypertension	15	36.6	7	17.1	18	43.9
Thrombocytopenia	17	41.5			17	41.5
Hypophosphatemia	16	39.0	4	9.8	16	39.0
Anemia	15	36.6			15	36.6
AST/SGOT	15	36.6	1	2.4	15	36.6
Neutropenia (ANC)	13	31.7	3	7.3	13	31.7
Rash	13	31.7			13	31.7
– Rash: acne/acneiform	6	14.6			6	14.6
– Rash: hand-foot skin reaction	4	9.8			4	9.8
– Rash: keratosis	1	2.4			1	2.4
– Rash/desquamation	1	2.4			1	2.4
– Hyperpigmentation	1	2.4			1	2.4
– Hypopigmentation	1	2.4			1	2.4
Muscle weakness	9	22.0	7	17.1	11	26.8
– Muscle weakness: generalized	6	14.6	5	12.2	8	19.5
– Muscle weakness: legs	4	9.8	3	7.3	6	14.6
– Muscle weakness: extraocular	1	2.4			1	2.4
Pain: headache	5	12.2	2	4.9	7	17.1
Alkaline phosphatase	6	14.6			6	14.6
Hypermagnesemia	6	14.6			6	14.6
Vomiting	5	12.2	1	2.4	6	14.6
ALT/SGPT	5	12.2			5	12.2
Hyperbilirubinemia	4	9.8			4	9.8
Vision-blurred vision	4	9.8			4	9.8
Creatinine	3	7.3			3	7.3
Hemorrhage, GI::Lower GI NOS	3	7.3			3	7.3
Hypernatremia	3	7.3			3	7.3
Allergic reaction/ hypersensitivity	2	4.9			2	4.9
Anorexia	2	4.9			2	4.9
Epistaxis	2	4.9			2	4.9
Dizziness	2	4.9			2	4.9
Hyponatremia	2	4.9			2	4.9
Infection: with grade 1–2 or unknown ANC	1	2.4	1	2.4	2	4.9
Nail changes	2	4.9			2	4.9
Perforation, GI			2	4.9	2	4.9

QTc: Corrected QT interval.

and cKIT as an attempt to further capitalize on the concept of targeting the tumor vasculature. Combined inhibition of the VEGFR and PDGFR enhances antitumor activity *in vivo*. PDGFR inhibition is hypothesized to exert its effect on pericytes, the cells that surround and support endothelial cells. These cells have abundant expression of PDGFR and require PDGF–PDGFR interaction for their normal function. We present the results of a Phase II trial of tandutinib combined with bevacizumab for recurrent glioblastomas to see if combined inhibition of VEGF, c-Kit and PDGF may result in improved tumor control. We detail the toxicity of combination therapy, including the neuromuscular junction pathology distinct to tandutinib as previously published [30].

Methods

• Study population

We enrolled 41 patient with at least 18 years of age and diagnosed with pathologically confirmed glioblastoma; patients with gliosarcoma were eligible, but none were enrolled. Radiographically confirmed tumor progression by MRI or computed tomography following standard external beam fractionated radiotherapy and temozolomide chemotherapy was required; prior bevacizumab use was prohibited. A minimum Karnofsky performance status of 60%, normal metabolic and end-organ function, and an expected survival of 2+ months were required for enrollment. Competent patients or their designated Power of Attorney/Health Care Proxy (POA/HCA) were required to sign the informed consent for this National Cancer Institute Institutional Review Board-approved trial. There were no limits on the number of prior therapies, but a minimum washout period of 2 or 4 weeks required after molecularly targeted or cytotoxic agents, respectively. A minimum of 4 weeks after radiation and full recovery after surgery were also required. Exclusion criteria included 1+ proteinuria, prolonged corrected QT interval (QTc), hypersensitivity to components of the study drugs, or significant medical illness. A stable dose of corticosteroids was required for 5+ days prior to the baseline MRI scan within 14 days of enrollment. Patients with acute intracranial hemorrhage determined by non-contrast-enhanced computed tomography or history of intracranial hemorrhage or stroke within 6 months were ineligible. Anticoagulation or antiplatelet therapy was prohibited.

• Study design

Progression-free survival at 6 months (PFS6) was the primary end point of this study. Using Kaplan–Meier methodology, we estimated PFS6 and associated 95% CI. Unless the date of progression after removal from study was verified, patients removed from study for toxicity were censored at their last evaluation on study, or start date of alternate therapy without disease progression. Target enrollment was 40 glioblastoma patients, powered to distinguish between a PFS6 of 30% for bevacizumab monotherapy and 50% expected with combination therapy of bevacizumab plus tandutinib. The historical value of 30% for PFS6 for glioblastomas based on two Phase II trials of bevacizumab, once in combination with irinotecan and the other as single agent [22,23]. Secondary end points included progression-free survival using Kaplan–Meier estimation; MRI response rates; and safety of combining tandutinib and bevacizumab in patients with recurrent malignant gliomas. Stoppage rules were also devised for excessive intracranial hemorrhage by the second cycle (8 weeks) at *a priori* accrual benchmarks.

• Treatment & patient assessment

Each cycle of therapy consisted of tandutinib monotherapy for 14 days, followed by combination tandutinib and bevacizumab. Tandutinib 500 mg b.i.d. was dosed orally, while bevacizumab 10 mg/kg was infused on day 1 and 15. Dose delays were permitted for reversible and preventable toxicity. Dosing was to continue until progression or dose-limited toxicity.

All patients underwent a perfusion MRI at baseline, within 48–96 h of their first dose of bevacizumab only, and every 4 weeks since enrollment thereafter. Peripheral blood was collected with each MRI perfusion scan to assess the phosphorylated GSK-3 levels from peripheral blood mononuclear cells. Blood counts were obtained every 2 weeks. A full metabolic screen, history, physical and neurological exam was performed prior to each cycle. MRI scans were assessed by two independent reviewers (J Sul, JA Butman) using the Response Assessment in Neuro-Oncology (RANO) criteria [31]. Specifically, stable or decreasing dose of corticosteroids and stable or improved fluid-attenuated inversion recovery (FLAIR) abnormality were required to be scored a complete response, partial response or stable disease based on complete, partial or no decrease in the enhancing tumor burden

Table 2. Treatment-related toxicities (cont.).

CTCAE v3.0 Term (n = 41)	Grade (frequency, percent)					
	1–2	%	3–4	%	Any	%
Urinary frequency/urgency	2	4.9			2	4.9
Voice changes	2	4.9			2	4.9
Hypoalbuminemia	1	2.4			1	2.4

QTc: Corrected QT interval.

based on standard post-gadolinium T1-weighted sequences. All MRI scans designated as partial or complete response were centrally reviewed (J Butman). Disease progression by RANO criteria was sufficient to terminate treatment, as was a determination of clinical progression in the absence of radiographic progression or any drug-related serious intracranial bleeding.

Results

• Study population

For the 41 patient enrolled between May 2008 and December 2009, the median age at enrollment was 55 (range 26–75). Women comprised 29% of our cohort. The median Karnofsky performance status was 90% (range 70–100%). The demographic data for our cohort are detailed in **Table 1**.

• Clinical end points

Patients were treated until progression or dose-limited toxicity for an average of 3.6 months, ranging 0.4–26.3 months. The 2-week MRI after the lead in phase of tandutinib revealed no early response to monotherapy. Of the 37 out of 41 (90%) patients evaluable for response, nine (24%) had partial response on combination bevacizumab and tandutinib. Median overall survival was 11.0 months, while median progression-free survival was 4.1 months. PFS6 was 23% (91% CI: 12–37%). The Kaplan–Meier survival curves are depicted in **Figure 1**, while response and survival data are listed in **Table 1**.

• Adverse events

All 41 enrolled patients experienced at least one treatment-related toxicity, listed by descending frequency in **Table 2**. The most common toxicities included diarrhea (78%), edema (58.5%), nausea (56.1%), lymphopenia (56.1%), leukopenia (51.2%), fatigue (51.2%), prolonged QTc (51.2%), proteinuria (46.3%), hypertension (43.9%), thrombocytopenia (41.5%), hypophosphatemia (39%), AST elevation (36.6%), anemia (36.6%), neutropenia (31.7%), rash (31.7%)

Figure 2. Tandutinib targets type III receptor tyrosine kinases. Tandutinib targets the family of type III receptor tyrosine kinases (RTKs), specifically c-Kit, PDGFR β and FLT3 [27]. Type III RTKs consist of an EC region with five immunoglobulin-like domains, a TM domain and intracellular JM and TK domains. Genetic alterations of *FLT3*, *PDGFR β* and *cKIT* have been linked to the pathogenesis and malignant transformation of various hematologic malignancies [32]. The key downstream targets of class III RTKs are the Ras-mediated Raf–MEK–ERK and PI3K–Akt–mTOR pathways, though mutated FLT3 also targets the STAT factors. These transcription factors promote cell proliferation, inhibited differentiation and/or genetic instability [33]. Diamond arrow: Inhibitory effect; Circle arrow: Site of genetic alteration; Triangle arrow: Activating effect. EC: Extracellular; ITD: Internal tandem duplication; JM: Juxtamembrane; TK: Tyrosine kinase; TM: Transmembrane.

and muscle weakness (26.8%). The most common grade ≥ 3 adverse events were hypertension (17.1%), muscle weakness (17.1%), lymphopenia (14.6%), and hypophosphatemia (9.8%). Six patients had grade ≥ 3 myasthenic-like muscle weakness associated with tandutinib with four having evidence on electromyography (EMG) of neuromuscular junction pathology.

Discussion

Glioma growth depends on angiogenesis induced by VEGF expression [11]. The objective of this

study was to assess the potential clinical benefit of combined inhibition of VEGF, c-Kit and PDGF. Bevacizumab targets inhibit angiogenesis by targeting VEGF [21]. As depicted in **Figure 2**, tandutinib targets the family of type III receptor tyrosine kinases, specifically FLT3, c-Kit and PDGFR β [27]. The intracellular domain is the site of various genetic alterations linked to the pathogenesis and malignant transformation of various hematologic malignancies [32]. The normal physiologic downstream effects include the Ras/Raf/MEK/ERK and Ras/PI3K/Akt

pathways, though mutated FLT3 also targets the STAT factors [33]. Activating mutations or upregulation of class type III receptor tyrosine kinases leads to proliferation, genetic instability and impaired cellular differentiation [33]. The effective dual inhibition of angiogenesis and proliferation was the rationale for combination therapy with bevacizumab and tandutinib in this Phase II study.

The Phase I and II trials of tandutinib showed minimal clinical benefit of monotherapy for relapsed and refractory AML, particularly those with blasts containing an activating ITD mutation of *FLT3* [28,29]. Tandutinib monotherapy showed no clinical benefit in metastatic renal cell carcinoma refractory to previous therapy with sunitinib or sorafenib [34]. Furthermore, PDGFR inhibition by tandutinib correlated with accelerated disease progression of bone metastases from prostate cancer [35]. Similarly, our trial of combination bevacizumab and tandutinib revealed response rates and survival similar to bevacizumab monotherapy, while no early responses were observed after 2 weeks of tandutinib monotherapy, suggesting tandutinib fails to enhance antitumor effects in recurrent glioblastomas. Our cohort was bevacizumab naive, not heavily pretreated and generally high functioning, so poor prognostic features are unlikely to explain the lack of response.

Tandutinib was also associated with multiple side effects. All patients experienced one or more treatment-related toxicities. Approximately 27–78% of patients experienced nausea, diarrhea, edema, fatigue, pancytopenias, prolonged QTc, hypophosphatemia, rash and muscle weakness mostly attributable to tandutinib, in addition to proteinuria, hypertension and AST elevation commonly seen with bevacizumab monotherapy [36]. As previously published, six patients had grade ≥ 3 myasthenic-like muscle weakness associated with tandutinib with four having evidence on EMG of neuromuscular junction pathology [30]. The onset of weakness occurred in <15 days in three (50%) patients, ranging from 3 to 112 days after initiation of tandutinib. Electrophysiologic studies in all six patients showed decremental response on repetitive nerve stimulation with immediate post-exercise repair along with increased jitter and blocking consistent with postsynaptic neuromuscular junction blockade. Four (66%) also had short duration motor unit potentials, while two (33%) had abnormal single-fiber EMG. The clinical

and electrophysiologic abnormalities improved with the termination or reduction in the dose of tandutinib, suggesting a dose-dependent neuromuscular pathology [30]. Similar muscle weakness was reported in doses ranging from 525 to 700 mg b.i.d. in the Phase I trials of tandutinib for acute myelogenous leukemia or myelodysplastic syndromes [28]. Unpublished *in vitro* studies by Millennium Pharmaceuticals revealed effects on nonselective CNS muscarinic nonselective receptors and muscle-type nicotinic acetylcholine receptors. High doses of tandutinib lead to tremors and incoordination in animal models. Tandutinib is thought to reversibly inhibit MuSK – a transmembrane polypeptide containing functional tyrosine kinase domains on the cytoplasmic surface at the postsynaptic neuromuscular junction. No other drug has been shown to interfere with MuSK function at the neuromuscular junction, including other anticancer FLT-3 inhibitors such as imatinib, nilotinib and dasatinib [30].

The search for more effective and durable control of glioblastomas has yielded multiple treatment options over the last decade. After maximal safe resection or stereotactic biopsy, standard treatment for newly diagnosed glioblastoma since 2005 includes involved field radiation with concurrent and adjuvant temozolomide for 6 or more months [37]. Tumor treating fields (NovoTTF) was approved in 2015 for use in combination with adjuvant temozolomide given improved overall survival compared with temozolomide alone in a pivotal Phase III randomized controlled trial [38]. There is no clear standard of care for recurrent glioblastomas, although FDA-approved options include novoTTF monotherapy (2011) [39], bevacizumab (2009) [22,40] and nitrosoureas monotherapy (1995). Combination bevacizumab plus lomustine proved more effective than either alone in recurrent glioblastoma patients [41]. Multiple combinations with bevacizumab, such as tandutinib as in our study, have not clearly shown improved response compared with bevacizumab monotherapy and often result in greater toxicity. Repeat surgery and radiotherapy with or without concurrent chemotherapy is reasonably tolerated and has shown some efficacy in select patients with recurrent glioblastoma [42–44]. Molecularly targeted agents or immunotherapy, such as rindopepimut vaccine for EGFR variant III mutations or dendritic cell vaccines targeting tumor-derived or generic tumor-specific antigens [45,46], have

shown promising results in early phase studies of recurrent glioblastomas, but await validation from ongoing Phase III studies. More effective molecularly based selection, novel targets and CNS-penetrating therapies remain the intense focus of clinical and translational research in order to personalize therapy and improve survival for patients with highly morbid and uniformly fatal glioblastomas.

Conclusion

Combination tandutinib and bevacizumab is an easily administered regimen for glioblastoma. The efficacy of combination therapy was comparable to that of monotherapy bevacizumab in patients with recurrent glioblastoma. Neuromuscular junction pathology is a distinct toxicity related to tandutinib.

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