An exploratory study of combination buspirone and melatonin SR in Major Depressive Disorder (MDD): A possible role for neurogenesis in drug discovery

Maurizio Fava a, Steven D. Targum b,*, Andrew A. Nierenberg a, Leo S. Bleicher b, Todd A. Carter b, Pamela C. Wedel b, René Hen c, d, Fred H. Gage e, Carrolee Barlow b

a Department of Psychiatry, Massachusetts General Hospital, Boston, MA, USA
b BrainCells Inc., San Diego, CA, USA
c Department of Psychiatry, Columbia University, NY, USA
d Department of Neuroscience, Columbia University, NY, USA
e Laboratory of Genetics, The Salk Institute, La Jolla, CA, USA

A R T I C L E   I N F O
Article history:
Received 2 April 2012
Received in revised form 8 August 2012
Accepted 10 August 2012

Keywords:
Major depressive disorder
Neurogenesis
5 HT1a agonists
Augmentation treatments

A B S T R A C T
We used in vitro neurogenesis-based human neural stem cell (hNSCs) assays and rodent in vivo behavioral assays to identify potential novel antidepressants. A combination of buspirone and melatonin displayed antidepressant activity in these assays whereas neither buspirone nor melatonin alone showed any antidepressant-like profile. After evaluating numerous combination ratios, we determined that low dose buspirone 15 mg combined with melatonin-SR 3 mg yielded optimal antidepressant efficacy in our pre-clinical platform. The low dose of buspirone suggested that antidepressant efficacy might be achieved with only minimal adverse event liability. Based on these data, we conducted an exploratory 6-week, multi-center, double-blind, randomized, placebo- and comparator-controlled study of the combination of buspirone and melatonin in subjects with acute Major Depressive Disorder (MDD). The combination treatment revealed a significant antidepressant response in subjects with MDD on several measures (Clinical Global Impression of Severity and Improvement, Inventory of Depressive Symptomatology) compared to either placebo or buspirone 15 mg monotherapy. These preliminary findings have clinical implications and suggest that a platform of pre-clinical neurogenesis matched with confirmatory behavioral assays may be useful as a drug discovery strategy.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Only 30% of patients with Major Depressive Disorder (MDD) achieve full remission of symptoms with currently available antidepressants (Blier and Ward, 2003; Rush et al., 2006; Trivedi et al., 2006; Rush, 2007; Nassir Ghaemi, 2008). These drugs are associated with adverse effects such as gastrointestinal symptoms, agitation, sleep disturbance, and sexual dysfunction that may affect treatment compliance. Consequently, there is still a clear need for antidepressant medications to treat MDD that possess enhanced efficacy, fewer adverse effects, and utilize different mechanisms of action than currently available medications (Rosker et al., 2004; Nassir Ghaemi, 2008; Rush et al., 2008). Historically, drug development relied upon extrapolations from the monoamine hypothesis or serendipity (Rosker et al., 2004; Kasper and Hamon, 2009), but some recent strategies have employed more rational pre-clinical foundations to investigate novel mechanisms of action.

One type of strategy to facilitate drug development for mood disorders may be a pre-clinical platform of neurogenesis-based assays (Aimone et al., 2010). It has been demonstrated that neural stem cells in the adult human brain undergo neurogenesis, and that the resulting new cells are integrated into neural circuits that can become fully functional neurons (Eriksson et al., 1998). Several studies have specifically linked hippocampal function and neurogenesis to mood disorders (Jacobs et al., 2000; Drevets, 2001; van Praag et al., 2002; Gold and Chrousos, 2002; Warner-Schmidt and Duman, 2006; Sahay and Hen, 2007; Boldrini et al., 2009; David et al., 2009; Lucassen et al., 2010; Dranovsky et al., 2011). It has been reported that reduced hippocampal volume in untreated depression may be reversed by treatment with antidepressant medications, and that blocking neurogenesis makes all FDA-approved antidepressants ineffective (Malberg et al., 2000; Sheline et al., 2003; Santarelli et al., 2003; Dranovsky and Hen, 2006; Sahay...
and Hen, 2007; Wang et al., 2008; Boldrini et al., 2009). These findings suggest that hippocampal neurogenesis may be implicated in the expression of depressive symptoms. It is important to note that the role of neurogenesis in the etiology of and recovery from depression is still not clear (Henn and Vollmayr, 2004; Gass and Henn, 2009; Hanson et al., 2011; Aimone et al., 2011). Besides mood, many other factors including stress, enriched environments, and exercise can influence hippocampal neurogenesis (Boldrini et al., 2009; Lucassen et al., 2010; Snyder et al., 2011; Hanson et al., 2011). Therefore, it is premature to draw any conclusions about the specific role despite strong support for some role for neurogenesis in depression. Nevertheless, the evidence to date does point to a different, intriguing approach for rational drug development for mood disorders based upon the pharmacological induction of neurogenesis (Sahay and Hen, 2007; Aimone et al., 2010; Dunan and Voleti, 2012).

BrainCells Inc. (BCI) developed an in vitro and in vivo neurogenesis platform to facilitate the identification of novel antidepressant treatment strategies, including drug combinations. Using this rationale for drug discovery, BCI studied a broad matrix of nonproprietary drugs to seek optimal drug combinations. Once a drug combination was identified, pharmacokinetic and dose optimization studies were conducted to identify the optimal dose range to maximize clinical efficacy and minimize the adverse event liability. Using this strategy, the combination of buspirone and melatonin was identified. This drug combination demonstrated both enhanced neurogenesis in the pre-clinical neurogenesis-based in vitro assays and antidepressant responses in the in vivo behavioral assays that were equivalent to a Serotonin Selective Reuptake Inhibitor (fluoxetine). Further, buspirone was effective in these assays at doses below those generally used in clinical practice for Generalized Anxiety Disorder (GAD) and well below doses reported for antidepressant efficacy in the literature (Schweizer et al., 1986; Robinson et al., 1990; Blier and Ward, 2003; Rickels et al., 2003). The lower dose of buspirone made the buspirone—melatonin combination of particular interest because of its potential clinical utility.

Buspirone is a partial 5-hydroxytryptamine 1A (5-HT1A) receptor agonist as well as a presynaptic dopamine antagonist (D2) and partial alpha12 receptor antagonist (Deakin, 1993; Blier et al., 1997; Stahl et al., 1998; Adeagbo et al., 2000). The potential of 5-HT1A receptor agonism has been studied extensively in MDD. A lack of normal responsiveness of the post-synaptic 5-HT1A receptor is implicated in the reduced level of serotonin available in the synapse as well as stimulation of pre-synaptic somatodendritic 5-HT1A receptors which inhibits serotonin synthesis and release (Deakin, 1993; Stahl et al., 1998; Blier and Ward, 2003). 5-HT1A agonists like buspirone, gepirone, and ipsapirone have been evaluated in several double-blind, placebo-controlled trials of MDD but have never received FDA approval (Rickels et al., 1990; Robinson et al., 1990; Rickels et al., 1991; Sramek et al., 1996; Wilcox et al., 1996; Heiser and Wilcox, 1998; Lapiere et al., 1998; Stahl et al., 1998; Alpert et al., 2004; Amsterdam et al., 2004; Keller et al., 2005; Bielski et al., 2008). A few studies suggested that buspirone doses above 40 mg per day were efficacious for acute MDD (Schweizer et al., 1986; Robinson et al., 1990). In fact, Blier and Ward (2003) contended that higher doses of azapirones were required in order to demonstrate efficacy in depression. However, a higher dose of buspirone has not been clinically practical because it can cause dizziness, drowsiness, headache, fatigue, nervousness, insomnia, light-headedness, cognitive impairment, as well as nausea, gastric distress, palpitations, nightmares, tremor, numbness (paresthesia), and blurred vision (Rickels et al., 2003). Consequently, further development of buspirone as an antidepressant has been restricted by the adverse event liability at higher doses.

Combination or augmentation treatment strategies have been endorsed by many clinicians and were employed as a fundamental part of the NIMH-sponsored sequenced treatment alternatives to relieve depression study, STAR*D (Thase et al., 1998; Fava and Rush, 2006; Rush, 2007). Buspirone has been used effectively as an adjunctive treatment with SSRIs in patients with MDD (Landen et al., 1998; Appelberg et al., 2001; Trivedi et al., 2006; Rush, 2007). Of course, the addition of buspirone to SSRIs still conveys the same adverse event liabilities as SSRI monotherapy. Therefore, combining 5-HT1A agonists with an alternative medication to the SSRI’s might be of clinical value. Ideally, a combination/augmentation strategy would allow lower doses of buspirone to be used in order to minimize the adverse event potential of the azapirones as well.

The FDA approved buspirone for the treatment of Generalized Anxiety Disorder (GAD) in 1986. The typical recommended dose of buspirone ranges between 20 and 60 mg/day in two divided doses, with 30 mg/day generally considered as the typical therapeutic dose for GAD (Rickels et al., 2003). As noted above, the pre-clinical

Fig. 1. Pre-clinical drug discovery platform of human in vitro and rodent in vivo assays with buspirone and melatonin to explore neuronal differentiation and antidepressant behavioral effects. A) Depiction of hNPC experimental flow: hNPC neurospheres were dissociated, plated in 96-well plates and allowed to differentiate for seven days in the presence of test compounds. Cells were fixed and stained for TuJ1, GFAP, and Hoechst, imaged, quantitated and analyzed for increases in neuronal or glial differentiation. B) Effect of serotonin, buspirone, and the combination of buspirone and melatonin on the differentiation of hNPCs in vivo: Cells staining positive for TuJ1 or GFAP were manually counted on a field-by-field basis as described in the methods. The percent increase in TuJ1 positive cells is indicated on left side Y-axis (solid bar) and on the right side for GFAP positive cells (hatched bar). Effects of serotonin are indicated in light blue, buspirone in dark blue, and the combination in green. The lower panels are representative images as indicated (Combo — buspirone and melatonin). The neuronal marker TuJ1 is shown in green (upper images), the glial marker GFAP is shown in red (lower images) and the nuclear stain Hoechst is shown in blue in both upper and lower panels. The scale bar indicates 50 μM. C) Heatmap representing the in vitro effects of varying concentration and ratios of melatonin on blocking buspirone mediated induction of GFAP positive cell formation: Scale is indicated by color ranging from red (high GFAP density) to yellow (low GFAP density). X-axis represents increasing concentrations of buspirone and the y-axis represents increasing concentrations of melatonin. D) Activity of the combination of buspirone and melatonin in NSF: Experimental design for assessing the effect of compounds on NSF and neurogenesis is shown in the upper figure. Arrows at the top indicate daily oral dosing of drugs or vehicle. Arrows at the bottom indicate experimental day of novelty suppressed feeding (NSF) assay or animal sacrifice, perfusion/fixation and subsequent immunohistochemistry. Shown in lower panel is the NSF assay data as a % of appropriate vehicle control (Latency, % Vehicle Y-axis). Treatment and dose in milligram per kilogram body weight (mpk) is indicated on the X-axis with fluoxetine (light blue), buspirone (dark blue), melatonin (yellow) or the combination of buspirone and melatonin (Combo-green). n indicates number of animals in each group. *indicates p < 0.01 for combination treatment and p < 0.05 for fluoxetine alone based on an unpaired two-tailed Student’s t-test comparing drug treatment versus appropriate vehicle control. E) Change in the number of NSC as assessed by Ki67 staining is shown as the total number of Ki67-positive cells per cubic mm (Ki-67+ Cells/mm³) within the dentate gyrus expressed as a % of appropriate vehicle control after 28 days of treatment. Dosing of rats was as described for Fig. 1D. Effects on Ki-67 positive cells are shown for fluoxetine (light blue), buspirone (dark blue), melatonin (yellow) and the combination of buspirone and melatonin (Combo-green). n indicates number of animals in each group. *indicates p < 0.01 and p < 0.05 value based on an unpaired two-tailed Student’s t-test comparing drug treatment versus appropriate vehicle control. The lower panels are representative images taken at 40X magnification using a confocal microscope. Insets are shown at 2X magnification. The mature neuronal marker NeuN is shown in red (indicating the granule cell layer of the dentate gyrus), and cells labeled with Ki-67 are shown in green. The scale bar indicates 50 μM. F) Diagram of the clinical study: Shown is a schematic of the study design. After successful completion of screening procedures, approximately 120 eligible patients were randomized at week 0 to one of three study treatment groups at a ratio of 2:1:1. The top line labeled buspirone (15 mg) + melatonin (3 mg) represents the combination treatment group; the buspirone (15 mg) alone and the placebo groups are shown on the lines below. Study treatment was administered once a day at bedtime (q.h.s.). All patients were evaluated every 2 weeks and the duration of the trail was 6 weeks.
neurogenesis-based data suggesting that buspirone doses below 20 mg combined with melatonin might yield an antidepressant effect has broad clinical implications. Consequently, we conducted a clinical trial to determine if this low dose buspirone–melatonin combination might yield antidepressant efficacy and minimize the dose-related side effects associated with higher doses of buspirone.

2. Methods

2.1. Pre-clinical drug discovery and dose optimization

We used neurogenesis-based in vitro screening assays using human neural progenitor cells (hNPCs) and specific rodent behavioral in vivo assays to create a unique pre-clinical platform for drug discovery (Appendix 1). Human NPCs isolated from intact brain grown as primary cells in culture retain their ability to produce additional progenitor cells for a restricted time (Fig. 1A). These hNPCs cells can be stimulated to differentiate into mature cells that phenotypically resemble neurons and express an early neuronal marker β-Tubulin III (TuJ1) or into cells that express markers for non-neuronal lineages, like astrocytes (Glia1 Fibrillary Acidic Protein—GFAP) in the absence of human serum. Exposure of hNPCs to serotonin increases the number of cells that differentiate into neurons consistent with a role for serotonin in promoting neurogenesis (Malberg et al., 2000; Sahay and Hen, 2007; Wang et al., 2008). Drugs like fluoxetine are very neurogenic in this assay platform. On the other hand, we found that buspirone caused hNPCs to differentiate into both neuronal and non-neuronal lineages (Fig. 1B). We postulated that the increased formation of non-neuronal cells might adversely affect the putative antidepressant efficacy of buspirone. Consequently, we sought a non-toxic drug that in combination with buspirone would suppress the buspirone-induced GFAP response while sustaining the buspirone-induced neurogenesis response. BCI examined over 60 different drugs in combination with buspirone to identify the best combination. We found that the addition of melatonin markedly reduced the non-neuronal differentiation produced by buspirone alone (Fig. 1B), although melatonin alone had no effect on hNPCs at all.

Subsequently, we evaluated a range of buspirone–melatonin combination ratios in vitro (Fig. 1C). Within the selected dosage ranges, a combination ratio of 1:10 (melatonin to buspirone) appeared to sufficiently suppress GFAP responses while sustaining the neurogenesis response.

Using the combination ratio derived from the in vitro assays as a guide, we applied these findings to determine dosing for the in vivo novelty suppressed feeding (NSF) assay. We aimed for the lowest possible dose range in animals and focused on achieving a mg/kg animal dosage (mpk) that could be scaled to clinically relevant human doses. The NSF assay is a conflict assay that assesses the balance between an animal’s desire to approach a palatable reward and a fear of entering an open space (Santarelli et al., 2003; Dranovsky and Hen, 2006). When administered chronically (but not acutely), antidepressants are typically active in the NSF assay, whereas inhibition of hippocampal neurogenesis blocks the activity of antidepressants (Santarelli et al., 2003). Fisher 344 rats were chronically dosed with the optimized buspirone–melatonin combination ratio in the NSF assay and studied for changes in behavior and hippocampal neurogenesis (Fig. 1D and E). Neither buspirone nor melatonin alone showed efficacy in either NSF or histological assessment of neurogenesis (examining the number of NSCs as assessed by Ki67 staining). However, the selected drug combination showed activity comparable to the marketed antidepressant fluoxetine on both the NSF assay (Fig. 1D) and on histological analysis (Fig. 1E).

It is well known that dose selection based upon either in vitro assays or in vivo animal studies have serious limits and can only approximate human dosing possibilities. Ultimately, using the pre-clinical neurogenesis data and available species comparisons of AUC (Area under the curve) and plasma Cmax values (maximum plasma concentration), we selected 3 mg of melatonin (sustained release) and 15 mg of buspirone as best dose estimates. The buspirone dose is below the typical recommended therapeutic range for anxiety and well below the previously reported 40–60 mg effective doses for MDD (Robinson et al., 1990; Rickels et al., 2003). Clearly, the lower buspirone dose suggests that an intriguing clinical benefit might be gained for patients with MDD. The typical clinical dose of melatonin ranges from approximately 1 to 10 mg/day.

2.2. Clinical trial methods

This nine-site study was conducted in the United States as an Investigator-Initiated Phase 2 Clinical Trial coordinated by Massachusetts General Hospital (MGH) and sponsored by BrainsCells Inc. (BCI, San Diego, California) which supplied study drug and matching placebo capsules. The protocol and informed consent forms were approved by a central Ethics Committee or by the Ethics Committee commonly used by the participating investigative site.

2.2.1. Study participants

Subjects between 18 and 65 years of age who met DSM-IV-TR criteria for Major Depressive Disorder (MDD), as determined by the Mini-International Neuropsychiatric Interview (M.I.N.I.) and psychiatric evaluation were eligible for this study (Sheehan et al., 1998). All subjects gave written documentation of informed consent before any study related procedures. Eligible subjects required a total score of 14 or higher on the 16-item Quick Inventory of Depressive Symptoms-Self-Rated scale (QIDS-SR16) at both the screening and baseline visits (Rush et al., 2003).

Female patients of childbearing potential were on a reliable, medically acceptable form of contraception for at least 30 days prior to screening and throughout the study. Subjects meeting criteria for other Axis-I disorders as their primary diagnosis, had a history of eating disorders, obsessive-compulsive disorder, psychotic disorder, bipolar disorder and/or mental retardation and those with alcohol or substance abuse or dependency were excluded. Use of antidepressant, antipsychotic, or anxiolytic medication or drugs with known psychotropic properties were prohibited for 1 week (4 weeks for fluoxetine) prior to screening and throughout the study. Subjects who used substances that are known inhibitors or inducers of CYP3A4 were excluded.

2.2.2. Study design and randomization procedure

This was a double-blind, randomized, placebo-controlled study using a 2:1:1 allocation between 3 treatment groups using a computer-generated centralized randomization schedule. Subjects received either: the study drug combination of buspirone immediate release (IR) 15 mg with melatonin slow release (SR) 3 mg; buspirone IR 15 mg as monotherapy; or matching placebo capsules for 6 weeks following randomization (Fig. 1F). Buspirone was obtained from Bristol Myers Squibb, melatonin SR was obtained from Mellen Medical Products, Inc. and the clinical trial material was prepared by Fisher Clinical Services.

Buspirone IR 15 mg tablets and melatonin SR 3 mg tablets were over-encapsulated for blinding purposes. Subjects took two capsules of study drug once a day at bedtime with or without food. Subjects, investigators, study staff and BCI personnel involved in the trial were blinded to study group assignment.
2.2.3. Study procedures

After subjects signed the informed consent, a M.I.N.I. diagnostic and psychiatric screening evaluation was done in addition to measurement of vital signs and routine blood and urine tests. The QIDS-SR$_{16}$ and Clinical Global Impression of Severity (CGI-S) were administered at screening, baseline and after 2, 4, and 6 weeks of treatment and the CGI-Improvement (CGI-I) was done after 2, 4, and 6 weeks (Rush et al., 2003; Guy, 1976; Targum et al., 2008). Additional efficacy assessments included the 30-item Inventory of Depressive Symptomatology-Clinical version (IDS-C30) and the Hamilton Rating Scale for Anxiety (HAM-A) that were done at baseline and after 6 weeks (Hamilton, 1959; Rush et al., 1996). Adverse events were recorded at each study visit.

2.2.4. Outcome measures

The primary endpoint in this exploratory study was the mean change from baseline of the CGI-I after 6 weeks of study drug treatment or the last available post-randomization CGI-I score available. Additional endpoints included change from baseline in the CGI-S, QIDS-SR$_{16}$, IDS-C$_{30}$ and HAM-A. In addition, responder analysis was carried out for the CGI-I and responder and remission analyses for the IDS-C$_{30}$ on the modified intent to treat (MITT) population. The CGI-I response was defined as an endpoint score of 1 or 2. The QIDS-SR$_{16}$ and IDS-C$_{30}$ response was defined as subjects obtaining scores less than or equal to half of their baseline score. Clinical remission was defined as an endpoint score of ≤5 for the QIDS-SR$_{16}$ and ≤11 for the IDS-C$_{30}$.

Safety assessments were based upon evaluation of vital signs and adverse events reported during the study.

2.2.5. Statistical analysis

Statistical analyses included Mixed-effects Model Repeated Measures (MMRM) for instruments that were assessed at multiple time points (CGI-S, CGI-I, QIDS-SR$_{16}$) in the MITT population using PROC mixed within SAS (Lieberman et al., 2005). In these analyses, the available data from each patient was used, without any imputation for missing values. Alternatively, for measures performed only at baseline and week 6 (IDS-C$_{30}$ and HAM-A), the efficacy analyses used an analysis of covariance (ANCOVA) model, with change from baseline as the dependent variable, the baseline value as a covariate, and treatment group as the factor with three. Point estimates represent the model-based estimate of the mean for this sampling of the population. A responder analysis was calculated for the CGI-I and responder and remission analyses for the IDS-C$_{30}$ and QIDS-SR$_{16}$ on the MITT population. Fisher’s Exact Test was used to determine the significance level of differences in response and remission rates across treatments. Paired t-tests were used to determine significance of changes in vital signs during the treatment phase of the study.

By design, the planned statistical analyses for this small study included a secondary pooling of the buspirone and placebo treatment groups on the expectation that these groups would not differ on the mean CGI-I at endpoint by more than 0.04 points.

3. Results

A total of 142 subjects consented, enrolled, and were randomized in the study. Eight subjects were randomized but never received study drug. The MITT and Safety populations were identical and consisted of 134 randomized patients (33 patients in the placebo group, 34 patients in the buspirone group, and 67 patients in the combination treatment group). 112 patients who received study drugs (83.6%) completed the study.

Demographic and baseline characteristics were similar among treatment groups (Table 1). The study population was predominantly female (64.9%). Patients had a mean age of 42.4 years, mean height of 66.3 inches, and a mean weight of 203.9 pounds. There were no differences in demographics across treatment groups.

There were no statistically significant differences between the baseline efficacy measures between treatment groups (Table 1).

### 3.1. Efficacy outcomes

The mean efficacy scores at baseline, point change estimates for CGI-I, and the mean changes from baseline for the other efficacy measures are shown in Table 1.
measures at week 6 by treatment group are presented in Table 2. The CGI-I improved significantly in the combination group compared to placebo (MMRM; \( p = 0.047 \)). Similarly, the CGI-S revealed statistically significant improvement in the combination treatment compared to either buspirone monotherapy (MMRM; \( p = 0.02 \)) or placebo (MMRM; \( p = 0.04 \)). Changes from baseline to week 6 in the QIDS-SR16, IDS-C30, and HAM-A also revealed clinical benefit for the combination treatment relative to the buspirone monotherapy or placebo groups. The combination treatment was significantly better than placebo on the IDS-C30 (ANCOVA; \( p = 0.034 \)), but not on the QIDS-SR16.

Comparison between the buspirone 15 mg monotherapy and placebo groups revealed no clinical differences on any measure. The mean endpoint CGI-I scores were 2.33 ± 0.97 (SD) for the combination treatment group, 2.79 ± 1.24 for the buspirone 15 mg group, and 2.83 ± 1.09 for the placebo group. The statistical analysis plan included a secondary pooling of the buspirone and placebo treatment groups if their respective mean CGI-I scores at endpoint were no greater than 0.04 points apart. In fact, the mean difference for the CGI-I endpoints for the buspirone and placebo groups was only 0.04. As revealed in Table 2, the pooling of these two groups enhanced the statistical significance in favor of the combination treatment. The CGI-I response rate was 58.2% in the combination group, 38.4% in the buspirone monotherapy group, and 33.6% in the placebo group. The advantage for the combination treatment approached statistical significance for both the buspirone (\( p = 0.0006 \)) and placebo groups (\( p = 0.055 \)). When the buspirone monotherapy and placebo groups are pooled versus the combination treatment, the difference does reach statistical significance (\( p = 0.024 \)).

As shown in Table 3, response and remission rates were also calculated for the IDS-C30, and QIDS-SR16. These remission rates reveal that the combination treatment was significantly better than buspirone 15 mg monotherapy on both the IDS-C30 (\( p = 0.045 \)) and the QIDS-SR16 (\( p = 0.0078 \)).

There was a significant improvement from baseline to week 6 on the mean Ham-A scores in the combination treatment group compared to placebo (\( p = 0.04 \)) and a non-significant trend for buspirone alone (\( p = 0.14 \)). Alternatively, buspirone 15 mg monotherapy revealed no clinical difference on the HAM-A compared to placebo (\( p = 0.62 \)) reflecting a no anxiolytic benefit at that low dose.

### 3.2. Safety and tolerability

30 subjects did not complete the study of who eight never received study drug. Among the non-completers, only eight subjects withdrew from the study due to adverse events, 6% in each of the treatment groups. There were no significant group differences noted amongst the 22 non-completers who received study drug.

The combination treatment was well tolerated; the frequency of treatment-emergent adverse events (TEAEs) was similar between the treatment groups (Table 4). No serious TEAEs were reported. Overall, 52.2% of the subjects in this study experienced some treatment emergent adverse events (TEAEs). 51.5% of subjects in the placebo group, 61.8% in the buspirone group, and 47.8% in the combination treatment group had TEAEs. Of the subjects experiencing TEAEs, 35.8% of these events were considered study drug related (33.3% of subjects in the placebo group, 44.1% in the buspirone group, and 32.8% in the combination treatment group). 15.7% of the TEAEs were assessed as mild in intensity, 16.4% as moderate in intensity, and 3.7% were assessed as severe in intensity.

### 4. Discussion

In this small exploratory study of subjects with acute MDD, low dose buspirone (15 mg) combined with melatonin-SR 3 mg yielded a significant antidepressant effect compared to placebo or buspirone monotherapy during 6-weeks of double-blind treatment. 58.2% of MDD subjects treated with the combination were CGI-I responders in contrast to only 36.4% of placebo subjects and 38.2% of subjects receiving only buspirone 15 mg. Similarly, significantly positive findings were noted for the CGI-S, IDS-C30, and HAM-A. These efficacy results compare favorably with reported results from other acute depression studies with approved antidepressant compounds and the STAR*D study (Trivedi et al., 2006; Rush et al., 2008). In retrospect, it would have been informative to include the Hamilton rating scale for depression (Ham-D) in the psychometrics as well.

The combination of buspirone and melatonin was well tolerated and safe. An analysis of the sleep items from the QIDS-SR16 revealed no significant difference in response between subjects who received the combination or placebo. This finding suggests that a possible melatonin effect on sleep was not the basis for the clinical improvement in the depressed subjects. This was a small double-blind, placebo-controlled proof-of-concept study. Buspirone was included as a third, separate treatment group to confirm the expected lack of antidepressant effect of this low dose. In fact, buspirone 15 mg monotherapy was not differentiated from placebo for depressive or anxiety symptoms in this study. Melatonin was not added as a fourth treatment group.
because it is not known to have antidepressant properties. However, the absence of a melatonin comparator is still a limitation in the interpretation of the results. The potential that 5-HT1a agonists might have antidepressant properties is not a new finding. Previous studies have suggested that doses of buspirone above 40 mg had antidepressant effects in MDD (Robinson et al., 1990; Rickels et al., 1991), and both gepirone and ipsapirone have been studied in MDD (Sramek et al., 1996; Wilcox et al., 1996; Blier et al., 1997; Heiser and Wilcox, 1998; Lapierre et al., 1998; Stahl et al., 1998; Alpert et al., 2004; Amsterdam et al., 2004; Keller et al., 2005; Bielski et al., 2008). Recently, vilazodone, an SSRI and partial 5-HT1a agonist was approved by the FDA (Rickels et al., 2009; Laughren et al., 2011). Regarding melatonin, agomelatine reveals melatonin receptor 1 and 2 partial agonism as well as serotonin 2C receptor antagonism. There have been several preclinical and clinical studies demonstrating the efficacy of agomelatine as a neurogenic agent and as a treatment for MDD (Eser et al., 2007; Kasper and Hamon, 2009; Bourin and Prica, 2009; Soumier et al., 2009). Of course, there are key differences in the receptor pharmacology between agomelatine and the combination of buspirone and melatonin. Buspirone is an agonist on the serotonin 1A receptor, does not affect serotonin 2C, and melatonin has agonistic properties at all known melatonin receptors including MT3 in addition to MT1 and MT2.

---

**Table 3**

<table>
<thead>
<tr>
<th>Responder variable</th>
<th>n</th>
<th>Responders (%)</th>
<th>Buspirone vs. placebo p-value</th>
<th>Combination (B + M)* vs. placebo p-value</th>
<th>(B + M)* vs. buspirone p-value</th>
<th>(B + M)* vs. pooled placebo &amp; buspirone p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGI-I at week 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>33</td>
<td>12 (36.36%)</td>
<td>1.0000</td>
<td>0.0554</td>
<td>0.0627</td>
<td>0.0242</td>
</tr>
<tr>
<td>Buspirone</td>
<td>34</td>
<td>13 (38.24%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combination (B + M)*</td>
<td>67</td>
<td>39 (58.21%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QIDS-SR16 at week 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>33</td>
<td>14 (42.42%)</td>
<td>0.8056</td>
<td>0.5245</td>
<td>0.2927</td>
<td>0.2979</td>
</tr>
<tr>
<td>Buspirone</td>
<td>34</td>
<td>13 (38.24%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combination (B + M)*</td>
<td>67</td>
<td>34 (50.75%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDS-C30 at week 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>32</td>
<td>10 (31.25%)</td>
<td>0.6029</td>
<td>0.1275</td>
<td>0.5053</td>
<td>0.1465</td>
</tr>
<tr>
<td>Buspirone</td>
<td>31</td>
<td>12 (38.71%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combination (B + M)*</td>
<td>60</td>
<td>29 (48.33%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fisher’s Exact Test was used to compare responses between groups for the responder and remission analyses. CGI-I responders had scores of 1 or 2. QIDS-SR16, and IDS-C30, responders had ≥50% improvement from baseline. QIDS-SR16 remission was defined as scores ≤5 and IDS-C30 remission as scores ≤11.

* Combination (B + M) indicates the group that was treated with both buspirone and melatonin.

---

**Table 4**

<table>
<thead>
<tr>
<th>TEAEs [n (%)]</th>
<th>Placebo (n = 33)</th>
<th>Buspirone 15 mg (n = 34)</th>
<th>Combination (B + M)* (n = 67)</th>
<th>Overall (n = 134)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with any TEAE</td>
<td>17 (51.5%)</td>
<td>21 (61.8%)</td>
<td>32 (47.8%)</td>
<td>70 (52.2%)</td>
</tr>
<tr>
<td>Patients with any TEAE related to drug</td>
<td>11 (33.3%)</td>
<td>15 (44.1%)</td>
<td>22 (32.8%)</td>
<td>48 (35.8%)</td>
</tr>
<tr>
<td>Patients with serious TEAEs</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Patients discontinued study due to TEAEs</td>
<td>2 (6.1%)</td>
<td>2 (5.9%)</td>
<td>4 (6.0%)</td>
<td>8 (6.0%)</td>
</tr>
<tr>
<td>Commonly occurring TEAEs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal disorders</td>
<td>7 (21.2%)</td>
<td>8 (23.5%)</td>
<td>14 (20.9%)</td>
<td>29 (21.6%)</td>
</tr>
<tr>
<td>Diarrhea*</td>
<td>2 (6.1%)</td>
<td>1 (2.9%)</td>
<td>6 (9.0%)</td>
<td>9 (6.7%)</td>
</tr>
<tr>
<td>Constipation*</td>
<td>0 (0.0%)</td>
<td>2 (5.9%)</td>
<td>1 (1.5%)</td>
<td>3 (2.2%)</td>
</tr>
<tr>
<td>Nervous system disorders</td>
<td>6 (18.2%)</td>
<td>7 (20.6%)</td>
<td>11 (16.4%)</td>
<td>24 (17.9%)</td>
</tr>
<tr>
<td>Dizziness*</td>
<td>0 (0.0%)</td>
<td>1 (2.9%)</td>
<td>4 (6.0%)</td>
<td>5 (3.7%)</td>
</tr>
<tr>
<td>Headache*</td>
<td>2 (6.1%)</td>
<td>3 (8.8%)</td>
<td>5 (7.5%)</td>
<td>10 (7.5%)</td>
</tr>
<tr>
<td>TEAEs of special interest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry mouth</td>
<td>1 (3.0%)</td>
<td>1 (2.9%)</td>
<td>2 (3.0%)</td>
<td>4 (3.0%)</td>
</tr>
<tr>
<td>Nausea*</td>
<td>2 (6.1%)</td>
<td>1 (2.9%)</td>
<td>4 (6.0%)</td>
<td>7 (5.2%)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>0 (0.0%)</td>
<td>1 (2.9%)</td>
<td>1 (1.5%)</td>
<td>2 (1.5%)</td>
</tr>
<tr>
<td>Middle insomnia</td>
<td>1 (3.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>1 (0.7%)</td>
</tr>
<tr>
<td>Terminal insomnia</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>1 (1.5%)</td>
<td>1 (0.7%)</td>
</tr>
</tbody>
</table>

Note: Related = definitely, probably, or possibly related to study drug and the n indicates the number of subjects in each group.

* Combination (B + M) indicates the group that was treated with both buspirone and melatonin.

b TEAEs occurring in >5% of patients in the combination (B + M) treatment group and higher than placebo.

c TEAEs occurring in >5% of patients in the buspirone group and higher than placebo.
The identification and dose optimization of the combination of buspirone and melatonin was facilitated by a preclinical neurogenesis-based drug discovery platform. The in vitro assays guided the selection and ratio of the combination for in vivo NSF assays. In turn, these pre-clinical studies informed the selection of clinically relevant doses for clinical trials.

The finding that adult humans retain the ability to generate new neurons in the dentate gyrus of the hippocampus throughout life offers the potential for therapeutic intervention (Eriksson et al., 1998). However, the specific role of hippocampal neurogenesis in the etiology or resolution of depression is not clear. The preclinical finding that all classes of marketed antidepressants promote an increase in neurogenesis specific to the dentate gyrus is consistent with the concept that enhancing neurogenesis in humans may be clinically meaningful (Dranovsky and Hen, 2006; Warner-Schmidt and Duman, 2006; Aimone et al., 2010). Further, suppressing neurogenesis blocks some of the effects of antidepressants in the in vivo NSF assay (Santarelli et al., 2003; Li et al., 2008). Hence, there appears to be a strong link between antidepressant efficacy and neurogenesis (Surget et al., 2008; David et al., 2009; Dranovsky et al., 2011). However, some recent studies imply that neurogenesis may not be involved at all (Henn and Vollmayr, 2004; Gass and Vollmayr, 2005; Hanson et al., 2009; Aimone et al., 2011). It is known that adult neurogenesis also decreases with stress in rodents and is enhanced by environmental enrichment or exercise, as well as the introduction of antidepressant medications (Boldrini et al., 2009; Lucassen et al., 2010; Dranovsky et al., 2011; Hanson et al., 2011). Further, animal studies do not always correlate neurogenesis levels with behavioral measurements of affective status (Holick et al., 2008; Miller et al., 2008). Boldrini et al. (2009) has suggested a broader view in that the observed impaired hippocampal plasticity associated with the pathogenesis of MDD is not merely due to impaired neurogenesis but to impaired cell connectivity and functional integration of brain circuitry regulating emotional responses as well.

Clearly, these preliminary clinical findings do not prove that neurogenesis is implicated in the antidepressant efficacy that was achieved in the trial. However, given the above limitations, it is still conceivable that the antidepressant effect of the combination treatment was achieved, in part, through sustained buspirone-induced neuronal proliferation and reversal by melatonin of the buspirone-induced stimulation of non-neuronal lineages (as measured by GFAP).

The precise nature of antidepressant-induced neurogenesis remains difficult to define, and most studies have relied on measures of proliferation assessed by incorporation of 5′-Bromo-2′-deoxyuridine (BrDU) or detection of Ki-67 in cells localized to the dentate gyrus and by confocal analysis for neuronal differentiation. These studies show that the majority (80–90%) of neural stem cells (NSCs) become neurons (Brown et al., 2003; Snyder et al., 2009). Formation of cell types other than neurons in vivo is difficult to assess, but newly developed in vitro techniques using human NPCs allowed us to study the effects of compounds on a variety of cell fates (Svendsen et al., 1998; Dranovsky et al., 2011). Exposure of hNPCs to serotonin increases the number of cells that differentiate into neurons, consistent with a role for serotonin in promoting neurogenesis (Wang et al., 2008). In contrast, buspirone increased not only the number of neuronal-positive cells but also the number of non-neuronal (GFAP positive) cells. Melatonin repressed buspirone-induced, GFAP-positive cell formation without eliminating the ability of buspirone to promote neuronal differentiation. Melatonin had no effect in the assays alone suggesting that melatonin works synergistically specifically with buspirone. Our data does not rule out that there are subtle effects of melatonin on neurogenesis in some settings and in certain experimental paradigms (Ramirez-Rodriguez et al., 2009). The combination of buspirone and melatonin had a robust effect in the NSF assay, whereas neither agent alone was effective. The combination also resulted in an increase in the total number of newborn hNSCs compared to either agent alone. Taken together, the in vitro and in vivo data showed that buspirone in combination with melatonin had a profile different from either agent alone.

This was a small exploratory study and the results must be interpreted with caution. Some reports suggest that high doses of buspirone above 40 mg may have antidepressant properties (Schweizer et al., 1986; Robinson et al., 1990). We found that melatonin-SR 3 mg enhanced the putative antidepressant properties of buspirone so that low dose buspirone (only 15 mg) taken in combination with melatonin yielded significant clinical improvement in MDD. In conjunction with the antidepressant efficacy, we found that the combination treatment had low adverse event liability. These findings have obvious clinical implications for the large number of subjects with MDD who do not respond adequately to currently available antidepressant medications. However, additional, larger studies evaluating combination buspirone and melatonin in MDD are needed to confirm these preliminary findings.

Contributors

Each listed author contributed to this paper.

Maurizio Fava, Steven D. Targum, Andrew A. Nierenberg, and Pamela C. Wedel contributed equally to the design, analysis, and execution of the clinical trial. Carrolee Barlow, Leo S. Bleicher, Todd A. Carter, René Hen, and Fred H. Gage contributed to the pre-clinical discovery platform, pre-clinical assays, study design and data analysis.

Role of funding source

BrainCells Inc. sponsored this investigator initiated trial conducted under the auspices of the Clinical trial Network (CTNI) at the Massachusetts General Hospital.

BrainCells Inc. supplied the study drug and matching placebo capsules and provided statistical analysis support.

Conflict of interest

Maurizio Fava, MD — Disclosures.

Research Support: Abbott Laboratories; Alkermes, Inc.; Aspect Medical Systems; AstraZeneca; BioResearch; BrainCells Inc.; Bristol-Myers Squibb; CeNeRx BioPharma; Cephalon; Clinical Trials Solutions, LLC; Clintara, LLC; Covance; Covidien; Eli Lilly and Company; EnVivo Pharmaceuticals, Inc.; Euthymics Bioscience, Inc.; Forest Pharmaceuticals, Inc.; Ganeden Biotech, Inc.; GlaxoSmithKline; Icon Clinical Research; i3 Innovus/Ingenix; Johnson & Johnson Pharmaceutical Research & Development; Lichtwer Pharma GmbH; Lorex Pharmaceuticals; National Alliance for Research on Schizophrenia & Depression (NARSAD); National Center for Complementary and Alternative Medicine (NCCAM); National Institute of Drug Abuse (NIDA); National Institute of Mental Health (NIMH); Novartis AG; Organon Pharmaceuticals; PamLab, LLC.; Pfizer Inc.; Pharmavite LLC; Photothera; Roche Pharmaceuticals; RCT Logic, LLC; Sanofi-Aventis US LLC; Shire; Solvay Pharmaceuticals, Inc.; Synthelabo; Wyeth-Ayerst Laboratories.

Advisory/Consulting: Abbott Laboratories; Alkermes, Inc.; Amarin Pharma Inc.; Aspect Medical Systems; AstraZeneca; Auspex Pharmaceuticals; Bayer AG; BioMarin Pharmaceuticals, Inc.; Biovail Corporation; BrainCells Inc; Bristol-Myers Squibb; CeNeRx BioPharma; Cephalon, Inc.; Clinical Trials Solutions, LLC; CNS...
Response, Inc.; Compellis Pharmaceuticals; Cypress Pharmaceuticals, Inc.; DiagnoSearch Life Sciences (P) Ltd.; Dov Pharmaceuticals, Inc.; Edgemont Pharmaceuticals, Inc.; Eisai Inc.; Eli Lilly and Company; EnVivo Pharmaceuticals, Inc.; ePharmaSolutions; EPIX Pharmaceuticals, Inc.; Euthymics Bioscience, Inc.; Fabre-Kramer Pharmaceuticals, Inc.; Forest Pharmaceuticals, Inc.; GenOmind, LLC; GlaxoSmithKline; Grunenthal GmbH; i3 Innovis/Ingenis; Janssen Pharmaceutical; Jazz Pharmaceuticals, Inc.; Johnson & Johnson Pharmaceutical Research & Development, LLC; Knoll Pharmaceutical Corp.; Labopharm Inc.; Lorex Pharmaceuticals; Lundbeck Inc.; MedAvante, Inc.; Merck & Co., Inc.; MSI Methylation Sciences, Inc.; Naurex, Inc.; Neuronetics, Inc.; NextWave Pharmaceuticals; Novartis AG; Nutrition 21; Organon Pharmaceuticals; Otsuka Pharmaceuticals; PamLab, LLC.; Pfizer Inc.; PharmaStar; Pharmavite LLC.; PharmoRx Therapeutics; Precision Human Biology Laboratory; Prexa Pharmaceuticals, Inc.; Puretech Ventures; PsychoGenics; Psylin Neurosciences, Inc.; Rexahn Pharmaceuticals, Inc.; Ridge Diagnostics, Inc.; Roche; RCT Logic, LLC; Sanofi-Aventis US LLC.; Sepracor Inc.; Servier Laboratories; Solvay Pharmaceuticals, Inc.; Somaxon Pharmaceuticals, Inc.; Somerset Pharmaceuticals, Inc.; Sunovion Pharmaceuticals; Supernus Pharmaceuticals, Inc.; Synthelabo; Takeda Pharmaceutical Company Limited; Tal Medical, Inc.; Tetragenex Pharmaceuticals, Inc.; TransForm Pharmaceuticals, Inc.; Transcept Pharmaceuticals, Inc.; Vanda Pharmaceuticals, Inc.;

**Speaking/Publishing:** Adamed, Co.; Advanced Meeting Partners; American Psychiatric Association; American Society of Clinical Psychopharmacology; AstraZeneca; Belvoir Media Group; Boehringer Ingelheim GmbH; Bristol-Myers Squibb; Cephalon, Inc.; CME Institute/Physicians Postgraduate Press, Inc.; Eli Lilly and Company; Forest Pharmaceuticals, Inc.; GlaxoSmithKline; Imomed, LLC.; MGH Psychiatry Academy/Primedia; MGH Psychiatry Academy/Reed Elsevier; Novartis AG; Organon Pharmaceuticals; Pfizer Inc.; PharmaStar; United BioSource, Corp.; Wyeth-Ayerst Laboratories.

**Equity Holdings:** Compellis.

**Royalty/patent, other income:** Patent for Sequential Parallel Comparison Design (SPCD) and patent application for a combination of azapirones and bupropion in Major Depressive Disorder (MDD), copyright royalties for the MGH Cognitive & Physical Functioning Questionnaire (CPFQ), Sexual Functioning Inventory (SFI), Antidepressant Treatment Response Questionnaire (ATRQ), Discontinuation-Emergent Signs & Symptoms (DESS), and SAFER. Patent for research and licensing of SPCD with RCT Logic; Lippincott, Williams & Wilkins; Wolkers Kluwer; World Scientific Publishing Co. Pte.Ltd.

**Steven D. Targum, M.D. Disclosures.**

**Current Professional Affiliations.**

Scientific Director, Clintara LLC, Consultant, Massachusetts General Hospital, Psychiatry (Boston, MA); Chief Medical Advisor, Prana Biotechnology Ltd (Melbourne, Aus); Acting Chief Medical officer, BranCells Inc. (San Diego, California); Chief Medical Officer, Methylation Sciences Inc. (Vancouver, BC, Canada); Chief Medical Officer, Functional Neuromodulation Inc. (Toronto, Canada).

**Equity interests**

Clintara LLC, Methylation Sciences Inc., Prana Biotechnology Ltd.

**Consultation/CDAs within the past three years.**


**Andrew A. Nierenberg, MD Financial Disclosures**

Dr. Nierenberg is a full time employee of the Massachusetts General Hospital (MGH) and has disclosed all external sources of revenue to Harvard Medical School and Partners Health care to the best of his knowledge and in accordance with current regulations. External activities were limited to no more than 8 h per week. In the past 36 months (as of July 6, 2010) he has served as a consultant to: Appliance Computing Inc. (Mindsite), Brain Cells, Inc., Brandeis University, Bristol Myers Squibb, Clintara, Dianippon Sumitomo (Now Sunovion), Eli Lilly and Company, EpiQ, Novartis, PamLabs, PGx Health, Shire, Schering-Plough, Takeda Pharmaceuticals, and Targacept. He has consulted through the MGH Clinical Trials Network and Institute (CTNI): Astra Zeneca, Brain Cells, Inc., Dianippon Sumitomo/Sepracor, Johnson and Johnson, Labopharm, Merck, Methylation Science, Novartis, PGx Health, Shire, Schering-Plough, Targacept, and Takeda/Lundbeck Pharmaceuticals. He has received grant/research support through MGH from AHRQ, Cephalon, NIMH, PamLabs, Pfizer Pharmaceuticals, and Shire. Dr. Nierenberg received honoraria or travel expenses including CME activities from: APSARD, Belvoir Publishing, University of Texas Southwestern Dallas, Hillside Hospital, American Drug Utilization Review, American Society for Clinical Psychopharmacology, Bayamon Region Psychiatric Society, San Juan, PR, Baystate Medical Center, Canadian Psychiatric Association, Columbia University, Douglas Hospital/McGill University, IMEDEX, International Society for Bipolar Disorders, Israel Society for Biological Psychiatry, John Hopkins University, MJ Consulting, New York State, Massachusetts Association of College Counselors, Medscape, MBL Publishing, Physicians Postgraduate Press, Slack Publishing, SUNY Buffalo, University of Florida, University of Miami, University of Wisconsin, University of Pisa, and SciMed. Dr. Nierenberg is a presenter for the Massachusetts General Hospital Psychiatry Academy (MGHPA). The education programs conducted by the MGHPA were supported through Independent Medical Education (IME) grants from the following pharmaceutical companies in 2008: Astra Zeneca, Eli Lilly, and Janssen Pharmaceuticals; in 2009 Astra Zeneca, Eli Lilly, and Bristol-Myers Squibb. No speaker bureaus or boards since 2003. Dr. Nierenberg owns stock options in Appliance Computing, Inc. and Brain Cells, Inc. Additional income is possible from Infomedic.com depending on overall revenues of the company but no revenue has been received to date. Through MGH, Dr. Nierenberg is named for copyrights to: the Clinical Positive Affect Scale and the MGH Structured Clinical Interview for the Montgomery Asberg Depression Scale exclusively licensed to the MGH Clinical Trials Network and Institute (CTNI).

**Pamela Wedel**

Employee of BrainCells Inc.

**Fred H Gage Disclosure**

Equity interest in BrainCells Inc., Advisory Board for BrainCells Inc., StemCells Inc., Ceregene.

**Rene Hen** disclosures

Advisory Board for BrainCells Inc.

**Leo Bleicher**

Former employee of BrainCells Inc.

**Carrcole Barlow**

Former employee of BrainCells Inc.

**Acknowledgements**

We would like to thank Eric R. Kandel, Mark H. Rapaport, Alan F. Schatzberg, Scott A. Small, Clive N. Svendsen, Alejandro R. Dearie 1.

1 These individuals were employees of BrainCells Inc at the time of this research.
Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jpsycho.2012.08.013

References


