Biomarker Profiling of NSI-189 Phosphate, a Neurogenic Compound, in Patients with Major Depressive Disorder (MDD) during a Phase Ib Randomized Double-Blind, Placebo-Controlled Trial

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Background

NSI-189 Phosphate, (4-benzylpiperazin-1-yl)-[2-(3-methyl-butylamino)pyridin-3-yl] methanone is a novel molecule developed by Neuralstem, Inc. for the treatment of MDD, based upon preclinical data demonstrating stimulation of neurogenesis of human hippocampus-derived neural stem cells *in vitro* and in mouse hippocampus *in vivo*.

In a Phase 1b, double-blind, placebo-controlled, multiple-ascending-dose study, patients with symptomatic MDD were randomized to receive NSI-189 40, 80, or 120 mg daily or placebo for 28 days.

<u>Aim:</u> Few biomarkers of antidepressant treatment response are in current use as an adjunct to clinical response measurements made using subjective clinical assessment tools. Our aim was to explore biomarkers in the MDDScore™ test panel for the ability to monitor efficacy during a trial of NSI-189.

Study Design

This was a Phase Ib, double-blind, randomized, placebo-controlled, multiple-dose study with 3 ascending cohorts. Twenty-four patients with MDD were recruited, with their diagnosis and illness severity confirmed through an independent, remote SAFER interview from the MGH CTNI raters.

Each cohort included at least 3 female subjects. Each patient underwent a screening for eligibility (Day -37 to Day -6 or -3), and eligible patients were admitted into the unit on Day -5 to complete antidepressant washout and undergo baseline assessments. Patients were randomized (3:1) to receive NSI-189 phosphate or placebo for 28 days. Cohort 1 received NSI-189 (or placebo) 40 mg QD; Cohort 2, NSI-189 (or placebo) 40 mg BID; Cohort 3, NSI-189 (or placebo) 40 mg TID.

During the 28-day, multiple-dose period, patients underwent traditional safety and pharmacokinetic assessments.

Assessments

During dosing and follow up, patients underwent traditional safety and pharmacokinetic assessments. Assessments included the CPFQ, CGI-S, C-SSRS, MADRS, and the SDQ. Plasma samples were tested by immunoassay for plasma levels of 10 biomarkers (A1AT, ApoC3, BDNF, Cortisol, EGF, MPO, Prolactin, Resistin, sTNFR2 and TSH) at 2 week intervals.

In our regression analysis, we used a reduction of greater or equal to 15.9 units in MADRS scores at Day 28 (end of treatment) as indicative of rapid clinical response to NSI-189 (Table 2).

Patient Population

Ethnic origin	Placebo	40 mg QD	40 mg BID	40 mg TID	*p value*
Caucasian	4	3	2	2	
African American	0	2	3	2	0.604†
Hispanic	2	1	1	1	
Asian	0	0	0	1	
Gender					
Male	5	2	3	2	0.262†
Female	1	4	3	4	
Age (yrs) mean	28.2	34	38.5	40.5	0.098‡
Standard Deviation	±4.75	±3.5	±12.94	±9.92	
MADRS, mean	25.2	26	26.83	24.83	0.636‡
Standard Deviation	± 2.93	±3.35	±2.64	±2.56	

†Statistical method used, X^2

‡Statistical method used, 1-way analysis of variance (ANOVA)

Methods

Each subject had a blood sample drawn at the indicated time points ranging from -5 to 84 days. Plasma was stored frozen at ≤-70°C until shipment to Ridge for measurement of ten plasma biomarkers (Alpha-1 Antitrypsin, Apolipoprotein C3, Brain Derived Neurotrophic Factor, Cortisol, Epidermal Growth Factor, Myeloperoxidase, Prolactin, Resistin, Soluble TNF Receptor II and Thyroid Stimulating Hormone) by immunoassay.

Results

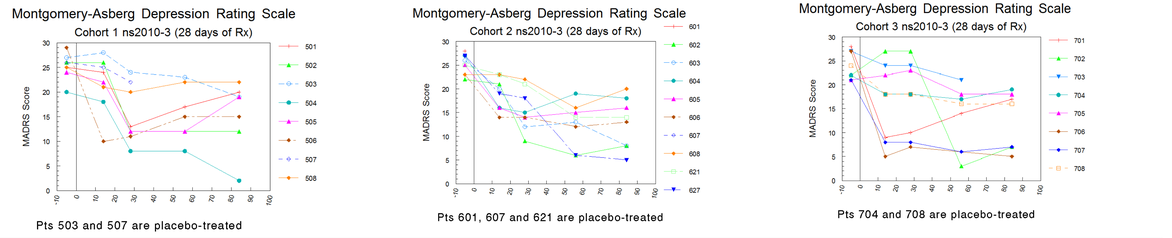

Figure 1. Montgomery-Åsberg Depression Scale assessments of individual patients in the three treatment cohorts. Note: Rapid and continued response post-treatment in some patients

Analyte	Placebo		40 mg QD		40 mg BID		40 mg TID	
	Mean	StDev	Mean	StDev	Mean	StDev	Mean	StDev
CORT	15.78	3.75	13.62	3.76	17.33	8.06	18.25	5.60
BDNF	3581.33	2084.57	3274.67	3123.11	2400.00	2521.10	5540.83	3770.76
EGF	62.00	31.34	104.17	62.82	63.50	71.81	78.00	39.36
MPO	89.00	48.78	69.00	26.80	59.00	10.90	72.83	22.23
PRL	13.95	12.14	8.57	7.04	9.15	3.92	12.35	6.46
RETN	5.18	0.91	5.30	1.57	6.78	6.22	9.28	3.94
A1AT	108.67	13.89	118.33	20.24	122.17	19.21	135.00	19.33
sTNFR2	2231.33	349.47	2130.50	299.88	2080.83	415.84	2520.67	1307.05
APOC3	11.48	6.08	12.75	7.06	11.45	5.29	14.23	3.26

Table 1. Baseline plasma biomarker measurements by cohort using Ridge LDT. No significant difference was observed between placebo and treated groups ($\alpha = 0.05$).

A Five Biomarker* Model Identifies Rapid Responders at Baseline

MADRS Group	Classification PR	RR	Total
Partial Responder (PR)	4	0	4
	100%	0%	
Rapid Responder (RR)	1	14	15
	7%	93%	

Table 2. Number of NSI-189 treated patients and percent classified into MADRS PR and RR groups

*BDNF, EGF, MPO, sTNFR2 and A1AT were used in the regression model

Linear Regression Model for Rapid Responders

BDNF, EGF, MPO sTNFR2 and A1AT are Key Analytes

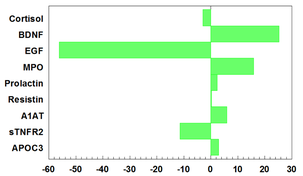

Figure 2. Standard coefficients for each marker from our linear regression model

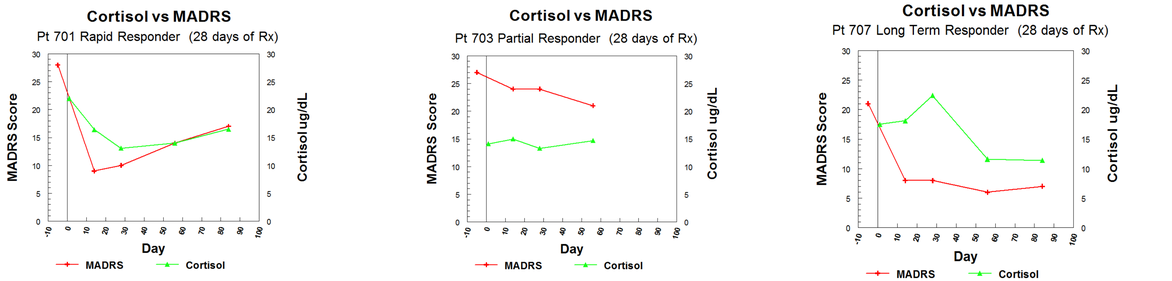

Figure 3. Time course of changes in MADRS scores and plasma cortisol levels in some representative patients in cohort 3. Classification into Rapid, Partial and Long-term Responder is based upon the time course of the fall in MADRS score during and/or post treatment. The timing of sample collection, impacts cortisol levels (diurnal variation).

Summary and Conclusions

A significant number of NSI-189 treated patients demonstrated clinical improvement by a reduction in total MADRS scores ≥15.9 points which was sustained beyond the active dosing period. At 8 weeks, this sustained drop was to a point at or near what is usually associated with SSRI remission (Thase et al. The British Journal of Psychiatry 199: 501-507. 2011). Analysis of the data on the plasma level of each individual biomarker at baseline (Day 1; Table 1) indicated that there were no significant differences between patients treated with NSI-189 or placebo prior to treatment. We developed a linear regression-based model utilizing baseline plasma levels of BDNF, EGF, MPO, sTNFR2 and A1AT which was able to identify a rapid response to NSI-189, consistent with therapeutic effects shown by the traditional clinical measures.

<u>Conclusions</u>: NSI-189 is rapidly and persistently efficacious. Preliminary analysis of this small sample set suggests that prediction of response to NSI-189 may be possible from baseline biomarker profiling.

***<u>Disclosures:</u>* Drs. Bilello and Thurmond are employees and stockholders of Ridge Diagnostics, Inc. Ms. Feng was a statistician employed by Ridge. Dr. Lev Gertsik was the PI of the study at CCT and is Medical Director at PAREXEL, Los Angeles. Drs. English and Ereshefsky are Senior Director and VP, Early Phase CNS, respectively at PAREXEL. Drs. Fava, Hoeppner, Mischoulon, Kinrys, and Freeman are associated with Harvard, MGH and the CTNI. Ms Flynn is Director of Clinical Trial Operations, MGH CTNI. Dr. Johe co-founded Neuralstem, Inc. and is its Chief Scientific Officer.**