PSYCHOPHARMACOLOGIC DRUGS ADVISORY COMMITTEE  
PDAC Meeting Date: 03 FEBRUARY 2016  

SPONSOR BRIEFING DOCUMENT  
BRINTELLIX (vortioxetine)  
Supplement for Cognitive Dysfunction in Major Depressive Disorder

Takeda Development Center Americas, Inc.  
One Takeda Parkway  
Deerfield, IL 60015  
USA

H. Lundbeck A/S  
2500 Valby  
Copenhagen, Denmark

ADVISORY COMMITTEE BRIEFING MATERIALS: AVAILABLE FOR PUBLIC RELEASE
1.0 EXECUTIVE SUMMARY

1.1 Synopsis

This briefing document summarizes data regarding the effects of Brintellix (vortioxetine) on improving cognitive dysfunction in major depressive disorder (MDD). The sponsor has submitted a supplemental New Drug Application (sNDA) to the US Food and Drug Administration (FDA) requesting that this information be added to the Clinical Studies section of the vortioxetine US prescribing information (USPI). Vortioxetine is an antidepressant that was approved for the treatment of MDD in the United States and Europe in 2013. Vortioxetine is now approved in 61 countries; an estimated 1.7 million patients have received vortioxetine.

Cognitive dysfunction is an essential feature of MDD; diminished ability to think or concentrate is part of the diagnostic criteria. Cognitive dysfunction in MDD has been documented both using subjective and objective measures, is associated with functional disability and adverse outcomes, and represents an unmet medical need.

There is growing evidence that cognitive symptoms are distinct from mood symptoms and that cognitive dysfunction in MDD may be considered a target for pharmacological treatment. Prior to the vortioxetine studies described herein, limited data existed on the effects of antidepressants on cognitive dysfunction in MDD; the vortioxetine program included the first large, well-controlled studies to comprehensively assess the effects of an antidepressant on cognitive dysfunction in MDD.

Vortioxetine has a distinct pharmacological profile among antidepressants, as evidenced by direct activity at serotonin (5-hydroxytryptamine [5-HT]) receptors in addition to serotonin transporter (SERT) inhibition. Nonclinical efficacy in models of cognition, human functional magnetic resonance imaging (fMRI) data in remitted MDD subjects, and efficacy data in subjects with MDD together support vortioxetine’s potential as a drug effective both for treating depressive symptoms and cognitive dysfunction.

The first clinical study (referred to as ELDERLY) to show positive effects of vortioxetine on cognitive dysfunction in MDD was in elderly subjects with major depression. In ELDERLY, cognitive measures (Digit Symbol Substitution Test [DSST] and Rey Auditory Verbal Learning Test [RAVLT]) were included as additional prespecified endpoints outside of the statistical testing hierarchy. This was followed by 2 large studies (referred to as FOCUS and CONNECT) in the adult population specifically designed to evaluate the effect of vortioxetine on cognitive dysfunction in MDD. FOCUS and CONNECT were randomized, 8-week, double-blind, placebo-controlled studies in subjects with MDD. Cognitive measures, including the DSST, were included as the multiplicity-controlled primary and key secondary endpoints. The DSST is a well-established objective neuropsychological test broadly sensitive to cognitive impairments affecting attention, processing speed, and executive function (including working memory).

In addition to its effects on mood symptoms, vortioxetine showed consistent beneficial effect on cognitive dysfunction as assessed using the DSST number of correct symbols (hereafter referred to as DSST) performance versus placebo across all 3 studies. Vortioxetine also showed an effect on a number of additional prespecified objective measures and subjective patient-reported...
measures, including cognitive function, functional capacity, and work limitations. Duloxetine, which was utilized as an active reference in CONNECT and ELDERLY, improved depressive symptoms but did not separate from placebo on the DSST.

In total, the data demonstrate that vortioxetine treatment leads to clinically meaningful improvements in cognitive dysfunction as well as effectively treating the affective symptoms in patients with MDD. The sponsor believes that these data represent important new information for physicians treating patients with MDD and should be included in the Clinical Studies section of the USPI.

1.2 Background

Vortioxetine, discovered by H. Lundbeck A/S and codeveloped with Takeda Pharmaceutical Company Ltd. (jointly the sponsor), is an approved antidepressant that is indicated for the treatment of MDD at doses of 5 to 20 mg/day. The clinical development program for MDD evaluated >3000 subjects with MDD. Originally approved in the United States and Europe in 2013, vortioxetine is now approved in 61 countries.

The sponsor has submitted an sNDA for vortioxetine with the aim of adding text in the Clinical Studies section of the USPI regarding the effect of vortioxetine, relative to placebo, on cognitive dysfunction in MDD, as assessed using the DSST. This application includes: data on the distinct pharmacological profile; nonclinical data in animal models of cognitive function; data from a clinical pharmacodynamic fMRI study in subjects remitted from depression; and data from 3 clinical studies in MDD subjects demonstrating a positive effect of vortioxetine on cognitive dysfunction in MDD.

During the course of the vortioxetine clinical studies assessing cognitive dysfunction in MDD, a number of meetings with the FDA, interactive workshops, and panel discussions have focused on the cognitive dysfunction in MDD, including an Institute of Medicine (IOM) workshop [1]. The FDA has indicated that a beneficial effect on cognitive dysfunction in MDD could represent a legitimate label claim; however, no formal FDA guidance has been issued. Regulatory agencies in >50 jurisdictions outside the United States, including Europe, have included data in the vortioxetine label to reflect the effect on aspects of cognitive dysfunction in MDD.

Duloxetine was included as an active reference to demonstrate assay sensitivity (regarding the antidepressant effect) in 2 of the clinical studies described and those data are presented in this document; the sponsor does not seek a comparative or superiority claim, nor is the intent to reference duloxetine data in the vortioxetine USPI.

1.3 Clinical Perspective on Cognitive Dysfunction in MDD

Symptoms of cognitive dysfunction (eg, difficulty in thinking, concentrating, or making decisions) are a core feature of major depressive episodes (MDEs), as defined in the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) [2]. Objective measures (neuropsychological tests) indicate that the cognitive domains affected in MDD include attention, processing speed, executive function, and learning and memory. Cognitive dysfunction can be found both in first-episode and in recurrent major depression, contributes to relapse risk,
and can worsen with recurrent MDEs [3-7]. Up to 50% of subjects who have otherwise experienced symptomatic relief with antidepressant treatment experience persistent cognitive symptoms or have measurable cognitive dysfunction [8]. Cognitive dysfunction is associated with work-related disability and adverse psychosocial outcomes in MDD [8-14], beyond the contributions of depressed mood.

The DSST is a well-established objective neuropsychological test broadly sensitive to cognitive impairments affecting attention, processing speed, and executive function (including working memory) and less vulnerable to the influence of mood than patient-reported cognitive measures. This brief test is among the most well studied cognitive measures and is reliable for detecting the presence of cognitive dysfunction and is sensitive to change over time [15,16]. DSST performance is strongly correlated with real world functional outcome and functional capacity in patients across a range of conditions [8,17] including MDD. In order to evaluate the effect on cognitive dysfunction in MDD, the DSST (symbol coding paradigm) was chosen as the key endpoint in the vortioxetine studies.

Improvement in depressive symptoms, which is expected in treatment studies with an effective antidepressant, can confound treatment effects on cognitive symptoms. To address this potential confounder, the treatment effects of vortioxetine and duloxetine were evaluated on both the DSST and the Montgomery-Åsberg Depression Rating Scale (MADRS). In addition, mediation (path) analysis was performed to determine the degree to which vortioxetine or duloxetine directly affected cognitive dysfunction after correcting for any effects mediated through improved depressive symptoms.

1.4 First Comprehensive Evaluation of an Antidepressant’s Effects on Cognitive Dysfunction in MDD

The evaluation of vortioxetine’s effect on cognitive dysfunction in MDD included over 1500 subjects in 3 studies, making it the most comprehensive antidepressant program to date to evaluate the improvement in cognitive dysfunction both in elderly and adult subjects with MDD. Within all 3 studies, both active treatments separated from placebo on depression scales.

As a result of vortioxetine’s distinct pharmacological profile compared to that of other antidepressants, a decision was made to investigate vortioxetine’s effect on cognitive dysfunction in MDD in a study in elderly subjects (ELDERLY) that was part of the original clinical registration program for MDD. In this 453-subject study of antidepressant efficacy in an elderly population, the DSST and RAVLT were included as prespecified, non–multiplicity-controlled endpoints; together these measures cover a wide range of cognitive domains impaired in MDD. The study was placebo controlled and included an active reference (duloxetine 60 mg, a well-studied antidepressant previously tested for cognitive effects in elderly MDD subjects [18]). Both vortioxetine and duloxetine showed antidepressant efficacy (separation from placebo on the MADRS) and effects on the RAVLT, a test that is sensitive to learning and memory. However, on the DSST, vortioxetine separated from placebo while duloxetine did not. This finding—an effect of duloxetine on the RAVLT but not the DSST—replicated a similar finding with duloxetine in elderly subjects [18].
On the basis of these results, it was decided to proceed with a thorough investigation to confirm vortioxetine’s effect on cognitive dysfunction in MDD, including evaluation of vortioxetine’s effects on cognitive measures in an adult MDD population in 2 large and well-controlled clinical studies:

- FOCUS was a 602-subject randomized, 8-week, controlled, pivotal study comparing vortioxetine 10 and 20 mg to placebo. The DSST and RAVLT (2 subscales) were used together in a composite primary endpoint and separately as multiplicity-controlled key secondary endpoints. On the primary (composite) endpoint and the DSST (the first key secondary endpoint in the testing hierarchy), vortioxetine was statistically significantly better than placebo. The difference between vortioxetine and placebo on the DSST replicated the result seen in ELDERLY.

- CONNECT was a 602-subject randomized, 8-week, controlled, pivotal study comparing vortioxetine (flexible dosing of 10 or 20 mg) and the active reference duloxetine (60 mg) to placebo. The DSST was the primary endpoint. As shown in FOCUS and ELDERLY, vortioxetine was statistically significantly better than placebo on the DSST. Duloxetine did not separate from placebo on the DSST.

The standardized effect sizes of vortioxetine on the DSST are shown in Figure 1.a, demonstrating a consistent beneficial effect of vortioxetine versus placebo on the DSST across the 3 studies.
Figure 1.a  Change From Baseline in DSST at Week 8, Standardized Effect Sizes Versus Placebo (FAS)—Across Studies

![Graph showing change from baseline in DSST at Week 8, standardized effect sizes versus placebo across studies for different doses of Vortioxetine and Duloxetine.]

* = p<0.05  *** = p<0.001 versus placebo.
† = p<0.05 versus placebo, nominal p-value.
Error bars indicate standard error (SE).
FAS = full analysis set, Flex = flexible, LOCF = last observation carried forward, MMRM = mixed model for repeated measures, ns = not significant.

In addition to the DSST, FOCUS and CONNECT included various prespecified, non–multiplicity-controlled endpoints, including additional neuropsychological tests (Table 1.a) and patient-reported assessments of cognitive function, as well as assessments of functional capacity and work limitations (CONNECT). The treatment effects on the additional neuropsychological tests varied, with the largest treatment effects observed in FOCUS, in accordance with the effect size observed for the DSST. In none of the studies was there evidence of a deleterious effect on cognition across a broad range of measures.
### Table 1.a Change From Baseline in Neuropsychological Tests at Week 8, Standardized Effect Sizes Versus Placebo (FAS)—Across Studies

<table>
<thead>
<tr>
<th>Test</th>
<th>Vortioxetine</th>
<th>Duloxetine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ELDERLY</td>
<td>FOCUS</td>
</tr>
<tr>
<td></td>
<td>5 mg</td>
<td>10 mg</td>
</tr>
<tr>
<td>DSST</td>
<td>0.27†</td>
<td>0.51***</td>
</tr>
<tr>
<td>RAVLT acquisition/learning</td>
<td>0.27†</td>
<td>0.23†</td>
</tr>
<tr>
<td>RAVLT delayed recall/memory</td>
<td>0.24†</td>
<td>0.31††</td>
</tr>
<tr>
<td>TMT-A</td>
<td>--</td>
<td>0.29††</td>
</tr>
<tr>
<td>TMT-B</td>
<td>--</td>
<td>0.29††</td>
</tr>
<tr>
<td>Stroop congruent</td>
<td>--</td>
<td>0.33††</td>
</tr>
<tr>
<td>Stroop incongruent</td>
<td>--</td>
<td>0.35††</td>
</tr>
<tr>
<td>GMLT</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>SRT</td>
<td>--</td>
<td>0.41†††</td>
</tr>
<tr>
<td>CRT</td>
<td>--</td>
<td>0.38†††</td>
</tr>
<tr>
<td>One-Back Test</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Analyses: FOCUS is MMRM; ELDERLY and CONNECT are LOCF (ie, primary analyses). *-=test not applied in this study, CRT=Choice Reaction Time Task, GMLT=Grotton Maze Learning Test, LOCF=last observation carried forward, MMRM=mixed model for repeated measures, SRT=Simple Reaction Time Task, Stroop= Stroop Color Naming Test, TMT=Trail Making Test.

*=p<0.05 (ELDERLY and CONNECT); *=p<0.025 (split level of significance in FOCUS per dose); **=p<0.01, ***=p<0.001 versus placebo; statistically significant p-values.

†=p<0.05, ††=p<0.01, †††=p<0.001 versus placebo; nominal p-values.

Vortioxetine also separated from placebo (nominal p<0.05) on subjective, patient-reported measures of cognitive symptoms, the Perceived Deficits Questionnaire (PDQ) and the Cognitive and Physical Functioning Questionnaire (CPFQ) analysis of subjects with baseline CPFQ >25 at Week 8; vortioxetine did not separate from placebo for the CPFQ analysis of all subjects. In addition, vortioxetine improved the time demands score of the Working Limitation Questionnaire (WLQ) and the composite score of the University of San Diego Performance-Based Skills Assessment (UPSA) (nominal p<0.05), which were included in CONNECT. Duloxetine separated from placebo for both PDQ and CPFQ (both the subgroup and all subjects), but not the UPSA or WLQ.

The profiles of vortioxetine and duloxetine on the MADRS and DSST illustrate that effects on mood and cognition can be differentiated in an acute MDD setting. The distinct effect of vortioxetine on cognitive dysfunction in MDD was further supported by adjusting for the correlation (mediation analysis) between changes in DSST and MADRS total score (improvement in depressive symptoms). The proportion of the direct effect on the DSST for vortioxetine was between 56% and 78% at Week 8. Thus, when adjusting for the effect on the...
MADRS total score, the majority of the effect of vortioxetine on cognitive deficits is retained, indicating a notable independent effect on the DSST.

The standardized effect size of the change from Baseline in DSST versus placebo for vortioxetine ranged from 0.25 to 0.52 across the studies. This level of improvement in DSST performance is consistent with the level of impairment reported in MDD subjects [4,19-21], the effect of agents that impair cognitive performance [22,23], and the effect size of approved therapeutics in psychiatry [24]. As performance on the DSST is strongly associated with functional recovery [8], it is expected that these improvements will be reflected in functionality, as evidenced by the vortioxetine effects on the UPSA and WLQ.

1.5 Conclusion

Cognitive dysfunction in MDD contributes significantly to functional disability and there are currently no established pharmacotherapies. Treatment of MDD should not be limited to focusing only on mood and somatic symptoms, but also target cognitive dysfunction with the ultimate aim of improving patient functionality.

The results from 3 large, prospective, well-controlled studies with vortioxetine consistently showed clinically meaningful improvement versus placebo on cognitive dysfunction, as assessed using the DSST. Overall, the vortioxetine treatment effects on the DSST and additional objective and subjective measures of cognitive dysfunction and functionality indicate a consistent and clinically meaningful effect. Additional mechanistic studies (nonclinical pharmacology studies and a human fMRI study) add to the weight of evidence supporting vortioxetine’s effects on cognitive dysfunction in MDD. The sponsor has proposed that the Clinical Studies section of the vortioxetine USPI be amended to adequately reflect this clinically meaningful information on cognitive dysfunction in MDD.
TABLE OF CONTENTS

1.0 EXECUTIVE SUMMARY ........................................................................................................... 2
  1.1 Synopsis .................................................................................................................................. 2
  1.2 Background ........................................................................................................................... 3
  1.3 Clinical Perspective on Cognitive Dysfunction in MDD ....................................................... 3
  1.4 First Comprehensive Evaluation of an Antidepressant’s Effects on Cognitive Dysfunction in MDD ......................................................................................................................... 4
  1.5 Conclusion .................................................................................................................................. 8

2.0 INTRODUCTION AND BACKGROUND ............................................................................... 17
  2.1 Development and Regulatory History of Vortioxetine ......................................................... 17
  2.2 Unmet Medical Need .............................................................................................................. 18
    2.2.1 Burden of Cognitive Dysfunction in Depression .......................................................... 18
    2.2.2 Functional Implications of Cognitive Dysfunction in MDD ........................................ 19
  2.3 Effects of Antidepressants on Cognitive Dysfunction in MDD ............................................. 20
  2.4 Measurement of Cognitive Function, Functional Capacity, and Work Limitations in MDD ............................................................................................................................ 21
    2.4.1 Background ........................................................................................................................ 21
    2.4.2 Measures Used to Assess Cognitive Function, Functional Capacity, and Work Limitations in the Vortioxetine Studies ................................................................................... 22
    2.4.3 Digit Symbol Substitution Test .......................................................................................... 23
    2.4.4 Performance-Based Functional Capacity .......................................................................... 26
    2.4.5 Patient-Reported Work Limitations .................................................................................. 26
    2.4.6 Patient-Reported Cognitive Function .............................................................................. 27
  2.5 Summary ................................................................................................................................ 27

3.0 VORTIOXETINE DEVELOPMENT PROGRAM FOR COGNITIVE DYSFUNCTION IN MDD .............................................................................................................................. 29
  3.1 Rationale for Development of Vortioxetine in Cognitive Dysfunction in MDD ................. 29
  3.2 Statistical Considerations ......................................................................................................... 31
    3.2.1 Analysis Sets ...................................................................................................................... 31
    3.2.2 Statistical Approaches ....................................................................................................... 32
    3.2.3 MADRS-Adjusted, Mediation, and Path Analyses ............................................................ 32
    3.2.4 Standardized Effect Sizes ................................................................................................. 32
  3.3 Hypothesis-Generating Study, ELDERLY (Study 12541A) ................................................... 32
    3.3.1 Study Design ..................................................................................................................... 33
    3.3.2 Endpoints .......................................................................................................................... 34
    3.3.3 Statistical Analyses ............................................................................................................ 34

ADVISORY COMMITTEE BRIEFING MATERIALS: AVAILABLE FOR PUBLIC RELEASE
Table 2.a Overview of the Assessment Tools........................................................................ 23
Table 3.a Subject Disposition—ELDERLY ............................................................................ 36
Table 3.b Demographics (All Treated Subjects)—ELDERLY .................................................. 36
Table 3.c Baseline Efficacy Scores (FAS)—ELDERLY ............................................................ 37
Table 3.d Analysis of Change From Baseline in Primary Endpoint, HAM-D24 Total
Score at Week 8 (FAS, ANCOVA, LOCF)—ELDERLY ..................................................... 37
Table 3.e Analysis of Change From Baseline in DSST at Week 8 (FAS, ANCOVA,
LOCF)—ELDERLY ........................................................................................................ 38
Table 3.f Analysis of Change From Baseline in RAVLT Acquisition/Learning at
Week 8 (FAS, ANCOVA, LOCF)—ELDERLY .................................................................. 38
Table 3.g Analysis of Change From Baseline in RAVLT Delayed Recall/Memory at
Week 8 (FAS, ANCOVA, LOCF)—ELDERLY .................................................................. 38
Table 3.h Subject Disposition—FOCUS .................................................................................. 43
Table 3.i Demographics (All Treated Subjects)—FOCUS ....................................................... 43
Table 3.j Baseline Efficacy Scores (FAS)—FOCUS ................................................................. 44
Table 3.k Analysis of Change From Baseline in Primary Endpoint, Composite Z-Score
at Week 8 (FAS, MMRM)—FOCUS ................................................................................ 44
Table 3.l Analysis of Change From Baseline in DSST at Week 8 (FAS, MMRM)—
FOCUS .......................................................................................................................... 45
Table 3.m Analysis of Change From Baseline in RAVLT Acquisition/Learning at Week
8 (FAS, MMRM)—FOCUS ............................................................................................. 46
Table 3.n Analysis of Change From Baseline in RAVLT Delayed Recall/Memory at Week
8 (FAS, MMRM)—FOCUS ............................................................................................. 46
Table 3.o Neuropsychological Tests at Week 8 (FAS, MMRM)—FOCUS ............................. 47
Table 3.p PDQ Scores at Week 8 (FAS, ANCOVA, LOCF)—FOCUS ..................................... 48
Table 3.q MADRS Total Score and CGI Scores at Week 8 (FAS, MMRM)—FOCUS .......... 48
Table 3.r Subject Disposition—CONNECT ........................................................................... 53
Table 3.s Demographics (All Randomized Subjects)—CONNECT ........................................ 54
Table 3.t Baseline Efficacy Scores (FAS)—CONNECT .......................................................... 54
Table 3.u Analysis of Change From Baseline in Primary Endpoint, DSST at Week 8
(FAS, ANCOVA, LOCF)—CONNECT ........................................................................... 55
Table 3.v Neuropsychological Tests at Week 8 (FAS, ANCOVA, LOCF)—CONNECT ... 56
Table 3.w PDQ Scores at Week 8 (FAS, MMRM)—CONNECT .............................................. 57
Table 3.x CPFQ Scores at Week 8 (FAS, MMRM)—CONNECT ............................................. 58
Table 3.y UPSA Scores at Week 8 (FAS, ANCOVA, LOCF)—CONNECT ........................... 59
Table 3.z WLQ Subscale Scores and Percentage of Productivity Loss Score in Working
Subjects at Week 8 (FAS, ANCOVA, LOCF)—CONNECT .............................................. 60
Table 3.aa MADRS Total Score and CGI Scores at Week 8 (FAS, MMRM)—CONNECT

Table 3.bb Binding Affinity of Vortioxetine in Recombinant Cell Lines/Cortex Tissue Expressing Human 5-HT Receptors and SERT

Table 3.cc Nonclinical Data Supporting the Effect of Vortioxetine on Enhancing Neurotransmission and Plasticity and Reversing Cognitive Deficits

Table 4.a Overview of TEAEs (All Treated Subjects)—FOCUS

Table 4.b Most Common TEAEs (Incidence ≥2% in Any Treatment Group)—FOCUS

Table 4.c Overview of TEAEs (All Treated Subjects)—CONNECT

Table 4.d Most Common TEAEs (Incidence ≥2% in Any Treatment Group)—CONNECT

Table 5.a Change From Baseline in Neuropsychological Tests at Week 8, Standardized Effect Sizes Versus Placebo (FAS)—Across Studies

LIST OF IN-TEXT FIGURES

Figure 1.a Change From Baseline in DSST at Week 8, Standardized Effect Sizes Versus Placebo (FAS)—Across Studies

Figure 2.a DSST Performance Change Resulting From Acute Pharmacologic Effects in Healthy Subjects and in MDD Relative to Healthy Control Subjects

Figure 3.a Pharmacological Profile of Vortioxetine

Figure 3.b Study Design—ELDERLY

Figure 3.c Study Design—FOCUS

Figure 3.d Change From Baseline in Neuropsychological Tests at Week 8, Standardized Effect Sizes Versus Placebo (FAS, MMRM)—FOCUS

Figure 3.e Study Design—CONNECT

Figure 3.f Change From Baseline in Neuropsychological Tests at Week 8, Standardized Effect Sizes Versus Placebo (FAS, ANCOVA, LOCF)—CONNECT

Figure 3.g UPSA Scores at Week 8 (FAS, ANCOVA, LOCF)—CONNECT

Figure 3.h WLQ Subscale Scores at Week 8 (FAS, ANCOVA, LOCF)—CONNECT

Figure 3.i Improvements in Depressive Symptoms at Week 8 (MADRS)(FAS, MMRM)

Figure 3.j Change From Baseline in DSST at Week 8, Standardized Effect Sizes Versus Placebo (FAS, ANCOVA, LOCF, Mediation Analysis)—All Studies

Figure 3.k N-Back Task in Remitted Subjects

Figure 5.a Change From Baseline in DSST at Week 8, Standardized Effect Sizes of Vortioxetine Versus Placebo (FAS)—Across Studies
LIST OF APPENDICES

Appendix A  Neuropsychological Tests in the Vortioxetine Studies
Appendix B  Digit Symbol Substitution Test: Psychometric Background
Appendix C  Additional Statistical Information
Appendix D  Sensitivity Analyses
Appendix E  Direct and Indirect Effect Sizes
Appendix F  fMRI Study (14137A): Methods and Materials
LIST OF ABBREVIATIONS

5-HT  5-hydroxytryptamine (serotonin)
5-HTx 5-hydroxytryptamine type x (receptor)
ANCOVA  analysis of covariance
BAC  blood alcohol concentration
BDI  Beck Depression Inventory
BOLD  blood-oxygen-level–dependent
CA 1 cornu ammonis 1
CGI-I  Clinical Global Impression of Improvement
CGI-S  Clinical Global Impression of Severity
CHMP  Committee for Medicinal Products for Human Use
CI  confidence interval
CPFQ  Cognitive and Physical Functioning Questionnaire
CRT  Choice Reaction Time Task
CT  Co-primary and Translation (forum)
DLPFC  dorsolateral prefrontal cortex
DMN  default mode network
DSM-5 (-III)  Diagnostic and Statistical Manual of Mental Disorders, 3rd (5th) Edition
DSM-IV-TR™  Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision
DSST  Digit Symbol Substitution Test
FAS  full analysis set
FDA  Food and Drug Administration
fMRI  functional magnetic resonance imaging
GABA  γ-aminobutyric acid
GMLT  Groton Maze Learning Test
HAM-Dxx  Hamilton Depression Rating Scale—xx items
IOM  Institute of Medicine
LOCF  last observation carried forward
LS  least squares
MADRS  Montgomery-Åsberg Depression Rating Scale
MATRICS  Measurement and Treatment Research to Improve Cognition in Schizophrenia
MCCB  MATRICS Consensus Cognitive Battery
MDD  major depressive disorder
MDE  major depressive episode
mITT  modified Intent-to-Treat
MMRM  mixed model for repeated measures
MMSE  Mini-Mental State Examination
MSIF  Multidimensional Scale of Independent Functioning
OC  observed cases
PDQ  Perceived Deficits Questionnaire
PFC  prefrontal cortex

ADVISORY COMMITTEE BRIEFING MATERIALS: AVAILABLE FOR PUBLIC RELEASE
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAVLT</td>
<td>Rey Auditory Verbal Learning Test</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SE</td>
<td>standard error</td>
</tr>
<tr>
<td>SERT</td>
<td>serotonin transporter</td>
</tr>
<tr>
<td>sNDA</td>
<td>supplemental New Drug Application</td>
</tr>
<tr>
<td>SNRI</td>
<td>serotonin-norepinephrine reuptake inhibitor</td>
</tr>
<tr>
<td>SRT</td>
<td>Simple Reaction Time Task</td>
</tr>
<tr>
<td>SSRI</td>
<td>selective serotonin reuptake inhibitor</td>
</tr>
<tr>
<td>STAR*D</td>
<td>Sequenced Treatment Alternatives to Relieve Depression</td>
</tr>
<tr>
<td>Stroop</td>
<td>Stroop Color Naming Test</td>
</tr>
<tr>
<td>TEAE</td>
<td>treatment-emergent adverse event</td>
</tr>
<tr>
<td>TMT</td>
<td>Trail Making Test</td>
</tr>
<tr>
<td>UPSA</td>
<td>University of San Diego Performance-Based Skills Assessment</td>
</tr>
<tr>
<td>UPSA-B</td>
<td>UPSA—Brief</td>
</tr>
<tr>
<td>UPSA-VIM</td>
<td>UPSA—Validation of Intermediate Measures</td>
</tr>
<tr>
<td>USPI</td>
<td>US prescribing information</td>
</tr>
<tr>
<td>WAIS</td>
<td>Wechsler Adult Intelligence Scale</td>
</tr>
<tr>
<td>WLQ</td>
<td>Working Limitation Questionnaire</td>
</tr>
</tbody>
</table>
CONVENTIONS

References to specific studies are as follows:

- Study 12541A is also referred to as ELDERLY.
- Study 14122A is also referred to as FOCUS.
- Study 202 is also referred to as CONNECT.
- Study 14137A is also referred to as the fMRI study.

Statistical conventions:

The 2 studies (FOCUS and CONNECT) with multiplicity-controlled cognition endpoints are referred to as pivotal studies. Multiplicity-controlled endpoints in these studies comprised a hierarchy of 1 primary endpoint and 2 or 3 key secondary endpoints. Results that were within the testing hierarchy and had a p-value less than the prespecified significance level (0.05 in CONNECT and 0.025 [for each dose] in FOCUS) were considered statistically significant, provided the preceding analysis in the testing hierarchy was statistically significant.

Additional endpoints were not multiplicity controlled and are considered supportive data. These include the additional endpoints in FOCUS and CONNECT and the cognition endpoints in ELDERLY, in which the testing hierarchy concerned depression endpoints. For these cognition endpoints, where p<0.05, the results were considered nominal.

The analyses presented in this document were prespecified unless otherwise noted. The exceptions are noted when presented in the text.
2.0 INTRODUCTION AND BACKGROUND

2.1 Development and Regulatory History of Vortioxetine

Vortioxetine was approved in the United States for the treatment of MDD on 30 September 2013. On 18 December 2013, vortioxetine was granted marketing authorization by the European Commission for the treatment of MDEs. As of 29 September 2015, vortioxetine has been granted marketing authorization in 61 countries for the treatment of MDD and an estimated 1.7 million patients have received vortioxetine. The approved therapeutic dose range is 5 to 20 mg/day.

The sponsor has submitted an sNDA for vortioxetine with the aim of adding text in the Clinical Studies section of the USPI regarding the effect of vortioxetine, relative to placebo, on cognitive dysfunction in MDD, as assessed using DSST.

This briefing document summarizes the data supporting the efficacy of vortioxetine in the treatment of cognitive dysfunction in MDD in addition to its antidepressant properties. These data include the results of Studies 14122A (referred to as FOCUS) and 202 (referred to as CONNECT), which were large and well-controlled studies conducted in support of the application, with primary measures of cognitive dysfunction in subjects with MDD. Supportive data from a study in elderly subjects with MDD (Study 12451A, referred to as ELDERLY), 14137A, an fMRI study comparing subjects remitted from MDD with healthy subjects, and nonclinical studies are also described.

At the time the FOCUS and CONNECT protocols were developed, the FDA viewed cognitive dysfunction as a symptom of MDD and regarded its treatment as one aspect of treating the overall disease. The FDA did not object to the studies proceeding, but stated that a regulatory path to support a claim was not clear at that time. Subsequently, the sponsor met with the Division of Psychiatry Products in February 2014 and February 2015 to understand the FDA’s view regarding the appropriateness of cognitive dysfunction in MDD as a development target for labeling. Concurrent with the FDA discussion, interactive public workshops, including an IOM workshop, and panel discussions were held that focused on cognitive dysfunction in MDD [1]. Participation at these meetings included individuals from academia, FDA, other regulatory agencies, and pharmaceutical companies. The data presented and discussions held at these meetings support the concept that cognitive dysfunction in MDD is an unmet need that results in functional consequences for patients and should be considered an appropriate therapeutic target.

At the FDA meeting in February 2015, the FDA indicated that a beneficial effect on cognitive dysfunction in MDD could be an appropriate label claim. Although no formal guidance has been issued with respect to study design or endpoints, the FDA encouraged the sponsor to submit a justification for the selection of the primary endpoint along with pertinent literature and other supportive evidence.

On 26 February 2015, the Committee for Medicinal Products for Human Use (CHMP) issued a positive opinion to update the product label for vortioxetine in the European Union, to include data on vortioxetine’s effects on certain aspects of cognitive dysfunction in MDD. To date, >50 jurisdictions have information regarding cognitive dysfunction in MDD included in the local vortioxetine label.
No new safety signals have been identified from the clinical studies conducted for this application or in the postmarketing experience, therefore the focus of this document is on the efficacy results, with a brief safety summary.

The sponsor has submitted an sNDA that seeks approval for a change to the Clinical Studies section of the vortioxetine USPI to include clinically important information for the prescriber and patient on the effect of vortioxetine, relative to placebo, on cognitive dysfunction in MDD, as assessed using the DSST. Although duloxetine was included as an active reference in 2 of the clinical studies described and those data are presented in this document, the sponsor does not seek a comparative or superiority claim, nor is the intent to reference duloxetine data in the vortioxetine USPI.

2.2 Unmet Medical Need

2.2.1 Burden of Cognitive Dysfunction in Depression

The 12-month prevalence of MDD in the United States is approximately 7% and the prevalence is approximately 1.5 times higher in women than in men [25]. The public health burden of MDD is substantial. The World Health Organization classified the disorder as the leading cause of years lost due to disability globally [26].

MDD is a complex illness that includes affective, somatic, and cognitive symptoms. Symptoms of cognitive dysfunction, such as difficulty in thinking, concentrating, and making decisions, have long been recognized as an intrinsic characteristic of MDD and are among the most persistent symptoms. Cognitive symptoms of MDD, namely the “diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others),” are among the diagnostic criteria for an MDE, already identified in DSM-III and remaining through to the current version (DSM-5 [2]).

Cognitive dysfunction is measured both by how the patient performs on objective neuropsychological tests and by how the patient subjectively experiences cognitive problems. There is evidence of poor agreement between a patient’s objectively measured cognitive function and the patient’s perception [27,28], with the latter being influenced by the nature and severity of depressive mood symptoms. Thus, it is important to capture both subjective and objective information as they represent different aspects of clinically relevant information.

Cognitive dysfunction can be present during the first episodes of depression, in recurrent depression, and in late-life depression [3-5]. Individuals with MDD often exhibit deficits in several domains of cognitive performance, including attention, processing speed, executive function, and learning and memory [1], assessed using objective neuropsychological tests [14,29,30]. In a meta-analysis of 13 studies in MDD (N=644 subjects), these domains were significantly worse in subjects experiencing their first MDE than in healthy controls subjects [4].

Although the true prevalence of cognitive dysfunction in MDD is unknown, the available evidence suggests that it is quite high. In a prospective study in which 267 subjects with MDD were followed for 3 years while receiving treatment, symptoms of cognitive dysfunction were present up to 94% of the time during the acute phase of MDD and up to 44% of the time during...
remission [6]. In another study, the Sequenced Treatment Alternatives to Relive Depression (STAR*D) study, a prospective, randomized, pragmatic clinical study of outpatients with nonpsychotic MDD (N=1426), the baseline prevalence of difficulty concentrating was 90%, and the prevalence of clinician-rated cognitive symptoms in patients with recurrent MDD was higher than that in patients experiencing their first MDE [31]. Furthermore, accumulating evidence suggests that cognitive dysfunction, as measured using objective neuropsychological tests, may persist even after mood symptoms resolve [32,33].

Cognitive dysfunction in MDD is, on average, of moderate severity and typically ranges from 0.2 to 0.7 (the standardized effect size; see Section 3.2.4) relative to healthy controls across a range of cognitive measurements [4,19-21]. Cognitive dysfunction in this range has also been observed in approximately 30% to 40% of remitted MDD patients [5,19], suggesting that, at least in some cases, this dysfunction persists independent of mood symptoms. A deficit of 0.2 to 0.7 is more modest than that, for example, in subjects with schizophrenia, who have impairments closer to 1.5 standard deviations (SD) lower than the normative means. However, unlike in schizophrenia, patients with MDD are more likely to have a history of functional success, are often still employed, and are generally attempting to maintain family and household responsibilities. In this context, a deficit with an effect size of 0.2 to 0.7 relative to healthy individuals, persisting over time, is likely to be disruptive, accounting for some of the increased absenteeism and presenteeism at work and placing at risk a patient’s ability to maintain employment or to meet other responsibilities without untoward consequences.

Several reviews have highlighted cognitive dysfunction as an unmet medical need in MDD due to its burden on patients and implications for functional recovery [4,5,12,13,34,35].

### 2.2.2 Functional Implications of Cognitive Dysfunction in MDD

A growing body of evidence from real-world studies demonstrates the impact of cognitive dysfunction on overall functioning in MDD. This research indicates that cognitive dysfunction in MDD negatively affects overall patient function including home, psychosocial, and occupational functioning [8,10-14,28,36-40].

Several studies have examined the association between cognitive dysfunction and community function cross-sectionally [28,36-39]. In an epidemiological survey of 21,000 individuals in 6 European countries [40], a strong relationship between MDD and impaired home and work functioning was reported. This association was largely mediated by the presence of patient-reported cognitive symptoms (difficulties with concentration, memory, understanding, and ability to think clearly). Smaller cross-sectional studies in patients with MDD support the observations from this survey. For example, Naismith et al [28] observed that poorer memory (both objectively and subjectively measured) was strongly correlated with worse overall functioning. In a separate study of patients with treatment-resistant depression, Gupta et al [36] reported statistically significant associations between 1) poorer executive function and lower adaptive competence, and 2) lower sustained attention and lower interpersonal competence and recreation. McCall and Dunn [37] reported moderate correlations between improved delayed memory and ability to perform activities of daily living in patients with severe MDD.
Two small studies examined the prospective association between cognitive dysfunction in MDD and functioning. The first study [8] in patients with MDD found that better cognitive functioning was associated with functional recovery 6 months post-hospitalization for an MDE (results were controlled for depression severity). Persistent disability in employment and independent living was associated with severity of residual cognitive dysfunction in MDD [8]. A second prospective cohort study of inpatients with MDD found that objective measures of executive function predicted functional outcome 3 months following hospital discharge [41].

Finally, a number of studies have found that objectively measured cognitive dysfunction across multiple cognitive domains in patients with MDD is correlated with impaired work function [38,39,42]. The first study found that poor cognitive function (objectively measured) across multiple cognitive domains was strongly related to unemployment status in subjects with MDD [38]. The second study found that objective measures of attention, executive function, and verbal memory were significantly correlated with a work subscale in subjects with MDD [39]. The third study was a large prospective study that found that patient-reported cognitive symptoms had a significant negative impact on work productivity and length of sick leave in a 2-year observational cohort study [42]; the influence of cognitive symptoms on work productivity was in addition to that of depression severity, suggesting that mood symptoms and cognitive dysfunction acted independently to influence occupational functioning in this study.

2.3 Effects of Antidepressants on Cognitive Dysfunction in MDD

There is a large body of evidence demonstrating the presence of significant residual cognitive dysfunction following improvement in mood symptoms, whether by pharmacotherapy, psychotherapy, or other means [13,35,43-45]. Two recently published reviews have evaluated the literature on cognitive dysfunction in MDD, focusing on meta-analyses and on relevant individual studies [13,35]. Both reviews found that the primary cognitive domains that are impaired in MDD are processing speed, attention, executive function, and learning and memory. They both conclude that cognitive symptoms are present in patients with MDD, have substantial impact on the consequences of the illness, including functional outcomes and workplace performance, are present as residual symptoms in a significant number of remitted subjects, and are not adequately treated by current antidepressant therapies.

Some antidepressants may actually contribute to cognitive dysfunction. Much of the evidence for this is derived from studies of classes of antidepressants, including those with anticholinergic or antihistaminergic activity, such as the tricyclic and tetracyclic antidepressants [30]. Repeated or long-term use of these classes of antidepressants may play a role in the impairment of cognitive function in depressed patients [29].

In contrast to potential deleterious effects on cognition, other literature suggests some newer antidepressants (selective serotonin reuptake inhibitors [SSRIs], serotonin-norepinephrine reuptake inhibitors [SNRIs]) may potentially treat cognitive dysfunction to a certain but limited degree in patients with MDD. In general, these studies were not designed to distinguish the direct effects of antidepressants on cognitive domains affected in cognitive dysfunction in MDD from indirect effects on cognition via improvements in mood [46-49]. Overall, the absence of
replication, small sample sizes, methodological constraints (including lack of a placebo control and within-group comparisons that only assess treatment effect compared to Baseline) limit the ability to generalize the results.

Studies of cognitive function with duloxetine (an SNRI hypothesized to have effect on cognitive dysfunction due to its noradrenergic properties) that have been conducted with larger sample sizes and greater methodological rigor have shown improvements in some cognitive domains. One double-blind, placebo-controlled study of elderly subjects with MDD [18] (duloxetine: n=207; placebo: n=104) showed statistically significant improvements in the duloxetine group on a composite cognitive measure consisting of 4 tests, including tests equivalent to the DSST (the key cognitive function endpoint in the vortioxetine studies) and the RAVLT, and 2 additional tests of attention and executive function. The 4 tests were selected because they assess aspects of cognition previously shown to be most impaired in patients with depression (verbal learning and memory, attention, executive function, and working memory). Duloxetine’s effect on the composite score was driven primarily by improvement in learning and memory (RAVLT equivalent) but not other domains, including those assessed by the symbol coding task (DSST equivalent).

In the vortioxetine ELDERLY study, duloxetine separated from placebo on the RAVLT but not on the DSST, consistent with the findings from the Raskin study of duloxetine effects on cognition being mainly limited to improvement in learning and memory [18]. Similarly, in the vortioxetine CONNECT study, duloxetine did not separate from placebo on a broad range of neuropsychological tests, including the DSST.

In summary, duloxetine has demonstrated improvement in some cognitive domains (learning and memory) in larger-scale, placebo-controlled studies, although the effects were only studied and shown in elderly patients. At the time the vortioxetine program was developed, duloxetine was the best characterized antidepressant for testing treatment effect on cognitive dysfunction in patients with MDD [50].

2.4 Measurement of Cognitive Function, Functional Capacity, and Work Limitations in MDD

2.4.1 Background

In clinical studies, including those in MDD, cognitive dysfunction is typically assessed using objective neuropsychological tests of cognitive performance to assess change and to distinguish between effects of change in mood from effects on cognition itself. Subjective measures, patient- or clinician-rated, are used to complement the findings of the objective tests. In addition, cognitive function represents a critical determinant of functional outcome in MDD, therefore it is also relevant to consider additional measures to evaluate functional capacity and work limitations.

A number of objective neuropsychological tests are available that are valid and sufficiently sensitive to detect clinically important cognitive dysfunction in MDD, as well as changes in...
cognitive function over time. Test scores may be based either on individual tests or on composite scores across tests (test batteries).

While most neuropsychological tests are designed to reflect performance on a particular domain, to varying degrees all tests are polyfactorial in that they are sensitive to the integrity of multiple cognitive operations. The DSST is among the most broadly polyfactorial tests and as a result, while not informative as to the particular domain(s) affected, it is among the most highly sensitive to the presence of overall dysfunction and to cognitive change. Appendix B provides additional detail on the DSST.

While battery composites have been used to measure change in some contexts (eg, Measurement and Treatment Research to Improve Cognition in Schizophrenia [MATRICS] Consensus Cognitive Battery [MCCB]), there are advantages to using a single polyfactorial test to detect change. For example, a single test is shorter than a full battery and therefore less vulnerable to the effect of subject fatigue, which may be particularly advantageous in depression.

Furthermore, basic psychometric principles and evidence from clinical studies have shown that increased test battery length does not always add explanatory power when the question studied is that of estimating the overall magnitude of cognitive dysfunction (in contrast to diagnostic specificity, for which longer batteries are generally relied upon). In schizophrenia studies, when cognitive test batteries are usually applied, the DSST appears to be the most sensitive individual test for the presence of cognitive impairment, explaining the majority (>80%) of the variance in much longer and more burdensome test batteries [17,51-53].

2.4.2 Measures Used to Assess Cognitive Function, Functional Capacity, and Work Limitations in the Vortioxetine Studies

The measures used to assess objective and subjective cognitive function, functional capacity, and work limitations in the vortioxetine studies are summarized in Table 2.a. The DSST was the key cognitive function endpoint in all 3 vortioxetine studies in which cognitive dysfunction in MDD was assessed.
# Table 2.a Overview of the Assessment Tools

<table>
<thead>
<tr>
<th>Objective, performance-based tests</th>
<th>ELDERLY</th>
<th>FOCUS</th>
<th>CONNECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurropsychological test performance</td>
<td>DSST (a), RAVLT</td>
<td>DSST, RAVLT, TMT, Stroop, CRT, SRT</td>
<td>DSST, TMT, Stroop, CRT, SRT, One-Back, GMLT</td>
</tr>
<tr>
<td>Functional capacity</td>
<td>--</td>
<td>--</td>
<td>UPSA (b)</td>
</tr>
</tbody>
</table>

| Subjective, patient-reported measures | | | |
|---------------------------------------|---------|------|
| Cognitive symptoms | -- | PDQ (c) | PDQ, CPFQ (c) |
| Work limitations | -- | -- | WLQ (d) |

CRT=Choice Reaction Time Task, SRT=Simple Reaction Time Task, GMLT=Groton Maze Learning Task, Stroop=Stroop Color Naming Test, TMT=Trail Making Test, -- = test not applied in this study.

(a) Described in Section 2.4.3. Other neuropsychological tests are described in Appendix A.
(b) Described in Section 2.4.4.
(c) Described in Section 2.4.6.
(d) Described in Section 2.4.5.

## 2.4.3 Digit Symbol Substitution Test

### 2.4.3.1 Overview

The DSST is among a number of validated neuropsychological tests with demonstrated sensitivity to cognitive dysfunction [21,54], and to clinically important change in cognitive dysfunction, as shown in experimental studies with compounds of known effect [21-23,55]. Originally developed for the purpose of understanding human associative learning [56], this test is often classified as a measure of information processing speed deficits [57,58], an assertion that has research support only in young healthy subjects. Extensive research into which cognitive domains underlie deficient DSST performance in clinical populations has implicated a broad range of cognitive functions, including not only associative learning and speed of processing but also attention and executive functions, including working memory [53,59-66].

The sponsor selected the DSST as the key endpoint because the DSST is not only sensitive to clinically important cognitive dysfunction in MDD, but importantly for clinical studies, it also possesses a variety of psychometric properties that make it ideal for detecting clinically meaningful change. Additional details regarding the DSST and its use in the vortioxetine studies are provided in Appendix B.

The DSST [67] is presented on a single piece of paper. The subject is instructed to write the symbol corresponding to a key at the top of the page below each number; the score is the number of correct symbols achieved in the time limit (either 90 or 120 seconds).

The DSST possesses properties that render it ideal among cognitive measures as a single efficacy assessment tool for evaluating the effect of treatment (change) on cognitive dysfunction in MDD:
• The DSST is among the most clinically useful neuropsychological tests in a wide range of clinical populations. This is because it is sensitive not only to the presence of cognitive dysfunction, but also to both improvement and worsening in cognitive function over time.

• The DSST has commonly been used in experimental medicine and other pharmacological studies. Its many advantages for clinical study use include those listed above as well as its brevity (low patient and administrator burden, thereby reducing the spurious effects of patient fatigue associated with prolonged testing, as well as administration or scoring errors) and its reliability. The extensive data accumulated over many years offer the opportunity to benchmark clinical changes to known experimental effects, and offer a means to improved understanding of the clinical meaningfulness of change in test performance [22,23,55].

• The DSST is an excellent choice among tests for measurement of change in that it is brief has high test-retest reliability, has modest practice effects, and has low correlations with education and cultural background [68].

• DSST performance is a predictor of functional outcome in patients with severe psychiatric disorders and correlates with real-world functional outcomes or proxies thereof [8,15-17,69].

2.4.3.2 Clinical Meaningfulness of Degree of Change in Cognitive Dysfunction as Measured Using the DSST

To understand the meaning of a particular effect size decrement in cognition, it is helpful to calibrate in more universal terms by considering the impact on a specific measure in healthy individuals of known (albeit transient) perturbations, such as sleep deprivation, elevated blood alcohol concentration (BAC), or effects of drugs with adverse cognitive effects. Meta-analyses indicate that relative to healthy control subjects, the deficit in MDD (expressed as standardized effect size) typically ranges from 0.2 to 0.7 across a variety of cognitive measurements, including the DSST [4,19-21]. In a recent meta-analysis combining samples from 22 depression studies and including 1904 subjects for whom DSST data were available [21], the standardized effect size for this measure was 0.55 lower (95% confidence interval [CI]=0.34-0.75) in subjects with MDD than in healthy control subjects. This magnitude of cognitive decline from a previously higher level would be expected to adversely impact performance to a degree that could result in an individual falling critically short on work and school demands, with substantial impact on daily living and potentially leading to disability.

The clinical meaningfulness of an observed change of a given magnitude can be gauged in reference to guidelines, such as a BAC of 0.08% specified in legal statutes around the world restricting drunken driving. Similar guidelines exist for extended periods of sustained wakefulness and for the use both of illegal and legal drugs in the context of cognitively demanding tasks where public safety may be affected, such as for pilots, truck drivers, and other heavy equipment operators, as well as physicians. In this context, it is interesting to note that a BAC of 0.088% yielded a DSST performance decrement versus placebo of 0.68 (standardized effect size; Figure 2.a) [22,23]. (This change is, of course, not the only effect intoxication has on an individual, but it is the measured impact on performance of the DSST). The DSST was also sensitive to the adverse cognitive effects of lorazepam and diphenhydramine [23,55]. An
important distinction between these circumstances and the case of MDD or other chronic conditions is that, in these settings, the changes observed on the DSST are transient and reversible. That is, once the sedative drug effects resolve, performance on the DSST returns to normal levels. This is in contrast to the case of MDD, where cognitive dysfunction may be chronic and persistent.

**Figure 2.a** DSST Performance Change Resulting From Acute Pharmacologic Effects in Healthy Subjects and in MDD Relative to Healthy Control Subjects

- Lorazepam (2 mg) (Pompeia, 2008)
- Diphenhydramine 150 mg (Roth et al, 1987)
- Alcohol BAC 0.088 (Mattila, 1998)
- MDD, relative to healthy controls (Snyder, 2013)


Data represent standardized effect sizes.

In summary, the cognitive dysfunction in MDD can be reliably captured with the DSST. Recognized benchmarks, such as the magnitude of deficit on the DSST that is associated with BACs beyond a safe limit for driving or performing cognitively demanding tasks, can be used to help appreciate the meaningfulness of the effect size seen in MDD. In addition, extensive published data using the DSST in experimental medicine and other pharmacological research provide a basis for understanding the clinical meaningfulness of the change in DSST performance associated with the illness and its treatment.

**2.4.3.3 Evidence for DSST Performance Correlation with Functional Outcome**

DSST performance correlates with real-world functional outcome or proxies thereof. For example, in schizophrenia and bipolar disorder, the UPSA, an objective measure of performance-based functional capacity (see Section 2.4.4) correlates with neuropsychological tests used to assess critical cognitive domains also known to be impaired in MDD (including digit symbol tasks). The DSST score had the single greatest correlation with the UPSA score, accounting for 27% of the variance in UPSA scores [69].
Furthermore, the DSST is a robust predictor of functional outcome across a range of conditions [15-17]. In patients with MDD studied 6 months after hospital discharge for an MDE, DSST performance was strongly associated with the magnitude of functional recovery in work, school, and independent living after adjusting for residual symptoms of depression and other medical disabilities [8]. The authors reported an odds ratio of 19.95 for the DSST association with subjects’ ratings on the 7-point overall global rating on the Multidimensional Scale of Independent Functioning (MSIF). Translated into practical terms, this indicates a 1 SD difference in DSST score increases the odds of a 1-point MSIF improvement by nearly 20-fold; for a 0.5 SD DSST difference, the increase in odds is 4.47. The association between DSST performance and residual disability demonstrated here indicates not only the importance of cognition on residual disability, but also the sensitivity of the DSST to a clinically meaningful outcome.

### 2.4.4 Performance-Based Functional Capacity

The UPSA is a performance-based measure of functional capacity that was originally developed for severe mental illness and has been used extensively to assess functional skill deficits in psychotic and other disorders, including bipolar disorder [69-71], comorbid posttraumatic stress disorder [72], major depression [73], personality disorder [74], substance abuse [75], Type II diabetes [76], mild cognitive impairment and AD [77,78], as well as healthy aging [78-80]. It carries a high level of face validity, as it evaluates performance accuracy on everyday tasks that are considered necessary for independent functioning in the community.

The UPSA–Validation Intermediate Measures (UPSA-VIM) is the complete UPSA and uses role-playing situations to evaluate skills in 5 areas (household, communication, finances, transportation, and planning recreational activities) as adapted from the MATRICS–Co-primary and Translation (CT) forum Validation of Intermediate Measures study [81]. The UPSA has shared variance with measures of cognitive performance, high interrater reliability, good utility as a repeated measure, and reasonable tolerability and practicality [82]. The UPSA–Brief (UPSA-B) [83] is a shorter version of the UPSA-VIM; it is used in non–English-speaking countries and consists of the 2 areas of functioning (communication and finances) that explain most of the variance of the UPSA-VIM [83]. As there is a strong correlation between UPSA-VIM and UPSA-B [83], in CONNECT the scores for the UPSA-VIM or UPSA-B were each standardized to a 100-point scale and used as the UPSA composite score in a combined analysis.

### 2.4.5 Patient-Reported Work Limitations

Given that depressed patients have higher levels of unemployment, absenteeism, and presenteeism than nondepressed individuals [84-86], work-related outcomes are important functional outcomes for MDD. The WLQ [87] is a patient-reported outcome measures that measures the degree to which employed individuals are experiencing on-the-job limitations due to health conditions. It has been used in a variety of disease areas, including depression and anxiety. It has 4 subscales that represent the percentage of time that a person was limited in particular job demands: time and scheduling demands, such as working without stopping or taking breaks and getting going easily at the beginning of the workday (WLQ Time); physical
work demands, such as sitting, standing, or staying in 1 position for longer than 15 minutes while
working and using hand-held equipment (for example, a telephone) (WLQ Physical); mental-
interpersonal work demands, such as thinking clearly when working and speaking with people in
person, in meetings, or on the telephone (WLQ Mental); and output demands, such as working
fast enough and doing work without making mistakes (WLQ Output). The scores can be
weighted and aggregated to generate a productivity loss index score.

2.4.6 Patient-Reported Cognitive Function

To complement the objective neuropsychological tests, cognitive function can also be measured
subjectively, eg, using patient questionnaires such as the PDQ and the Cognitive and Physical
Functioning Questionnaire (CPFQ). These questionnaires allow for the assessment of cognitive
symptoms based on subjects’ experiences in their daily lives, in contrast to neuropsychological
tests that evaluate the objective nature and severity of the deficit in a controlled setting.

The PDQ [88] assesses cognitive functioning in 4 domains, which are reflected in four 5-item
subscales: attention/concentration, retrospective memory, prospective memory, and
planning/organization. Subjects rate how frequently they have experienced each symptom over
the past month on a scale ranging from 0 (never) to 4 (almost always). The total score of the
20 items ranges from 0 to 80; lower scores reflect better patient-reported cognitive function.
Qualitative research in patients with MDD confirms that the items in the PDQ are relevant to the
patients’ experience with cognitive dysfunction [89]. In subjects with depression, increased
severity of depression was associated with more cognitive dysfunction (ie, higher PDQ total
scores), with the attention/concentration and planning/organization subscales demonstrating the
greatest difference between severely depressed subjects and those with no depression symptoms
[90].

The CPFQ [91,92] is a 7-item scale that is used to assess cognitive and physical symptoms
associated with depression that are routinely observed in clinical practice. Subjects rate their
current cognitive function compared with their “normal” function on a scale from 1 (greater than
normal) to 6 (totally absent). The total score ranges from 7 to 42; higher scores represent greater
patient-reported dysfunction. Two subscale scores can be calculated: physical dimension,
composed of items 1 to 3 (motivation/interest/enthusiasm, wakefulness/alertness and energy) and
cognitive dimension, composed of items 4 to 7 (ability to focus/sustain attention, ability to
remember/recall information, ability to find words, and sharpness/mental acuity). Changes in the
CPFQ scores are influenced by the level of cognitive symptoms at Baseline. Therefore, when
evaluating changes, a cut-off may be used to exclude subjects with minimal or no cognitive
symptoms at Baseline. In CONNECT, subjects with a CPFQ total score >25 at Baseline were
included in CPFQ analyses (based on experience with the CPFQ in untreated depressed subjects,
where the mean CPFQ score was 26 [93]).

2.5 Summary

Vortioxetine is an antidepressant approved in the United States and European Union in 2013; to
date, it has been granted marketing authorization in 61 countries. Over 1.7 million patients have

ADVISORY COMMITTEE BRIEFING MATERIALS: AVAILABLE FOR PUBLIC RELEASE
received vortioxetine. To date, >50 jurisdictions have information regarding cognitive dysfunction in MDD included in the local vortioxetine label.

Cognitive dysfunction is frequently present in patients suffering from acute MDD and involves the domains of attention, processing speed, executive functioning, and learning and memory.

Current treatments for depression help to improve mood symptoms, but leave many patients with residual symptomatology, including cognitive dysfunction (reported at 30% to 44%). Cognitive dysfunction in MDD can be persistent and is associated with worse functional outcomes, suggesting that full recovery may not be possible without addressing cognitive dysfunction.

Evaluations based on objective neuropsychological tests are less vulnerable to the influence of the patient’s mood than subjective reports are and are therefore more useful to distinguish between effects on change in mood from effects on cognition itself. The DSST is a sufficient, valid, and sensitive objective measure of cognitive dysfunction in many settings, including MDD. DSST performance is a predictor of functional outcome in patients with severe psychiatric disorders and correlates with real-world functional outcomes or proxies thereof.
3.0 VORTIOXETINE DEVELOPMENT PROGRAM FOR COGNITIVE DYSFUNCTION IN MDD

3.1 Rationale for Development of Vortioxetine in Cognitive Dysfunction in MDD

Vortioxetine is thought to work through a combination of 2 pharmacological modes of action: a direct effect on 5-HT receptor activity as well as 5-HT reuptake inhibition. In vitro studies indicate that vortioxetine is a 5-hydroxytryptamine type 3 (5-HT3), 5-HT7, and 5-HT1D receptor antagonist, a 5-HT1B receptor partial agonist, a 5-HT1A receptor agonist, and an inhibitor of SERT (Figure 3.a; see also Section 3.7.1).

Figure 3.a Pharmacological Profile of Vortioxetine

As a result of vortioxetine’s distinct and potentially relevant pharmacological profile compared to that of other antidepressants, a decision was made to investigate vortioxetine’s effect on cognitive dysfunction in MDD in a study in elderly subjects (ELDERLY) that was part of the original MDD development program.

- In ELDERLY, the primary endpoint assessed depressive symptoms and the additional prespecified endpoints (not multiplicity controlled) included 2 objective neuropsychological tests—the DSST (processing speed, attention, and executive function [including working memory]) and the RAVLT (learning and memory).
- The selection of these 2 tests was based on the study by Raskin et al [18]. That study examined the effect of duloxetine on cognitive dysfunction in MDD in an elderly population using 4 neuropsychological tests. Duloxetine showed positive effects versus placebo on the RAVLT-equivalent test, but not on any of the 3 other more executive function–demanding tests, including a symbol coding test equivalent to the DSST. Given the pharmacological profile of vortioxetine, it was relevant to explore the potential clinical effects of vortioxetine.
across a broad range of cognitive domains relevant in MDD beyond learning and memory; therefore, the DSST was included in addition to the RAVLT.

- **ELDERLY** was considered an appropriate study for this evaluation, since cognitive dysfunction in MDD is known to be even more pronounced in the elderly than in adults.

- Duloxetine was selected as the active reference in ELDERLY based on its antidepressant effect in the elderly [18]. It was also considered an appropriate reference for exploring effects on cognitive dysfunction because of the positive effects shown on learning and memory [18].

- The results of ELDERLY showed that both vortioxetine and duloxetine improved depressive symptoms and had a positive effect (nominal p<0.05) on the RAVLT; in addition, only vortioxetine had a positive effect (nominal p<0.05) on the DSST. These results are consistent with the known antidepressant effect of both compounds, replicate published findings with duloxetine [18], and suggested that vortioxetine has an effect on a broad range of cognitive domains relevant in MDD.

On the basis of these results, it was decided to proceed with a thorough investigation to confirm vortioxetine’s effect on cognitive dysfunction in MDD. The approach included evaluation of vortioxetine’s effects on cognitive measures in an adult MDD population in 2 large and well-controlled clinical studies, additional nonclinical studies, and a mechanistic evaluation in an fMRI study in remitted MDD subjects. These studies were designed in the absence of precedent or regulatory guidance on evaluating cognitive dysfunction in MDD, but in consultation with experts in MDD as well as in cognitive testing.

The 2 large and well-controlled studies in adults were the pivotal studies FOCUS and CONNECT. Both studies:

- Had the primary objective to assess the effect of vortioxetine versus placebo on cognitive dysfunction in MDD.

- Used the DSST either as the primary endpoint or as part of a composite primary endpoint/multiplicity-controlled key secondary endpoint to objectively measure improvement of cognitive dysfunction in MDD.

- Included additional neuropsychological tests to further evaluate vortioxetine’s effects on cognitive dysfunction in MDD.

- Were placebo-controlled clinical studies in acute MDD to allow assessment of cognitive dysfunction in addition to the antidepressant effect.

- Included subjective measures of cognitive function to evaluate treatment effects on subjects’ perception of their cognitive symptoms.
Thus, the 2 studies were generally very similar, although some differences did exist. Study aims specific to FOCUS were to:

- Replicate in a general adult population with MDD the results on the DSST and the RAVLT observed in ELDERLY; therefore, the primary efficacy endpoint was the composite score for these 2 tests, with subsequent emphasis on the DSST.
- Investigate early treatment effects on cognitive dysfunction via a Week 1 assessment of cognitive performance.

Study aims specific to CONNECT were to:

- Replicate the results of FOCUS on the DSST in an adult population.
- Further evaluate the distinct effects of vortioxetine on cognitive function observed in ELDERLY through inclusion of duloxetine as an active reference. The duloxetine dose (60 mg/day) is the recommended dose; the USPI for duloxetine states that there is no evidence that higher doses will give additional benefit, while some adverse events are dose dependent.
- Support the clinical relevance of the improvement in cognitive dysfunction by assessing functionality using a performance-based measure of functional capacity (UPSA) as well as a patient-reported work limitations questionnaire (WLQ) and additional patient-reported measures of cognitive dysfunction.

In parallel with the 2 pivotal clinical studies, additional nonclinical studies and an fMRI study were conducted:

- Nonclinical studies were conducted to extend the understanding of vortioxetine’s distinct cognition-enhancing effects not shown with SSRIs/SNRIs.
- The fMRI study in remitted MDD subjects was conducted to evaluate the effect of vortioxetine on activity in brain regions relevant for cognitive performance in MDD.

3.2 Statistical Considerations

The classification of the endpoints in each study—whether primary, key secondary, or additional prespecified, and whether or not its analysis was multiplicity controlled—is described in the individual study sections and summarized in Appendix C.

3.2.1 Analysis Sets

Because the primary endpoints in the clinical studies were assessed as changes from Baseline, the full analysis set (FAS) in each study was defined to include the subjects who were randomized, received at least 1 dose of study drug, and had at least 1 post-Baseline assessment of the primary efficacy variable(s). The FAS is equivalent to a modified Intent-to-Treat (mITT) population.
3.2.2 Statistical Approaches

For the 2 studies (ELDERLY and CONNECT) with only 1 post-Baseline (Week 8) assessment of the DSST, the Week 8 assessment was used for statistical analysis; thus, the observed cases (OC) and last observation carried forward (LOCF) analyses were identical. An analysis of covariance (ANCOVA) model with site, treatment, and baseline value was applied.

For FOCUS, which had 2 post-Baseline assessments of the DSST, a mixed model for repeated measures (MMRM) analysis was chosen. The MMRM analysis included terms for baseline value and site and had a freely varying mean structure and an unstructured covariance matrix.

Endpoints assessed more than once post-Baseline were analyzed by MMRM. Endpoints assessed only once post Baseline were analyzed by ANCOVA, LOCF.

3.2.3 MADRS-Adjusted, Mediation, and Path Analyses

The vortioxetine studies assessing cognitive dysfunction in MDD were conducted in acutely depressed subjects. Improvement in depressive symptoms can confound treatment effects on cognitive dysfunction, especially in subjective measures. To obtain an estimate of the degree to which the effect of treatment on the DSST could be attributed to alleviation of depressive symptoms (indirect effect), a MADRS-adjusted analysis was performed by adding the change from Baseline in MADRS total score as a covariate (mediator) in the ANCOVA model. The baseline value for the MADRS total score was also added to the model as a covariate. In the path analysis that estimated the proportion of cognitive dysfunction improvement that was due to improvement in depressive symptoms, the above analysis served as the first part, whereas the separate analysis of MADRS served as the second part.

The results of these analyses are not presented in the individual studies but are discussed across studies in Section 3.6 (as ANCOVA, LOCF).

Additional information on mediation (path) analysis is presented in Appendix C.

3.2.4 Standardized Effect Sizes

The estimated differences from placebo were standardized to allow for comparisons of the magnitude of the effect sizes across studies, endpoints, and different versions of endpoints. These standardized effect sizes (also known as Cohen’s d) were calculated using the least squares (LS) means and the standard errors (SEs) from the linear models. The methods are described in Appendix C.

3.3 Hypothesis-Generating Study, ELDERLY (Study 12541A)

ELDERLY was specifically designed to assess the effect of vortioxetine on depressive symptoms as the primary endpoint and had improvements of objective cognitive function prespecified as additional endpoints without multiplicity control; the results of this early study provided the first clinical evidence for the hypothesis that vortioxetine could exert beneficial effects on cognitive dysfunction in MDD.
3.3.1 Study Design

ELDERLY was a randomized, double-blind, parallel-group, placebo-controlled, 8-week study in elderly subjects, conducted in Asia, Australia, Canada, Europe, South Africa, and the United States. The subjects were randomized equally to treatment with placebo, vortioxetine 5 mg, or duloxetine 60 mg. The study design is illustrated in Figure 3.b.

Figure 3.b Study Design—ELDERLY

Depressive symptoms (primary endpoint) assessed at Screening, Baseline, and Weeks 1, 2, 4, 6, and 8. Cognitive assessments (additional prespecified endpoints) administered at Baseline and Week 8.

Eligible subjects:
- Were men and women aged ≥65 years.
- Had a primary diagnosis of MDE within MDD according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR™) criteria [94].
- Had a MADRS total score ≥26, current MDE with a duration ≥4 weeks, and at least 1 MDE before the age of 60 years.
- Had Mini Mental State Examination [MMSE] [95] score ≥24 in order to exclude the confounding effects of dementia.
- Had not previously failed to respond to duloxetine.
3.3.2 Endpoints

3.3.2.1 Primary, Multiplicity-Controlled Endpoint
The primary endpoint was the Hamilton Depression Scale-24 item (HAM-D24) total score at Week 8 [96].

3.3.2.2 Additional Prespecified, Non–Multiplicity-Controlled Endpoints
Depression endpoints included:
- MADRS total score.
- Clinical Global Impression of Severity (CGI-S) score.
- Clinical Global Impression of Improvement (CGI-I) score.

Cognition endpoints included:
- DSST.
- RAVLT.

The Wechsler Adult Intelligence Scale (WAIS)-III DSST 120-sec original version was used for these elderly subjects.

3.3.3 Statistical Analyses

3.3.3.1 Sample Size Calculation
Approximately 450 subjects were planned for enrollment in the study. With 150 subjects per treatment group and an SD of 8, the power to detect a true treatment effect of 2.64 in the change from Baseline to Week 8 on the HAM-D24 would be at least 80%, using a 0.05 level of significance and a standard ANCOVA (t-distributed contrast). The treatment effect of 2.64 corresponds to a standardized effect size of 0.33.

3.3.3.2 Multiplicity-Controlled Analyses
The primary efficacy analysis was an ANCOVA of the change from Baseline in HAM-D24 total score at Week 8 (FAS, LOCF) for vortioxetine compared to placebo, with treatment and site as factors and the baseline HAM-D24 total score as a covariate. The potential influence of covariates was investigated within the ANCOVA model by adding main terms for covariates and interaction terms with treatment. The covariates included site, country, sex, and baseline HAM-D24 total score.

The primary efficacy analysis was repeated on OC data, using both ANCOVA and MMRM. The analyses were repeated for duloxetine versus placebo, but outside the testing hierarchy.
3.3.3.3 Additional Prespecified, Non-Multiplicity-Controlled Analyses

Additional prespecified analyses for depression included the change from Baseline in the MADRS total score, the CGI-S score, and the CGI-I score [97].

The DSST and RAVLT were analyzed to assess objective cognitive function.

In the analyses of efficacy variables that were only assessed at Baseline and Week 8, any assessment ≥2 days post-Baseline was considered a Week 8 assessment; therefore, the OC and LOCF results are identical.

Analyses outside the testing hierarchy were tested at the 0.05 level of significance; all p-values from such analyses are nominal.

Comparisons of the active reference duloxetine versus placebo were also performed outside the testing strategy at a nominal significance level of 0.05.

3.3.4 Disposition, Demographics, and Baseline Results

A total of 453 subjects were randomized to the 8-week treatment period (Table 3.a). Of these, 452 were treated (received at least 1 dose of study drug) and 448 of those subjects had at least 1 valid post-Baseline assessment of the primary efficacy variable (HAM-D24 total score). The FAS used in the efficacy analyses comprised the latter 448 subjects. Of the 452 treated subjects, 392 completed the study.
Table 3.a  Subject Disposition—ELDERLY

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Vortioxetine 5 mg</th>
<th>Duloxetine 60 mg</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomized</td>
<td>145</td>
<td>157</td>
<td>151</td>
<td>453</td>
</tr>
<tr>
<td>Treated</td>
<td>145</td>
<td>156</td>
<td>151</td>
<td>452</td>
</tr>
<tr>
<td>Completed</td>
<td>128 (88.3)</td>
<td>136 (87.2)</td>
<td>128 (84.8)</td>
<td>392 (86.7)</td>
</tr>
<tr>
<td>Early termination</td>
<td>17 (11.7)</td>
<td>20 (12.8)</td>
<td>23 (15.2)</td>
<td>60 (13.3)</td>
</tr>
</tbody>
</table>

Reason for early termination
- Adverse event: 6 (4.1) | 10 (6.4) | 15 (9.9) | 31 (6.9)
- Lack of efficacy: 7 (4.8) | 2 (1.3) | 0 | 9 (2.0)
- Protocol deviations: 3 (2.1) | 3 (1.9) | 2 (1.3) | 8 (1.8)
- Withdrawal of consent: 1 (0.7) | 2 (1.3) | 2 (1.3) | 5 (1.1)
- Lost to follow-up: 0 | 0 | 2 (1.3) | 2 (0.4)
- Other: 0 | 3 (1.9) | 2 (1.3) | 5 (1.1)

FAS | 145 | 155 | 148 | 448

(a) Percentages are based on all treated subjects.

Overall, the mean age (SD) at Baseline was 71(5) years; 66% of the subjects were female. The majority of the subjects were Caucasian (95%), 4% were Black, and <1% were Asian (Table 3.b).

Table 3.b  Demographics (All Treated Subjects)—ELDERLY

<table>
<thead>
<tr>
<th></th>
<th>Placebo N=145</th>
<th>Vortioxetine 5 mg N=156</th>
<th>Duloxetine 60 mg N=151</th>
<th>Total N=452</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>55 (37.9)</td>
<td>49 (31.4)</td>
<td>51 (33.8)</td>
<td>155 (34.3)</td>
</tr>
<tr>
<td>Female</td>
<td>90 (62.1)</td>
<td>107 (68.6)</td>
<td>100 (66.2)</td>
<td>297 (65.7)</td>
</tr>
<tr>
<td>Age: Mean (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian (a)</td>
<td>70.3 (4.4)</td>
<td>70.5 (4.8)</td>
<td>70.9 (5.5)</td>
<td>70.6 (4.9)</td>
</tr>
<tr>
<td>Black</td>
<td>4 (2.8)</td>
<td>8 (5.1)</td>
<td>6 (4.0)</td>
<td>18 (4.0)</td>
</tr>
<tr>
<td>Asian</td>
<td>1 (0.7)</td>
<td>1 (0.6)</td>
<td>1 (0.7)</td>
<td>3 (0.7)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (0.7)</td>
<td>2 (1.3)</td>
<td>0 (0.0)</td>
<td>3 (0.7)</td>
</tr>
</tbody>
</table>

(a) Including Hispanic.

Overall, at Baseline, the subjects had moderate to severe MDD based on the MADRS total scores and were moderately to markedly ill based on the CGI-S scores (Table 3.c).
Table 3.c  Baseline Efficacy Scores (FAS)—ELDERLY

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo (N=145)</th>
<th>Vortioxetine 5 mg (N=155)</th>
<th>Duloxetine 60 mg (N=148)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MADRS total score (a)</td>
<td>30.3 (3.2)</td>
<td>30.7 (3.6)</td>
<td>30.4 (3.1)</td>
</tr>
<tr>
<td>HAM-D24 (b)</td>
<td>29.4 (5.1)</td>
<td>29.2 (5.0)</td>
<td>28.5 (4.9)</td>
</tr>
<tr>
<td>CGI-S</td>
<td>4.7 (0.7)</td>
<td>4.8 (0.7)</td>
<td>4.7 (0.8)</td>
</tr>
<tr>
<td>DSST (c)</td>
<td>44.6 (17.9)</td>
<td>45.2 (17.9)</td>
<td>46.3 (18.3)</td>
</tr>
<tr>
<td>RAVLT acquisition/learning (c)</td>
<td>21.8 (6.0)</td>
<td>22.3 (6.4)</td>
<td>22.0 (6.7)</td>
</tr>
<tr>
<td>RAVLT delayed recall/memory (c)</td>
<td>6.2 (3.0)</td>
<td>6.6 (3.2)</td>
<td>6.5 (3.1)</td>
</tr>
</tbody>
</table>

Data are mean (SD).
(a) A MADRS total score ≥26 at Baseline was an inclusion criterion.
(b) Primary endpoint in this MDD study.
(c) Cognition endpoints.

There were no meaningful imbalances in demographic or baseline values between the treatment groups.

The mean exposure to study drug ranged from 50 to 53 days (median: 56 days). Approximately 87% of the subjects in each treatment group received study drug for 50-63 days in the 8-week treatment period.

3.3.5 Testing Hierarchy: Primary Analysis Results, Depression

The outcome of ELDERLY was positive: vortioxetine 5 mg was statistically significantly better than placebo in reducing the HAM-D24 total score at Week 8, with LS mean difference from placebo of 3.3 points (Table 3.d). Duloxetine 60 mg separated from placebo (nominal p<0.05) with LS mean difference from placebo of 5.5 points.

Table 3.d Analysis of Change From Baseline in Primary Endpoint, HAM-D24 Total Score at Week 8 (FAS, ANCOVA, LOCF)—ELDERLY

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline Mean</th>
<th>Change From Baseline</th>
<th>Difference From Placebo</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>LS Mean</td>
<td>SE</td>
<td>LS Mean Lower 95% CI Upper 95% CI</td>
</tr>
<tr>
<td>Placebo</td>
<td>29.37</td>
<td>-10.3</td>
<td>0.76</td>
<td>-3.32   -5.31 -1.34 0.001*</td>
</tr>
<tr>
<td>VOR 5 mg</td>
<td>29.24</td>
<td>-13.7</td>
<td>0.74</td>
<td>-5.48   -7.50 -3.46 &lt;0.0001†</td>
</tr>
<tr>
<td>DUL 60 mg</td>
<td>28.51</td>
<td>-15.8</td>
<td>0.75</td>
<td>-3.22   -5.24 -1.20 0.002*</td>
</tr>
</tbody>
</table>

DUL=duloxetine, VOR=vortioxetine.
* = p<0.05, †= nominal p<0.05.

3.3.6 Efficacy Results Outside the Testing Hierarchy: Cognition

Vortioxetine separated from placebo (nominal p<0.05) on the DSST, but duloxetine did not (Table 3.e). The mean differences from placebo for vortioxetine and duloxetine on the DSST were 2.79 and 0.77, respectively.
Table 3.e  **Analysis of Change From Baseline in DSST at Week 8 (FAS, ANCOVA, LOCF)—ELDERLY**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline Mean</th>
<th>Change From Baseline</th>
<th>Difference From Placebo</th>
<th>Nominal p-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>LS Mean</td>
<td>SE</td>
<td>LS Mean</td>
<td>Lower 95% CI</td>
</tr>
<tr>
<td>Placebo</td>
<td>144</td>
<td>1.51</td>
<td>0.92</td>
<td>2.79</td>
<td>0.40</td>
</tr>
<tr>
<td>VOR 5 mg</td>
<td>153</td>
<td>4.30</td>
<td>0.89</td>
<td>2.79</td>
<td>0.40</td>
</tr>
<tr>
<td>DUL 60 mg</td>
<td>144</td>
<td>2.28</td>
<td>0.91</td>
<td>0.77</td>
<td>-1.67</td>
</tr>
</tbody>
</table>

VOR=vortioxetine, DUL=duloxetine.

For the cognition variable RAVLT, both vortioxetine and duloxetine separated from placebo (nominal p<0.05) on both the acquisition/learning and delayed recall/memory measures (Table 3.f and Table 3.g).

Table 3.f  **Analysis of Change From Baseline in RAVLT Acquisition/Learning at Week 8 (FAS, ANCOVA, LOCF)—ELDERLY**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline Mean</th>
<th>Change From Baseline</th>
<th>Difference From Placebo</th>
<th>Nominal p-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>LS Mean</td>
<td>SE</td>
<td>LS Mean</td>
<td>Lower 95% CI</td>
</tr>
<tr>
<td>Placebo</td>
<td>144</td>
<td>2.31</td>
<td>0.37</td>
<td>1.14</td>
<td>0.17</td>
</tr>
<tr>
<td>VOR 5 mg</td>
<td>152</td>
<td>3.45</td>
<td>0.36</td>
<td>1.41</td>
<td>0.42</td>
</tr>
<tr>
<td>DUL 60 mg</td>
<td>144</td>
<td>3.72</td>
<td>0.37</td>
<td>1.41</td>
<td>0.42</td>
</tr>
</tbody>
</table>

VOR=vortioxetine, DUL=duloxetine.

Table 3.g  **Analysis of Change From Baseline in RAVLT Delayed Recall/Memory at Week 8 (FAS, ANCOVA, LOCF)—ELDERLY**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline Mean</th>
<th>Change From Baseline</th>
<th>Difference From Placebo</th>
<th>Nominal p-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>LS Mean</td>
<td>SE</td>
<td>LS Mean</td>
<td>Lower 95% CI</td>
</tr>
<tr>
<td>Placebo</td>
<td>144</td>
<td>0.94</td>
<td>0.17</td>
<td>0.47</td>
<td>0.02</td>
</tr>
<tr>
<td>VOR 5 mg</td>
<td>152</td>
<td>1.58</td>
<td>0.17</td>
<td>0.47</td>
<td>0.02</td>
</tr>
<tr>
<td>DUL 60 mg</td>
<td>143</td>
<td>1.42</td>
<td>0.17</td>
<td>0.64</td>
<td>0.18</td>
</tr>
</tbody>
</table>

VOR=vortioxetine, DUL=duloxetine.

3.3.7  **Summary of Results for ELDERLY**

Both vortioxetine (statistically significantly different from placebo [p<0.05]) and duloxetine (nominal p<0.05 versus placebo) showed antidepressant effect on the HAM-D24 at Week 8. Vortioxetine separated from placebo (nominal p<0.05) on objective cognitive performance as assessed using the DSST and RAVLT. Duloxetine separated from placebo (nominal p<0.05) on the RAVLT but not the DSST.
The effect of duloxetine on the RAVLT, as well as the lack of effect of duloxetine on the DSST while still alleviating mood symptoms was consistent with results in published data [18]. The findings that both vortioxetine and duloxetine had a treatment effect on depression measures but only vortioxetine had a treatment effect on the DSST suggest that vortioxetine’s effects on cognitive dysfunction are distinct from improvements in depressive symptoms.

3.4 Pivotal Study—FOCUS (Study 14122A)

FOCUS was designed to confirm the potential effect of vortioxetine on cognitive dysfunction in adults with MDD. The objectives were to evaluate the efficacy of vortioxetine (10 or 20 mg/day) versus placebo on cognitive dysfunction and the efficacy on the subjects’ depressive symptoms.

3.4.1 Study Design

FOCUS was a randomized, double-blind, parallel-group, placebo-controlled, 8-week study conducted in Australia, Canada, Europe, Mexico, South Africa, and the United States. The subjects were randomized equally to treatment with vortioxetine (a fixed dose of 10 or 20 mg/day) or placebo. The subjects who were randomized to 20 mg/day vortioxetine were uptitrated from 10 mg/day at the end of the first week of treatment. The study design is illustrated in Figure 3.c.

Figure 3.c Study Design—FOCUS

Cognitive assessments (primary and key secondary endpoints) administered at Baseline and Weeks 1 and 8. Depressive symptoms (additional prespecified endpoints) assessed at Screening, Baseline, and Weeks 1, 4, and 8.
Eligible subjects:
- Were men and women, aged ≥18 and ≤65 years, with a primary diagnosis of recurrent MDE within MDD.
- Had a MADRS total score ≥26 and had a duration of current MDE ≥3 months.
- Had a score <70 on the DSST and <42 on the RAVLT (acquisition/learning) and <14 on the RAVLT (delayed recall/memory) at the Baseline Visit (to improve sensitivity to change).

3.4.2 Endpoints

3.4.2.1 Primary, Multiplicity-Controlled Endpoint

The primary efficacy endpoint was the change from Baseline to Week 8 in the composite z-score, defined as the weighted sum of the individual subject z-scores on the DSST (WAIS®-III DSST 90-sec test period, adopted to improve sensitivity to change in adults) and RAVLT (acquisition/learning and delayed recall/memory). (The DSST and RAVLT were key secondary endpoints, see below.)

Each individual composite z-score was the weighted sum of the 3 standardized test scores. The DSST and RAVLT were equally weighted, thus the DSST was assigned a weight of 0.5 and the 2 test subscores of the RAVLT (acquisition/learning and delayed recall/memory) were each assigned a weight of 0.25.

3.4.2.2 Key Secondary, Multiplicity-Controlled Endpoints

The key secondary endpoints were change from Baseline at Week 8 in the individual tests making up the composite:
- DSST.
- RAVLT acquisition/learning.
- RAVLT delayed recall/memory.

3.4.2.3 Additional Prespecified, Non-Multiplicity-Controlled Endpoints

Additional endpoints used to assess cognitive function objectively were the change from Baseline to Week 8 in the following neuropsychological tests:
- Trail Making Test (TMT) -A and -B.
- Stroop Color Naming Test (Stroop).
- Simple Reaction Time Task (SRT).
- Choice Reaction Time Task (CRT).

The PDQ was used to assess patient-reported cognitive function subjectively.
Depressive symptoms at Week 8 were assessed for the:

- Change from Baseline in MADRS total score.
- Change from Baseline in CGI-S score.
- CGI-I score.

### 3.4.3 Statistical Analyses

#### 3.4.3.1 Sample Size Calculation

Within each dose, the significance level was set to 0.025, thus keeping an overall significance level of 0.05. Based on the results of ELDERLY, the treatment difference between placebo and vortioxetine was assumed to be 0.25 for each dose on the composite z-score change from Baseline, while the SD was assumed to be 0.68. With these assumptions and an expected withdrawal rate of 20%, 200 subjects per treatment group provided a power of approximately 90% for finding at least 1 dose significant at Week 8 and a power of approximately 85% for finding a specific dose significant. Thus, a total of 600 subjects (200 subjects per treatment group) were planned for enrollment.

#### 3.4.3.2 Multiplicity-Controlled Analyses

The following were prespecified endpoints within the testing hierarchy.

The primary efficacy analysis was a comparison of the composite z-score for vortioxetine 10 or 20 mg/day versus that for placebo at Week 8, using an MMRM analysis. The model included terms for pooled site (accounting for variation by country), baseline composite z-score, baseline composite z-score–by–visit interaction, and treatment-by-visit interaction.

Hierarchical testing was performed in 2 sequences (10 or 20 mg/day) with a significance level of 0.025 for each sequence; the analyses were versus placebo at Week 8, in the following order:

- Primary endpoint, change from Baseline in the composite z-score (MMRM) of DSST+RAVLT.

- Key secondary endpoints:
  - Change from Baseline in DSST (MMRM).
  - Change from Baseline in RAVLT acquisition/learning (MMRM).
  - Change from Baseline in RAVLT delayed recall/memory (MMRM).

#### 3.4.3.3 Additional Prespecified, Non–Multiplicity-Controlled Analyses

Additional prespecified endpoints were analyzed at a nominal significance level of 0.05. All comparisons were versus placebo:
• The analyses of the objective neuropsychological tests—TMT A and B, Stroop, SRT, and CRT—were by MMRM (OC) at Weeks 1 and 8. For each endpoint, its baseline score was used as a covariate in the analyses.

• Subjectively reported cognitive function, PDQ, was analyzed at Week 8 by ANCOVA (LOCF).

• The analyses of depressive symptoms—MADRS, CGI-S, and CGI—were by MMRM (OC) at Weeks 1, 4 and 8. (Because it is a measure of improvement, there was no baseline assessment of CGI-I; therefore CGI-I score was analyzed rather than change from Baseline, using the baseline CGI-S score as a covariate.)

As sensitivity analyses, analyses of the composite z-score and DSST were repeated as ANCOVA (LOCF and OC) at Weeks 1 and 8.

3.4.4 Disposition, Demographics, and Baseline Results

A total of 877 subjects were screened, 602 of whom were randomized to the 8-week Treatment Period (Table 3.h). Of these, 598 were treated (received at least 1 dose of study drug) and 591 of those subjects had at least 1 valid post-Baseline assessment of the DSST, RAVLT (learning/acquisition), and RAVLT (memory/delayed recall). The FAS used in the efficacy analyses comprised the latter 591 subjects. Of the 598 treated subjects, 514 completed the study.
Table 3.h  Subject Disposition—FOCUS

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Vortioxetine 10 mg</th>
<th>Vortioxetine 20 mg</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomized</td>
<td>198</td>
<td>197</td>
<td>207</td>
<td>602</td>
</tr>
<tr>
<td>Treated</td>
<td>196</td>
<td>195</td>
<td>207</td>
<td>598</td>
</tr>
<tr>
<td>Completed study</td>
<td>163 (83.2)</td>
<td>173 (88.7)</td>
<td>178 (86.0)</td>
<td>514 (86.0)</td>
</tr>
<tr>
<td>Early termination</td>
<td>33 (16.8)</td>
<td>22 (11.3)</td>
<td>29 (14.0)</td>
<td>84 (14.0)</td>
</tr>
</tbody>
</table>

Primary reason for early termination
- Adverse event(s)
  - Placebo: 8 (4.1)
  - Vortioxetine 10 mg: 7 (3.6)
  - Vortioxetine 20 mg: 11 (5.3)
  - Total: 26 (4.3)
- Lack of efficacy
  - Placebo: 10 (5.1)
  - Vortioxetine 10 mg: 2 (1.0)
  - Vortioxetine 20 mg: 2 (1.0)
  - Total: 14 (2.3)
- Noncompliance with study drug
  - Placebo: 0
  - Vortioxetine 10 mg: 1 (0.5)
  - Vortioxetine 20 mg: 0
  - Total: 1 (0.2)
- Protocol violation
  - Placebo: 0
  - Vortioxetine 10 mg: 2 (1.0)
  - Vortioxetine 20 mg: 4 (1.9)
  - Total: 6 (1.0)
- Withdrawal of consent
  - Placebo: 7 (3.6)
  - Vortioxetine 10 mg: 3 (1.5)
  - Vortioxetine 20 mg: 5 (2.4)
  - Total: 15 (2.5)
- Lost to follow-up
  - Placebo: 3 (1.5)
  - Vortioxetine 10 mg: 3 (1.5)
  - Vortioxetine 20 mg: 5 (2.4)
  - Total: 11 (1.8)
- Administrative or other reason(s)
  - Placebo: 5 (2.6)
  - Vortioxetine 10 mg: 4 (2.1)
  - Vortioxetine 20 mg: 2 (1.0)
  - Total: 11 (1.8)

FAS 194 193 204 591

(a) Percentages based on all treated subjects.

Overall, the mean age (SD) of the subjects at Baseline was 46 (12) years; 66% were female. The majority of the subjects (95%) were Caucasian, 3% were Black, and 0.5% were Asian (Table 3.i).

Table 3.i  Demographics (All Treated Subjects)—FOCUS

<table>
<thead>
<tr>
<th></th>
<th>Placebo N=196</th>
<th>Vortioxetine 10 mg N=195</th>
<th>Vortioxetine 20 mg N=207</th>
<th>Total N=598</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex: N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>67 (34.2)</td>
<td>61 (31.3)</td>
<td>74 (35.7)</td>
<td>202 (33.8)</td>
</tr>
<tr>
<td>Female</td>
<td>129 (65.8)</td>
<td>134 (68.7)</td>
<td>133 (64.3)</td>
<td>396 (66.2)</td>
</tr>
<tr>
<td>Age: Mean (SD)</td>
<td>45.55 (12.13)</td>
<td>45.44 (12.17)</td>
<td>46.09 (11.80)</td>
<td>45.70 (12.01)</td>
</tr>
<tr>
<td>Race: N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>188 (95.9)</td>
<td>183 (93.8)</td>
<td>194 (93.7)</td>
<td>565 (94.5)</td>
</tr>
<tr>
<td>Black (a)</td>
<td>6 (3.1)</td>
<td>5 (2.6)</td>
<td>7 (3.4)</td>
<td>18 (3.0)</td>
</tr>
<tr>
<td>Asian</td>
<td>0</td>
<td>2 (1.0)</td>
<td>1 (0.5)</td>
<td>3 (0.5)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (1.0)</td>
<td>5 (2.6)</td>
<td>5 (2.4)</td>
<td>12 (2.0)</td>
</tr>
</tbody>
</table>

(a) Or African American.

The distribution of MADRS total scores at Baseline indicated that the subjects had moderate to severe MDD (a MADRS total score ≥26 at Baseline was an inclusion criterion) and the distribution of CGI-S scores at Baseline indicated that the subjects were moderately to markedly ill (Table 3.j).
### Table 3.j  Baseline Efficacy Scores (FAS)—FOCUS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo (N=194)</th>
<th>Vortioxetine 10 mg (N=193)</th>
<th>Vortioxetine 20 mg (N=204)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MADRS total score</td>
<td>31.3 (3.75)</td>
<td>31.6 (3.77)</td>
<td>31.7 (3.53)</td>
</tr>
<tr>
<td>CGI-S score</td>
<td>4.6 (0.63)</td>
<td>4.6 (0.62)</td>
<td>4.6 (0.58)</td>
</tr>
<tr>
<td>DSST total score</td>
<td>42.4 (13.76)</td>
<td>42.0 (12.56)</td>
<td>41.6 (12.72)</td>
</tr>
<tr>
<td>RAVLT acquisition /learning</td>
<td>22.1 (5.70)</td>
<td>22.3 (5.76)</td>
<td>22.6 (5.88)</td>
</tr>
<tr>
<td>RAVLT delayed recall/memory</td>
<td>5.7 (2.82)</td>
<td>5.8 (2.82)</td>
<td>6.1 (3.14)</td>
</tr>
<tr>
<td>PDQ total score</td>
<td>39.8 (11.89)</td>
<td>41.4 (11.50)</td>
<td>41.1 (12.11)</td>
</tr>
</tbody>
</table>

Data are mean (SD).

There were no meaningful imbalances in demographic or baseline values among the treatment groups.

The mean exposure to the study drug ranged from 51 to 53 days (median 56 days) across treatment groups. Approximately 87% of the subjects in each treatment group received study drug for 50 to 63 days in the 8-week Treatment Period.

#### 3.4.5 Testing Hierarchy: Primary and Key Secondary Efficacy Analyses Results

The subjects in both vortioxetine groups improved statistically significantly more on cognitive performance than those in the placebo group did. The mean difference from placebo in the primary endpoint, composite z-score at Week 8, was 0.36 points in favor of vortioxetine 10 mg/day (p<0.001) and 0.33 points in favor of vortioxetine 20 mg/day (p<0.001) (Table 3.k).

### Table 3.k  Analysis of Change From Baseline in Primary Endpoint, Composite Z-Score at Week 8 (FAS, MMRM)—FOCUS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline Mean</th>
<th>Change From Baseline</th>
<th>Difference From Placebo</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>LS Mean</td>
<td>SE</td>
<td>LS Mean</td>
</tr>
<tr>
<td>Placebo</td>
<td>-0.007</td>
<td>178</td>
<td>-0.24</td>
<td>0.05</td>
</tr>
<tr>
<td>VOR 10 mg</td>
<td>-0.009</td>
<td>180</td>
<td>0.13</td>
<td>0.05</td>
</tr>
<tr>
<td>VOR 20 mg</td>
<td>0.015</td>
<td>187</td>
<td>0.10</td>
<td>0.05</td>
</tr>
</tbody>
</table>

VOR=vortioxetine.

In the sensitivity analyses of the primary efficacy endpoint, the results were overall similar to that of the primary efficacy analysis (see Appendix D).

The subjects in both vortioxetine groups also improved statistically significantly more (4.2 points) than those in the placebo group did in the next prespecified analysis, the multiplicity-controlled key secondary efficacy analysis of the DSST (p<0.001) (Table 3.l). Vortioxetine (both doses) also separated from placebo (nominal p<0.05) at Week 1 using the DSST.
### Table 3.1 Analysis of Change From Baseline in DSST at Week 8 (FAS, MMRM)—FOCUS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline Mean</th>
<th>Change From Baseline</th>
<th>Difference From Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>42.38</td>
<td>179 4.83 0.63</td>
<td>4.20 2.50 5.90 &lt;0.0001 0.51</td>
</tr>
<tr>
<td>VOR 10 mg</td>
<td>41.96</td>
<td>180 9.03 0.63</td>
<td>4.26 2.57 5.94 &lt;0.0001 0.52</td>
</tr>
<tr>
<td>VOR 20 mg</td>
<td>42.61</td>
<td>187 9.09 0.61</td>
<td></td>
</tr>
</tbody>
</table>

VOR=vortioxetine.

In the sensitivity analyses of the DSST, the results were overall similar to that of the primary efficacy analysis (see Appendix D).

The testing hierarchy stopped with a nonsignificant p-value (p≥0.025) in the 10 and 20 mg groups while testing the RAVLT acquisition/learning score (Table 3.m); the p-values for subsequent steps are nominal. However, the subjects in both dose groups improved more than those in the placebo group (nominal p<0.05) on the RAVLT delayed recall/memory score, and in the 10 mg group on the RAVLT acquisition/learning score (Table 3.m and Table 3.n).
Table 3.m Analysis of Change From Baseline in RAVLT Acquisition/Learning at Week 8 (FAS, MMRM)—FOCUS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline Mean</th>
<th>Change From Baseline</th>
<th>Difference From Placebo</th>
<th>Nominal p-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>22.14</td>
<td>3.06</td>
<td>0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VOR 10 mg</td>
<td>22.34</td>
<td>4.08</td>
<td>0.34</td>
<td>1.02</td>
<td>0.11</td>
</tr>
<tr>
<td>VOR 20 mg</td>
<td>22.64</td>
<td>3.65</td>
<td>0.33</td>
<td>0.59</td>
<td>-0.31</td>
</tr>
</tbody>
</table>

VOR=vortioxetine.

Table 3.n Analysis of Change From Baseline in RAVLT Delayed Recall/Memory at Week 8 (FAS, MMRM)—FOCUS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline Mean</th>
<th>Change From Baseline</th>
<th>Difference From Placebo</th>
<th>Nominal p-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>5.70</td>
<td>0.91</td>
<td>0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VOR 10 mg</td>
<td>5.76</td>
<td>1.63</td>
<td>0.18</td>
<td>0.71</td>
<td>0.24</td>
</tr>
<tr>
<td>VOR 20 mg</td>
<td>6.05</td>
<td>1.56</td>
<td>0.17</td>
<td>0.65</td>
<td>0.17</td>
</tr>
</tbody>
</table>

VOR=vortioxetine.

3.4.6 Efficacy Results Outside the Testing Hierarchy

3.4.6.1 Additional Objective Neuropsychological Tests of Cognitive Function

The effect of vortioxetine on cognitive performance was further assessed using additional neuropsychological tests. The results of these analyses are summarized in Table 3.o. With the exception of vortioxetine 20 mg on the CRT, vortioxetine 10 and 20 mg separated from placebo (nominal p<0.05) on all cognitive tests.
Table 3.o  Neuropsychological Tests at Week 8 (FAS, MMRM)—FOCUS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Difference From Placebo at Week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vortioxetine 10 mg/day</td>
</tr>
<tr>
<td></td>
<td>LS Mean 95% CI Effect Size LS Mean 95% CI Effect Size</td>
</tr>
<tr>
<td>Δ TMT–A</td>
<td>-3.76†† -6.45; -1.08 0.29</td>
</tr>
<tr>
<td>Δ TMT–B</td>
<td>-7.57†† -12.93; -2.20 0.29</td>
</tr>
<tr>
<td>Δ Stroop congruent</td>
<td>-4.00†† -6.50; -1.49 0.33</td>
</tr>
<tr>
<td>Δ Stroop incongruent</td>
<td>-6.75†† -10.76; -2.74 0.35</td>
</tr>
<tr>
<td>Δ SRT</td>
<td>-0.046††† -0.07; -0.02 0.41</td>
</tr>
<tr>
<td>Δ CRT</td>
<td>-0.032††† -0.05; -0.01 0.38</td>
</tr>
</tbody>
</table>

Δ=change from Baseline.
† p<0.05, †† p<0.01, ††† p<0.001 versus placebo (all nominal).
A negative LS mean value indicates advantages compared to placebo.

The standardized effect sizes for all the objective neuropsychological tests are shown in Figure 3.d.

Figure 3.d  Change From Baseline in Neuropsychological Tests at Week 8, Standardized Effect Sizes Versus Placebo (FAS, MMRM)—FOCUS

Acq=acquisition/learning, Delay=delayed recall/memory, Con=congruent, Incon=incongruent.
Error bars indicate SE.
***=p<0.001 versus placebo (statistically significant).
† p<0.05; †† p<0.01; ††† p<0.001 versus placebo (all nominal).

ADVISORY COMMITTEE BRIEFING MATERIALS: AVAILABLE FOR PUBLIC RELEASE
3.4.6.2 Patient-Reported Cognitive Symptoms

Both vortioxetine groups separated from the placebo group in self-reported cognitive symptoms as assessed using the PDQ total score and PDQ attention/concentration and planning/organization combined subscore at Week 8 (Table 3.p).

Table 3.p PDQ Scores at Week 8 (FAS, ANCOVA, LOCF)—FOCUS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vortioxetine 10 mg/day</th>
<th>Vortioxetine 20 mg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LS Mean</td>
<td>95% CI</td>
</tr>
<tr>
<td>Δ PDQ total score</td>
<td>-4.40††† (a)</td>
<td>-6.75, -2.06</td>
</tr>
<tr>
<td>Δ PDQ attention/concentration and planning/organization combined (c)</td>
<td>-2.55†††</td>
<td>-3.94, -1.17</td>
</tr>
</tbody>
</table>

Δ=change from Baseline. ††† p<0.001 versus placebo (all nominal).
(a) Effect size = 0.39.
(b) Effect size = 0.50.
(c) This analysis was added post hoc to FOCUS because the same analysis had been prespecified in CONNECT (key secondary endpoint).
Note: LS Mean values are presented; a negative LS mean value indicates advantages compared to placebo.

3.4.6.3 Depressive Symptoms

The results for the depressive symptom variables are summarized in Table 3.q. The vortioxetine groups separated from the placebo group on the MADRS total score and the clinical global impression variables (CGI-S score and CGI-I score) at Week 8. Vortioxetine also separated from placebo (nominal p<0.05) on depressive symptoms at Week 1.

Table 3.q MADRS Total Score and CGI Scores at Week 8 (FAS, MMRM)—FOCUS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vortioxetine 10 mg/day</th>
<th>Vortioxetine 20 mg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LS Mean</td>
<td>95% CI</td>
</tr>
<tr>
<td>Δ MADRS total score</td>
<td>-4.70†††</td>
<td>-6.45, -2.96</td>
</tr>
<tr>
<td>Δ CGI-S score</td>
<td>-0.65†††</td>
<td>-0.88, -0.42</td>
</tr>
<tr>
<td>CGI-I score</td>
<td>-0.61†††</td>
<td>-0.81, -0.40</td>
</tr>
</tbody>
</table>

Δ=change from Baseline.
††† nominal p<0.001 versus placebo.
Note: LS Mean values are presented; a negative LS mean value indicates advantages compared to placebo.

3.4.7 Summary of Results for FOCUS

- Vortioxetine was statistically significantly better than placebo on the primary endpoint assessing objective cognitive dysfunction (composite z-score of DSST and RAVLT) at Week 8 (10 mg: p<0.001 and 20 mg: p<0.001).
Vortioxetine was statistically significantly better than placebo on the key secondary endpoint, the DSST at Week 8 (10 mg: p<0.001 and 20 mg: p<0.001).

The testing hierarchy stopped with nonsignificant p-values in both dose groups while testing the RAVLT acquisition/learning score. However, the subjects in both dose groups improved more than those in the placebo group (nominal p<0.05) on the RAVLT delayed recall/memory score, and in the 10 mg group on the RAVLT acquisition/learning score.

Vortioxetine also consistently separated from placebo (nominal p<0.05) on all the remaining neuropsychological tests at both 10 and 20 mg (TMT-A, TMT-B, Stroop, and SRT) and at 10 mg on the CRT at Week 8, as well as on the PDQ, which measured self-reported cognitive symptoms.

Vortioxetine 10 and 20 mg separated from placebo (nominal p<0.05) on depressive symptoms at Week 8.

Vortioxetine separated from placebo (nominal p<0.05) both on the DSST and on depressive symptoms already at Week 1.

3.5 Pivotal Study, CONNECT (Study 202)

CONNECT was designed to confirm the potential effect of vortioxetine on cognitive dysfunction in adults with MDD. The objectives of CONNECT were to evaluate the efficacy of vortioxetine (10 or 20 mg/day) versus placebo on cognitive dysfunction and functionality in subjects with MDD and the efficacy on the subject’s depressive symptoms and global clinical status. Duloxetine 60 mg/day was included as an active reference for depressive symptoms, and to enable further evaluation of the potentially distinct profile of vortioxetine on cognitive measures, as previously observed in ELDERLY.

3.5.1 Study Design

CONNECT was a randomized, double-blind, parallel-group, placebo-controlled and active-reference, 8-week study conducted in Europe and the United States. The subjects were randomized equally to treatment with vortioxetine (a flexible dose of 10 or 20 mg/day), placebo, or a fixed dose of duloxetine 60 mg/day. The investigator, based on his or her judgment, could request a dose increase beginning at the end of the first week of treatment. However, to protect the blind, the interactive voice response (drug dispensing) system increased the dose only for subjects in the vortioxetine group. The study design is illustrated in Figure 3.e.
Figure 3.e Study Design—CONNECT

Cognitive assessments (primary endpoint) administered at Baseline and Week 8. Depressive symptoms (additional prespecified endpoint) assessed at Screening, Baseline, and Weeks 1, 4, and 8.

Eligible subjects:

- Were men and women, aged ≥18 and ≤65 years, with a primary diagnosis of recurrent MDE within MDD.
- Reported subjective cognitive dysfunction at Baseline, such as difficulties concentrating, slow thinking, and difficulties in learning or remembering new things.
- Had a MADRS total score ≥26 and a duration of current MDE ≥3 months.
- Had a score <70 on the DSST at the Baseline Visit (to improve sensitivity to change).
- Had not previously failed to respond to duloxetine.

3.5.2 Endpoints

3.5.2.1 Primary, Multiplicity-Controlled Endpoint

The primary efficacy endpoint was the change from Baseline in DSST (WAIS®-III DSST 90-sec test period, adopted to improve sensitivity to change in adults) after 8 weeks of treatment.

3.5.2.2 Key Secondary, Multiplicity-Controlled Endpoints

The key secondary endpoints were, at Week 8:
• Change from Baseline in PDQ attention/concentration and planning/organization combined subscore.
• CGI-I score.

3.5.2.3 Additional Prespecified, Non–Multiplicity-Controlled Endpoints

Additional endpoints used to assess cognitive function objectively were the change from Baseline to Week 8 in the following neuropsychological tests:

• TMT A and B.
• Stroop.
• SRT.
• CRT.
• Groton Maze Learning Task (GMLT).
• One-Back Test.

The PDQ and CPFQ were used to assess patient-reported cognitive symptoms subjectively. (PDQ scores other than attention/concentration and planning/organization combined subscore were not multiplicity controlled.)

The UPSA composite score and the WLQ percentage of productivity loss and subscores were assessed at Week 8 and compared to Baseline.

Depressive symptoms at Week 8 were assessed for the:

• Change from Baseline in MADRS total score.
• Change from Baseline in CGI-S score.
• CGI-I score.

3.5.3 Statistical Analyses

3.5.3.1 Sample Size Calculation

The sample size estimate was based on the observed treatment effect from ELDERLY, in which the difference in the change from Baseline to Week 8 in DSST between vortioxetine and placebo was 3.3 with SD=10.8, after excluding the subjects with a baseline DSST≥70.

Assuming SD=10.8 for the change from Baseline in DSST at Week 8 and a 15% withdrawal rate, a total of 600 subjects (200 per treatment group) was sufficient to achieve at least 80% power to detect a difference of 3.3 in the change from Baseline in DSST between vortioxetine and placebo by a 2-sample t-test with a 2-sided significance level of 0.05.
3.5.3.2 Multiplicity-Controlled Analyses

The following were prespecified endpoints within the testing hierarchy.

The primary efficacy analysis was a comparison of the change from Baseline in DSST for vortioxetine 10 or 20 mg/day versus that for placebo at Week 8, using ANCOVA and OC. Pooled study sites and the baseline value of the endpoint were used as covariates; the study sites accounted for variability between countries.

As a sensitivity analysis, the efficacy analyses on the DSST were repeated using data for Week 8 completers only.

Hierarchical testing was performed in 1 sequence (10/20 mg/day) with a significance level of 0.05; the analyses were versus placebo at Week 8, in the following order:

- Primary endpoint, change from Baseline in DSST (ANCOVA, OC). (Any assessment ≥2 days post-Baseline was considered a Week 8 assessment; therefore, the OC and LOCF results are identical.)
- The key secondary endpoints were:
  - Change from Baseline in PDQ attention/concentration and planning/organization combined subscore (MMRM).
  - CGI-I score (MMRM), with baseline CGI-S value as a covariate.

The analyses were repeated for duloxetine versus placebo, but outside the testing hierarchy.

3.5.3.3 Additional Prespecified, Non-Multiplicity-Controlled Analyses

Additional prespecified endpoints were analyzed at a nominal significance level of 0.05. All comparisons were versus placebo:

- The analyses of the objective neuropsychological tests—TMT A and B, Stroop, SRT, CRT, GMLT, and the One-Back Test—were by ANCOVA at Week 8 (LOCF). For each endpoint, its baseline score was used as a covariate in the analyses.
- Subjectively reported cognitive function, measured by PDQ and CPFQ, were analyzed at Weeks 4 and 8 by MMRM. For CPFQ, separate analyses were conducted of the total population and a more impaired subpopulation with baseline CPFQ >25.
- Performance-based functional capacity, measured by UPSA composite score, was analyzed at Week 8 by ANCOVA (LOCF). Patient-reported work limitations, measured by WLQ percent productivity loss and WLQ subscores, were analyzed by ANCOVA at Week 8 (LOCF).
- The analyses of depressive symptoms—MADRS, CGI-S, and CGI—were by MMRM (OC) and ANCOVA (LOCF and OC) at Weeks 1, 4, and 8. For each endpoint, its baseline score was used as a covariate in the analyses. (Because it is a measure of improvement, there was no baseline assessment of the CGI-I; therefore, the CGI-I score was analyzed rather than change from Baseline, using the baseline CGI-S score as a covariate.)
In the analyses of efficacy variables that were only assessed at Baseline and Week 8, any assessment ≥2 days post-Baseline was considered a Week 8 assessment; therefore, the OC and LOCF results are identical.

3.5.4 Disposition, Demographics, and Baseline Results

A total of 885 subjects were screened, 602 of whom were randomized to the 8-week Treatment Period (Table 3.r). Of these, 594 were treated (received at least 1 dose of study drug) and 529 of those subjects had at least 1 valid post-Baseline assessment of the primary efficacy variable (DSST). The FAS used in the efficacy analyses comprised the latter 529 subjects. Of the 594 treated subjects, 508 completed the study.

Table 3.r  Subject Disposition—CONNECT

<table>
<thead>
<tr>
<th>Number of Subjects (%)</th>
<th>Placebo</th>
<th>Vortioxetine 10 or 20 mg/day</th>
<th>Duloxetine 60 mg/day</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Randomized</td>
<td>194</td>
<td>198</td>
<td>210</td>
<td>602</td>
</tr>
<tr>
<td>Treated</td>
<td>191</td>
<td>196</td>
<td>207</td>
<td>594</td>
</tr>
<tr>
<td>Completed study</td>
<td>164 (84.5)</td>
<td>168 (84.8)</td>
<td>176 (83.8)</td>
<td>508 (84.8)</td>
</tr>
<tr>
<td>Early termination</td>
<td>30 (15.5)</td>
<td>30 (15.2)</td>
<td>34 (16.2)</td>
<td>94 (15.6)</td>
</tr>
</tbody>
</table>

Primary reason for early termination

| Pretreatment event or adverse event | 6 (3.1) | 6 (3.0) | 12 (5.7) | 24 (4.0) |
| Major protocol deviation          | 4 (2.1) | 3 (1.5) | 3 (1.4)  | 10 (1.7) |
| Lost to follow-up                 | 6 (3.1) | 10 (5.1)| 8 (3.8)  | 24 (4.0) |
| Voluntary withdrawal              | 8 (4.1) | 9 (4.5)| 6 (2.9)  | 23 (3.8) |
| Study termination                 | 0       | 0       | 0        | 0       |
| Pregnancy                         | 0       | 0       | 0        | 0       |
| Lack of efficacy                  | 6 (3.1) | 1 (0.5)| 5 (2.4)  | 12 (2.0) |
| Other reason(s)                   | 0       | 1 (0.5)| 0        | 1 (0.2) |

FAS: 167 175 187 529

(a) Percentages based on all randomized subjects.

Overall, the mean age (SD) of the subjects at Baseline was 45 (12) years; 65% were female. The majority of the subjects (85.7%) were Caucasian, 12.5% were Black, and 1.3% were Asian (Table 3.s).
### Table 3.s Demographics (All Randomized Subjects)—CONNECT

<table>
<thead>
<tr>
<th></th>
<th>Placebo N=194</th>
<th>Vortioxetine 10 or 20 mg N=198</th>
<th>Duloxetine 60 mg N=210</th>
<th>Total N=602</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex: N (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>75 (38.7)</td>
<td>63 (31.8)</td>
<td>72 (34.3)</td>
<td>210 (34.9)</td>
</tr>
<tr>
<td>Female</td>
<td>119 (61.3)</td>
<td>135 (68.2)</td>
<td>138 (65.7)</td>
<td>392 (65.1)</td>
</tr>
<tr>
<td><strong>Age: Mean (SD)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45.0 (12.07)</td>
<td>44.2 (12.21)</td>
<td>45.7 (11.46)</td>
<td>45.0 (11.90)</td>
</tr>
<tr>
<td><strong>Race: N (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian (a)</td>
<td>171 (88.1)</td>
<td>169 (85.4)</td>
<td>176 (83.8)</td>
<td>516 (85.7)</td>
</tr>
<tr>
<td>Black (b)</td>
<td>20 (10.3)</td>
<td>28 (14.1)</td>
<td>27 (12.9)</td>
<td>75 (12.5)</td>
</tr>
<tr>
<td>Asian</td>
<td>1 (0.5)</td>
<td>1 (0.5)</td>
<td>6 (2.9)</td>
<td>8 (1.3)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (1.0)</td>
<td>0</td>
<td>1 (0.5)</td>
<td>3 (0.5)</td>
</tr>
</tbody>
</table>

(a) Including Hispanic.
(b) Or African American.

The distribution of MADRS total scores at Baseline indicated that the subjects had moderate to severe MDD (a MADRS total score  \( \geq 26 \) at Baseline was an inclusion criterion) and the distribution of CGI-S scores at Baseline indicated that the subjects were moderately to markedly ill (Table 3.t).

### Table 3.t Baseline Efficacy Scores (FAS)—CONNECT

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo N=167</th>
<th>Vortioxetine 10 or 20 mg/day N=175</th>
<th>Duloxetine 60 mg/day N=187</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSST</td>
<td>43.0 (12.28)</td>
<td>42.1 (11.93)</td>
<td>42.8 (12.20)</td>
</tr>
<tr>
<td>MADRS total score</td>
<td>31.9 (3.89)</td>
<td>31.3 (3.94)</td>
<td>31.6 (3.85)</td>
</tr>
<tr>
<td>CGI-S score</td>
<td>4.6 (0.59)</td>
<td>4.6 (0.62)</td>
<td>4.6 (0.59)</td>
</tr>
<tr>
<td>UPSA composite score</td>
<td>76.5 (13.10)</td>
<td>78.1 (12.71)</td>
<td>78.7 (12.82)</td>
</tr>
<tr>
<td>WLQ percent of productivity loss score</td>
<td>14.1 (3.90)</td>
<td>13.0 (4.34)</td>
<td>13.7 (4.68)</td>
</tr>
<tr>
<td>PDQ total score</td>
<td>43.9 (10.62)</td>
<td>43.5 (10.89)</td>
<td>41.2 (12.56)</td>
</tr>
<tr>
<td>CPFQ total score</td>
<td>30.2 (4.47)</td>
<td>29.5 (5.27)</td>
<td>29.3 (4.86)</td>
</tr>
</tbody>
</table>

Data are mean (SD).

There were no meaningful imbalances in demographic or Baseline values among the treatment groups.

The mean exposure to study drug ranged from 52 to 54 days across treatment groups (median: 56 days). A range of 66% to 70% of the subjects across the treatment groups received study drug for \( \geq 56 \) days in the 8-week Treatment Period.
3.5.5 Testing Hierarchy: Primary and Key Secondary Efficacy Analyses Results

The subjects in the vortioxetine group improved statistically significantly more on cognitive performance as measured by the DSST than those in the placebo group did. The mean difference from placebo in the primary efficacy analysis of the change from Baseline in DSST at Week 8 was 1.75 points in favor of vortioxetine 10 or 20 mg/day (p<0.05) (Table 3.u). Duloxetine did not separate from placebo (nominal p≥0.05, outside the testing hierarchy). In the sensitivity analysis of completers the results overall were similar to those of the primary efficacy analysis (see Appendix D).

Table 3.u Analysis of Change From Baseline in Primary Endpoint, DSST at Week 8 (FAS, ANCOVA, LOCF)—CONNECT

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline Mean</th>
<th>N</th>
<th>Change From Baseline</th>
<th>LS Mean</th>
<th>SE</th>
<th>Difference From Placebo</th>
<th>LS Mean</th>
<th>Lower 95% CI</th>
<th>Upper 95% CI</th>
<th>p-value</th>
<th>Effect Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>43.0</td>
<td>167</td>
<td>Change From Baseline</td>
<td>2.85</td>
<td>0.54</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VOR 10 or 20 mg</td>
<td>42.1</td>
<td>175</td>
<td></td>
<td>4.60</td>
<td>0.53</td>
<td>1.75</td>
<td>0.28</td>
<td>3.21</td>
<td>0.019</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>DUL 60 mg</td>
<td>42.8</td>
<td>187</td>
<td></td>
<td>4.06</td>
<td>0.51</td>
<td>1.21</td>
<td>-0.23</td>
<td>2.65</td>
<td>0.099 (a)</td>
<td>0.18</td>
<td></td>
</tr>
</tbody>
</table>

VOR=vortioxetine, DUL=duloxetine.
(a) Nominal p-value.

The testing hierarchy applied only to the vortioxetine group. These subjects improved statistically significantly more than those in the placebo group did in both multiplicity-controlled key secondary efficacy analyses, the PDQ attention/concentration and planning/organization combined subscore (p<0.01) and the CGI-I score at Week 8 (p<0.05) (see Sections 3.5.6.1 and 3.5.6.5). Duloxetine also separated from placebo on these 2 measures (nominal p<0.05).

3.5.6 Efficacy Results Outside the Testing Hierarchy

3.5.6.1 Additional Objective Neuropsychological Tests of Cognitive Function

The effect of vortioxetine on cognitive performance was further assessed using additional neuropsychological tests. The results of these analyses are summarized in Table 3.v. With the exception of the TMT-B (nominal p<0.001), vortioxetine did not separate from placebo (nominal p≥0.05) on any of the additional neuropsychological tests. Duloxetine did not separate from placebo on any of these tests.
Table 3.v  Neuropsychological Tests at Week 8 (FAS, ANCOVA, LOCF)—CONNECT

<table>
<thead>
<tr>
<th></th>
<th>Vortioxetine 10 or 20 mg</th>
<th>Duloxetine 60 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Δ TMT–A</strong></td>
<td>-1.05</td>
<td>-1.41</td>
</tr>
<tr>
<td><strong>Δ TMT–B</strong></td>
<td>-9.67†††</td>
<td>-5.54</td>
</tr>
<tr>
<td>Δ Stroop congruent</td>
<td>1.07</td>
<td>-0.18</td>
</tr>
<tr>
<td>Δ Stroop incongruent</td>
<td>-0.05</td>
<td>-1.72</td>
</tr>
<tr>
<td>Δ GMLT</td>
<td>-1.94</td>
<td>-1.67</td>
</tr>
<tr>
<td>Δ SRT</td>
<td>-0.017</td>
<td>-0.006</td>
</tr>
<tr>
<td>Δ CRT</td>
<td>-0.014</td>
<td>-0.007</td>
</tr>
</tbody>
</table>
| Δ One-Back Task             | -0.006                   | -0.003           | 0.11

Note: LS Mean values are presented. A negative LS mean value indicates advantages compared to placebo.

The standardized effect sizes for all the objective neuropsychological tests are shown in Figure 3.f.

Figure 3.f  Change From Baseline in Neuropsychological Tests at Week 8, Standardized Effect Sizes Versus Placebo (FAS, ANCOVA, LOCF)—CONNECT

Con=congruent, Incon=incongruent.
Error bars indicate SE.
* p<0.05 versus placebo (statistically significant).
††† p<0.001 versus placebo (nominal).

ADVISORY COMMITTEE BRIEFING MATERIALS: AVAILABLE FOR PUBLIC RELEASE
3.5.6.2 Patient-Reported Cognitive Symptoms

The improvements observed on the objective measures of cognitive function were complemented by improvements in self-reported cognitive symptoms, as measured using the PDQ and CPFQ.

The subjects in both the vortioxetine and duloxetine groups reported greater improvements in self-reported cognitive symptoms as shown by separation from placebo (nominal p-values) in the change from Baseline in the PDQ total score and PDQ attention/concentration and planning/organization combined subscores at Week 8 (Table 3.w).

Table 3.w  PDQ Scores at Week 8 (FAS, MMRM)—CONNECT

<table>
<thead>
<tr>
<th>Difference From Placebo at Week 8</th>
<th>Vortioxetine 10-20 mg</th>
<th>Duloxetine 60 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LS Mean</td>
<td>95% CI</td>
</tr>
<tr>
<td>Δ PDQ total score</td>
<td>-4.2†† (a)</td>
<td>(-6.9, -1.6)</td>
</tr>
<tr>
<td>Δ PDQ attention/concentration and planning/organization combined subscore (c)</td>
<td>-2.6**</td>
<td>(-4.1, -1.0)</td>
</tr>
</tbody>
</table>

Δ=change from Baseline.

**p<0.01 (statistically significant); ††p<0.01, ††† p<0.001 versus placebo (both nominal).

(a) Effect size = 0.33.
(b) Effect size = 0.44.
(c) Included in the testing hierarchy as a key secondary efficacy endpoint.

Note: LS means are presented; a negative LS mean value indicates advantages compared to placebo.

In the CPFQ analysis of the change from Baseline at Week 8 in the total population, duloxetine, but not vortioxetine, separated from placebo (nominal p<0.05) in the CPFQ total score (Table 3.x). In the subjects with significant symptoms, ie, a CPFQ total score >25 at Baseline (81% of subjects), both vortioxetine and duloxetine separated from placebo (nominal p<0.05) in reducing the total score.
### Table 3.x  CPFQ Scores at Week 8 (FAS, MMRM)—CONNECT

<table>
<thead>
<tr>
<th></th>
<th>Vortioxetine 10-20 mg</th>
<th>Duloxetine 60 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ CPFQ total score, all subjects</td>
<td>-1.2 (−2.6, 0.2)</td>
<td>-1.7† (−3.1, -0.4)</td>
</tr>
<tr>
<td>Δ CPFQ total score, subjects with baseline CPFQ&gt;25</td>
<td>-1.7† (−3.3, -0.1)</td>
<td>-1.8† (−3.4, -0.2)</td>
</tr>
</tbody>
</table>

Δ=change from Baseline.
† p<0.05 versus placebo (nominal).
Note: LS means are presented; a negative LS mean value indicates advantages compared to placebo.

#### 3.5.6.3  Performance-Based Functional Capacity

Vortioxetine improved subjects’ functional capacity (nominal p<0.001, FAS, ANCOVA, OC versus placebo), as assessed using the UPSA composite score at Week 8 (Table 3.y and Figure 3.g). Duloxetine did not separate from placebo (nominal p≥0.05).
Table 3.y  UPSA Scores at Week 8 (FAS, ANCOVA, LOCF)—CONNECT

<table>
<thead>
<tr>
<th></th>
<th>Vortioxetine 10-20 mg</th>
<th>Duloxetine 60 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difference From Placebo at Week 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS Mean</td>
<td>2.94††† (a)</td>
<td>0.38 (b)</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.35, 4.52</td>
<td>-1.19, 1.94</td>
</tr>
</tbody>
</table>

Δ=change from Baseline.
††† p<0.001 versus placebo (nominal).
(a) Effect size = 0.39.
(b) Effect size = 0.05.

Figure 3.g  UPSA Scores at Week 8 (FAS, ANCOVA, LOCF)—CONNECT

3.5.6.4 Patient-Reported Work Limitations

In working subjects (40% of the sample) vortioxetine separated from placebo (nominal p<0.05) in decreasing the time demands score (eg, difficulties in working the required number of hours or starting on the job when arriving) (Table 3.z and Figure 3.h). Vortioxetine did not improve the physical demands, mental-interpersonal demands, or output demands subscale scores, nor the productivity loss index score. Duloxetine did not separate from placebo on any WLQ subscale or the productivity loss index score.
Table 3.z  WLQ Subscale Scores and Percentage of Productivity Loss Score in Working Subjects at Week 8 (FAS, ANCOVA, LOCF)—CONNECT

<table>
<thead>
<tr>
<th>WLQ Assessment</th>
<th>Difference From Placebo at Week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vortioxetine 10 or 20 mg/day</td>
</tr>
<tr>
<td></td>
<td>LS Mean</td>
</tr>
<tr>
<td>WLQ subscale score</td>
<td></td>
</tr>
<tr>
<td>Δ Time demands</td>
<td>-8.13†</td>
</tr>
<tr>
<td>Δ Physical demands</td>
<td>0.36</td>
</tr>
<tr>
<td>Δ Mental-interpersonal demands</td>
<td>-5.39</td>
</tr>
<tr>
<td>Δ Output demands</td>
<td>-7.60</td>
</tr>
<tr>
<td>Δ % of productivity loss index score</td>
<td>-1.35</td>
</tr>
</tbody>
</table>

Δ=change from Baseline.
†=p<0.05 (nominal).
Mean values are presented.
Note: A negative LS mean value indicates advantages compared to placebo.

Figure 3.h  WLQ Subscale Scores at Week 8 (FAS, ANCOVA, LOCF)—CONNECT

3.5.6.5  Depressive Symptoms

Vortioxetine and duloxetine separated from placebo (nominal p<0.05) in the depressive symptom score (MADRS total score) and clinical global impression variables (CGI-S and CGI-I scores) at Week 8 (Table 3.aa).
Table 3.aa MADRS Total Score and CGI Scores at Week 8 (FAS, MMRM)—CONNECT

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vortioxetine 10 or 20 mg/day</th>
<th>Duloxetine 60 mg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ MADRS total score</td>
<td>-2.3†</td>
<td>-3.3†††</td>
</tr>
<tr>
<td>Δ CGI-S score</td>
<td>-0.32†</td>
<td>-0.47†††</td>
</tr>
<tr>
<td>CGI-I score</td>
<td>-0.29**</td>
<td>-0.40†††</td>
</tr>
</tbody>
</table>

\(\Delta=\text{change from Baseline.}\)

† nominal \(p<0.05\), ††† nominal \(p<0.001\) versus placebo.

**statistically significant \(p<0.01\) versus placebo.

Note: A negative LS mean value indicates advantages compared to placebo.

3.5.7 Summary of Results for CONNECT

- Vortioxetine was statistically significantly better than placebo on the primary endpoint, objective cognitive performance as assessed using the DSST (\(p=0.019\)).
- Vortioxetine was statistically significantly better than placebo on the key secondary endpoints, the PDQ attention/concentration and planning/organization combined subscore (\(p<0.01\)) and the CGI-I score (\(p<0.01\)) at Week 8.
- With the exception of the TMT-B (nominal \(p<0.001\)), vortioxetine did not separate from placebo (nominal \(p\geq0.05\)) on any of the additional neuropsychological tests.
- Duloxetine separated from placebo on the key secondary endpoints, the PDQ attention/concentration and planning/organization combined subscore (nominal \(p<0.01\)) and the CGI-I score (nominal \(p<0.05\)) at Week 8, but did not separate from placebo (\(p\geq0.05\)) on any of the neuropsychological tests, including the DSST.
- In addition to its effect on cognitive dysfunction, vortioxetine improved subjects’ functional capacity, as assessed using the UPSA composite score at Week 8 (nominal \(p<0.05\)). Duloxetine did not separate from placebo on the UPSA.
- Vortioxetine also improved the time demand aspect (nominal \(p<0.05\)) but not other subscales of self-reported work productivity (WLQ) in working subjects (approximately 40% of the total population at Week 8). Duloxetine did not separate from placebo on any subscale.
- Vortioxetine also separated from placebo on patient-reported measures of cognitive symptoms (PDQ and CPFQ [subjects with baseline CPFQ >25]) at Week 8; it did not separate in the CPFQ analysis of all subjects. Duloxetine separated from placebo for both PDQ and CPFQ, both the subgroup and all subjects.
- Vortioxetine and duloxetine both separated from placebo (nominal \(p<0.05\)) on depressive symptoms (change from Baseline on the MADRS total score at Week 8).
3.6 Mood-Independent Effect on Cognitive Dysfunction Across Studies

As expected, in all studies, vortioxetine and duloxetine (where included) improved depressive symptoms (as assessed using the MADRS; Figure 3.i).

Figure 3.i Improvements in Depressive Symptoms at Week 8 (MADRS)(FAS, MMRM)

The vortioxetine studies assessing cognitive dysfunction in MDD were conducted in acutely depressed subjects. Improvement in depressive symptoms can confound treatment effects on cognitive dysfunction, especially in subjective measures. To support that vortioxetine’s effect on cognitive dysfunction in MDD was not just due to improvement in mood, 2 different approaches were used across the 3 studies (ELDERLY, FOCUS, and CONNECT):

- The profiles of vortioxetine and duloxetine on the MADRS and DSST were compared for potential differential effects on mood and cognition.
- The MADRS-adjusted effect on the DSST was determined using a mediation analysis:
  - After adjusting for the effect on depressive symptoms (MADRS).
  - To assess the proportion of the effect on cognitive performance that cannot be explained by the improvement in depression (direct effect).

The distinct effect of vortioxetine on cognitive dysfunction is supported by the results of ELDERLY and CONNECT, in which duloxetine was included as an active reference.

- The first indication of a differential effect of vortioxetine vs duloxetine on cognition was observed in elderly, where both agents were efficacious on depression (MADRS; nominal p<0.05), as well as on the RAVLT (nominal p<0.05), but only vortioxetine demonstrated an effect on the DSST (nominal p<0.05).
Additional evidence of differential effects were observed in CONNECT, in which vortioxetine was statistically significantly different (p<0.05) from placebo on DSST; duloxetine did not separate from placebo on DSST. In addition, only vortioxetine separated from placebo (nominal p<0.05) on cognitive performance (TMT-B) and performance-based functional capacity (UPSA).

The mood-independent effect of vortioxetine on the DSST was further supported by adjusting for the correlation (mediation analysis) between changes in DSST and MADRS total score across the 3 studies. Figure 3.1 illustrates the proportion of the total effect that is mediated through the improvement in depressive symptoms (indirect effect) and the proportion that can be distinguished from the effect on mood as assessed using the MADRS (direct effect). Across the 3 studies, the magnitude of the adjusted standardized effect size for vortioxetine was between 0.19 and 0.32, and the proportion of the direct effect on the DSST for vortioxetine was between 56% and 78% at Week 8 (Appendix E). Thus, across all 3 studies, when adjusting for the effect on the MADRS total score, the majority of the effect of vortioxetine on cognitive deficits is retained, indicating a mood-independent effect on the DSST.

**Figure 3.1** Change From Baseline in DSST at Week 8, Standardized Effect Sizes Versus Placebo (FAS, ANCOVA, LOCF, Mediation Analysis)—All Studies

![Graph showing standardized effect sizes](image)

VOR=vortioxetine, DUL=duloxetine.

Note: Mediation analysis was post hoc in ELDERLY.
3.7 Supportive Pharmacology Information

Evidence from in vitro studies, nonclinical pharmacology, and a human fMRI study provide additional evidence regarding the unique profile of vortioxetine.

3.7.1 Effects of Vortioxetine in In Vitro and Animal Studies of Cognitive Function

5-HT mediates its wide range of physiological functions through interactions with multiple receptor subtypes. Fifteen subtypes have been identified, 12 of which have been shown to mediate functional responses in tissues [98]. Most of these 5-HT receptor subtypes have been associated with depression (reviewed in [99]). 5-HT receptors expressed on serotonergic neurons (autoreceptors) are intimately involved in the control of serotonergic neurotransmission, whereas 5-HT receptors expressed on nonserotonergic neurons (heteroreceptors) additionally play important modulatory roles on the activity of other neurotransmitter systems [99]. Glutamate and γ-aminobutyric acid (GABA) are the brain’s primary excitatory and inhibitory neurotransmitter systems, respectively, and are key regulators of cognitive processing. Most 5-HT receptor subtypes are expressed on glutamatergic and GABAergic neurons; expression is widespread but varies between brain regions and neuronal cell types [100,101]. The 5-HT3 receptor is unique, since it has only been identified on GABAergic cells [102]. Thus, drugs with 5-HT3 receptor antagonistic effects may have the potential to reduce the inhibitory control that GABAergic interneurons exert on glutamatergic pyramidal neurons and thereby enhance cognitive processing. In vitro and in vivo nonclinical studies indicate that vortioxetine’s primary biological targets are the SERT and the 5-HT1A, 5-HT1B, 5-HT1D, 5-HT3, and 5-HT7 receptors (Figure 3.a and Table 3.bb) [103]. Thus, vortioxetine has the potential to modulate glutamatergic and GABAergic neurotransmission through direct actions on various 5-HT receptors in a manner different than that of SSRIs and SNRIs.

Table 3.bb Binding Affinity of Vortioxetine in Recombinant Cell Lines/Cortex Tissue Expressing Human 5-HT Receptors and SERT

<table>
<thead>
<tr>
<th>5-HT3 Receptor Antagonism</th>
<th>5-HT7 Receptor Antagonism</th>
<th>5-HT1D Receptor Antagonism</th>
<th>5-HT1B Partial Agonism</th>
<th>5-HT1A Agonism</th>
<th>SERT Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.7</td>
<td>18</td>
<td>54</td>
<td>33</td>
<td>15</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Data are Ki (nmol/L).

Animal electrophysiology studies have shown that vortioxetine at clinically meaningful levels of SERT occupancy (50%-90%), but not an SSRI, enhanced glutamatergic output from pyramidal neurons in key brain structures involved in cognitive processing (eg, the prefrontal cortex [PFC] and hippocampus). Vortioxetine increased the discharge rate of pyramidal neurons in the PFC of rats [104] and counteracted the 5-HT–induced suppression of output from pyramidal neurons in the cornu ammonis 1 (CA1) layer of rat hippocampal slices [105]. The electrophysiology studies also indicated that vortioxetine’s increase of pyramidal neuron output is at least partly ascribed to its 5-HT3 receptor antagonistic effects [104,106]. Further to this, electrophysiology studies in rat hippocampal slices (CA1 Striatum Radiatum) and in vivo studies in rat PFC showed that...
vortioxetine reduced the activity of GABAergic interneurons through antagonism at 5-HT3 receptors [107,108]. Taken together, the electrophysiology studies strongly support that vortioxetine increases glutamatergic signaling through a reduction of the GABAergic inhibitory control of glutamatergic pyramidal neurons. The enhanced glutamate signaling achieved with vortioxetine compared to an SSRI led to a significant increase in the magnitude of theta-burst–induced long-term potentiation in the CA1 area of rat hippocampus slices and significantly increased theta oscillation in vivo, 2 effects that both are indicators of enhanced synaptic plasticity and cognitive function [105].

Additional studies, including cell proliferation studies in the hippocampal dentate gyrus [109], dendritic remodeling and spine morphology studies in the hippocampus CA1 area in rats [110,111] [112], and gene expression studies in middle-aged mice [113] all showed that vortioxetine had superior effects on neuronal plasticity compared to an SSRI [109,112]. In addition to vortioxetine increasing glutamate signaling, animal microdialysis studies showed that vortioxetine at doses in the upper end of the clinically meaningful range (>80% SERT occupancy) also enhanced extracellular norepinephrine, dopamine, acetylcholine, and histamine levels in brain areas of importance for cognitive processing, such as the hippocampus and PFC [103,114,115]. These neurotransmitters have all been associated with procognitive effects, and might, therefore, also contribute to the positive cognitive profile of vortioxetine [116].

Studies of vortioxetine in rodent cognition models that mimic aspects of attention, learning and memory, and executive function also suggest that vortioxetine has a procognitive profile different from those of SSRIs and SNRIs [103]. Quantitative electroencephalographic studies in rats suggest that vortioxetine, unlike escitalopram or duloxetine, activates cortical circuitries involved in cognitive processing [117]. Furthermore, vortioxetine reversed cognitive deficits in rodent models that used a variety of cognitive disruptors including time, low brain 5-HT levels, stress, age, the N-methyl-D-aspartate receptor antagonists dizocilpine and phencyclidine, and the cholinergic muscarinic receptor antagonist scopolamine [103].

In conclusion, these data provide a mechanistic rationale for why vortioxetine has the potential to positively affect cognitive function. These data also support that the direct activity of vortioxetine at 5-HT receptors is essential for its pharmacodynamic profile. The nonclinical mechanistic data supporting the effect of vortioxetine on cognitive dysfunction is summarized in Table 3.cc.
Table 3.cc  Nonclinical Data Supporting the Effect of Vortioxetine on Enhancing Neurotransmission and Plasticity and Reversing Cognitive Deficits

<table>
<thead>
<tr>
<th>Effect</th>
<th>Vortioxetine</th>
<th>SSRI</th>
<th>SNRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enhances neuronal plasticity and synaptic transmission; activates</td>
<td>+</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td>brain circuitries involved in cognitive processing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reversal of age-associated decrease of plasticity</td>
<td>+</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td>Dendritic spines maturation with presynaptic contacts</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Long-term potentiation</td>
<td>+</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td>Activation of pyramidal neurons and inhibition of interneurons</td>
<td>+</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td>Increased cortical EEG power (δ, θ, γ)</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Reverses deficits in animal models of cognitive function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serotonin depletion</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ovariectomy</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Age</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Flinders Sensitive Line, genetic depression model</td>
<td>+</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td>Stress</td>
<td>+</td>
<td>+</td>
<td>NT</td>
</tr>
<tr>
<td>Time</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
</tbody>
</table>

0=no effect, NT=not tested, +=effective.
EEG=electroencephalogram.

3.7.2 Clinical Pharmacodynamic Imaging Study With Vortioxetine

Study 14137A was a parallel-group, placebo-controlled study conducted in the United Kingdom to explore the effects of vortioxetine (20 mg/day for 13 days) compared to placebo on the blood-oxygen-level–dependent (BOLD) signal in fMRI in brain regions associated with working memory (executive function) in subjects remitted from depression by DSM-IV-TR™ criteria (N=48, 24 each on vortioxetine or placebo) who had suffered from at least 2 previous MDEs and reported subjective cognitive dysfunction. A healthy subject cohort also randomized to vortioxetine or placebo was included as a control (N=48). The main objective of the study was to explore the effect of vortioxetine on the BOLD signal in remitted subjects during performance of the N-back task. The BOLD signal is considered a proxy of the brain activity required to perform a task. For the purposes of this document, the material presented here focuses on the imaging results obtained while performing the N-back task. The methods for this study are detailed in Appendix F.

The N-back task is an effortful test of attention and working memory. It is associated with activation of the dorsolateral prefrontal cortex (DLPFC) and decrease in hippocampal activity. This can be interpreted as a shift of processing resources to areas subserving working memory (eg, the DLPFC) from areas involved in episodic memory (the hippocampus). In subjects remitted from depression, working memory performance appears to require a greater recruitment of frontal activity and be associated with a less efficient reduction in hippocampal activity [118,119]. Thus, a region of interest within the DLPFC and anterior cingulate cortex (selected based on Harvard-Oxford Anatomical maps) was prespecified for primary analysis of the effects of vortioxetine in the N-back task.

ADVISORY COMMITTEE BRIEFING MATERIALS: AVAILABLE FOR PUBLIC RELEASE
3.7.2.1  Results

The healthy control and remitted groups were generally well-matched on baseline characteristics and neuropsychological test performance, with the remitted group scoring higher than the control group on trait anxiety, HAM-D17, Beck Depression Inventory (BDI), and the PDQ.

N-back Performance

There was no difference in baseline accuracy between the subject groups either across all levels of N-back complexity or as a function of complexity. As expected, increasing complexity was associated with reduced accuracy and increased latency. There were no significant subject and/or treatment group differences in the change in performance of this task when analyzing all subjects together, or the subject groups separately. Therefore neural responses can be assessed in the absence of performance confounds.

fMRI Effect of Task

Across groups, performance of the N-back task was associated with increased neural responses in bilateral DLPFC, rostral medial PFC and bilateral posterior parietal cortex. At the same time, the default mode network (DMN) was deactivated during the N-back compared to the 0-back in the inferior medial PFC, cingulate cortex, and hippocampi. This network of response was not significantly different in the remitted subjects compared to healthy controls. Increasing load was associated with linearly increased activation in the DLPFC, rostral medial PFC, and posterior parietal lobe with deactivation in the DMN including hippocampus, where remitted subjects displayed a greater differentiation between the various levels of complexity than control subjects.

3.7.2.2  fMRI Effect of Treatment

Across all subjects, vortioxetine significantly reduced BOLD signal within the predefined anatomical masks for the right DLPFC and left hippocampus; there was no effect of BDI scores when added as a covariate. There was no treatment-by-group interaction (remitted versus healthy control subjects); when the 2 groups were analyzed separately, the treatment effects within the predefined anatomical masks remained significant in the remitted group but not in the healthy control group (Figure 3.k). Similar findings were observed in the linear response analysis.
3.7.2.3 Discussion

The reduction in activity in the DLPFC and cingulate cortex during performance of the N-back task and improved attenuation in the hippocampus suggest vortioxetine improved the efficiency of circuits underlying cognitive dysfunction. At the group level, the findings were significant in remitted MDD but not healthy control subjects. The effects were obtained in subjects with very low levels of depressive symptoms and were not related to change in depressive symptoms during the study.

Overall, the results of this study are consistent with vortioxetine’s effects in neural pathways important in cognitive function. Given that these subjects had few or no depressive symptoms, these results suggest an effect on cognitive function independent of depressive symptoms.
4.0 SAFETY RESULTS

The emphasis of the sNDA submitted is on efficacy. However, a brief summary of safety results is provide here. Because the safety data from ELDERLY were included in the original registration program and are part of the data resulting in the current USPI, those data are not included.

4.1 FOCUS

An overview of treatment-emergent adverse events (TEAEs) in FOCUS is presented in Table 4.a.

Table 4.a Overview of TEAEs (All Treated Subjects)—FOCUS

<table>
<thead>
<tr>
<th></th>
<th>Placebo (N=196)</th>
<th>Vortioxetine 10 mg (N=195)</th>
<th>Vortioxetine 20 mg (N=207)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Events</td>
<td>130</td>
<td>180</td>
<td>219</td>
</tr>
<tr>
<td>Subjects</td>
<td>76 (38.8)</td>
<td>95 (48.7)</td>
<td>109 (52.7)</td>
</tr>
<tr>
<td>Leading to discontinuation</td>
<td>8 (4.1)</td>
<td>7 (3.6)</td>
<td>10 (4.8)</td>
</tr>
<tr>
<td>Serious TEAEs</td>
<td>2 (1.0)</td>
<td>0</td>
<td>2 (1.0)</td>
</tr>
<tr>
<td>Deaths</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

No deaths occurred during the study. Two subjects in the placebo group (hiatus hernia and cholecystitis chronic) and 2 subjects in the vortioxetine 20 mg group (type I diabetes mellitus and arterial hypertension) had serious TEAEs. The incidence of TEAEs leading to discontinuation was similar in all 3 treatment groups (approximately 4%). The TEAEs leading to discontinuation of ≥2 subjects any treatment group were depression (2 subjects) and disturbance in attention (2 subjects) in the placebo group, and nausea (4 subjects), headache (2 subjects), and hypertension (2 subjects) in the vortioxetine 20 mg group.

The most common TEAEs in the study are shown in Table 4.b.
Table 4.b  Most Common TEAEs (Incidence ≥2% in Any Treatment Group)—FOCUS

<table>
<thead>
<tr>
<th></th>
<th>Placebo (N=196)</th>
<th>Vortioxetine 10 mg (N=195)</th>
<th>Vortioxetine 20 mg (N=207)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects with any TEAE</td>
<td>75 (38.3)</td>
<td>90 (46.2)</td>
<td>109 (52.7)</td>
</tr>
<tr>
<td>Nausea</td>
<td>8 (4.1)</td>
<td>32 (16.4)</td>
<td>43 (20.8)</td>
</tr>
<tr>
<td>Headache</td>
<td>14 (7.1)</td>
<td>16 (8.2)</td>
<td>26 (12.6)</td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>10 (5.1)</td>
<td>7 (3.6)</td>
<td>9 (4.3)</td>
</tr>
<tr>
<td>Somnolence</td>
<td>1 (0.5)</td>
<td>1 (0.5)</td>
<td>8 (3.9)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2 (1.0)</td>
<td>4 (2.1)</td>
<td>7 (3.4)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>7 (3.6)</td>
<td>8 (4.1)</td>
<td>7 (3.4)</td>
</tr>
<tr>
<td>Dry mouth</td>
<td>6 (3.1)</td>
<td>4 (2.1)</td>
<td>7 (3.4)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0</td>
<td>5 (2.6)</td>
<td>7 (3.4)</td>
</tr>
<tr>
<td>Viral upper respiratory tract infection</td>
<td>7 (3.6)</td>
<td>5 (2.6)</td>
<td>3 (1.4)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>1 (0.5)</td>
<td>5 (2.6)</td>
<td>2 (1.0)</td>
</tr>
<tr>
<td>Accidental overdose</td>
<td>0</td>
<td>5 (2.6)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Depression</td>
<td>4 (2.0)</td>
<td>1 (0.5)</td>
<td>0</td>
</tr>
</tbody>
</table>

TEAEs are ordered by decreasing incidence in the vortioxetine 20 mg column.

Vortioxetine was not associated with any clinically important changes in laboratory test parameters in serum chemistry, hematology, and urinalysis.

4.2 CONNECT

An overview of TEAEs in CONNECT is presented in Table 4.c.

Table 4.c  Overview of TEAEs (All Treated Subjects)—CONNECT

<table>
<thead>
<tr>
<th></th>
<th>Placebo (N=191)</th>
<th>Vortioxetine 10 or 20 mg (N=196)</th>
<th>Duloxetine 60 mg (N=207)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Events</td>
<td>162</td>
<td>235</td>
<td>323</td>
</tr>
<tr>
<td>Subjects</td>
<td>85 (44.5)</td>
<td>117 (59.7)</td>
<td>119 (57.5)</td>
</tr>
<tr>
<td>Leading to discontinuation</td>
<td>7 (3.7)</td>
<td>7 (3.6)</td>
<td>13 (6.3)</td>
</tr>
<tr>
<td>Serious TEAEs</td>
<td>2 (1.0)</td>
<td>1 (0.5)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Deaths</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

No deaths occurred during the study. Two subjects in the placebo group (acute myocardial infarction and depression), 1 in the vortioxetine group (suicide attempt), and 1 in the duloxetine group (1 subject with both craniocerebral injury and subdural hematoma) had serious TEAEs. The incidence of TEAEs leading to discontinuation was approximately 4% in the placebo and vortioxetine groups and approximately 6% in the duloxetine group. The TEAEs leading to
Brintellix (Vortioxetine)
Advisory Committee Meeting Briefing Document

Discontinuation of ≥2 subjects in any treatment group were depression (4 subjects in the placebo group), fatigue (1 subject in the vortioxetine group and 3 in the duloxetine group), and nausea (2 subjects each in both the vortioxetine and duloxetine groups).

The most common TEAEs in CONNECT are shown in Table 4.d.

### Table 4.d Most Common TEAEs (Incidence ≥2% in Any Treatment Group)—CONNECT

<table>
<thead>
<tr>
<th>TEAE</th>
<th>Placebo (N=191)</th>
<th>Vortioxetine 10/20 mg (N=196)</th>
<th>Duloxetine 60 mg (N=207)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects with any TEAEs</td>
<td>85 (44.5)</td>
<td>117 (59.7)</td>
<td>119 (57.5)</td>
</tr>
<tr>
<td>Nausea</td>
<td>8 (4.2)</td>
<td>40 (20.4)</td>
<td>43 (20.8)</td>
</tr>
<tr>
<td>Headache</td>
<td>16 (8.4)</td>
<td>20 (10.2)</td>
<td>24 (11.6)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>5 (2.6)</td>
<td>11 (5.6)</td>
<td>6 (2.9)</td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>11 (5.8)</td>
<td>7 (3.6)</td>
<td>8 (3.9)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1 (0.5)</td>
<td>7 (3.6)</td>
<td>9 (4.3)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>5 (2.6)</td>
<td>6 (3.1)</td>
<td>11 (5.3)</td>
</tr>
<tr>
<td>Dry mouth</td>
<td>9 (4.7)</td>
<td>6 (3.1)</td>
<td>16 (7.7)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>4 (2.1)</td>
<td>6 (3.1)</td>
<td>10 (4.8)</td>
</tr>
<tr>
<td>Constipation</td>
<td>2 (1.0)</td>
<td>6 (3.1)</td>
<td>4 (1.9)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>1 (0.5)</td>
<td>4 (2.0)</td>
<td>3 (1.4)</td>
</tr>
<tr>
<td>Increased appetite</td>
<td>2 (1.0)</td>
<td>4 (2.0)</td>
<td>4 (1.9)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>5 (2.6)</td>
<td>4 (2.0)</td>
<td>3 (1.4)</td>
</tr>
<tr>
<td>Somnolence</td>
<td>1 (0.5)</td>
<td>4 (2.0)</td>
<td>7 (3.4)</td>
</tr>
<tr>
<td>Depression</td>
<td>5 (2.6)</td>
<td>3 (1.5)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>Tension</td>
<td>5 (2.6)</td>
<td>2 (1.0)</td>
<td>2 (1.0)</td>
</tr>
<tr>
<td>Chills</td>
<td>0</td>
<td>1 (0.5)</td>
<td>5 (2.4)</td>
</tr>
<tr>
<td>Influenza</td>
<td>0</td>
<td>1 (0.5)</td>
<td>5 (2.4)</td>
</tr>
<tr>
<td>Hyperhidrosis</td>
<td>3 (1.6)</td>
<td>1 (0.5)</td>
<td>5 (2.4)</td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>5 (2.6)</td>
<td>1 (0.5)</td>
<td>1 (0.5)</td>
</tr>
</tbody>
</table>

Note: TEAEs are arranged in descending order of frequency in the vortioxetine column.

Vortioxetine was not associated with any clinically important changes in laboratory test parameters in serum chemistry, hematology, and urinalysis.

### 4.3 Overall Safety Profile

Overall, the safety profile of vortioxetine with respect to TEAEs in these 2 studies does not substantively differ from that in the USPI. Nausea was the most common TEAE during the 8-week Treatment Period in the vortioxetine treatment group(s) (FOCUS: 16% of the subjects treated with 10 mg and 21% of the subjects treated with 20 mg [Table 4.b]; CONNECT: 20% of
the subjects treated with flexible doses [10 or 20 mg; Table 4.d]). These incidences are consistent with those in the previous vortioxetine studies, as reflected in the USPI.

### 4.4 Postmarketing Experience

Vortioxetine was first approved in the United States on 30 September 2013. As of the Periodic Safety Update Report data lock point, 29 September 2015, vortioxetine had been authorized in 61 countries and launched in 36 countries.

As of 29 September 2015, the cumulative exposure from postmarketing sources based on the amount of vortioxetine that has been shipped to wholesalers and delivered samples is 834,087 patient years. Furthermore, it is assumed that all sales and samples have been consumed by patients.

Cumulatively, the Global Safety Database contains 5751 adverse reactions from marketed experience with vortioxetine: 653 serious and 5098 nonserious.

The most commonly reported adverse events were nausea (n=757), pruritus (n=217), and vomiting (n=211).

The most commonly reported serious adverse events were suicidal ideation (n=107), nausea (n=23), serotonin syndrome (n=23), and completed suicide (n=21).

No new safety signals have been identified from the postmarketing experience. The events nausea, pruritus, and vomiting were seen more often for vortioxetine-treated subjects than for placebo-treated subjects in the clinical development program and are all listed in the USPI.

No new signals have been identified from the postmarketing experience.
5.0 DISCUSSION

The sponsor has submitted an sNDA for vortioxetine with the aim of adding text in the Clinical Studies section of the USPI regarding the effect of vortioxetine, relative to placebo, on cognitive dysfunction in MDD, as assessed using DSST.

Cognitive dysfunction is present in many patients suffering from acute MDD [31], with cognitive symptoms often persisting after remission of mood symptoms [32,33]. Cognitive function is considered among the critical determinants of functional outcome in MDD [8,28,36-41].

Cognitive dysfunction can be subjectively experienced, objectively measured, or both. Objective neuropsychological tests can measure changes in cognitive performance and have important advantages, including being less susceptible to bias from mood state compared to patient-reported measures. The DSST, the key endpoint in the vortioxetine studies in this application [67], is a validated neuropsychological test used in a wide range of clinical populations, including patients with MDD. Cognitive function as assessed using the DSST robustly correlates with functional capacity and real-world functional outcomes [8,15-17,21,51-53,69]. Further, performance on the DSST explains a large proportion of the variance seen in large neuropsychological test batteries.

In vitro and animal data have shown vortioxetine to have a distinct pharmacological profile with the potential to improve cognitive dysfunction in MDD. The effect of vortioxetine on cognitive dysfunction in MDD was studied in 3 large clinical studies and an fMRI study. These studies were designed in the absence of formal guidance from FDA on the development of pharmacological compounds to improve cognitive dysfunction in MDD and with limited precedent in the field. The studies were designed in consultation with experts in MDD as well as in cognitive testing and guided by experience from the vortioxetine MDD registration program and literature on cognitive dysfunction in MDD. The antidepressant duloxetine has been studied in cognitive dysfunction in MDD and was included in ELDERLY and CONNECT as an active reference and benchmark for vortioxetine. However, the program was not designed to demonstrate superiority over duloxetine.

The demographic and clinical characteristics of the subjects who participated in these studies were similar to those in the MDD registration program. As expected, both vortioxetine and duloxetine demonstrated favorable effects on measures of depression, verifying that the effects of cognitive dysfunction in MDD were studied in the appropriate population.

On the DSST, vortioxetine demonstrated a treatment effect in ELDERLY (nominal p<0.05). The favorable vortioxetine effect on the DSST was confirmed by statistically significant effects of vortioxetine versus placebo in FOCUS and CONNECT. Duloxetine did not separate from placebo on the DSST in either ELDERLY or CONNECT.

The standardized effect size of the change from Baseline in DSST versus placebo is presented in Figure 5.a for the 3 individual studies and ranged from 0.25 to 0.52 across the studies. This level of improvement in DSST performance associated with vortioxetine therapy is considered to be clinically meaningful for the following reasons:
• The magnitude of cognitive dysfunction in MDD compared to healthy subjects across a range of cognitive measurements is reported to be between 0.2 and 0.7 [4,19-21]. Specifically on the DSST, the reported effect size is 0.55 [21].

• Cognitive deficits of similar magnitude have been reported in conditions that are readily recognized as reflecting impaired cognitive performance, such as legal intoxication (standardized effect size on DSST performance of 0.68) [22,23] or diphenhydramine or lorazepam use (standardized effect size on DSST performance of ~0.3 and ~0.5, respectively).

• Performance on the DSST is strongly associated with the magnitude of functional recovery in work, school, and the community [8]. As many patients with MDD continue to work or remain in school, even a small magnitude of cognitive impairment from a previously higher level, if persistent, can impact work or school performance and activities of daily living.

• Standardized effect sizes in the range observed are consistent with clinically meaningful effects and are similar to those reported with registered pharmacological agents in psychiatry [24].
Additional objective cognitive measures were also included across the studies. The treatment effects on these measures varied, with the greatest treatment effects observed in FOCUS (Table 5.a). In none of the studies was there evidence of a deleterious effect of vortioxetine on cognition across a broad range of measures.
**Table 5.a**  
Change From Baseline in Neuropsychological Tests at Week 8, Standardized Effect Sizes Versus Placebo (FAS)—Across Studies

<table>
<thead>
<tr>
<th>Test</th>
<th>Vortioxetine</th>
<th>Duloxetine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ELDERLY</td>
<td>CONNECT</td>
</tr>
<tr>
<td></td>
<td>5 mg</td>
<td>10 mg</td>
</tr>
<tr>
<td>DSST</td>
<td>0.27†</td>
<td>0.51***</td>
</tr>
<tr>
<td>RAVLT acquisition/learning</td>
<td>0.27†</td>
<td>0.23†</td>
</tr>
<tr>
<td>RAVLT delayed recall/memory</td>
<td>0.24†</td>
<td>0.31††</td>
</tr>
<tr>
<td>TMT-A</td>
<td>--</td>
<td>0.29††</td>
</tr>
<tr>
<td>TMT-B</td>
<td>--</td>
<td>0.29††</td>
</tr>
<tr>
<td>Stroop congruent</td>
<td>--</td>
<td>0.33††</td>
</tr>
<tr>
<td>Stroop incongruent</td>
<td>--</td>
<td>0.35††</td>
</tr>
<tr>
<td>GMLT</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>SRT</td>
<td>--</td>
<td>0.41†††</td>
</tr>
<tr>
<td>CRT</td>
<td>--</td>
<td>0.38†††</td>
</tr>
<tr>
<td>One-Back Test</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Analyses: FOCUS is MMRM; ELDERLY and CONNECT are LOCF (ie, primary analyses).

--- = test not applied in this study.

*=p<0.05 (ELDERLY and CONNECT); *=p<0.025 (split level of significance in FOCUS per dose), **=p<0.01, ***=p<0.001 versus placebo; statistically significant p-values.

†=p<0.05, ††=p<0.01, †††=p<0.001 versus placebo; nominal p-values.

Patient-reported cognitive symptoms, while particularly useful to gauge changes that are important to patients, are also known to be highly correlated with mood [1,27]. FOCUS and CONNECT included a patient-reported measure of cognitive symptoms, the PDQ. Both vortioxetine and duloxetine treatment were associated with improvements in the PDQ total score in both studies. On the CPFQ, included in CONNECT, both vortioxetine and duloxetine improved the total score in subjects with significant symptoms (total scores >25 at Baseline), while duloxetine was also associated with improvement in CPFQ scores in the overall population (all nominal p<0.05).

Functional capacity and work limitations are particularly important outcomes in MDD, given the need to preserve employment or educational performance in subjects often in their formative or prime employment years [1]. CONNECT included the UPSA and WLQ, which measure functional capacity and work limitations, respectively. Vortioxetine treatment was associated with improvement in UPSA composite score (standardized effect size of 0.39, nominal p<0.001). In working subjects, vortioxetine treatment was also associated with improvement in patient-reported work limitations as measured using the WLQ time demands subscale (nominal p<0.05). Duloxetine did not separate from placebo on UPSA or WLQ. These data support that vortioxetine has a distinct profile with respect to improving functional capacity.
The vortioxetine studies assessing cognitive dysfunction in MDD were conducted in acutely depressed subjects. Improvement in depressive symptoms can confound treatment effects on cognitive dysfunction, especially in subjective measures. The profiles of vortioxetine and duloxetine on the MADRS and DSST illustrate that effects on mood and cognition can be differentiated in an acute MDD setting. Both agents show an antidepressant effect; however, unlike vortioxetine, duloxetine does not show an effect on the DSST. The numerically greater treatment effect of duloxetine relative to vortioxetine on the MADRS but smaller effect on the DSST is consistent with the view that the effect on objectively measured cognitive dysfunction is to a large degree not mediated by improved mood.

In addition, mediation analyses were conducted to quantify the effects of vortioxetine on cognitive dysfunction by adjusting for effects mediated through improved depressive symptoms. The results of these analyses show that the majority of the vortioxetine treatment effect on the DSST is not mediated by improved depressive symptoms, ie, is a direct effect.

In summary, the distinct effects of vortioxetine on cognitive dysfunction in MDD, as measured by the DSST, were demonstrated and replicated in large clinical studies and supported by treatment effects on additional objective and subjective measures of cognitive function, functional capacity, and work limitations. The effects of vortioxetine on objective cognitive measures appear to be largely independent of improvements in mood. The distinct clinical profile of vortioxetine is supported by its pharmacological profile, animal data demonstrating effects in models of cognitive function that are different from those for SSRIs and SNRIs, and the data from a clinical pharmacodynamic fMRI study in subjects remitted from depression.

Beyond mood symptoms, the treatment of MDD should also target cognitive dysfunction with the ultimate aim of improving real-world outcomes. Through its effects on cognitive dysfunction, in addition to improvement in mood symptoms, vortioxetine addresses an unmet medical need in MDD. These clinically meaningful treatment effects support the proposed additions to the Clinical Studies section of the USPI.
6.0 REFERENCES


ADVISORY COMMITTEE BRIEFING MATERIALS: AVAILABLE FOR PUBLIC RELEASE


108. Schweimer J, Li Y, Sanchez C, Sharp T. In vivo electrophysiological evidence for the targeting of 5-HT3 expressing cortical interneurons by the novel multimodal

ADVISORY COMMITTEE BRIEFING MATERIALS: AVAILABLE FOR PUBLIC RELEASE
antidepressant vortioxetine [Poster]. Society for Neuroscience; October 17-21, 2015; Chicago, IL. Poster No. 410.23/K36.


111. Waller JA, Sanchez C. Vortioxetine promotes maturation of dendritic spines-An in vitro study in hippocampal cultures [Abstract]. Biological Psychiatry 2015;77(9):58S.


LIST OF APPENDICES

Appendix A  Neuropsychological Tests in the Vortioxetine Studies ............................................. 2
Appendix B  Digit Symbol Substitution Test: Psychometric Background ............................................. 3
Appendix C  Additional Statistical Information ...................................................................................... 8
Appendix D  Sensitivity Analyses .............................................................................................................. 12
Appendix E  Direct and Indirect Effect Sizes ......................................................................................... 15
Appendix F  fMRI Study (14137A): Methods and Materials ................................................................. 16
### Appendix A  Neuropsychological Tests in the Vortioxetine Studies

The neuropsychological tests used for the objective measurement of cognitive function are described below.

<table>
<thead>
<tr>
<th>Test</th>
<th>Impacted by Dysfunction in These Cognitive Domains</th>
<th>Task Description and Scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSST</td>
<td>Speed of processing, attention, executive function (including working memory)</td>
<td>Using a pencil, and working as quickly as possible, copy the matching symbol shown on the key displayed across the top of the page into the box under each numeral. Measure: number of correct symbols copied within the time limit of either 90- or 120 sec (depending upon which version of the test is used). (Score range is 0 to 133.)</td>
</tr>
<tr>
<td>RAVLT</td>
<td>Verbal learning and memory</td>
<td>Recall as many words as possible from a list of 15 words read aloud. This process using the same word list is repeated 3 times. Measure: sum of total number of words recalled across 3 trials (acquisition/learning). (Score range is 0-45.) Recall as many words as possible from the list after a 20-30 min delay. Measure: total number of words recalled (delayed recall/memory). (Score range is 0-15.)</td>
</tr>
<tr>
<td>TMT-A, TMT-B</td>
<td>Attention, speed of processing (TMT-A) Executive function, cognitive flexibility (TMT-B)</td>
<td>Using a pencil, draw lines to connect circles numbered 1 to 25 (TMT-A), and to connect circles alternating numbers (1 to 13) with letters (A to L) (TMT-B), in ascending order, as fast as possible. Measure: For each part of the test, the number of seconds required for completion.</td>
</tr>
<tr>
<td>Stroop</td>
<td>Speed of processing (Stroop congruent) Executive function, response inhibition (Stroop incongruent)</td>
<td>Read words representing names of colors that are printed in the same color ink as the word name (congruent), and in a different color ink (incongruent), as quickly as possible. Measure: number of seconds required to complete each part of the test.</td>
</tr>
<tr>
<td>CRT, SRT</td>
<td>Psychomotor speed (SRT) Attention (CRT)</td>
<td>Press a key as soon as a playing card appears on a computer screen (simple reaction time, SRT), and only if the card is red (choice reaction time, CRT). Measure: mean latency of correct responses (log10 msec).</td>
</tr>
<tr>
<td>One-back Task</td>
<td>Attention, working memory</td>
<td>Press a key after deciding whether a playing card appearing on a computer screen is identical to the one just before. Measure: mean latency of correct responses (log10 msec).</td>
</tr>
<tr>
<td>GMLT</td>
<td>Executive function, learning</td>
<td>Uncover a 28-step hidden pathway through trial and error learning on a computer touch screen. Measure: total number of errors made after completing the same pathway 5 times.</td>
</tr>
</tbody>
</table>
Appendix B  Digit Symbol Substitution Test: Psychometric Background

B.1 Instrument

The Digit Symbol Substitution Test (DSST) is an objective paper and pencil neuropsychological test. The test consists of rows containing blank squares that are each paired with a randomly assigned number from 1 to 9 and is presented to subjects on a single piece of paper. Above the rows is a printed key that pairs each number with a different symbol. The subject must draw a symbol below each digit as indicated by the key presented with each digit. Following completion at the end of the limited time period in which the test is administered, the number of correct symbols substituted for digits is counted.

The completion of the DSST requires a variety of cognitive functions including incidental short-term memory, perceptual organization, visuo-motor coordination, and selective attention [1]. The WAIS-III 120-second version of the DSST was used in ELDERLY (Study 12541A). In FOCUS (Study 14122A) and CONNECT (Study 202) the WAIS-III version was used but subjects were only allowed 90 seconds for completion to improve sensitivity to change.

B.2 Instructions for Use

The paper and pencil version of the DSST was used across all 3 studies. Administration time for completion and scoring of the DSST is approximately 5 minutes. General instructions for completing the DSST include having the test be timed on a stop-watch, providing a smooth drawing surface or a flat surface on which the Digit Symbol Coding page can be placed, and introducing the test by stating, “In this test, I’m going to ask you to copy some symbols.” Examinees should be encouraged to work as fast as they can, and should not be discouraged from making spontaneous corrections unless they do so repeatedly and performance is impeded. Examinees should be reminded if necessary to start at the beginning of a new row once having completed the prior row of symbols. Examinees should not skip any rows.

B.3 Training Method and Materials Used for Questionnaire Administration

The DSST was administered for a given subject by the same examiner at approximately the same time of day. The test was administered by a psychologist or other site personnel with previous experience in the application of neuropsychological tests. A test training session was organized for investigators prior to study start across all studies. Only certified examiners were authorized to administer the DSST to subjects.

To ensure satisfactory training of DSST administrators and quality execution with regards to data collection, DSST administrators were required to comply with certain requirements prior to study participation in all 3 studies. All DSST administrators were required to successfully complete the full scope of training requirements before subject rating. DSST administrators who successfully completed all requirements were approved for study participation by the sponsor and/or its designee before site enrollment began.
B.4 Content Validity

MDD is a complex illness that includes affective, somatic, and cognitive symptoms. Symptoms of cognitive dysfunction, such as difficulty in thinking, concentrating, and making decisions, have long been recognized as an intrinsic characteristic of MDD and are among the most persistent symptoms. Cognitive symptoms of MDD, namely the “diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others),” are among the diagnostic criteria for an MDE already identified in the Diagnostic and Statistical Manual of Mental Disorders (DSM)-III and remaining through to the current version [2].

Cognitive dysfunction is present during the first episode of depression, in recurrent depression, and in late-life depression [3-5]. Individuals with MDD often exhibit deficits in several domains of cognitive performance including attention, processing speed, executive function, and learning and memory [6], and are assessed using objective neuropsychological tests [7-9]. In a meta-analysis of 13 studies in MDD (N=644 subjects), these domains were significantly worse in subjects experiencing their first MDE than in healthy controls [4].

The DSST was originally developed to understand human associative learning [10]. Extensive research into which cognitive processes underlie deficient DSST performance in clinical populations has implicated a broad range of cognitive functions including not only associative learning and speed of processing, but also attention and executive functions such as working memory, which are the cognitive domains affected in MDD [11-18].

B.5 Assessment of Other Measurement Properties

B.5.1 Reliability

According to the WAIS-R manual [1], the test-retest reliability estimate for the DSST was 0.82 among middle-aged adults, demonstrating substantial agreement between test and retest [19]. Similarly, Lezak et al. [20] reported DSST stability coefficients in the range of 0.83 to 0.86 in healthy adults, but noted that stability varies by clinical population. Additionally, repeated administration of the DSST over 3 months did not result in noticeable practice effects. The DSST does not have notable education effects on test performance [21]. Practice effects under normal conditions are modest [22] and do not interfere with comparisons in the presence of placebo. Age-corrected norms are available.

B.5.2 Construct Validity

DSST scores are strongly correlated with functional outcomes in patients with MDD and across a range of conditions [23,24]. In patients with MDD studied 6 months after hospital discharge for an MDE, DSST performance was strongly associated with the magnitude of functional recovery in work, school, and the community after adjusting for residual symptoms of depression and other medical disabilities [23]. In this study functional recovery was measured using the 7-point overall global rating on the Multidimensional Scale of Independent Functioning (MSIF). The divergent validity of the DSST was demonstrated by Storanit [25], who found that the DSST did not correlate with verbal ability.
B.5.3 Known Groups

Over the past decade, the DSST has demonstrated known-groups validity in MDD and other important patient populations including differentiation in group changes over time. A recent meta-analysis that focused on executive dysfunction and estimated the magnitude of impairment relative to healthy controls on a wide array of neuropsychological tests concluded that “MDD is reliably associated with impaired performance on neuropsychological measures of executive functioning” [26]. In that report, a combined 1904 subjects across 22 studies overall provided data on the DSST, yielding an impairment of moderate magnitude with mean effect size (Cohen’s d) relative to healthy controls of 0.55 (P<0.001) (CI=0.34-0.75). Similar findings have been reported in elderly depressed patients (eg, where DSST performance is worse in early compared with late onset of disease [p<0.04; [27]). In demonstrating change over time, a meta-analysis of 3 placebo-controlled studies of the effect of statins on cognition demonstrated an important change over time in overall cognition improvement from statins as measured by the DSST (mean change difference =1.65; p=0.05; [28]). During a 4-month aerobic exercise conditioning program, Dustman et al [29] found significant pre-post differences (p<0.001) in the aerobic exercise group, but no statistically significant differences (p>0.05) over time in the exercise or nonexercise controls.

B.6 Language Translation and Cultural Adaptation

Linguistically and culturally validated translations of the DSST were used, where needed, in the languages of all the participating countries and provided by the sponsor. All translations were conducted in accordance with the ISPOR guidelines [30].

B.7 Data Collection Method

The paper and pencil version of the DSST was used across all studies. The total performance score on the DSST was calculated by counting the total number of correct symbols for each subject.

B.8 References


Appendix C  Additional Statistical Information

C.1. Assessments

The primary analysis in the 3 studies was based on FAS. As a sensitivity analysis, pattern mixture models using standard SAS STAT procedures were performed to address the issue of data missing not at random. The results of these analyses were robust and indicated no evidence that the missing at random assumption had been violated.

The multiplicity-controlled endpoints in the 3 studies comprised a hierarchy of 1 primary endpoint and 2 or 3 key secondary endpoints. Results that were within the testing hierarchy and had a p-value less than the predefined significance level for the study (p<0.05 for ELDERLY and CONNECT, p<0.025 for each dose in FOCUS) were considered statistically significant.

The results from additional endpoints were not multiplicity controlled and are considered supportive. These include the results for the cognition endpoints in ELDERLY, in which the hierarchical testing strategy concerned depression endpoints.

Nearly all the analyses presented in this document were prespecified. The exceptions are noted when presented in the text.

This following table describes the classification of the endpoints in each study—whether primary, key secondary, or additional prespecified—and whether or not its analysis was multiplicity controlled.
<table>
<thead>
<tr>
<th>Study</th>
<th>ELDERLY</th>
<th>FOCUS</th>
<th>CONNECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuropsychological tests of cognitive function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSST</td>
<td>Prespecified, not multiplicity controlled</td>
<td>Key secondary, multiplicity controlled (within doses)</td>
<td>Primary, multiplicity controlled</td>
</tr>
<tr>
<td>RAVLT</td>
<td>Prespecified, not multiplicity controlled</td>
<td>Key secondary, multiplicity controlled (within doses)</td>
<td></td>
</tr>
<tr>
<td>Composite of DSST and RAVLT</td>
<td></td>
<td>Primary, multiplicity controlled (within doses)</td>
<td></td>
</tr>
<tr>
<td>TMT-A and -B, Stroop Tests (congruent and incongruent), SRT/Detection Task, CRT/Identification Task</td>
<td>Prespecified, not multiplicity controlled</td>
<td>Prespecified, not multiplicity controlled</td>
<td>Prespecified, not multiplicity controlled</td>
</tr>
<tr>
<td>GMLT, OBT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient-reported cognitive symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDQ</td>
<td>Prespecified, not multiplicity controlled</td>
<td>Key secondary endpoint, multiplicity controlled (b)</td>
<td>Prespecified, not multiplicity controlled</td>
</tr>
<tr>
<td>CPFQ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depressive symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAM-D24</td>
<td>Primary, multiplicity controlled (a)</td>
<td>Prespecified, not multiplicity controlled</td>
<td>Prespecified, not multiplicity controlled</td>
</tr>
<tr>
<td>MADRS, CGI-S</td>
<td>Prespecified, not multiplicity controlled</td>
<td>Prespecified, not multiplicity controlled</td>
<td>Prespecified, not multiplicity controlled</td>
</tr>
<tr>
<td>CGI-I (c)</td>
<td>Prespecified, not multiplicity controlled</td>
<td>Prespecified, not multiplicity controlled</td>
<td>Key secondary endpoint, multiplicity controlled</td>
</tr>
<tr>
<td>Performance-based functional capacity</td>
<td></td>
<td></td>
<td>Prespecified, not multiplicity controlled</td>
</tr>
<tr>
<td>UPSA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient-Reported Work Productivity</td>
<td></td>
<td></td>
<td>Prespecified, not multiplicity controlled</td>
</tr>
<tr>
<td>WLQ</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Each test was implemented at a Baseline and Week 8, unless otherwise noted.
(a) Also for earlier time points.
(b) PDQ attention/concentration plus planning/organization combined subscore was a multiplicity-controlled, key secondary endpoint in CONNECT. Other PDQ subscores and total score were prespecified, not multiplicity controlled.
(c) No baseline for CGI-I.

C.2. MADRS-Adjusted, Mediation, and Path Analyses

To evaluate the mediation of the effects on DSST by the changes in depressive symptoms (MADRS total score), mediation (path) analyses were performed. Mediation analysis is a general
term for statistical analyses of causal dependencies among a set of variables. The statistical modeling can involve methodology ranging from simple ANCOVA and regression analyses to complex structural equation modeling involving latent variables.

For the vortioxetine studies, a straightforward approach was used, involving only 3 measured variables (cognition [DSST], depression, and treatment) analyzed with LOCF. The analyses were used to discern the effect of vortioxetine on cognitive dysfunction after correcting for the effect on depressive symptoms and to determine the proportion of the treatment effect on cognitive dysfunction that was a direct treatment effect (ie, not mediated through an improvement in depressive symptoms). The direct and indirect effects are expressed as percentages of the total effect (direct effect + indirect effect). The figure summarizes this analysis.

\[
\gamma_1, \gamma_2, \text{ and } \gamma_3 \text{ are described below.}\]

In order to obtain an estimate of the effect of treatment on DSST that could not simply be attributed to alleviation of depressive symptoms (ie, mood-independent effect), MADRS-adjusted analyses were performed by adding the change from Baseline in MADRS total score as a covariate in the ANCOVA model. The baseline value for MADRS was also added to the model as a covariate. This model also served as the first part of a path analysis in which the proportion of cognitive dysfunction improvement due to improvement of depression was analyzed adjusting the analysis of the change from Baseline in DSST for the change from Baseline in the MADRS total score.

In the model, treatment is assumed to have a direct effect both on the change in depression (\(\gamma_2\) in the figure) and on the change in cognition (\(\gamma_1\)). In addition, the change in depression is assumed to be linked to the change in cognition (\(\gamma_3\)). Consequently, treatment has an indirect effect on cognition given by \(\gamma_2 \times \gamma_3\). The total effect (direct+indirect) of treatment on cognition is \(\gamma_1 + \gamma_2 \times \gamma_3\) and the proportion of direct effect is \(\gamma_1 / (\gamma_1 + \gamma_2 \times \gamma_3)\).
In practice, the parameters were estimated using standard ANCOVA models:

\[ M1: \Delta DSST = \gamma_1 \times \text{Treatment} + \gamma_3 \times \Delta \text{MADRS} + \text{Covariates} \]

\[ M2: \Delta \text{MADRS} = \gamma_2 \times \text{Treatment} + \text{Covariates} \]

The covariates included in the models were the baseline values for DSST and MADRS and site.

### C.3. Calculating Standardized Effect Sizes

The treatment effects were standardized allowing for comparisons of the magnitude of the effect sizes between studies and different endpoints. These standardized effect sizes (also known as Cohen’s d) were calculated for differences from placebo using the LS means and the SEs from the linear models. Each effect size \( (d) \) was calculated as:

\[
d = \frac{\hat{\mu}_t - \hat{\mu}_p}{s_p}
\]

where \( \hat{\mu}_t \) and \( \hat{\mu}_p \) are the LS means for the active treatment group and placebo group, respectively, from the linear model. \( s_p \) is the pooled SD from both treatments which is calculated per the following equation:

\[
s_p = \frac{SE_t}{\sqrt{(1/n_t + 1/n_p)}}
\]

where \( n_t \) and \( n_p \) are the observed numbers of subjects from the active treatment group and the placebo group, respectively, and \( SE_t \) is the standard error associated with the difference of the LS means from the linear model.
Appendix D  Sensitivity Analyses

D.1. Sensitivity Analyses in FOCUS

In the sensitivity analyses of the primary efficacy endpoint (composite z-score analyzed as OC and LOCF), the results were overall similar to that of the primary efficacy analysis (FOCUS, Tables 42 and 43, respectively). The same analyses for the DSST were likewise similar to the MMRM analysis (FOCUS, Tables 51 and 52, respectively).

Table 42  Analysis of Composite Z-score (FAS, OC, ANCOVA)

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Baseline N</th>
<th>Mean</th>
<th>Week</th>
<th>Difference to PBO</th>
<th>95% CI</th>
<th>Lower</th>
<th>Upper p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBO</td>
<td>194</td>
<td>-0.007</td>
<td>1</td>
<td>0.083</td>
<td>0.050</td>
<td>0.23</td>
<td>0.1361</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>-0.226</td>
<td>1</td>
<td>0.054</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VOR 10 mg</td>
<td>193</td>
<td>-0.009</td>
<td>1</td>
<td>0.018</td>
<td>0.060</td>
<td>0.338</td>
<td>0.48 &lt;.0001</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.112</td>
<td>1</td>
<td>0.056</td>
<td>0.072</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>VOR 20 mg</td>
<td>204</td>
<td>0.015</td>
<td>1</td>
<td>0.074</td>
<td>0.049</td>
<td>0.157</td>
<td>0.29 0.0192</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.065</td>
<td>1</td>
<td>0.052</td>
<td>0.072</td>
<td>0.17</td>
<td>0.45 &lt;.0001</td>
</tr>
</tbody>
</table>

Table 43  Analysis of Composite Z-score (FAS, LOCF, ANCOVA)

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Baseline N</th>
<th>Mean</th>
<th>Week</th>
<th>Difference to PBO</th>
<th>95% CI</th>
<th>Lower</th>
<th>Upper p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBO</td>
<td>194</td>
<td>-0.007</td>
<td>1</td>
<td>0.083</td>
<td>0.050</td>
<td>0.23</td>
<td>0.1361</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>-0.281</td>
<td>1</td>
<td>0.052</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VOR 10 mg</td>
<td>193</td>
<td>-0.009</td>
<td>1</td>
<td>0.018</td>
<td>0.068</td>
<td>0.101</td>
<td>0.23 0.1361</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.077</td>
<td>1</td>
<td>0.056</td>
<td>0.070</td>
<td>0.22</td>
<td>0.50 &lt;.0001</td>
</tr>
<tr>
<td>VOR 20 mg</td>
<td>204</td>
<td>0.015</td>
<td>1</td>
<td>0.074</td>
<td>0.049</td>
<td>0.157</td>
<td>0.29 0.0192</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.035</td>
<td>1</td>
<td>0.050</td>
<td>0.069</td>
<td>0.18</td>
<td>0.45 &lt;.0001</td>
</tr>
</tbody>
</table>

ADVISORY COMMITTEE BRIEFING MATERIALS: AVAILABLE FOR PUBLIC RELEASE
### Table 51  Analysis of Change from Baseline in DSST Number of Correct Symbols (FAS, OC, ANCOVA)

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Baseline</th>
<th>Difference to PBO</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>Week</td>
</tr>
<tr>
<td>PBO</td>
<td>194</td>
<td>42.38</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>178</td>
<td>4.69</td>
</tr>
<tr>
<td>VOR 10 mg</td>
<td>193</td>
<td>41.96</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>180</td>
<td>8.61</td>
</tr>
<tr>
<td>VOR 20 mg</td>
<td>204</td>
<td>41.61</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>187</td>
<td>8.84</td>
</tr>
</tbody>
</table>

### Table 52  Analysis of Change from Baseline in DSST Number of Correct Symbols (FAS, LOCF, ANCOVA)

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Baseline</th>
<th>Difference to PBO</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>Week</td>
</tr>
<tr>
<td>PBO</td>
<td>194</td>
<td>42.38</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>194</td>
<td>4.41</td>
</tr>
<tr>
<td>VOR 10 mg</td>
<td>193</td>
<td>41.96</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>190</td>
<td>8.37</td>
</tr>
<tr>
<td>VOR 20 mg</td>
<td>204</td>
<td>41.61</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>204</td>
<td>8.41</td>
</tr>
</tbody>
</table>
D.2. Sensitivity Analyses in CONNECT

Sensitivity analyses (FAS, ANCOVA, LOCF of completers) confirmed the results of the primary efficacy analysis.

| Analysis of Change from Baseline in DSST Total Number of Correct Symbols by Study Visit Subjects Completed Double-Blind Study Full Analysis Set |
|---|---|---|
| | Placebo (N=161) | Lu AA21004 (N=167) | Duloxetine (N=175) |
| Baseline Value | | | |
| N | 161 | 167 | 175 |
| LS Mean (SE) | 42.65 (0.866) | 42.34 (0.851) | 42.24 (0.828) |
| F-Value, Active vs Placebo | 0.664 | 0.605 |
| Change from Baseline at Week 8/Final Visit | | | |
| N | 161 | 167 | 175 |
| LS Mean (SE) | 2.02 (0.553) | 4.68 (0.544) | 4.28 (0.529) |
| F-Value, Active vs Placebo | 0.029 | 0.094 |
| LS Mean Differences (SE) from Placebo 95% CI for Differences | 1.66 (0.762) (0.17, 3.16) | 1.26 (0.753) (-0.22, 2.74) |
Appendix E  Direct and Indirect Effect Sizes

The effect of vortioxetine on DSST cannot be explained solely by the improvement on MADRS. The table shows the effect on DSST (difference from placebo on change from Baseline at Week 8) for total effect and the effect not mediated through the MADRS (FAS, ANCOVA, LOCF). As shown, the magnitude of the adjusted standardized effect size for vortioxetine was between 0.19 and 0.32 across the studies at Week 8, and the proportion of the direct effect on the DSST for vortioxetine was between 56% and 78% at Week 8.

| DSST Change From Baseline at Week 8 (FAS, ANCOVA, LOCF)—Difference From Placebo; Standardized Effect Size |
|---------------------------------------------------------------|---------------------------------------------------------------|
| Vortioxetine                                                  | Duloxetine                                                   |
| ELDERLY                                                       | FOCUS                                                        | CONNECT                       |
| 5 mg                                                         | 10 mg                                                        | 20 mg                         | 10/20 mg                      | 60 mg                        | 60 mg                        |
| Subjects (n)                                                  | 152                                                          | 193                            | 204                          | 175                          | 144                          | 187                          |
| Total effect (a)                                              | 0.27†                                                        | 0.48 †††                        | 0.48 †††                      | 0.25*                        | 0.07                         | 0.18                         |
| Direct treatment effect (a) (after correction for effect of MADRS) | 0.20                                                          | 0.32††                         | 0.27††                        | 0.19                         | -0.02                        | 0.09                         |
| Proportion (%)                                                | 78                                                           | 66                             | 56                           | 75                           | NA                           | 48                           |
| 95% CI                                                       | 49,106                                                       | 47, 84                         | 34, 78                       | 49, 102                      | NA                           | -16, 113                      |

Statistically significant *p<0.05.
Nominal †p<0.05, ††p<0.01, †††p<0.001.
NA=Not applicable. Because of the very small total effect for duloxetine versus placebo and its estimated negative direct effect, the proportion of direct effect and its associated CI could not be calculated reliably.

(a) Standardized effect size.
Appendix F  fMRI Study (14137A): Methods and Materials

F.1 Subjects

Ninety-six subjects were enrolled and all completed the study. All were recruited by advertisement and assessed for the presence of current and past psychiatric disorders using the Structured Clinical Interview for DSM-IV [1].

Forty-eight remitted MDD subjects (27 women), mean age 35.6 years (range 20-53 years) were eligible. They were included only if they met criteria for having had at least 2 previous episodes of MDD, which included treatment with an antidepressant or recognized psychotherapy (for at least 1 episode), reported present cognitive dysfunction/difficulties (such as difficulty concentrating, slow thinking, difficulty in learning new things and/or remembering things), had no current Axis I disorder and were unmedicated for at least 6 weeks prior to inclusion into the study.

Forty-eight healthy control subjects (26 women), mean age 34.1 years (range 20-53 years), were recruited with no current or past history of MDD, never treated for depression with drugs or psychotherapy, no history of depression in a biological parent or other first-degree relative, and no present cognitive dysfunction/difficulties.

For all subjects, current mood was assessed with the HAMD-17 [2] and only subjects scoring ≤7 were included. All subjects were right-handed and had normal or corrected-to-normal vision. Subjects with any neurological disorders were excluded. Subjects were also required to be medically healthy, as determined from medical history and physical examination. A drug screen was taken to exclude current use of illicit drugs. Subjects were all nonsmokers or light smokers (<10 cigarettes a day) and had low or moderate levels of caffeine intake.

Subjects were recruited from 3 academic sites in the United Kingdom (the Universities of Manchester and Oxford and the Institute of Psychiatry in London). All subjects gave full informed written consent to the study, which was approved by a National Health Service ethics committee, and received an honorarium for their participation.

F.2 Experimental Design

In a randomized, double-blind, parallel-group, placebo-controlled study, subjects received a daily dose of vortioxetine (20 mg) or placebo for 13 or 14 days. Subjects were instructed to take 1 capsule per day at their normal waking time around 06:00-09:00. Measures (BOLD fMRI, neuropsychological assessments, questionnaires) were taken before (Baseline) and on days 12 to 14 of drug administration (Posttreatment).

F.3 Questionnaire Measures

The National Adult Reading Test (NART; clinician rated [3] was used to estimate IQ at the screening session. Subject-rated baseline measures of anxiety (Spielberger State-Trait Anxiety Inventory [STAI] [4]), subjective mood (Positive and Negative Affect Schedule [PANAS] [5], subjective symptoms of depression (Beck Depression Inventory, BDI-II [6]), and total score on the perceived cognitive deficits questionnaire (PDQ [7] were assessed for all subjects.
HAM-D17 (clinician rated) was also assessed. These scales were reassessed on the test day after 13 to 14 days of vortioxetine or placebo treatment.

**F.4 N-Back Working Memory Task Design**

While in the scanner subjects completed a letter variant of the N-back task [8]. Working memory was manipulated by using 3 levels of complexity: blocks of 1-, 2-, or 3-back trials. Briefly, subjects were required to indicate whether a letter presented on screen (the ‘target’ stimulus) matched a previously presented letter (the ‘cue’ stimulus). The cue stimulus was defined as the letter presented either 1, 2, or 3 screens previously for the 1-, 2-, and 3-back blocks, respectively. To minimize visual and phonological strategies, phonologically closed letters presented in upper and lower case were used. Thus, the following characters were presented: b, B, d, D, g, G, p, P, t, T, v, V. Subjects were instructed to ignore the case of letters and respond by pressing the right or left button if the target was identical or different from the cue, respectively.

Subjects also performed a sensorimotor control task (0-back) during which they were required to respond positively when a prespecified letter (x, X) was displayed and negatively when other letters were displayed. All blocks consisted of a sequence of 10 consonants varying in case. Letters were presented for 500msec with a fixed interstimulus interval of 1500 msec. Prior to each task block, an instruction screen (0-, 1-, 2-, or 3-back) was presented for 2000 msec. A 4000 msec blank screen separated the instructions from the onset of the first letter. Task blocks were separated by 8000 msec of fixation cross. Four blocks of each condition were presented in a fixed pseudo-random order (0-, 1-, 2-, 3-, 1-, 3-, 2-, 0-, 2-, 1-, 0-, 3-, 1-, 0-, 3-, 2-back). All conditions were matched for the number of target and upper-/lower-case letters presented. Stimuli were presented on a personal computer running a custom written visual basic program and projected onto an opaque screen within the scanner room, which subjects viewed using angled mirrors. Subjects’ responses were made via an MRI-compatible button box. Both accuracy and response latency were recorded. Subjects received training with another set of stimuli to ensure they fully understood the requirements during a study visit scheduled 5 to 10 days before the first dosing day and again immediately before scanning. During training, subjects were exposed to single blocks of the 0-back, 1-back, 2-back, and 3-back conditions. The same blocks were repeated until the subjects indicated that they understood the nature of the task.

**F.5 fMRI Data Acquisition**

Imaging data were collected using scanners located at each of the 3 academic sites: a Siemens TIM Trio 3T located at the Oxford Centre for Magnetic Resonance (OCMR), University of Oxford; a 3T Philips Achieva located at the Wellcome Trust Clinical Research Facility (WTCRF) and Salford Royal NHS Foundation Trust, University of Manchester; and a 3T GE MR750 located in the Institute of Psychiatry of King’s College London. Functional imaging consisted of 42 T2*-weighted echo-planar image (EPI) axial oblique slices that began at the cerebral vertex and encompassed the entire cerebrum and the majority of the cerebellum. Acquisition parameters were as follows: repetition time (TR)/echo time (TE) =3000 msec/35 msec, flip angle =90°, field of view/matrix size =224 x 224/64 x 64, slice thickness =3mm with a 0.3mm gap between slices. These parameters were selected to optimize the signal across the entire volume of acquisition for all sites and scanners in the study. The
initial EPI volumes in each session were discarded to avoid T1 equilibration effects. A high-resolution T1-weighted sequence was used to facilitate later coregistration of the fMRI data into standard space (site-specific settings for the parameters of the structural scan were used to produce optimal performance tailored to each machine).

**F.6 fMRI Data Analysis**

fMRI data were preprocessed and analyzed using FSL, version 5.0.5 [9]. Preprocessing included motion correction using within-subject image realignment [10], non-brain removal [11], spatial smoothing using a Gaussian kernel (5 mm full-width-half-maximum), high pass temporal filtering (with a cutoff of 150 sec). First level statistical analysis was carried out in individual subject space with the resulting statistical maps being spatially registered onto a standard Montreal Neurological Institute (MNI) template, refined using nonlinear registration [12].

Analyses of data from both the baseline and posttreatment sessions of individual subjects were performed using the general linear model with local autocorrelation [13]. Four explanatory variables were included in the model: regressors representing the presentation of 0-, 1-, 2-, and 3-back blocks. These explanatory variables were convolved with a hemodynamic response function. An additional control regressor representing periods of task instructions was also included in the model alongside the temporal derivatives of each regressor. Contrasts of the parameter estimates from this model were then created to identify voxels which responded to (a) the main effect of task (ie, N [1, 2, or 3]-back versus 0-back) and (b) the linear effect of task load (ie, a linear increase in activation from 0- to 3-back).

The within-subject change in these contrast images between Posttreatment and Baseline was then calculated using a fixed-effects model. Finally, the degree to which treatment, subject group, and the interaction between treatment and subject group influenced the change in activation from Baseline to Posttreatment was assessed by combining the results of the within-subject analyses in random-effects analyses performed across all subjects [14], with the effect of treatment then tested within each subject group separately. We included gender and site as prespecified covariates in the third level analyses to minimize the potential impact of these variables on between-group comparisons. The effects of subject group at Baseline only were also examined at the group level using a random-effects model.

Significant activations across the whole brain were identified using cluster-based thresholding of statistical images with a height threshold of Z =2.3 and a (whole-brain) corrected spatial extent threshold of p<0.05. Small volume corrections were applied to explore activation within predefined anatomical masks in the bilateral dorsolateral prefrontal cortex (DLPFC), bilateral hippocampus, anterior cingulate cortex, and precuneus. The DLPFC anatomical masks were defined as 10 mm radius spheres centered on activations identified in the N-back meta-analysis of neuroimaging studies of the N-back task by Owen et al [15]. The coordinates used for the center of the right and left masks were [40, 32, 30] and [-44, 18, 22], respectively. The other regions were defined as voxels with >50% probability of lying within the relevant structure as defined by the Harvard-Oxford Cortical/Subcortical anatomical atlases. Finally, in order to exclude the influence of change in symptoms of depression on the neuroimaging outcomes, the group level analysis was repeated with the change in BDI between Baseline and Posttreatment.
for each subject included as an additional covariate. In order to illustrate significant treatment effects a mean % signal change was extracted from the identified clusters. The maximum z-score within each cluster was reported alongside the coordinates of the voxel with that score. The predefined primary analysis was a comparison of BOLD activity, within the anatomical structural masks, between the treatment groups in remitted subjects. Both this primary analysis and additional comparisons using all subjects were defined in a statistical analysis plan, which was finalized before database lock.

F.7 Behavioral, Questionnaire, and Demographic Data

Baseline demographic, questionnaire, and neuropsychological data were analyzed using analyses of variance (ANOVAs) or logistic regression with subject group (control versus remitted) and treatment group (vortioxetine versus placebo) as between-subject factors. Baseline and change from baseline N-back response accuracy and latency were analyzed using repeated-measures ANOVAs with complexity (0-, 1-, 2-, or 3-back) as the within-subject factor. Change from Baseline in the questionnaire and neuropsychological tests were analyzed using univariate ANOVAs in which the dependent variable was the task/questionnaire score after treatment. Treatment group and subject group were included as between-subject factors with the baseline score on the task/questionnaire included as a covariate. In all the above analyses, gender and site were included as additional between-subject factors. As done in the neuroimaging analysis, the effect of treatment was tested across all subjects and then independently in the control and remitted groups. Significant interactions were followed up using standard post hoc tests.

F.8 References


