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ANK3 as a Novel Genetic Biomarker for Liafensine in Treatment-Resistant Depression
The ENLIGHTEN Randomized Clinical Trial

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IMPORTANCE This study represents a first successful use of a genetic biomarker to select potential responders in a prospective study in psychiatry. Liafensine, a triple reuptake inhibitor, may become a new precision medicine for treatment-resistant depression (TRD), a major unmet medical need.

OBJECTIVE To determine whether ANK3-positive patients with TRD benefit from a 1-mg and/or 2-mg daily oral dose of liafensine, compared with placebo, in a clinical trial.

DESIGN, SETTING, AND PARTICIPANTS A novel pharmacogenomic biomarker, ANK3, was discovered as a predictor of liafensine's efficacy retrospectively. In this biomarker-guided, randomized, double-blind, placebo-controlled, phase 2b clinical trial conducted at 58 sites from July 2022 through March 2024, 1967 patients were assessed for eligibility and 189 ANK3-positive patients with TRD were randomized. Key exclusion criteria included specified disorders, concomitant medications, or organ dysfunction. Investigators, patients, raters, and sponsors were blinded to ANK3 status and treatment. Data analysis was performed from March 26 to April 23, 2024.

INTERVENTIONS Patients were randomized 1:1:1 to once-daily oral liafensine, 1 mg; once-daily oral liafensine, 2 mg; or oral placebo once daily.

MAIN OUTCOMES AND MEASURES The primary outcome was the Montgomery-Åsberg Depression Rating Scale (MADRS) total score change from baseline to day 42.

RESULTS Of the 189 ANK3-positive patients with TRD who were randomized, 188 received study drug (mean [SD] age, 43.2 [14.8] years; 119 [63.3%] female), and 186 had at least 1 dose of study drug and 1 postrandomization efficacy evaluation. The mean (SE) MADRS score change in these patients was −15.4 (0.9) for liafensine (including both 1- and 2-mg doses) vs −11.0 (1.3) for placebo (mean treatment difference, −4.4; 95% CI, −7.6 to −1.3; $P = .006$). Statistically significant improvements were also seen in all secondary end points. Adverse events were tolerable, with low rates of meaningful events. Adverse events leading to discontinuation of treatment occurred in 5 patients (4.0%) receiving liafensine and 9 (14.1%) receiving placebo.

CONCLUSIONS AND RELEVANCE Liafensine was efficacious and well tolerated in ANK3-positive patients with TRD, with clinically meaningful and statistically significant improvements over placebo suggesting ANK3 as a predictive genetic biomarker for liafensine. This represents a first successful prospective genetic biomarker-guided trial in psychiatry.

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Visual Abstract

Supplemental content

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Major depressive disorder (MDD) is a leading cause of disability,[1,2] and treatment-resistant depression (TRD) represents a major unmet medical need with high rates of suicide and comorbidities.[3-5] It is estimated 30% to 55% of patients with MDD meet the criteria for TRD (2 antidepressants failed).[6,7] Currently, there are only 2 approved drugs for TRD: Symbyax (olanzapine and fluoxetine; Eli Lilly and Co) and Spravato (esketamine; Janssen). Both have demonstrated efficacy, but with notable adverse reactions.[8,9] Additionally, esketamine is a Schedule III controlled substance, available only through a risk evaluation and mitigation strategy program. Novel therapeutic approaches with better risk-benefit profiles are needed to address this unmet need.

The monoamine hypothesis of depression postulates dysfunction of the serotonin, norepinephrine, and dopamine systems in the brain.[10-12] Patients with TRD often fail to respond to selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs). Thus, an inhibitor of all 3 monoamine transporters, a triple reuptake inhibitor, may present a new treatment option. Liafensine, a potent and selective triple reuptake inhibitor, does not display many of the adverse effects associated with existing TRD therapies.[7,8,12-14] However, it failed to demonstrate efficacy in 2 prior large-scale phase 2b trials in non-biomarker-selected patients.[13,14]

Given the clear mechanism of action and the complexities of TRD, we hypothesized that a subset of patients might benefit from liafensine. A genome-wide scan using germline DNA extracted from blood samples of patients from the prior phase 2b trials demonstrated a strong correlation between single-nucleotide polymorphism rs12217173 status and efficacy in patients treated with liafensine.[15] Single-nucleotide polymorphism rs12217173, also named DGM4 (Denovo Genomic Marker 4), resides in the *ANK3* gene. The *ANK3* gene product is a scaffolding protein primarily expressed in the nervous system, plays an important role in neuronal signaling through modulation of cell membrane proteins, and is linked to psychiatric diseases, including depression.[16-19] Approximately 20% of patients were ANK3 biomarker positive. Liafensine demonstrated significant efficacy compared with the control in this subset. The remaining 80% of patients, who were ANK3 negative, showed no difference between liafensine vs control. Thus, the objective of the present biomarker-guided trial was to prospectively confirm liafensine's efficacy in ANK3-positive patients. While liafensine did not exhibit efficacy at any dose in non-biomarker-selected patients in the prior studies,[13] a dose response was observed in ANK3-positive patients from 0.25 mg up to 1 mg, then similar efficacy was observed between 1 mg and 2 mg.[15] Thus, 1-mg and 2-mg doses were evaluated in the current study, with the primary end point using the combined results to increase statistical power.

Key Points

Question Does the newly discovered ANK3 pharmacogenomic biomarker predict the response of patients with treatment-resistant depression (TRD) to liafensine, a triple reuptake inhibitor, despite failure in a non–biomarker-selected TRD patient population in prior phase 2b trials?

Findings In this randomized clinical trial including 189 ANK3-positive patients with TRD, liafensine demonstrated a 4.4-point Montgomery-Åsberg Depression Rating Scale improvement over placebo, a clinically meaningful and statistically significant difference.

Meaning This represents a first successful genetic biomarker-guided clinical trial in psychiatry, advancing a new treatment for TRD and providing a new path for developing precision medicines in the field.

Methods

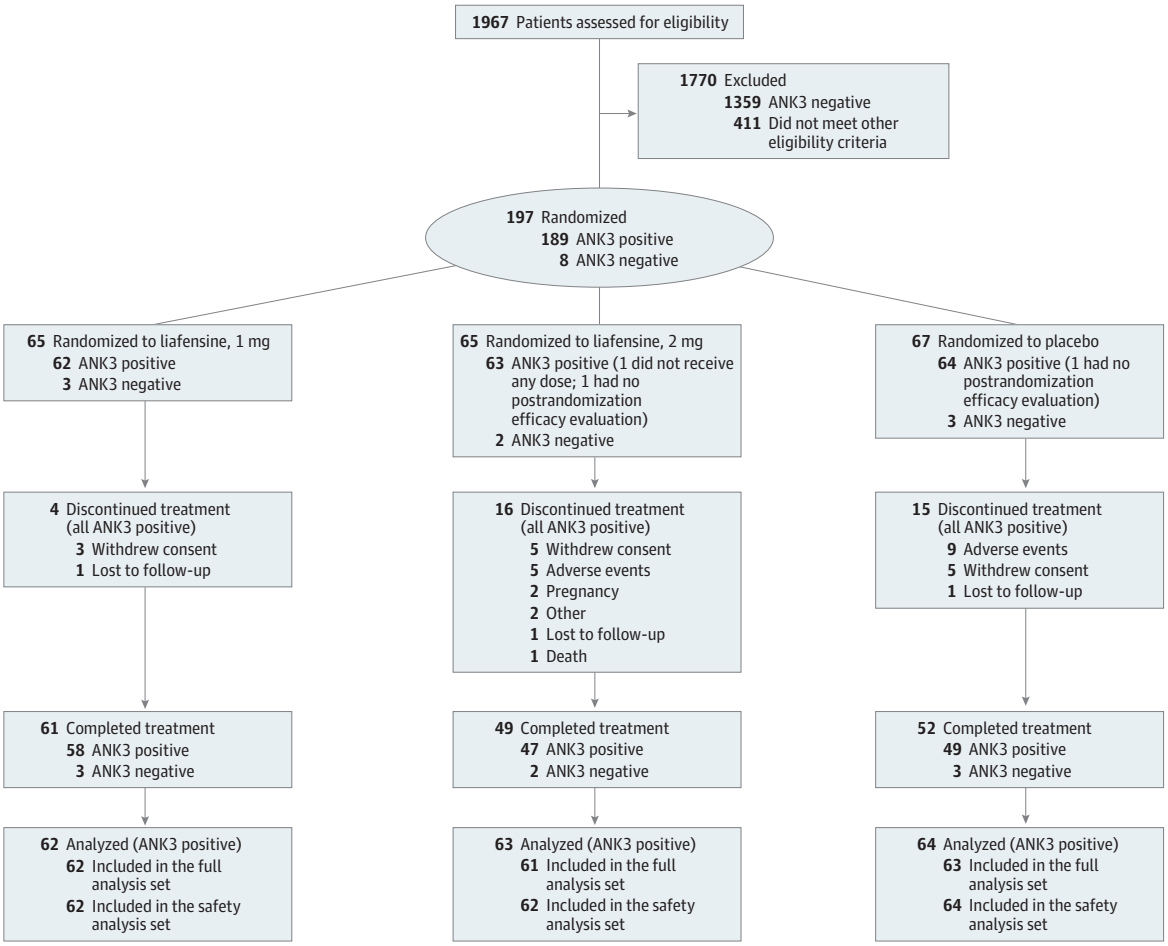
Trial Design

The ENLIGHTEN trial was a 3-arm (liafensine, 1 mg once daily; liafensine, 2 mg once daily; or placebo once daily, orally for 6 weeks), randomized, double-blind, placebo-controlled, multicenter, phase 2b clinical trial that assessed the efficacy and safety of liafensine in ANK3-positive patients with TRD. The trial protocol is available in Supplement 1. The trial was conducted at 58 sites in the United States, Canada, and China from July 2022 to March 2024, when the last patient completed follow-up. Approval was obtained from each institutional review board, and written informed consent was obtained from each patient. The sponsor was Denovo Biopharma LLC. IQVIA supervised the trial and collected the data. The trial was conducted in accordance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice guideline. The Consolidated Standards of Reporting Trials (CONSORT) reporting guidelines were followed. Data analysis was performed from March 26 to April 23, 2024.

Participants

Adults aged 18 to 70 years were eligible if they met *Diagnostic and Statistical Manual of Mental Disorders* (Fifth Edition) criteria for MDD, without psychotic features, and the diagnostic criteria for TRD in accordance with Massachusetts General Hospital Antidepressant Treatment Response Questionnaire (5-year version). Per this standard definition and US Food and Drug Administration (FDA) guidance, primary inclusion criteria were that patients must have failed at least 2 antidepressants in different pharmacological classes, given at labeled effective doses for at least 6 weeks, and have a 17-item Hamilton Depression Rating Scale total score of at least 21 (moderate). Patients were required to wash out of other antidepressants for 5 half-lives and of specified psychiatric medications and CYP3A4 inducers prior to baseline. The primary exclusion criteria included significant suicide risk or specified psychiatric and medical disorders, medications, or organ dysfunction with the potential to confound study conduct, safety, or interpretation. Full entry criteria are described in the protocol (Supplement 1). Recruitment was conducted through physician referrals, online advertisements, and print advertisements.

Figure 1. CONSORT Flow Diagram of Participants Through the Trial



Randomization

Patients were randomized to the 3 groups in a 1:1:1 ratio (**Figure 1**). The objective of the study was to assess the effects of liafensine in ANK3-positive patients; however, a small number of ANK3-negative patients (30 patients) were to be enrolled for exploratory observation. A blinded interactive response technology system by Suvoda LLC was used for randomization. This correctly assorted patients, but the algorithm inadvertently enrolled fewer (8) ANK3-negative patients than planned (blinded to the study team). As the study was prospectively designed to test the efficacy of liafensine only in ANK3-positive patients, this did not affect the analyses or conclusions of the study. Study participants, investigators, raters, and the sponsor were blinded to ANK3 status and treatment assignment, and a written plan for maintaining the blind, extensive rater training, and indistinguishable investigational products were used to help maintain blinding. The investigational product was a blinded carton containing 2 bottles of identical tablets. The 1-mg, 2-mg, and placebo cartons consisted of 1 bottle of liafensine, 1 mg, and 1 bottle of placebo; 2 bottles of liafensine, 1 mg; and 2 bottles of placebo, respectively. The study drug was manufactured and tested by Patheon Inc and kitted by Fisher Clinical Services Inc in accordance with the Current Good Manufacturing Practice standards.

Assessments

ANK3 biomarker status was determined by a real-time polymerase chain reaction–based assay to genotype germline DNA extracted from whole blood in a Clinical Laboratory Improvement Amendments–certified laboratory.

Efficacy measures were performed at days −1 (baseline), 7, 14, 28, and 42 for the Montgomery-Åsberg Depression Rating Scale (MADRS) (range, 0-60) and Clinical Global Impression–Severity (CGI-S) scale (range, 1-7); at days 7, 14, 28, and 42 for the Clinical Global Impression–Improvement (CGI-I) scale (range, 1-7); and at baseline and day 42 for the Sheehan Disability Scale (SDS) (range, 0-30). This was followed by 2 posttreatment safety visits at day 14 and day 28. Higher scores indicate more severe symptoms on all scales. Assessments were performed at the investigational sites by blinded raters.

Safety was assessed on the basis of reported adverse events; the Discontinuation-Emergent Signs and Symptoms Scale; physical, neurological, and psychiatric examinations, including the Columbia-Suicide Severity Rating Scale; vital signs;

weight; 12-lead electrocardiograms; laboratory test results; pregnancy test results (individuals of childbearing potential only); and concomitant medication assessments. Blood samples were collected for pharmacokinetic analysis.

Outcomes

The primary objective was to assess the superiority of liafensine (combined 1-mg and 2-mg doses) vs placebo in MADRS total score change from baseline to day 42 in ANK3-positive patients. The secondary efficacy objectives were (in ANK3-positive patients) change from baseline to day 42 in CGI-S score, CGI-I score at day 42, and change from baseline to day 42 in SDS score. Safety end points included adverse events by type, frequency, severity, timing, seriousness, and relationship to study therapy and changes in the other safety assessments.

Sample Size Calculation

To conduct the analysis for the primary end point, a sample size of 47 ANK3-positive patients per group would be sufficient to detect a 4.5-unit difference with 80% power at the 2-sided .05 α level, assuming a standard deviation (SD) of 9 units. The treatment effect of 4.5 units and SD of 9 units were based on the retrospective analyses of the prior trials. To compensate for dropouts, approximately 150 ANK3-positive patients (50 per group) were to be randomized.

Statistical Analysis

The efficacy analyses were performed on randomized ANK3-positive patients who received at least 1 dose of study drug and had a postrandomization efficacy evaluation (full analysis set).

The primary analysis of MADRS score was performed using the mixed model for repeated measures with imputation based on the missing-at-random assumption. Hence, missing values were imputed with nonmissing values in the same treatment group. A fully conditional specification method with predictive mean matching was used in the imputation of missing MADRS score changes as continuous variables.[20] The model included the fixed effects of treatment, visit, treatment-by-visit interaction, treatment by baseline MADRS total score, baseline MADRS total score, and region. The combined liafensine, 1 mg and 2 mg, group was first compared with placebo in ANK3-positive patients. After the significance from the combined group was established, the effects of 1 mg vs placebo and 2 mg vs placebo were compared.

The CGI-S score change from baseline was analyzed using the mixed model for repeated measures without imputation, with similar factors as for MADRS. The CGI-I score was analyzed without imputation using the Cochran-Mantel-Haenszel test stratified by region. The SDS score change from baseline to day 42 was evaluated without imputation using the analysis of covariance model with treatment, baseline SDS score, and region.

The safety analysis set consisted of ANK3-positive patients who received at least 1 dose of study drug. Safety and tolerability data were summarized descriptively. An independent data monitoring committee was established to review data to ensure safety of patients.

SAS version 9.4 (SAS Institute) statistical software was used for analyses. A 2-sided $P < .05$ was considered statistically significant.

Results

Patients

Of the 189 ANK3-positive patients, 188 (mean [SD] age, 43.2 [14.8] years; 119 [63.3%] female) qualified for the safety analysis set for demographic characteristics (self-identified sex, ethnicity, and race), baseline characteristics, treatment compliance, and safety outcomes, and 186 qualified for the full analysis set (Figure 1). As shown in **Table 1**, the baseline 17-item Hamilton Depression Rating Scale, MADRS, and CGI-S scores were similar across all groups, with an overall mean (SD) score of 25.1 (3.2), 33.0 (6.1), and 4.8 (0.6), respectively. The median (IQR) duration of MDD was 7.8 (3.0-19.7) years. On average, patients had used approximately 3 antidepressants previously (mean [SD], 3.2 [1.9] prior antidepressants), and the median (IQR) duration of use was approximately 2.6 (1.4-4.9) years (additional information is available in eTables 1 and 2 in Supplement 2). As shown in Figure 1, more liafensine-treated patients (105 [84.0%]) completed treatment than patients who received placebo (49 [76.6%]). Five liafensine-treated patients (4.0%) discontinued treatment due to adverse events vs 9 (14.1%) for those who received placebo. The mean (SD) overall study treatment compliance rates were comparable: 97.5% (7.5%) for liafensine-treated patients and 98.5% (4.1%) for patients who received placebo.

Primary Outcomes

The primary end point of MADRS total score change from baseline at day 42 in ANK3-positive patients (**Table 2**, **Figure 2**A; eTable 3 and eFigure in Supplement 2) showed a clinically meaningful and statistically significant improvement of 4.4 points (95% CI, −7.6 to −1.3; $P = .006$; 40% improvement) for the combined liafensine, 1 mg and 2 mg, group over placebo at the 2-sided .05 α level (mean [SE] MADRS score change, −15.4 [0.9] for the combined liafensine group vs −11.0 [1.3] for the placebo group). Additionally, both the 1-mg and 2-mg liafensine groups alone demonstrated similar statistically significant MADRS improvements (1-mg liafensine: mean treatment difference, −4.4; 95% CI, −7.9 to −0.8; $P = .02$; 2-mg liafensine: mean treatment difference, −4.5; 95% CI, −8.1 to −0.9; $P = .02$). Standardized effect size was 0.42 for the combined 1-mg and 2-mg liafensine group (0.41 for 1 mg to 0.44 for 2 mg). Improvements in MADRS scores were observed during the first week of liafensine treatment (eTable 4 in Supplement 2), indicating rapid onset of symptom relief, although this could be augmented by the initial placebo effect. Analysis using a pattern-mixture model with imputation based on missing not at random (eTable 5 in Supplement 2) as well as analysis without imputation (eTable 6 in Supplement 2) both demonstrated statistically robust efficacy. The P values were adjusted and controlled for multiplicity using a closed testing procedure.[21] MADRS scores at other time points and all other

Table 1. Baseline Characteristics of Study Participants

Characteristic	Liafensine, 1 mg (n = 62)	Liafensine, 2 mg (n = 62)	Liafensine, 1 mg + 2 mg (n = 124)	Placebo (n = 64)	Total (N = 188)
Age, mean (SD), y	43.5 (15.6)	42.0 (14.0)	42.8 (14.8)	44.2 (15.0)	43.2 (14.8)
Sex, No. (%)					
Female	41 (66.1)	43 (69.4)	84 (67.7)	35 (54.7)	119 (63.3)
Male	21 (33.9)	19 (30.6)	40 (32.3)	29 (45.3)	69 (36.7)
Ethnicity, No. (%)[a]					
Hispanic or Latino	10 (16.1)	13 (21.0)	23 (18.5)	9 (14.1)	32 (17.0)
Non-Hispanic or non-Latino	52 (83.9)	49 (79.0)	101 (81.5)	55 (85.9)	156 (83.0)
Race, No. (%)[a]					
Asian	31 (50.0)	31 (50.0)	62 (50.0)	31 (48.4)	93 (49.5)
Black or African American	1 (1.6)	0	1 (0.8)	2 (3.1)	3 (1.6)
White	30 (48.4)	31 (50.0)	61 (49.2)	30 (46.9)	91 (48.4)
Other	0	0	0	1 (1.6)	1 (0.5)
HAMD-17 score, mean (SD)	24.6 (2.5)	25.1 (3.5)	24.8 (3.1)	25.5 (3.3)	25.1 (3.2)
MADRS score, mean (SD)	33.2 (6.1)	32.7 (5.9)	32.9 (6.0)	33.1 (6.4)	33.0 (6.1)
CGI-S scale score, mean (SD)	4.8 (0.5)	4.7 (0.7)	4.7 (0.6)	4.8 (0.7)	4.8 (0.6)
MDD duration, median (IQR), y	9.6 (3.7-24.1)	6.7 (3.5-14.5)	9.1 (3.5-19.8)	7.0 (2.3-17.6)	7.8 (3.0-19.7)
Prior antidepressants used, mean (SD), No./patient	3.3 (2.3)	3.0 (1.4)	3.2 (1.9)	3.4 (2.0)	3.2 (1.9)
Duration of prior antidepressant use, median (IQR), y	2.3 (1.3-4.9)	3.1 (1.8-4.6)	2.5 (1.5-4.8)	3.0 (1.4-5.0)	2.6 (1.4-4.9)

Abbreviations: CGI-S, Clinical Global Impression–Severity; HAMD-17, 17-item Hamilton Depression Rating Scale; MADRS, Montgomery-Åsberg Depression Rating Scale; MDD, major depressive disorder.

[a] Ethnicity and race data were self-reported. For race, other could include American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, multiple races, or unknown.

efficacy end points were not controlled for multiplicity, with nominal *P* values provided for exploratory purpose only.

Secondary Outcomes

Liafensine was also superior in all secondary end points (Table 2). For the combined 1-mg and 2-mg liafensine group, the CGI-S score mean treatment difference compared with placebo was −0.4 (95% CI, −0.8 to −0.1; nominal *P* = .02) (Figure 2B). For CGI-I, the mean (SD) score was 2.3 (1.0) for the combined 1-mg and 2-mg liafensine group vs 2.9 (1.3) for placebo (Figure 2C); the analysis of the categorical CGI-I scores had a nominal *P* = .003. The SDS score mean treatment difference for the combined 1-mg and 2-mg liafensine group was −2.4 (95% CI, −4.8 to −0.1; nominal *P* = .04). The correlations of MADRS scores vs CGI-S, CGI-I, and SDS scores were also analyzed for all visits (eTable 7 in Supplement 2). Exploratory analyses of response rate (eTable 8 in Supplement 2), remission rate (eTable 9 in Supplement 2), and anhedonia (eTable 10 in Supplement 2) all show liafensine as superior to placebo. Exploratory analyses involving ANK3-negative patients were not conducted due to the small number of these patients enrolled.

Safety

Table 3 provides treatment-emergent adverse events (TEAEs). The proportion of patients who reported at least 1 TEAE was comparable between liafensine-treated and placebo groups (71 of 124 patients [57.3%] and 36 of 64 patients [56.3%], respectively). Adverse events were generally tolerable, with low rates of meaningful events. The most common (≥10%) TEAEs were nausea (16 patients [12.9%]), headache (15 patients [12.1%]), and constipation (13 patients [10.5%]) in patients receiving liafensine (1-mg and 2-mg combined group) and headache (9 patients [14.1%]) and nausea (7 patients [10.9%]) in those receiving placebo. There were 0, 5, and 9 patients in the 1-mg liafensine, 2-mg liafensine, and placebo groups, respectively, who withdrew from the study due to TEAEs (eTable 11 in Supplement 2).

No cases of sedation, dissociation, respiratory depression, serotonin syndrome, or neuroleptic malignant syndrome were reported. The incidences of the following were low and similar for the liafensine-treated patients vs those who received placebo: hyperglycemia (1.6% vs 1.6%), blood glucose increased (0% vs 1.6%), hyperlipidemia (hypercholesterolemia) (0.8% vs 1.6%), increased cholesterol (0.8% vs 1.6%), weight increased (0.8% vs 1.6%), sexual dysfunction (3.2% vs 0%), and suicidal ideation (0.8% vs 1.6%). Additionally, no clinically meaningful body weight changes were observed for patients who received liafensine vs placebo (mean [SD] change, −0.16 [2.03] kg vs 0.07 [1.89] kg at day 42, respectively).

Eight serious TEAEs were reported in 5 patients: 3 (2.4%) with liafensine and 2 (3.1%) with placebo (eTable 12 in Supplement 2). One death was reported in the liafensine, 2 mg, group with unknown cause and undetermined relationship to study drug (the site personnel noticed that the patient did not return for follow-up and later found an obituary with no cause of death provided and no other information available). All other serious adverse events were deemed unrelated to the study treatment.

Table 2. Primary and Secondary Efficacy Results

Efficacy end point	Liafensine, 1 mg (n = 62)	Liafensine, 2 mg (n = 61)	Liafensine, 1 mg + 2 mg (n = 123)	Placebo (n = 63)
Primary: MADRS total score change from baseline				
LS mean change (SE)[a]				
Wk 1	−6.9 (0.8)	−7.2 (0.8)	−7.0 (0.6)	−4.4 (0.8)
Wk 2	−10.9 (1.1)	−9.2 (1.2)	−10.0 (0.8)	−8.7 (1.1)
Wk 4	−12.5 (1.2)	−11.0 (1.2)	−11.8 (0.9)	−9.1 (1.2)
Wk 6	−15.4 (1.2)	−15.5 (1.3)	−15.4 (0.9)	−11.0 (1.3)
LS mean treatment difference (SE) [95% CI] at wk 6[a]	−4.4 (1.81) [−7.9 to −0.8]	−4.5 (1.8) [−8.1 to −0.9]	−4.4 (1.6) [−7.6 to −1.3]	NA
2-sided *P* value	.02	.02	.006	NA
Effect size, Cohen *d*	0.41	0.44	0.42	NA
Key secondary: CGI-S score change from baseline				
LS mean change (SE)[a]				
Wk 1	−0.5 (0.1)	−0.6 (0.1)	−0.5 (0.1)	−0.3 (0.1)
Wk 2	−1.0 (0.1)	−0.9 (0.1)	−0.9 (0.1)	−0.7 (0.1)
Wk 4	−1.2 (0.1)	−1.1 (0.1)	−1.2 (0.1)	−0.7 (0.1)
Wk 6	−1.5 (0.2)	−1.5 (0.2)	−1.5 (0.1)	−1.1 (0.2)
LS mean treatment difference (SE) [95% CI] at wk 6[a]	−0.5 (0.2) [−0.9 to 0.0]	−0.4 (0.2) [−0.9 to 0.0]	−0.4 (0.2) [−0.8 to −0.1]	NA
2-sided *P* value	.03	.05	.02	NA
Effect size, Cohen *d*	0.38	0.38	0.38	NA
Secondary: CGI-I score				
Score, mean (SD)				
Wk 1	3.3 (1.0)	3.1 (0.9)	3.2 (0.9)	3.5 (0.8)
Wk 2	2.9 (1.0)	3.1 (1.1)	3.0 (1.0)	3.2 (0.9)
Wk 4	2.7 (1.0)	2.8 (1.1)	2.8 (1.1)	3.1 (1.2)
Wk 6	2.3 (0.9)	2.4 (1.1)	2.3 (1.0)	2.9 (1.3)
Score at wk 6, No. (%)				
1, Very much improved since the initiation of treatment	11 (19.0)	7 (14.3)	18 (16.8)	5 (10.0)
2, Much improved	23 (39.7)	25 (51.0)	48 (44.9)	18 (36.0)
3, Minimally improved	21 (36.2)	11 (22.4)	32 (29.9)	10 (20.0)
4, No change from baseline, the initiation of treatment	2 (3.4)	3 (6.1)	5 (4.7)	12 (24.0)
5, Minimally worse	1 (1.7)	2 (4.1)	3 (2.8)	3 (6.0)
6, Much worse	0	1 (2.0)	1 (0.9)	2 (4.0)
7, Very much worse since the initiation of treatment	0	0	0	0
2-sided *P* value[b]	.004	.04	.003	NA
Odds ratio vs placebo (95% CI)	2.49 (1.24 to 5.03)	2.39 (1.15 to 4.96)	2.44 (1.31 to 4.56)	NA
Secondary: SDS score change from baseline				
LS mean change (SE) at wk 6[c]	−9.9 (0.9)	−8.5 (1.0)	−9.2 (0.7)	−6.8 (1.0)
LS mean treatment difference (SE) [95% CI] at wk 6[c]	−3.2 (1.3) [−5.8 to −0.5]	−1.7 (1.4) [−4.5 to 1.0]	−2.4 (1.2) [−4.8 to −0.1]	NA
2-sided *P* value[c]	.02	.21	.04	NA
Effect size, Cohen *d*	0.38	0.23	0.31	NA

Abbreviations: CGI-I, Clinical Global Impression–Improvement; CGI-S, Clinical Global Impression–Severity; LS, least squares; MADRS, Montgomery-Åsberg Depression Rating Scale; NA, not applicable; SDS, Sheehan Disability Scale.

[a] LS mean results are estimated means from the mixed model for repeated measures analyses.

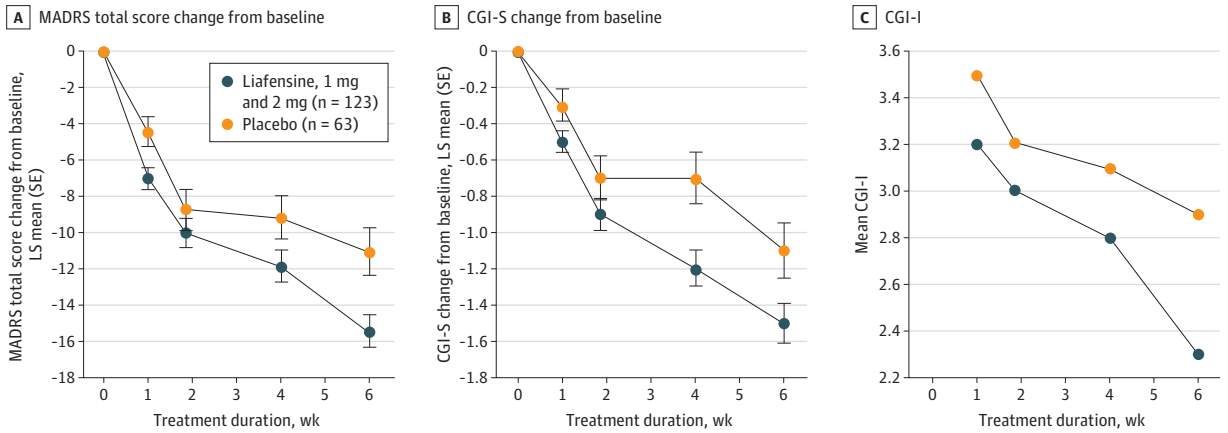
[b] Cochran-Mantel-Haenszel test.

[c] Analysis of covariance.

Discussion

Liafensine demonstrated a significant improvement in MADRS scores in ANK3-positive patients, increasing over time with a 4.4-point improvement over placebo by day 42, and this trend may suggest further improvement in efficacy with longer treatment. As liafensine targets membrane transporters of serotonin, norepinephrine, and dopamine and the *ANK3* gene product links the cytoskeleton to membrane transporters, there is a plausible mechanistic link between liafensine and *ANK3*. The equivalent efficacy in prior studies between 1 mg and 2 mg was recapitulated here (MADRS score improvements of 4.4 and 4.5 points). However, the discontinuation rate at 1 mg was lower than that at 2 mg, which is similar to the placebo group (Figure 1). This is consistent with SSRI studies demonstrating flat dose-response curves for efficacy but increasing discontinuation rates due to adverse effects at higher doses.[22] Thus,

Figure 2. Efficacy Results



CGI-I indicates Clinical Global Impression–Improvement; CGI-S, Clinical Global Impression–Severity; LS, least squares; MADRS, Montgomery-Åsberg Depression Rating Scale.

Table 3. Safety Results Showing Treatment-Emergent Adverse Event (TEAE) Overview and TEAEs in at Least 5% (by Preferred Term) of Patients Receiving Liafensine or Placebo

TEAE[a]	No. (%)			
	Liafensine, 1 mg (n = 62)	Liafensine, 2 mg (n = 62)	Liafensine, 1 mg + 2 mg (n = 124)	Placebo (n = 64)
≥1 TEAE	35 (56.5)	36 (58.1)	71 (57.3)	36 (56.3)
Maximum severity				
Mild	23 (37.1)	23 (37.1)	46 (37.1)	20 (31.3)
Moderate	12 (19.4)	11 (17.7)	23 (18.5)	13 (20.3)
Severe	0	2 (3.2)	2 (1.6)	3 (4.7)
≥1 Study drug-related TEAE	27 (43.5)	21 (33.9)	48 (38.7)	26 (40.6)
≥1 Serious TEAE	0	3 (4.8)	3 (2.4)	2 (3.1)
≥1 Study drug-related serious TEAE	0	0	0	0
TEAE leading to discontinuation of treatment	0	5 (8.1)	5 (4.0)	9 (14.1)
TEAE leading to study drug being on hold	0	1 (1.6)	1 (0.8)	0
TEAE leading to death	0	1 (1.6)	1 (0.8)	0
Adverse events of special interest, Hy law	0	0	0	0
Nervous system disorders	19 (30.6)	14 (22.6)	33 (26.6)	14 (21.9)
Headache	11 (17.7)	4 (6.5)	15 (12.1)	9 (14.1)
Somnolence	5 (8.1)	6 (9.7)	11 (8.9)	1 (1.6)
Dizziness	7 (11.3)	2 (3.2)	9 (7.3)	4 (6.3)
Gastrointestinal disorders	19 (30.6)	13 (21.0)	32 (25.8)	10 (15.6)
Nausea	11 (17.7)	5 (8.1)	16 (12.9)	7 (10.9)
Constipation	7 (11.3)	6 (9.7)	13 (10.5)	0
Dry mouth	4 (6.5)	1 (1.6)	5 (4.0)	0
Abdominal distention	4 (6.5)	0	4 (3.2)	0
Psychiatric disorders	10 (16.1)	8 (12.9)	18 (14.5)	9 (14.1)
Anxiety	5 (8.1)	0	5 (4.0)	2 (3.1)
Depression	0	1 (1.6)	1 (0.8)	5 (7.8)
Infections and infestations	8 (12.9)	6 (9.7)	14 (11.3)	5 (7.8)
Influenza	4 (6.5)	1 (1.6)	5 (4.0)	1 (1.6)
Upper respiratory tract infection	1 (1.6)	4 (6.5)	5 (4.0)	1 (1.6)
General disorders and administration-site conditions	7 (11.3)	6 (9.7)	13 (10.5)	1 (1.6)
Fatigue	4 (6.5)	1 (1.6)	5 (4.0)	1 (1.6)
Metabolism and nutrition disorders	6 (9.7)	7 (11.3)	13 (10.5)	4 (6.3)
Decreased appetite	4 (6.5)	6 (9.7)	10 (8.1)	2 (3.1)
Skin and subcutaneous tissue disorders	5 (8.1)	3 (4.8)	8 (6.5)	5 (7.8)
Pruritus	1 (1.6)	0	1 (0.8)	4 (6.3)

[a] TEAEs are shown by severity or by system organ class or preferred term (Medical Dictionary for Regulatory Activities [MedDRA] version 25.0).

1 mg will be used in future studies. The effect sizes of 0.42 in this study indicate clinical relevance, which, for example, is higher than those (ranging from 0.23-0.32) of the short-term phase 3 studies in the New Drug Application for esketamine as an adjunctive therapy.[23] These findings closely replicated the analyses of ANK3-positive patients from the prior phase 2b trials (4.7-point improvement).[15] The prior studies were conducted in North America, Europe, South America, and Africa, while the current study was run in North America and Asia. Thus, the results are expected to be generalizable to global TRD populations. Treatment differences (liafensine vs placebo) in CGI-S and SDS score changes from baseline as well as CGI-I score are also statistically significant with meaningful improvements, as were exploratory outcomes, such as response, remission, and anhedonia subscale.

Cumulative safety data include 1487 individuals exposed to liafensine, 482 for 6 months or longer and 218 for 12 months or longer. Adverse events reported here were similar to those in historical liafensine studies[13,14] and indicate that liafensine is safe and well tolerated. Although in the current study there was 1 death of unknown cause, there were no liafensine-related deaths in more than 1300 individuals exposed to liafensine in prior studies. The morbid safety issues associated with the drugs currently used to treat TRD, or many frequent with SSRIs and SNRIs, either were not observed or were reported with low incidence in liafensine-treated patients. In particular, data from liafensine studies do not indicate abuse liability, sedation, dissociative symptoms, respiratory depression, metabolic dysfunction, weight gain, or notable sexual dysfunction. Thus, liafensine represents the potential for an improved risk-benefit profile using precision medicine in ANK3-positive patients with TRD.

That liafensine had previously failed in 2 large phase 2b trials (346 in study CN162006 and 502 in study CN162007) in non–biomarker-selected patients with TRD[13] and in ANK3-negative patient subsets[15] indicates a lack of efficacy in biomarker-negative patients and that these patients should not be further studied per the FDA enrichment guidance.[24] In fact, the pivotal studies of most approved drugs with companion diagnostics focused on biomarker-positive patients only.[25]

A number of studies have been conducted to discover predictive biomarkers for psychiatric drugs, but most of them have not been confirmed in a prospective study.[26] Currently, most pharmacogenomic testing for antidepressants focuses on differences in drug metabolism due to cytochrome P450 enzyme variations. However, these tests lead to small and sometimes nonpersistent outcome differences.[27,28] One possible difficulty in finding biomarkers for antidepressants may be due to the complicated genetics of depression; for example, one study found more than 600 loci associated with depression.[29] This indicates that 1 drug is not optimal for all patients with such diverse genetic backgrounds, and it raises a concern there might not be a single-gene biomarker that can predict drug efficacy in depression like those companion diagnostics approved in oncology. However, this work suggests that a single locus, if identified correctly, could be successfully used to predict antidepressant response, which represents major progress in advancing precision medicine in psychiatry.

Limitations

This study has several limitations. Despite extensive literature supporting the *ANK3* gene's role in neuronal transport proteins, the precise mechanism of affecting liafensine is not yet fully characterized. Additionally, although the 6-week study duration is consistent with FDA guidance for approval, long-term efficacy is yet to be investigated. Blinding integrity was not formally evaluated, which introduces uncertainty regarding the potential for expectation or observer bias in subjective outcomes; however, extensive measures were used to maintain blinding, and liafensine is not associated with high-frequency adverse effects or recognizable symptoms expected to interfere with blinding.

Conclusions

The favorable efficacy and safety outcomes in this phase 2b trial demonstrate that liafensine could be a promising new therapy for ANK3-positive patients with TRD. The findings support continued development of liafensine, 1 mg once daily, for TRD using the ANK3 biomarker in another similar pivotal (phase 3) study, potentially leading to marketing approval.

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Correction: This article was corrected on October 29, 2025, to clarify in the Conflict of Interest Disclosures section that the patent pending for Dr Luo was a global patent with the World Intellectual Property Organization.

Author Contributions: Drs G. Wang and Spear had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: G. Wang, Spear, Alphs, Chen, Huang, Lu, Nemeroff, Luo.
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Conflict of Interest Disclosures: Dr G. Wang reported consulting fees from Boehringer Ingelheim, Janssen, and Otsuka Pharmaceutical Co Ltd and being chairman of the China Association for Mental Health outside the submitted work. Dr Spear reported employment, salary, and equity from Denovo Biopharma during the conduct of the study and outside the submitted work. Dr Alphs reported employment, salary, and equity from Denovo Biopharma during the conduct of the study; and personal fees from NetraMark outside the submitted work. Dr Chen reported employment, salary, and equity from Denovo Biopharma during the conduct of the study and outside the submitted work. Dr Huang reported employment, salary, and equity from Denovo

Biopharma during the conduct of the study and outside the submitted work. Dr Lu reported employment, salary, and equity from Denovo Biopharma during the conduct of the study and outside the submitted work. Dr Doostzadeh reported employment, salary, and equity from Denovo Biopharma during the conduct of the study and outside the submitted work. Dr Wu reported employment, salary, and equity from Denovo Biopharma during the conduct of the study and outside the submitted work. Dr Patel reported grants from Denovo Biopharma during the conduct of the study. Dr Nemeroff reported personal fees and stock from Denovo Biopharma during the conduct of the study; and grants from the National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Texas Child Mental Health Consortium, and National Institute of Mental Health, personal fees from ANeuroTech, Abbott Laboratories, Signant Health, Janssen, BioXcel Therapeutics, Silo Pharma, Engrail Therapeutics, Clexio Biosciences, EcoR1 Capital, EmbarkNeuro, Galen Mental Health, GoodCap Pharmaceuticals, ITI, LUCY Scientific Discovery, Relmada Therapeutics, Sage Therapeutics, Senseye, Precisement Health, Autobahn Therapeutics, EMA Wellness, Heading Health, SynapseBio, Ninnion, Pasithea, Skyland Trails, Denovo Biopharma, Acadia, Alvogen, Reunion Neuroscience, Neurocrine Bioscience, Kivira Health LLC, and Laureate Institute for Brain Health, and stock from Corcept Therapeutics, EMA Wellness, Precisement Health, Relmada Therapeutics, Signant Health, Galen Mental Health, Senseye, and Kivira Health outside the submitted work; in addition, Dr Nemeroff had a patent for method and devices for transdermal delivery of lithium licensed to US 6,375,990B1, a patent for method of assessing antidepressant drug therapy via transport inhibition of monoamine neurotransmitters by ex vivo assay licensed to US 7,148,027B2, and a patent for compounds, compositions, methods of synthesis, and methods of treatment (CRF receptor binding ligand) licensed to US 8,551,996 B2. Dr Luo reported employment, salary, and equity from Denovo Biopharma during the conduct of the study and outside the submitted work; in addition, Dr Luo on behalf of Denovo Biopharma had a global patent for the ANK3 biomarker for liafensine tested in this study with the World Intellectual Property Organization (WIPO) for WO2021/178166A pending, which was also filed in many other countries and regions. No other disclosures were reported.

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