Neuromuscular junction toxicity with tandutinib induces a myasthenic-like syndrome

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ABSTRACT

Background: Tandutinib (MLN 518, Millennium Pharmaceuticals, Cambridge, MA) is an orally active multitargeted tyrosine kinase inhibitor that is currently under evaluation for the treatment of glioblastoma and has been used in the treatment of leukemia. In prior clinical and animal studies, a dose-dependent muscular weakness has been observed with this drug, though the etiology of the weakness has not been defined.

Methods: Standard neurophysiologic techniques, including repetitive nerve stimulation, needle EMG, and single-fiber EMG, were used to evaluate patients who developed weakness while being treated with tandutinib and bevacizumab (Avastin, Genentech, South San Francisco, CA) for glioblastoma (NCT00667394).

Results: Six patients were observed to develop a reversible weakness that correlated with the administration of the tandutinib. The onset of weakness after starting tandutinib occurred within 3 to 112 days and in less than 15 days in 3 patients. Electrophysiologic studies showed that all patients developed abnormal repetitive nerve stimulation studies. Four patients had short duration motor unit potentials. Two of these patients also had abnormal single-fiber EMG, as did a third patient who did not have standard needle EMG. The clinical and electrophysiologic abnormalities improved with the termination or reduction in the dose of tandutinib.

Conclusion: These observations suggest that tandutinib is toxic to the neuromuscular junction, possibly by reversibly binding to a molecule on the postsynaptic acetylcholine receptor complex.

Classification of evidence: This study provides Class III evidence that tandutinib 500 mg twice daily induces reversible muscle weakness and electrophysiologic changes consistent with neuromuscular junction dysfunction. *Neurology*® 2011;76:236-241

GLOSSARY

ACh = acetylcholine; **CMAP** = compound muscle action potential; **FLT3** = FMS-like tyrosine kinase-3; **GBM** = glioblastoma; **MUP** = motor unit potential; **MuSK** = muscle-specific kinase; **PDGF** = platelet-derived growth factor; **RNS** = repetitive nerve stimulation; **SFEMG** = single-fiber EMG.

Tandutinib is a small molecule that inhibits tyrosine kinase and is being studied as a targeted agent in the treatment of glioblastoma (GBM), though previously studied in the treatment of leukemia.¹ Tandutinib inhibits cellular proliferation and induces apoptosis through inhibition of tyrosine kinases such as FMS-like tyrosine kinase-3 (FLT3), c-Kit, and platelet-derived growth factor (PDGF).² Specifically, the disruption of PDGF and c-Kit pathways may be useful for inducing apoptosis in glioma cells³ and inhibiting tumor angiogenesis.^{4,5}

Preclinical and clinical studies have shown that tandutinib has effects on the nervous system. In vitro toxicology studies have demonstrated that the drug has activity against the nonselective CNS muscarinic nonselective receptors and muscle-type nicotinic acetylcholine receptor (Millennium Pharmaceuticals, unpublished data). In animal studies, high doses of oral tandutinib produced tremor and incoordination. Phase I clinical trials in patients with acute myelogenous

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leukemia or myelodysplastic syndromes found that the primary dose limiting toxicity was generalized muscle weakness and fatigue when using doses in the range 525 mg and 700 mg twice a day.⁶ These symptoms resolved within 24 to 72 hours after discontinuing the drug.

In this report, we present a series of 6 patients with GBM being treated with tandutinib and bevacizumab according to protocol (NCT00667394) who subsequently developed weakness and abnormal neurophysiologic findings. The findings corroborate earlier reports of a reversible weakness related to tandutinib administration, and more specifically examine the effect of tandutinib on the neuromuscular junction.

METHODS **Standard protocol approvals, registrations, and patient consents.** All subjects signed a written consent and were enrolled into the protocol—A Phase 2 Trial of Tandutinib in Combination with Bevacizumab for Treating Patients with Recurrent High-Grade Glioma (NCT00667394)—which was approved by the National Cancer Institute's institutional review board at the NIH. This study provides Class III evidence that tandutinib 500 mg twice daily induces reversible muscle weakness and electrophysiologic changes consistent with neuromuscular junction dysfunction.

Treatment protocol. For the first cycle of treatment, oral tandutinib was started on day 1, with a dose of 500 mg twice daily for 6 weeks. Bevacizumab infusions began on day 15 and were administered every 2 weeks in all subjects. The subsequent cycles were the same except only 4 weeks in duration. Forty subjects have been enrolled in the clinical trial but only subjects who developed clinical signs of neuromuscular weakness were referred to the EMG laboratory and presented in this article.

Neurophysiologic studies. Neurophysiologic studies were performed only on subjects who developed signs of neuromuscular weakness and not prospectively on all subjects in the clinical trial. The subjects were evaluated by standard nerve conduction techniques⁷ on a Viking Select Machine, Viasys, Madison, WI. Repetitive nerve stimulation (RNS) studies were performed at 3 Hz for 5 stimuli at rest and at 0 seconds, 30 seconds, and 1, 2, 3, and 5 minutes after 1 minute of exercise. Needle EMG, using concentric EMG needle, was performed on proximal and distal limb muscles. Single-fiber EMG (SFEMG) using a SFEMG needle was performed on the extensor digitorum communis, if indicated. Motor unit potential (MUP) stability was not formally assessed during standard concentric needle examination, but was quantitated by SFEMG.

RESULTS The clinical findings of the 6 patients show that they all developed facial weakness, neck weakness, and proximal greater than distal limb weakness while on the treatment protocol (table 1). None of the patients had diplopia or dysphagia. The patients studied had no known prior neuromuscular

disorders. Patient 2 had a history of treated hypothyroidism and steroid-induced diabetes and had the most rapid onset of disease. These 2 endocrine disorders were not present in the other patients. All patients were on dexamethasone to control vasogenic edema related to GBM and seizure medication, generally levetiracetam. Acetylcholine receptor antibodies, striated muscle antibodies, and voltage-gated calcium channel antibodies were negative in patients 2 and 3. Muscle-specific kinase (MuSK) antibodies were studied only in patient 6 and were negative. Two patients needed to discontinue tandutinib permanently after recurrence of muscle weakness on reduced tandutinib doses, but other patients were able to tolerate reintroduction of drug at lower doses without significant clinical weakness. Bevacizumab infusion was not temporally associated with the onset of weakness except in patient 4, whose weakness improved after lowering the tandutinib dose, though he continued the same bevacizumab dose.

The electrophysiologic findings from the 6 patients are summarized in table 2 (see tables e-1 and e-2 on the *Neurology*[®] Web site at www.neurology.org). Abnormal decremental responses were observed in all patients with RNS and improved with the discontinuation of the tandutinib in 3 patients evaluated after being off drug for 1 week (figure). There was abnormal jitter with blocking in the 3 patients evaluated by SFEMG. One subject continued to have relatively mild increased jitter on SFEMG after being off tandutinib for 1 week even though the RNS had normalized. Needle EMG showed small short-duration motor units in 4 patients while on tandutinib that may have been related to neuromuscular junction blockade or a myopathy. The temporal onset of the needle EMG findings with tandutinib administration was not ascertained since no patient had needle EMG studies prior to drug or after discontinuing drug.

The standard nerve conduction studies showed low peroneal amplitudes in patients 1, 2, 4, and 5 on initial studies with normal distal latencies and conduction velocities with normal sensory responses for age (table e-1). The median or ulnar motor nerve conduction studies were normal except for prolonged distal latency in patient 4 and an isolated ulnar neuropathy in patient 2. In patient 2, there was 35% decline in the median compound muscle action potential (CMAP) and a decline in the facial CMAP over the 3 studies. The other 2 patients with postdrug studies did not show a similar decline in amplitude.

DISCUSSION The findings in this small series of patients suggest that tandutinib causes a reversible

Table 1 Summary of clinical findings

Patient	Age, y/sex/race/diagnosis	Symptoms (day of exam)	Tandutinib treatment (dose in mg/day)	Muscle testing (MRC)					
				Face	Neck	Proximal arm (R/L)	Distal arm (R/L)	Proximal leg (R/L)	Distal leg (R/L)
1	60/M/Asian/R GBM	Day 14: Onset of head droop and leg weakness Day 56: Sx resolved Day 100: Recurrence of weakness on lower dose	Day 1-43: 1,000 Day 56-100: 600 Day >100: Drug discontinued	4	Flex 4 Ext 3	4/4	4-5/4-5	4/4	5/5
2	61/M/Asian/R GBM	Day 3: Onset of head droop and leg weakness Day 14: Sx resolved	Day 1-7: 1000 Day 15-29: 600	5 4+	Flex 4 Ext 5 Flex 4+ Ext 5	5/4 5/4+	5/4 5/4	4/4 4+/4+	4-5/4-5 5/4-5
		Day 29: Recurrence of weakness on lower dose	Day >29: Drug discontinued	4	Flex 4 Ext 5	5/4	5/4	4/4	4-5/4-5
3	50/M/Asian/R GBM	Day 10: Onset of head droop, arm and leg weakness Day 21: Sx resolved 1 week off drug	Day 1-15: 1,000 Day 22-70: 600 Day >70: Drug discontinued	4 5	Flex 4 Ext 5 Flex 5 Ext 5	5/4 5/5	4-5/4-5 5/5	5/5 5/5	5/5 5/5
4	55/M/Caucasian/L GBM	Day 30: Onset of leg and arm weakness Day 43: Sx resolved 1 week off drug	Day 1-36: 1,000 Day 43-61: 600 Day >161: On drug	3	Flex 4	3/3 5/5	4/4 5/5	4/4 4/5	4-5/4-5 5/5
5	54/M/Caucasian/R GBM	Day 112: Onset of leg weakness Day 133: Sx resolved 1 week off drug	Day 1-126: 1,000 Day 133-175: 400; on drug	4	Flex 4 Ext 5	4/4-	4-5/3-4	3/3	5/5
6	60/M/Caucasian/L GBM	Day 63: Onset of leg weakness	Day 1-77: 1,000 Day >77: On drug	4/5	Flex 4 Ext 5	4/4	5/5	4/4	5/5

Abbreviations: Ext = neck extensors; Flex = neck flexors; GBM = glioblastoma; MRC = Medical Research Council (5/5 strength testing with 5 = full strength and 0 = no muscle movement); sx = symptoms.

inhibition of the postsynaptic neuromuscular junction. The neurophysiologic findings of a decremental response on repetitive nerve stimulation with immediate postexercise repair along with increased jitter and blocking are classic findings for postsynaptic neuromuscular junction blockade. The onset of clinical symptoms of weakness occurs within 3 to 112 days of initiating tandutinib. A cumulative effect with prolonged administration was observed in one patient (no. 2) who had more abnormalities in his second study while on a lower dose of tandutinib. The relatively rapid onset of symptoms following the initiation of tandutinib suggests that this agent has a direct effect on the neuromuscular junction rather than generating an immune-mediated response. The second drug in the study, bevacizumab, is an anti-angiogenesis factor that has previously been studied in GBM and has not been associated with weakness.⁸ The bevacizumab biweekly regimen did not appear to correlate with worsening of weakness and one patient (no. 2) had onset of weakness prior to initiating bevacizumab. Most patients had been on dexamethasone for control of vasogenic edema and could have had some steroid myopathy prior to the tandutinib and bevacizumab protocol. The specific effect of the

combined steroid myopathy and neuromuscular junction drug effect is not known but weakness has been seen in other clinical trials using tandutinib in the absence of steroids.

A potential target of tandutinib is MuSK in the postsynaptic neuromuscular junction receptor complex. Antibodies to MuSK are associated with a form of myasthenia gravis that has a predilection for the bulbar muscles as well generalized weakness⁹ and it is possible that nonantigenic modulation of the MuSK protein may also result in a defect in neuromuscular transmission. MuSK is a transmembrane polypeptide containing functional tyrosine kinase domains on the cytoplasmic surface and several immunoglobulin-like protein subunits on the outer membrane surface. It is thought to be involved in acetylcholine (ACh) receptor clustering on the postsynaptic membrane and have a role in maintaining the integrity of the neuromuscular junction.¹⁰ As a tyrosine protein kinase, MuSK protein may be susceptible to inhibition by tandutinib. The resulting change in phosphorylation of the ACh receptor may lead to destabilization of the ACh receptor complex and rearrangement of the receptor architecture at the junctional folds with subsequent, reduced safety factor for neuromuscular

Table 2 Summary of neurophysiologic findings

Patient	Neurodiagnostic studies	RNS (% decrement of amplitude)		SFEMG (EDC)	EMG	
		Hand	Face		Qualitative	Muscle (no. of mu) and mu duration
1	Day 43 (full dose)	Right ulnar Pre 16% Post 0 sec 11% Post 2 min 21% Post 5 min 20% <i>Abnormal</i>	Right facial Pre 26% Post 0 sec 28% Post 2 min 32% Post 5 min 30% <i>Abnormal</i>	MCD 73 μ s (16 pairs); Blocking 56% Abnormal individual pairs: 12/16 <i>Abnormal</i>	Trapezius: nl mu Deltoid: small mu FDI: nl mu Vastus L: small mu Tibialis ant: nl mu C5/C7 parasp: nl mu	Deltoid (18) 7.4 ms Vastus L (17) 10.1 ms <i>Abnormal</i>
2	Day 7 (full dose)	Right median Pre 6% Post 0 sec 1% Post 2 min 4% Post 4 min 5% <i>Abnormal</i>	Right facial Pre 7% Post 0 sec 1% Post 2 min 5% Post 6 min 9% <i>Abnormal</i>	MCD 104 μ s (8 pairs) Blocking 88% Abnormal individual pairs: 7/8 <i>Abnormal</i>	Trapezius: nl mu Deltoid: small mu FDI: nl mu Iliacus: small mu Vastus L: nl mu Tibialis ant: nl mu	Deltoid (10) 12.0 ms Iliacus (14) 7.7 ms <i>Abnormal</i>
	Day 29 (low dose)	<u>Right median</u> Pre 7% Post 0 sec 4% Post 2 min 9% Post 5 min 8% <i>Abnormal</i>	Right facial abnormal Pre 19% Post 0 sec 12% Post 2 min 15% Post 5 min 14% <i>Abnormal</i>	MCD 149 μ s (3 pairs) Blocking 100% Abnormal individual pairs: 3/3 <i>Abnormal</i>	ND	ND
	Day 36 (1 week off drug)	Right median Pre 3% Post 0 sec 4% Post 2 min 1% Post 5 min 1% <i>Normal</i>	Right facial Pre 1% Post 0 sec 2% Post 2 min 0% Post 5 min 0% <i>Normal</i>	ND	ND	ND
3	Day 15 (full dose)	Right ulnar Pre 14% Post 0 sec 7% Post 2 min 20% Post 5 min 18% <i>Abnormal</i>	Right facial Pre 35% Post 0 sec 25% Post 2 min 33% Post 5 min 32% <i>Abnormal</i>	ND	Trapezius: nl Deltoid: nl FDI: nl Vastus L: nl Tibialis ant: nl C7 parasp: CRD	ND
	Day 22 (1 week off drug)	Right ulnar Pre 2% Post 0 sec 0% Post 2 min 5% Post 5 min 5% <i>Normal</i>	Right facial Pre 11% Post 0 sec 9% Post 2 min 12% Post 5 min 12% <i>Abnormal</i>	MCD 38 μ s (20 pairs) Blocking 5% Abnormal individual pairs: 2/20 <i>Abnormal</i>	ND	ND
4	Day 36 (full dose)	Left ulnar Pre 28% Post 0 sec 20% Post 2 min 26% Post 5 min 25% <i>Abnormal</i>	Left facial Pre 42% <i>Abnormal</i>	ND	Deltoid: small mu FDI: nl Vastus L: nl Tibialis ant: nl	Deltoid (20) 6.4 ms <i>Abnormal</i>
	Day 43 (1 week off drug)	Left ulnar Pre 13% Post 0 sec 9% Post 2 min 17% Post 5 min 13% <i>Abnormal</i>	Left facial Pre 43% <i>Abnormal</i>	ND	ND	
5	Day 126 (full dose)	Left ulnar Pre 15% Post 0 sec 11% Post 2 min 17% Post 5 min 17% <i>Abnormal</i>	Left facial Pre 47% <i>Abnormal</i>	ND	Deltoid: nl FDI: nl Vastus lat nl	ND
6	Day 70 (full dose)	Left ulnar Pre 20% Post 0 sec 12% Post 3 min 19% Post 5 min 21% <i>Abnormal</i>	Left facial Pre 37% Post 0 sec 31% Post 3 min 41% Post 5 min 46% <i>Abnormal</i>	ND	Deltoid: small mu FDI: small mu	Deltoid (6) 7.9 ms FDI (6) 10.4 ms <i>Abnormal</i>

Abbreviations: CRD = complex repetitive discharges; EDC = extensor digitorum communis; FDI = first dorsal interossei; MCD = mean consecutive difference; mu = motor units; ND = not done; nl = normal; parasp = paraspinal muscle; Post 0 = immediately following exercise; Post 2 min = 2 minutes post 1-minute exercise; Post 5 min = 5 minutes following 1-minute exercise; Pre = pre-exercise repetitive nerve stimulation; RNS = repetitive nerve stimulation; SFEMG = single-fiber EMG; tibialis ant = tibialis anterior; vastus L = vastus lateralis.

transmission. Tandutinib is related to the FLT-3 inhibitors, imatinib, nilotinib, and dasatinib, which reportedly do not interact with MuSK,¹¹ but similar studies have not been performed with tandutinib.

Other tandutinib targets could include an effect on PDGF and its receptor since these have also been observed to be colocalized to the acetylcholine receptor at the neuromuscular junction.¹² However, alter-

RNS of the ulnar nerve recording at the adductor digiti minimi (ADM) (A, B) and facial nerve recording at the nasalis (C, D) was performed on patient 3. Initial study (A, C) was performed while patient 3 was on tandutinib. Follow-up study (B, D) was performed while the same patient was off tandutinib for 1 week. All studies in the figure were performed by stimulating the ulnar nerve or facial nerve at 3 Hz for 5 stimuli prior to 1-minute exercise. While on tandutinib, the decremental response was 14% for the ADM and 36% for the nasalis. After the tandutinib was discontinued for 1 week, the decremental response at rest was 5% for the ADM and 11% for the nasalis. The amplitudes increased off the tandutinib for patient 3 but not in the other patients with serial studies (patients 2 and 4).

ation of PDGF has not been associated with any defects in neuromuscular transmission.

Many drugs have been associated with inducing myasthenia gravis or worsening the clinical symptoms of myasthenia gravis.¹³ To date, no drug has been identified as specifically interfering with MuSK function at the neuromuscular junction. Further studies are needed to determine if tandutinib is affecting MuSK or some other component of the neuromuscular junction.

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DISCLOSURE

Dr. Lehky, Dr. Iwamoto, and Dr. Kreisl report no disclosures. Dr. Floeter serves on the editorial board of *Muscle and Nerve*. Dr. Fine reports no disclosures.

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