A Randomized Placebo-Controlled Multicenter Trial of Monotherapy with TNX-601 ER* (Tianeptine Hemioxalate Extended-Release Tablets) for Treatment of Major Depressive Disorder (MDD)

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* TNX-601 ER is an investigational new drug and has not been approved for any indication

INTRODUCTION

In the US in 2020, ~21 million adults experienced at least 1 major depressive episode (MDE). Despite an array of antidepressants, mostly targeting monoaminergic (MA) neurotransmitters, only ~20% achieve remission. Importantly, MA antidepressants have intolerable side effects for many, including sexual dysfunction, agitation, insomnia with daytime sedation, weight gain, and mild cognitive impairment.

Tianeptine is a unique non-MA antidepressant used across Europe, Asia, and Latin America, first marketed in France in 1989. There are over 30 years of studies demonstrating that tianeptine's efficacy is comparable to both the SSRIs and TCAs. Tianeptine also appears to be much more tolerable than MA antidepressants, without significant sexual side effects, adverse effects on sleep, or weight gain. Other unique advantages of tianeptine include anxiolysis without sedation, and pro-cognitive effects.

Until recently, the mechanism of action was elusive and distinctive, lacking direct effects on CNS receptors and transporters. The predominant hypothesis of its mechanism of action in depression has been that it indirectly modulates the glutamatergic system, thereby reversing the adverse effects of stress on hippocampal function, stimulating new interneural connections, and restoring neuroplasticity through synaptogenesis.[1] Subsequently, it has been discovered that the enhancement of these connections is also a common therapeutic principle for traditional antidepressants, which modulate neuroplasticity indirectly by modulating synaptic neurotransmitters.

Tianeptine and its main metabolite MC5 are also weak μ-opioid receptor agonists, presenting an abuse liability if illicitly misused in large quantities (typically 8-80X therapeutic dose daily[2]). Prescribed for depression, incidence of misuse is approximately 0.1%,[3] suggesting low abuse liability when used as antidepressant under clinical care.

A novel once-daily extended-release formulation of tianeptine hemioxalate, TNX-601 ER, is being developed by Tonix Pharmaceuticals under an investigational new drug (IND) in the US. A randomized, placebo-controlled, multicenter Phase 2 trial for the treatment of major depressive disorder (MDD) is currently enrolling across about 30 US sites. The methods of this trial will be presented, and the enrollment status updated. Additionally, several critical new findings from discovery and nonclinical work will be presented which clarify tianeptine's unique molecular mechanism of action in treatment of depression.

[1]McEwen BS, et al. Mol Psychiatry. 2010;15(3):237-249. [2]Lauhan R, et al. Psychosomatics. 2018;59(6), 547-553. [3]Haute Authorite de Sante; Transparency Committee Opinion 5 Dec 2012.

Pharmacokinetics and Formulation

Table 1	TNX-601 ER 39.4 mg (fasted) N=12	TNX-601 ER 39.4 mg (fed) N=12	TNX-601 ER 39.4 mg (fasted) N=12	TNX-601 ER 39.4 mg (fed) N=12
Parameter (Mean)	Tianeptine		Metabolite MC5	
$AUC_{0\text{-}24}$ (ng.h/mL)	2040	1990	1220	1270
$AUC_{0\text{-}last}$ (ng.h/mL)	2300	2060	1750	1700
F_{rel} $AUC_{0\text{-}last}$ (%)	89.22 [81.59, 97.56], p=0.043		97.02 [90.55, 103.96], p=0.45	
$AUC_{0\text{-}inf}$ (ng.h/mL)	2360	2230	2030	1830
F_{rel} $AUC_{0\text{-}inf}$ (%)	92.81 [84.63, 101.77], p=0.17		93.57 [86.25, 101.51], p=0.17	
C_{max} (ng/mL)	230	321	76.3	102
F_{rel} C_{max} (%)	139.70 [114.19, 170.91], p=0.013		134.19 [117.30, 153.51], p=0.002	
AUC_{extrap} (%)	1.944	1.691	6.821	6.198
T_{max} (h)[a]	3.500	5.000	8.042	8.000
$T_{1/2}$ (h)	6.874	5.050	11.306	11.175
Vz/F (L)	150	116	*ND	*ND

AUC area under the curve; C_{max}, max. observed conc. (MOC); F_{rel}, relative bioavailability; T_{max}, time of MOC; $T_{1/2}$, apparent elimination half-life; Vz/F, apparent volume of distribution (for plasma tianeptine only); *ND, no data

TNX-601 ER was formulated with attention to potential abuse deterrent properties: lower solubility of hemioxalate salt (reduced extraction efficiency); microcrystalline cellulose as compression aid and compressed at >100 Newtons (difficulty crushing to fine particles for efficient insufflation or extraction); and inclusion of high molecular weight gel-forming polymers (poor "syringe-ability"/injectability).

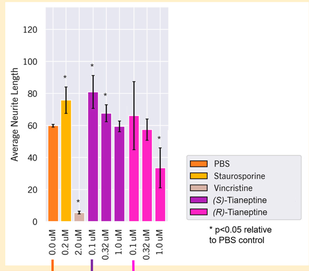
Mechanism of Action of Racemic Tianeptine in Depression and Potential of Single Isomer, TNX-4300#, in Psychiatric and Neurodegenerative Conditions

CNS Activities: Tianeptine, *(S)*- & *(R)*-Isomers

	Racemic-Tianeptine	*(S)*-Tianeptine TNX-4300	*(R)*-Tianeptine
μ-Opioid Receptor	+	-	+
PPAR-β/δ	+	+	-
PPAR-α	-	-	-
PPAR-γ	+	+	+
FST	+	-	+
Neurite Outgrowth	ND	+	-

ND, no data

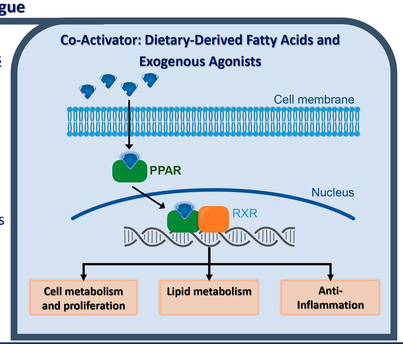
Treatment with 0.1 and 0.32 μM *(S)*-Tianeptine Significantly Increased Average Neurite Length

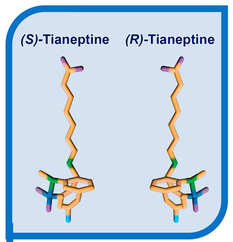


Tianeptine is a Polyunsaturated Fatty Acid (PUFA) Analogue

PUFAs and Analogues:
Distinct Ligand-Target Interactions with PUFA Binding Proteins

- Peroxisome proliferator-activated receptors (PPARs) are members of the ligand-activated transcription factors of nuclear receptors
- Three isotypes: PPAR-α, PPAR-β/δ, and PPAR-γ
- Regulate important cellular metabolic and proliferative functions, lipid metabolism and anti-inflammatory effects
- Natural ligands for PPAR-β/δ include PUFAs (e.g., docosahexaenoic acid, arachidonic acid) and their metabolites
- In brain, high levels PPAR-β/δ in hypothalamus and hippocampus
- Chronic stress reduces PPAR-β/δ levels, whereas overexpression or activation of hippocampal PPAR-β/δ produces antidepressant-like effects



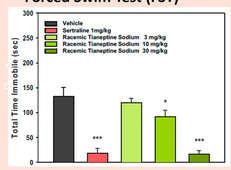


Tianeptine is 50:50 Mixture of Mirror Image Isomers

μ-Opioid Activity Studies of Tianeptine and its Pure *(S)*- and *(R)*-Isomers

- The >99.9% pure Tianeptine (*S*)-Isomer shows ***no activity*** in μ-opioid receptor agonism (β-arrestin and cAMP) studies
- In contrast, Tianeptine *(R)*-isomer has β-arrestin recruitment EC_{50}=1.873 μM and cAMP inhibition EC_{50}=0.044 μM
- Tianeptine (*S*)-Isomer in development by Tonix, TNX-4300 (estianeptine) for psychiatric and neurodegenerative conditions

Racemic Tianeptine in the Forced Swim Test (FST)

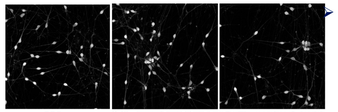


*p<0.05, ***p<0.001 compared to vehicle

(S)-Tianeptine Significantly Increases Neurite Length in Culture

Neurite network dynamics tested in plated human iPSC-derived glutamatergic neurons

- Increase in average neurite length in culture for *(S)*-Tianeptine indicates plastogenic effects which are independent μ-opioid activity (μ-opioid activity only in *(R)*-isomer)
- Consistent with neuroregenerative effects of tianeptine on dendritic connectivity of glutamatergic neurons in hippocampus CA3 region, identified by McEwen & Coworkers



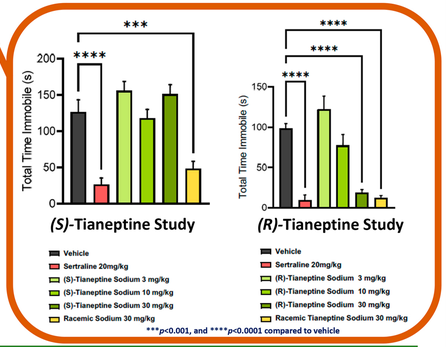
Tianeptine Reduces Immobility in Murine Forced Swim Test

Similar Acute Effects Reported by Samuels & Co-Workers, 2017^

- Samuels et al found no effect of tianeptine on FST activity in μ–opioid receptor KO mouse or in mice pretreated with opioid antagonists, indicating behavioral effect was μ–opioid mediated
- Their murine PK studies demonstrated tianeptine rapidly metabolized and nearly eliminated from murine plasma and brain after 1 hour (the time after tianeptine their FST was performed); whereas MC5 metabolite detectable for at least 8 hours
- Authors suggest MC5 is expected to play a major role in mediating the behavioral effects on FST in mice

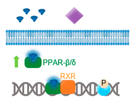
^Samuels BA, et al. Neuropsychopharmacology. 2017;42(10):2052-2063

(S)-Tianeptine not active in FST, *(R)*-Tianeptine is active

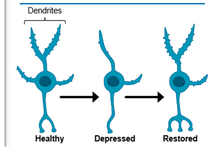


Tianeptine Restores Neuroplasticity After Stressor-Induced Atrophy

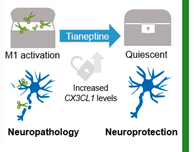
(S)-Tianeptine activation of PPAR-β/δ appears related to tianeptine's known effect on reducing inflammation and encouraging cell growth and development



Tianeptine administration reversed stress-induced dendrite shrinkage in hippocampal CA3 dendrites



CX3CL1 levels were normalized by tianeptine treatment reflecting effects on microglia



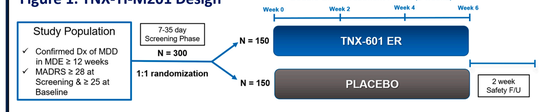
Tianeptine activation of PPAR-β/δ regulates the expression of genes that stimulate dendrite arborization and reduce proinflammatory microglia activation. By these activities, tianeptine is thought to treat depression at the neuroplastic and neurogenerative levels[1-4]

1. Ji MJ, et al. *Int J Neuropsychopharmacol.* 2015;19(1):pyv083. 2. Liu K, et al. *Clin Exp Pharmacol Physiol.* 2017;44(6):664-670. 3. Magariños AM, et al. *Eur J Pharmacol.* 1999;371(2-3):113-122. 4. Trojan E, et al. *Front Pharmacol.* 2017;8:779.

Phase 2 Study

UPLIFT Study

Figure 1: TNX-TI-M201 Design



- **Visits (6):** Screening, Baseline, Week 2, Week 4, Week 6, & Week 8 Safety Follow-Up
- **Eligibility:**
  - Ages 18-65 meeting a DSM-5 diagnosis of MDD, with current MDE > 12 weeks
  - Screening Montgomery-Åsberg Depression Rating Scale (MADRS) ≥ 28
  - Baseline MADRS <25 and >25% change from Screening score are both exclusionary
  - No acute or confounding medical conditions
  - No lifetime bipolar, psychotic, or antisocial or borderline personality disorder
  - No current obsessive-compulsive disorder, PTSD, or anorexia nervosa
  - No past year alcohol or substance use disorder; no lifetime opioid or sedative-hypnotic use disorder
  - No use of antidepressants, antipsychotics, mood stabilizers, stimulants, benzodiazepines, buspirone, or anticonvulsants (exception for migraineurs)
  - Greater than mild traumatic brain injury by history is exclusionary
- **Investigational Product (IP):** TNX-601 ER (tianeptine hemioxalate extended-release tablets) 39.4 mg; subjects should take 1 tablet daily in morning with breakfast/food.
- **Primary Endpoint:** change from baseline (CFB) in MADRS total score at Week 6
- **Key Secondaries:** CFB in Clinical Global Impression – Improvement score at Week 6; CFB in Sheehan Disability Scale total score at Week 6
- **Safety Measures:** AEs/SAEs; physical/neuro exams; clinical labs; vital signs; ECG; Columbia-Suicide Severity Rating Scale (C-SSRS); Changes in Sexual Functioning Questionnaire short form (CSFQ-14); Misuse, Abuse, Diversion Drug Event Reporting System (MADDERS®) and Subjective Opiate Withdrawal Scale (SOWS) to monitor for abuse, diversion, withdrawal
- Statistical Analyses: mixed model repeated measures (MMRM) approach with covariates that include fixed categorical effects of treatment, site, visit, and treatment by visit interaction; and continuous fixed covariates of baseline score and baseline score by visit interaction. Missing data will be imputed via multiple imputation (MI)
- Phase 1 PK parameters for the developed once-daily TNX-601 ER formulation are in **Table 1**:

CONCLUSIONS

- **First participant enrolled on 14 March 2023; to date, ~15% have been randomized**
- **Interim analysis to occur upon first 50% patient completed, estimated in Q4 2023**
- **As illustrated in the Mechanism of Action panel, isolation of pure enantiomers of tianeptine has led to a new understanding of its mechanism of action as an antidepressant and future potential in other CNS conditions; tianeptine is a PPAR-β/δ and PPAR-γ agonist, binding intracellularly and activating transcription factors; tianeptine's unique properties with respect to restoration of neuroplasticity are explained by the PPAR mechanism, as are the lack of typical monoaminergic side effects such as sexual dysfunction and weight gain; anti-inflammatory, metabolic, and neuroprotective effects suggest potential uses in neurodegenerative conditions such as Alzheimer's disease**
- **"Follow on" development of pure *(S)*-tianeptine isomer (TNX-4300#), which is devoid of μ–opioid receptor activity, offers potential for expanded dose range in other CNS conditions without abuse liability at high doses of the racemic**

TNX-4300 is a pre-clinical stage investigational new drug and has not been approved for any indication

ACKNOWLEDGEMENTS

Other Tonix scientists who contributed significantly to the studies on mechanism and formulation development include:

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