A multicenter ascending dose, double blind, placebo-controlled study of NAP (AL-108) in chronic schizophrenia

V. 1.0
6/01/2007

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Background

AL-108 is an intranasal drug product containing NAP, an 8 amino-acid peptide (Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln; NAPVSIPQ, MW=824.9) fragment of the much larger (approx. 124KD) Activity-Dependent Neuroprotective Protein (ADNP), which participates in neurodevelopment and neuroprotection. In mice, ADNP knockouts are lethal exhibiting CNS dysgenesis. ADNP mediates its effects in part through interaction with microtubules. Because of its large size, ADNP is assumed to not penetrate the BBB and thus cannot be used pharmacologically. NAP was chosen because it represents the epitope most associated with microtubule interaction and neuroprotection. NAP is absorbed following IV or intranasal administration, and has been shown to cross the BBB.

Rationale for NAP treatment: tubulin function in brain function

The cytoskeleton plays a key role in maintaining the highly asymmetrical shape and structural polarity of neurons that are essential for neuronal physiology. The cytoskeleton is made up of microfilaments, intermediate filaments and microtubules. Microfilaments (4-9 nm diameter) are made up of actin monomers and they function mainly to provide mechanical support and locomotion to the cell. Intermediate filaments are cytoplasmic fibers of ~10nm diameter. They provide supporting framework within the cell. Microtubules (~24nm diameter) consist of tubulin and microtubule associate proteins. They function to transport nutrients and chemical messengers along the cell. Neurofibrillar tangles are twisted bundles of neurofibrils formed when the microtubule-associated protein, tau, dissociates from microtubules and clusters to form an insoluble mass. Under normal conditions tau binds to microtubules, stabilizing neuronal structure and integrity.

Hyperphosphorylation of tau is assumed to be the cause for the formation of neurofibrillary tangles. Although neurofibrillary tangles are most associated with cognitive dysfunction in Alzheimers disease, some increase in neurofibrillary pathology has also been reported in schizophrenia, potentially as consequence of antipsychotic medication (1). Thus, mechanisms underlying microtubular function may be relevant to schizophrenia as well. In association with tubulin polymerization into microtubules, NAP influences tau dynamics by increasing the ratio of non-phosphorylated tau to phosphorylated tau, implying a dynamic process of cellular maintenance of the microtubular network, which is essential for the survival of the cell.

In brain, tubulin frameworks are stabilized by recently described STOP proteins (2) (aka MAP6). Linkages to allelic variation in STOP genes has been reported in schizophrenia, along with altered STOP protein expression in some brain regions (3). STOP knockdown mice show disturbances in dopaminergic neurotransmission (4) along with deficits in PPI and hypermotility that were partially reversed with clozapine (5). Thus, neuropathological features of schizophrenia may be due, in part, to abnormal STOP-related stabilization of microtubular structure, and NAP may stabilize STOP-related abnormal neurophysiological processes in schizophrenia.
**In vitro effects**

The primary in vitro support for use of NAP is effectiveness in stimulating neurite outgrowth based upon interaction with tubulin. Concentrations of NAP most associated with this effect are in the range of 10 fM (6). In schizophrenia, several studies have reported decreased spine density along with decreased expression of neurite-related proteins (7-9). Further, abnormal neuronal shape, loss of dendrites and spines, and irregular distribution of neuronal elongations occur in specific brain areas of schizophrenic patients have all been reported (10, 11). To the extent that these abnormalities reflect tubulin-related pathology and to the extent that such deficits underlie cognitive dysfunction in schizophrenia, the present treatment approach may be effective. In tissue cultures, NAP stimulates out-growth of neurites in hippocampal and cortical cells (6). This might be relevant to schizophrenia, as many studies have indicated that cellular size is small in these brain areas in schizophrenia.

**Animal studies**

The majority of animal studies using NAP have been conducted using chronic administration in neurodegeneration. However, some studies support a relatively acute effect of NAP on cognition as well. Thus, improvements in escape latency are observed in rats treated with NAP even in non-degenerative conditions (12). The same dose of NAP ameliorated the effects of the cholinotoxin AF64A, suggesting potential effectiveness in animal models of cholinergic toxicity.

In rodents, NAP improved short-term memory assessed using the Morris water maze (13), and reduced anxiety as measured by increased percentage of time spent in the open arms of the elevated plus maze (14). It is argued that anti-anxiety effects of NAP may be relevant to cognitive dysfunction in schizophrenia as many patients with schizophrenia suffer from anxiety (15). If NAP improves cognition and decreases anxiety in schizophrenia, this would be highly desirable.

The triple transgenic mouse model expressing mutant APP (Swedish), tau (P301L), and presenilin-1 (M146V) develops both neurofibrillary tangles and amyloid beta plaques in a progressive fashion (16). Treatment of 12-month-old animals with an intranasal dose of 2 µg/day (~0.07 mg/kg/day) for 3 months resulted in a 70% decrease in phosphorylated tau at Ser202/Thr205, Thr231, and Ser202 residues (17). Histological examination of the hippocampal CA1 region confirmed AL-108 treatment resulted in a reduction of phosphorylated tau. Treatment of 9-month-old animals with an intranasal dose of 0.5 µg/day (~0.017 mg/kg/day) for 3 months resulted in a 20% decrease in both amyloid beta 1-40 and 1-42 levels as well as a 30% to 40% decrease in phosphorylated tau. Phosphorylation of tau results in destabilization of microtubules and therefore a loss of neuronal function. In a separate study, chronic nasal administration of AL-108 over several months resulted in enhancement in the Morris spatial navigation test in a mouse model of tauopathy (tau over-expression) (18). Therefore, the reduction of tau phosphorylation by AL-108 treatment may promote an improvement of cognitive performance and prevention of neurofibrillary tangles.

VIP (Vasoactive-Intestinal Peptide) mediates expression of the NAP-containing protein, ADNP, and rats exposed to VIP had improvements in their sexual behavior (19). As
sexual behavior is impaired in schizophrenia (20), improvements in sexual behavior might improve patients' quality of life.

Finally, NAP is involved in the inflammatory process, and recent studies have linked schizophrenia to autoimmune diseases (21, 22).

**Human studies:**

The safety, tolerability, and pharmacokinetics of AL-108 have been evaluated in two Phase 1 studies in a total of 62 subjects. Both healthy adult (18 to 45 years of age) and healthy elderly subjects (50 to 85 years of age) have received AL-108 in doses up to 15 mg once daily (QD) and BID.

In the initial study of 30 adults (18 to 45 years of age), subjects were randomized to receive one of five doses; a single subject in each dosing cohort received placebo. The dose groups were 1, 3, 10, 12.5, and 15 mg of AL-108 or placebo administered intranasally. The mean age was 31 years (range 19 to 44 years) and all subjects completed the study.

Treatment administration appeared to be safe and was well tolerated. Approximately half of all subjects (47%) reported a total of 32 adverse events (AEs). Of those subjects who reported AEs, 70% were in the AL-108 treatment group and 30% were in the placebo group. A higher percentage of subjects in the placebo group (4/5, 80%) reported an AE compared with the AL-108 group (10/25, 40%). Most AEs (60%) occurred within 7 days of treatment administration; all resolved by the end of the study.

No serious adverse events (SAEs) were reported; all events were mild in severity with the exception of one episode of dizziness (moderate), which was experienced by a subject in the placebo group during a blood draw. Nine AEs were considered by the Investigator to have a suspected relationship to study drug; of these, 6 were headaches.

The most frequently reported AE was headache. Nine occurrences of headache were reported by 5/25 (20%) subjects in the AL-108 group and 3/5 (60%) subjects in the placebo group. All of the headaches reported by subjects in the placebo group and half of headaches reported by subjects in the AL-108 group occurred on the day of study drug administration.

The body systems with the most reports of AEs were the nervous system (11 events) and the respiratory system (8 events). The most frequently reported event in the CNS body system was headache (9/11 events). Respiratory system events reported included cough, nasal congestion, pharyngolaryngeal pain, rhinorrhea, sinus congestion, sinus pain, and sneezing. All of these events were reported at Day 10 or later post-dose; none were considered associated with AL-108 or placebo administration.

None of the post-dose chemistry, hematology, or urinalysis laboratory results were considered clinically significant by the Principal Investigator with the exception of one value for total bilirubin for a subject in the AL-108 treatment group. The value was elevated (2 mg) at the 24-hour blood draw and returned to normal by Day 21.

There were no clinically significant changes in vital signs or ECGs measured in any subject during the observation period.
Plasma levels of AL-108 were below the limit of quantification (<0.75 ng/mL) for all intranasal doses up to 12.5 mg. Of the subjects receiving 15 mg of AL-108 intranasally, 3/5 subjects had detectable plasma levels, with a maximum plasma concentration ($C_{\text{max}}$) of 1.24 to 1.57 ng/mL and time to peak plasma concentration ($T_{\text{max}}$) of 10 to 40 minutes. The area under the plasma concentration time curve (last) ($\text{AUC}_{\text{last}}$) varied from 17.7 to 153.2 ng/mL*min.

In the phase 1b study of healthy elderly adults (55 to 85 years of age), 32 subjects were enrolled in one of four dose groups: 10 mg QD, 15 mg QD, 10 mg BID, and 15 mg BID for a total of 7 days. Subjects were randomized to treatment with either the study drug or placebo in a 6:2 ratio. All subjects completed the study per the protocol.

This study was ongoing at the time of preparation of this protocol and thus has not been unblinded. All subjects have been treated and follow-up is completed. Based on data available for all treatment groups, 15 of the 32 subjects (47%) reported 42 AEs. Subjects in the 10 mg QD dose group reported the most AEs (22 AEs reported by 5/8 subjects). Subjects in the 15 mg QD dose group reported the fewest AEs (6 AEs reported by 3/8 subjects). No SAEs were reported. Of the 43 reported events, 18 had a suspected relationship to study treatment. At the time of the protocol preparation, treatment association had not been determined for 7 events. All events with the exception of 1 event of heartburn were rated as mild in severity.

The body system with the most reports of AEs was the body-as-a-whole system. Nine subjects reported 13 events; reported events included generalized body aches, leg pains, leg cramps, and neck and shoulder pain. Four subjects in the 10 mg QD dose group reported 6 events; 3 subjects in the 15 mg QD dose group reported 3 events; and 1 subject in each of the BID dose groups each reported 2 events. Of the 13 events, 8 were suspected to be related to study treatment; 3 were not suspected. At the time of the protocol preparation, treatment association had not been determined by the Investigator for 2 events.

Ten events occurring in the gastrointestinal body system were reported by 6 subjects and included nausea (3 events) and heartburn (2 events). Two events of nausea were suspected of being related to study treatment by the Investigator; 2 had not been evaluated. These events were reported by subjects in all dose groups.

Three subjects reported 7 events related to the upper respiratory tract, such as runny nose, cough, sinus congestion, and sore throat. These events were reported on Day 4 by 2 subjects, 1 in the 15 mg QD dose group and 1 in the 10 mg BID dose group. Events reported as related to the CNS body system were headache, dizziness, and drowsiness. Four subjects, one in each dose group, reported headaches: 3 subjects reported drowsiness and 2 subjects reported dizziness. All events were mild in intensity and 6 of the 9 events were suspected of being related to treatment.

All events with the exception of the 3 in the same subject were reported during the 7 days of treatment. Runny nose, watery eyes, and a sinus infection occurring on Day 20 were reported by 1 subject in the 10 mg QD dose group.

No clinically significant laboratory values or clinical laboratory AEs have been reported to date during this study.
In both phase 1 studies of AL-108, all doses of study drug administered have been safe and well tolerated.

Dose considerations

In general, NAP is effective at extremely low concentrations. Thus, NAP induces rapid microtubular reorganization at concentration of $10^{-15}$ to $10^{-10}$ M (23). In tissue culture, optimal effects of NAP on neurite outgrowth have been observed with doses in the range of 100 aM to 1 pM ($10^{-16}$ to $10^{-12}$ M).

Effective doses in rodents for i.n. administration are reported to be in range of 2-300 ug/kg (~5-75 ug/rat). Following intranasal administration of radioactive NAP in rodents, peak concentrations were obtained 60 min after administration. Behavioral effects were associated with brain concentrations in the range of 45 fmol/g (~45 pM). Levels of intact NAP fall to 12% of total radioactivity within 30 min, and 2% of total radioactivity within 60 min. Thus, persistent activation may not be required for beneficial effect. Following i.v. administration in rodents, a more linear relationship between CSF and plasma levels were obtained, with plasma concentrations of ~150 ng/mL (~180 nM) corresponding to CSF concentrations of ~30 ng/mL (~36 nM). CSF concentrations were approx. 0.14 X plasma concentrations.

The 5 mg QD AL-108 dose was selected on the basis of preclinical pharmacology. In the triple transgenic mouse model, an intranasal dose of 2 µg/day (~0.07 mg/kg) for 3 months was found to significantly reduce the pathological hallmarks of this model (Matsuoka et al. 2006). This preclinical dose of 0.07 mg/kg would correspond to ~5 mg/day for a 70 kg human, the lower dose proposed in this study.

The 15 mg BID AL-108 dose to be studied was selected based on clinical tolerability as established in Phase 1 studies. In a Phase 1a study of both healthy adult (18 to 45 yrs) subjects, single doses of AL-108 up to 15 mg were safe and well tolerated. In a multiple ascending dose Phase 1b study, dosing of healthy elderly (50 to 85 yrs) subjects, AL-108 at 10 mg/day (10 mg QD), 15 mg/day, 20 mg/day (10 mg BID) or 30 mg/day (15 mg BID) for 7 days was well tolerated. Preliminary pharmacokinetic (PK) data from the phase 1b study (AL-108-102) also supports the 15 mg dose selection. This dose resulted in a plasma $C_{\text{max}}$ of ~1.3 ng/mL, an AUC of ~50 ng/mL*min, and a plasma half-life of ~30 minutes. A plasma $C_{\text{max}}$ of 1.3 ng/mL is approximately 1.6 nM. In nonclinical rat PK studies, cerebrospinal fluid (CSF) levels of AL-108 were approximately 15% to 20% of plasma levels, suggesting that a concentration of ~0.3 nM might be expected in human CSF (the pharmacodynamic compartment) after a 15 mg dose, which represents a concentration several orders of magnitude greater than that needed for in vitro neuroprotection.

Phase of development

AL-108 is administered in the US under IND 77,081 to Allon Therapeutics Inc. The nonclinical safety and toxicity profile of AL-108 has been characterized in rat and dog by a series of safety pharmacology studies, and by single- and repeat-dose toxicity studies. AL-108 was administered by both the nasal and intravenous (IV) routes.
AL-108 had minimal effect on vital organ function. Overall effects on the CNS were limited to piloerection in mice at intraperitoneal doses of 10, 100, 1000, and 2000 mg/kg in male mice and 2000 mg/kg in female mice, and lethargy in male mice at 2000 mg/kg. No adverse behavioral effects were seen in rats following IV administration of doses up to 100 mg/kg.

Cardiovascular and pulmonary safety pharmacology was evaluated in vivo and effects of AL-108 indicated a low risk. In vivo, IV doses up to 100 mg/kg had no effect on cardiovascular parameters including electrocardiograms (ECG), and mean systolic and diastolic blood pressure and heart rate in beagle dogs. In the respiratory system evaluation, there were no clinical signs or effects on ventilatory parameters in dogs treated with IV doses up to 100 mg/kg.

Single escalating IV doses of AL-108 were well tolerated by male dogs up to 500 mg/kg, and a single intranasal administration of 500 mg (~50 mg/kg) was well tolerated. Repeat intranasal administration of AL-108 for 28-, 30- and 90-days in rat was well tolerated and there were no toxicological findings at doses up to 40 mg/day (20 mg twice daily [BID]) for 28 days and 1 mg/day for 90 days. In dog, daily intranasal administration of a minimum dose of 12.5 mg/kg/day for 90 consecutive days was found to be the no observed effect level (NOEL).

Repeat IV administration of AL-108 for 14-days in rat and dog was well tolerated and there were no toxicological findings at doses up to 300 mg/kg/day in rat and 150 mg/kg/day in dog. Clinical signs included increased vocalization in rats at 100 and 300 mg/kg/day and a dose-dependent increase in licking, vocalization, and salivation in dog.

**Protocol**

The study consists of a 12-week multicenter, double blind randomized clinical trial of two doses of AL-108 (5 and 30 mg/day i.n) vs. placebo in the treatment of persistent cognitive dysfunction in schizophrenia. The primary outcome measure will consist of the composite score of the MATRICS neuropsychological battery. Secondary outcome measures will include scores on symptom, functional outcome, and safety assessments.

**Inclusion Criteria:**

1. DSM-IV/DSM-IV-TR diagnosis of schizophrenia
2. Capable of providing informed consent
3. Males and Females
4. Age: 18 - 55 years
5. Caucasian or Non-Caucasian
6. Subjects will be treated with one of the following second generation antipsychotics: risperidone, olanzapine, quetiapine, ziprasidone, or aripiprazole or paliperidone for the previous two months, with no change in dose in the last month, and/or with injectable depot antipsychotics (fluphenazine or haloperidol decanoate) with no change in last 3 months.
7. Subjects will meet the following symptom criteria:
   a. Brief Psychiatric Rating Scale (BPRS) Hallucinatory Behavior or Unusual Thought Content item scores ≤ 4
b. BPRS Conceptual Disorganization item score ≤ 4

c. All Scale for the Assessment of Negative Symptoms (SANS) global items ≤ 3

d. Simpson-Angus Scale total score ≤ 6

e. Calgary Depression Scale total score ≤ 10

8. Subjects will meet the following cognitive performance criteria:
   a. Performance less than the maximum cutoff (in parentheses) for ONE of the following MCCB tests: i.) Letter-number span (20); ii.) HVLT total (31); and iii.) CPT d-prime (3.47)
   b. Able to complete the baseline MCCB validly as assessed by Chief Neuropsychologist or NP tester
   c. Raw score of 6 or greater on the WTAR

Exclusion Criteria:

1. Current treatment with oral conventional antipsychotics (e.g. fluphenazine, haloperidol) or clozapine.
2. Subjects with a DSM-IV diagnosis of alcohol or substance abuse (other than nicotine) within the last month or a DSM-IV diagnosis of alcohol or substance dependence (other than nicotine) within the last 6 months
3. Subjects with a history of significant head injury/trauma, as defined by one or more of the following:
   a. Loss of consciousness (LOC) for more than 1 hour
   b. Recurring seizures resulting from the head injury
   c. Clear cognitive sequelae of the injury
   d. Cognitive rehabilitation following the injury
4. Subjects with a clinically significant neurological, metabolic, hepatic, hematological, pulmonary, cardiovascular, gastrointestinal, and/or urological disorder (e.g. unstable angina, decompensated congestive heart failure, CNS infection or history of HIV seropositivity), which would pose a risk to the patient if they were to participate in the study or that might confound the results of the study. Active medical conditions that are minor or well controlled are not exclusionary if they do not affect risk to the patient or the study results. For example, the following are not exclusionary: a) stable and well controlled hypertension (BP normally <160/95 for at least 3 months); b) asthma (no serious attacks in the past year); c) hypothyroidism (TSH within normal limits for at least 1 year); and d) Type II diabetes (subjects with a reported HgbA1c outside of normal limits within the last 6 months should be reviewed with the study site investigator). Site Investigators who are uncertain about a specific patient or condition should consult the Principal Investigator for clearance prior to enrollment.
5. Clinically significant abnormalities in physical examination, ECG, or laboratory assessments.
6. Clinically significant renal disease (e.g. chronic renal insufficiency with GFR <60, inflammatory disease requiring medication, acute renal failure).
7. Pregnant women or women of child-bearing potential, who are either not surgically-sterile or using appropriate methods of birth control. Women of child-
bearing potential must have a negative serum β-hCG pregnancy test at the screening visit.
8. Women who are breast-feeding
9. Prior participation in a clinical trial of investigational medication within 60 days.

Research Design:

The study will be conducted in the Treatment Units for Research on Neurocognition and Schizophrenia (TURNS) study network, which is comprised of seven sites: Columbia University School of Medicine (P.I.: Jeffrey Lieberman, M.D.); Duke University School of Medicine (P.I.: Joseph McEvoy, M.D.); Harvard University School of Medicine (P.I.: Donald Goff, M.D.); Maryland Psychiatric Research Center (MPRC) (P.I.: Robert W. Buchanan, M.D.); Nathan Kline Institute (P.I.: Daniel Javitt, M.D.) University of California Los Angeles School of Medicine (P.I.: Steve Marder, M.D.); and Washington University School of Medicine (P.I.: John Csernansky, M.D.). The TURNS is a NIMH-funded contract for the evaluation of new compounds for the treatment of cognitive impairments in schizophrenia (HHSN 27820044 1003C; P.I.: Steve Marder, M.D.).

Because AL-108 is available in only a single concentration, subjects will be randomized to one of four arms in order to maintain blind: high dose AL-108 (30 mg/day, 15 mg BID = 3 sprays per nostril, twice a day or 12 sprays total per day), low dose AL-108 (5 mg/day = 1 spray per nostril daily), high dose placebo (3 sprays per nostril, twice a day) or low dose placebo (1 spray per nostril daily). Within each arm, doses will be fixed for 12 weeks. Randomization will be 1/3 to high dose AL-108, 1/3 to low dose AL-108, and 1/3 to placebo, of which 50% (1/6 of total group) will receive “low dose” regimen and 50% will receive (“high dose”). Thus, ½ of patients will be receiving 6 sprays BID (12 sprays per day) and ½ will be receiving 2 sprays daily, maintaining blind. 2/3 of individuals on either treatment regimen will be receiving active treatment and 1/3 placebo. It is expected that the placebo groups will be collapsed for purposes of statistical analysis, following prior inspection of data for similarity between groups.

The total sample will consist of 60 clinically stable patients with DSM-IV-TR schizophrenia, with 20 subjects randomized to each of three groups (high dose AL-108, low dose AL-108, placebo). A best estimate diagnostic approach will be utilized, in which information from the Structured Clinical Interview for DSM-IV (24) is supplemented by information from family informants, previous psychiatrists, and medical records to generate a diagnosis. The projected number of subjects to be recruited from each site is 10-30, depending upon number of participating centers.

Subjects may be treated with any second generation antipsychotic (oral or injectable), or with depot first or second generation antipsychotics. Subjects must have been treated with the same oral antipsychotic for 2 months, with no change in dose for the previous month. Or have been treated with an injectable depot medication with no change in dose for 3 months. Subjects who are receiving concurrent medications will be able to remain on those medications during the course of the study. If a subject is receiving other medications, they must be on the same dose for the previous month.
Procedures:

**Neurocognitive Assessments:** The NIMH MATRICS Neuropsychological Battery will be used to assess cognitive function. The NIMH MATRICS Neuropsychological Battery is comprised of measures of: a) working memory; b) attention/vigilance; c) verbal memory; d) visual memory; e) processing speed; f) problem solving; and g) social cognition. The MATRICS battery is designed to be completed in 90 minutes or less.

**Clinical Assessments:** The symptom assessments will include the Brief Psychiatric Rating Scale (BPRS); Scale for the Assessment of Negative Symptoms (SANS); Calgary Depression Scale (CDS); and Clinical Global Impression Scale (CGI).

i) BPRS: the four positive symptom items - conceptual disorganization, suspiciousness, hallucinatory behavior, and unusual thought content - will be used to measure positive psychotic symptoms.

ii) SANS: the SANS total score, minus the global items, inappropriate affect, poverty of content of speech, and attention items, will be used to measure negative symptoms. The inappropriate affect, poverty of content of speech, and attention items are excluded as lacking construct validity and because factor analytic study results suggest that these items are not closely related to negative symptoms.

iii) CDS: the CDS total score will be used to measure depressive symptoms.

iv) CGI: the CGI severity of illness item will be used to assess global changes

**Functional Assessments:** The functional assessments will include the UCSD Performance-Based Skills Assessment (UPSA) and the Schizophrenia Cognition Rating Scale (SCoRS).

i) UPSA: is designed to assess skills in five areas: household chores, communication, finance, transportation, and planning recreational activities. Subjects are asked to perform tasks in each of these areas and scored according to their ability to complete the task. The UPSA takes 25 - 30 minutes to administer.

ii) SCoRS: is a rating scale designed to elicit information from the subject and informant on the level of cognitive function of the subject. The subject and informant versions both have 20 items. Subject and informant interviews take from 10 - 15 minutes to complete.

**General Safety Assessments:** The safety assessments will include the Simpson-Angus Extrapyramidal Symptom Rating Scale (SAS); Abnormal Involuntary Movement Scale (AIMS); and Side Effect Checklist (SEC).

i) SAS: a modified 11-item version of the SAS will be used to assess EPS.

ii) AIMS: is a 12-item scale, with 7 items designed to assess abnormal facial, oral, extremity, and trunk movements; 3 global judgment items; and 2 current dental status items.

iii) SEC: is designed to assess vital signs and commonly occurring antipsychotic side effects (see Appendix).

Subjects will be asked about adverse events at each visit, and instructed to call the study site should they experience adverse effects at any point in the study. Any serious adverse experience, including death due to any cause, which occurs to any subject
entered into this study or within 14 days following cessation of treatment, whether or not related to the investigational product, will be reported to Allon Therapeutics within 24 hours. Vital signs will be monitored at each safety assessment.

**Compound-specific Safety Assessments:** Because AL-108 is administered intranasally, smell testing before and following treatment will insure lack of effect of the compound on nasal function. The Smell Identification Test (SIT, formerly UPSIT) consists of 40-odorants in 4 booklets. The smell test is administered at baseline, at the end of treatment (Week 12). The data is collected as the raw score from each of the four booklets and then the study coordinator adds the score to determine the total score. Using the nomogram determines the percentile for the subject and then using the nomogram to adjust for age and gender records whether the subject is normosmic, microanosmic etc.

The SIT will also be administered at study midpoint (week 6) to detect potential adverse effects. Initial testing will be done with a 3 item version of the test, which has a sensitivity of 99% for detecting anosmia when used with a cutoff value of 2 (25). For patients scoring a 2 or 3 on the test, no further screening will be necessary. Those scoring 0 or 1 will have full 40 item test administered.

On the 40-item test, a change of 2 or more categories on the SIT (i.e. normal to moderate anosmia, mild to severe anosmia, or moderate to total anosmia) will be considered an adverse event. If this happens, the subject will have medication held for 48 hours and then be retested. If retest shows return to prior level of function, patient will be restarted on medication and retained in the protocol. However, SIT will then be repeated biweekly (weeks 8 and 10, in addition to already scheduled end of treatment administration). If retest fails to show return, subject will be discontinued. SIT will be readministered at 2 week intervals until recovery is seen, or until original end of study (week 12).

In addition, a nasal irritation assessment will be done concomitant with vital signs assessments as per assessment schedule. The nasal irritation CRF will capture both subjective and objective evidence of irritation on a 1-4 scale, as well as information regarding whether the subject shows signs of a cold or allergy at time of testing.

For subjective complains, example questions will be as follows: Does your nose itch or burn more than usual? Does it hurt more than usual when you sneeze or blow your nose? Do you find yourself scratching it more than usual? How often does it hurt or itch? Does it itch more after you take your medication? How long does it last? Scoring will be None, Mild (more than average, but within usual range), Moderate (noticeable to patient but no significant distress), or Severe (persistent itching, burning or pain, or symptoms for which patient feels symptomatic treatment is needed).

For objective complaints, examination will be via penlight or other appropriate light source, and will evaluate whether there is redness, erythema, swelling or irritation. Ratings will be none, mild (may be upper limit of normal), moderate (patchy erythema, outside normal range), or severe (marked erythema or irritation sufficient to require referral for topical intervention).
Presence/absence of cold/allergy will be based on question and observation. Ratings will be on 1-4 scale, with 4 indicating that condition is sufficiently severe that it may interfere with medication administration.

If subject is observed to have a cold or allergy at the time of scheduled SIT testing, it will be deferred to such time as a more reliable estimate can be obtained.

**Physical and Laboratory Safety Assessments:** Physical and laboratory assessments will be performed as detailed in Appendix I.

Abnormal laboratory findings or other abnormal assessments (e.g., ECGs, vital signs) that are judged by the investigator as clinically significant should be recorded as AEs. Clinically significant abnormal laboratory findings or other abnormal assessments that are detected after the start of study treatment, or that are present before the start of study treatment and worsen after study treatment, are considered AEs.

Clinically significant abnormal laboratory findings or other abnormal assessments that are associated with a disease (unless judged by the investigator as more severe than expected for the subject's condition) or that are present before the start of study treatment and do not worsen after study treatment are not included as AEs.

Investigators will exercise medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant, and grounds for stopping or withholding medication.

**Assessment schedule:** Assessments will be performed as per attached schedule of assessments (Table 1).

**Study phases**

The study will be conducted in successive phases, as follows:

**Recruitment:** Recruitment for potential subjects will be performed by reviewing subject records to determine eligibility based on the inclusion and exclusion criteria. This initial recruitment screening requires a partial privacy waiver approved by the IRB. Once qualifying records have been identified, potential subjects will be informed individually and/or in a group setting about the study. Those who express initial interest will be provided with additional information about the study, including the purpose of the study, a description of the procedures, and the overall length of the study. The length of the evaluation phase; the length of the double-blind study period; an explanation of double-blind and how it is determined; a list of the risks and side effects; expectations of the study participant, including all study tests and assessments; how to withdraw from the study; and what to do in case of a potential side effect will also be explained to potential subjects. Only subjects who are considered capable of understanding the risk/benefit considerations and providing written informed consent will be considered eligible for participation in the study. Capacity to provide informed consent will be assessed, if necessary, by a licensed mental health professional unrelated to the research team. Following consent, subjects will be screened for continued eligibility for the study.

**Screening:** Following recruitment, subjects will be screened for continuing
eligibility for the study, as per assessment schedule (Table 1). Subjects will undergo baseline diagnostic; medical, including a physical examination; vital signs; EKG; CBC; complete metabolic panel including liver function tests, electrolytes, glucose, BUN/creatinine; T3/T4; antipsychotic levels; urinalysis; urine toxicology; psychiatric symptom ratings; motor side effect scales. All women of child bearing potential will have a pregnancy test.

**2-Week Stabilization Phase:** In the 2-week stabilization phase, additional measures to be collected will include functional outcome measures, smell testing, a nasal exam, neuropsychological test battery and reading test and a checklist of side effects. Vital signs, psychiatric symptom ratings and motor side effects scales will be repeated. Randomization will occur after all stabilization week 1 assessments have been completed. Because subjects will have different treatment regimens depending upon randomization, no placebo treatment will be administered during the stabilization phase.

**Randomization:** Subjects will be randomly assigned to placebo or experimental treatment within strata defined by site. Study medication will be labeled with randomization numbers and shipped by Allon to the pharmacist at the Maryland Psychiatric Research Center. After the MPRC pharmacist is notified regarding a subject’s randomization, the pharmacist will dispense the medication for the subject and ship it to the study site. A research pharmacist or designated research staff member at each site will be responsible for storing the medication and giving it to the subject at the scheduled study visit. An emergency unblinding tool will reside in the NKI data management system. Designated staff will log into the randomization database and request treatment assignment for a specific subject. An electronic audit trail will be kept of all such unblinding requests.

**Double-Blind Treatment Phase:** The double-blind treatment phase will be 12 weeks. Subjects will be randomized to either high or low dose AL-108, or placebo. Each subject will be given detailed instructions on how to use the nasal spray device following randomization at the end of the stabilization phase. In addition, sites may provide memory aids or follow-up contacts based on subjects’ needs in order to optimize compliance with study drug administration and study procedures. Sites will determine the frequency of this contact. Assessments will be performed as per assessment schedule. Each assessment will be performed plus or minus 1 day from the target date. Subjects will be assessed at each visit for vital signs, adverse events, concomitant medications, and side effects.

**Maintenance of the Blind:** Study medication will be dispensed on a weekly basis for the first two weeks, then biweekly for the remainder of the study. Subjects will be given three extra days of medication per visit in case of a missed appointment. The blind will be broken only if a medical emergency requires this information. In case of medical emergency, the blind will be broken only for the individual subject involved. If this occurs, the subject will be withdrawn from the study. All raters, investigators and other staff will be blind to treatment assignment except for the pharmacist. The pharmacist does not participate in assessing any of the primary symptom or side effect dependent variables and conveys no information about treatment assignment to subjects or staff except in a medical emergency.
Compliance: Subjects taking 75% of their assigned medication will be considered compliant. The 75% criterion ensures that subjects will receive adequate treatment to evaluate the comparative efficacy of AL-108 and placebo. Tear off portion of medication labels (Appendix II) will be entered into CRF upon dispensing of medication. Compliance will be monitored at each scheduled visit by the weighing of spray units (if possible) and subject interviews. At each scheduled visit, medications will be dispensed only after compliance is assessed and all assessments are completed. If a subject is observed to have a compliance problem, then this will be discussed with the subject and a plan formulated to bring the subject back within the compliance parameter. The plan may include contacting the subject's caretaker or scheduling increased clinic visits. These monitoring procedures have resulted in high levels of compliance. Compliance patterns will be carefully monitored in each treatment group and will be described as part of any presentation of study results.

Concomitant Medication: All illicit drugs and the following concomitant medications are prohibited during the study:

Prohibited Psychotropics:
- amphetamines, methylphenidate or other stimulants
- herbal preparations with possible psychotropic effects (e.g., St. Johns wort, kava-kava, Valerian, S-Adenosyl Methionine [SAMe])
- MAOIs
- phenobarbital and other barbiturates

All prohibited medications must be discontinued at least 1 week prior to randomization. Patients should be withdrawn from these medications in a manner that is consistent with labeling recommendations and conventional medical practice.

Overdose Management: No overdoses with AL-108 have been observed. The lethal dose of AL-108 remains largely unknown. No specific recommendations with regard to overdose can be made at this time. Further, AL-108 is a peptide, and so would be destroyed following oral administration. Following severe intranasal overdose (1/2 bottle or more), the subject should be evaluated at a local emergency service and examined both for nasal disturbances and general medical condition. Any abnormalities should be treated symptomatically. As the drug is rapidly cleared from plasma, no specific interventions for inactivation are required. Saline lavage of the nostrils and topical intervention should be considered for nasal irritation.

Sample Size and Data Analysis:

1. Primary Analysis:

The MATRICS battery consists of one or more tests to assess functioning in each of seven cognitive domains: speed of processing, attention/vigilance, working memory (verbal and nonverbal), verbal learning and memory, visual learning and memory, reasoning and problem solving and social cognition. The primary outcome for this trial will be the average of the z-scores for all MATRICS domains. Neurocognitive testing will be conducted at baseline, and weeks 6 and 12. Data reduction for analysis of neurocognitive testing will use these steps: i) individual neurocognitive test scores at baseline and follow-up will be converted to z-scores using the formula: \( z = \frac{\text{individual score} - \text{mean at baseline}}{\text{standard deviation at baseline}} \).
score – PASS normal mean)/PASS normal SD; and ii) z-scores within the pre-specified
cognitive domains measured by more than one test will be averaged to obtain a domain-
specific z-score. We will then calculate an overall average z-score for each participant at
each testing occasion, by averaging the domain-specific z-scores.

Some participants may only complete partial test batteries. While it would be
possible to calculate an average z-score only on the non-missing test scores, this
procedure would implicitly assume scores on the missing tests equaled the average of the
scores on the non-missing tests. While there is substantial correlation in test results
across cognitive domains, the assumption that all tests have a similar average level is
doubtful: subjects with schizophrenia have a distinctive and variable profile of level of
deficits across cognitive domains. Before calculating the overall average z-score, z-
scores for missing domains will be imputed using the pooled data from all treatment
groups. Briefly, imputed values will be calculated from the predicted values for the
missing domain z-score, given the non-missing domain scores, with an added random
component to take account of variability around the expected value in the subject
population (Schafer 1997). Multiple imputations (n=5) will be performed yielding
different Acompleted@ data sets. The analysis described below will be performed in each
completed data set, and the analysis results combined, taking account of the between-
imputation variability in estimates (Little and Rubin, 2002). These computations will be
performed with the SAS7 procedures MI and MIANALYZE.

Treatments will be compared on trends over time (linear slope) in average
neurocognitive z-scores, using a mixed model for repeated measures. Treatment groups
will be compared on an intent to treat basis, using all available follow-up data from each
subject, analyzed according to their original treatment assignment. An advantage of the
mixed model slope analysis is that subjects with only one follow-up measurement, or
subjects who had an end of study measurement completed other than at the scheduled
times, can be included in the analysis taking into account their trend in scores when they
terminated participation, rather than carrying the last value forward.

To test the primary hypothesis, we will fit a mixed model for repeated measures,
using the model:

average z-score = intercept + treatment + treatment x time

The treatment x time interaction term in this model tests for an average difference in
change in z-scores, between placebo and the two dose levels of active treatment, and will
be used to test the null hypotheses that these two differences were equal to zero.

The proposed study will use two-sided tests to compare two doses of active
treatment to a single placebo control. Because of the potentially U-shaped dose response
curve, we will also compare effects of the two active doses. To control the Type I error
rate, we will use Westfall’s (1997) procedure [Westfall PH, Multiple testing of general
Using Westfall’s procedure, with pairwise tests among 3 groups, to maintain an overall
alpha level at at alpha=0.05, each test is conducted at 0.05/2, since there at most only 2
logically independent null hypotheses which can be falsified among the three tests (that
is, if Group 1 = Group 2, and Group 2 = Group 3, then Group 1 = Group 3 follows
logically and is not an independent hypothesis).
a. **Time course of treatment response**: The primary analysis assumes that treatment response follows an approximately linear increasing trend over time. Secondary analyses relax this assumption to use repeated measures ANCOVA to explore treatment differences in changes from baseline at weeks 6 and 12 weeks) to obtain more information on time course of treatment response (if any) to guide planning of future studies.

b. **Other cognitive domains**: None

c. **Effects of race/ethnicity and gender**: We will repeat the primary analysis discussed above with added terms for race/ethnicity and male/female gender and their interactions with time and treatment, in order to explore whether sex or racial/ethnic differences may exist in neurocognitive effects of the study treatments.

2. **Statistical power for primary analysis**:

   Sample size and statistical power for the proposed analysis was approximated using a formula proposed by Diggle, Liang and Zeger (26) for two-sample test comparing slopes for m repeated measures with n participants per group may be computed using the formula: 
   
   \[ n = \frac{2[z_\alpha + z_\beta]^2 \sigma^2 (1-R)}{m s^2_x d^2} \]
   
   where \( d \) = the difference in slopes, \( R \) is the intra-class correlation among the repeated measures, \( \sigma^2 \) is the cross-sectional variance of the scores at each time point, \( s^2_x \) is the variance of the measurement times, and \( z_\alpha \) and \( z_\beta \) are the percentiles of the normal distribution corresponding to the specified Type I error and power; e.g., for two-sided tests with Type I error = 0.05 and power = 0.80, \( z_\alpha = 1.96 \) and \( z_\beta = 0.842 \), respectively. If we standardize the time scale to run from 0 to 1, with 4 equally spaced measurements, \( s^2_x = 17/64 \), and \( d \) becomes equal to the expected difference between treatments in mean change in scores at the end of the study. The difference in change in scores divided by the cross-sectional standard deviation of the scores (\( d/\sigma \)) provides a convenient measure of the effect size of active treatment versus placebo. We may solve the power formula above to calculate the detectable effect size, \( d/\sigma = \left[ \frac{2[z_\alpha + z_\beta]^2 (1-R)}{mn s^2_x} \right]^{1/2} \). To correct for multiple comparisons of two dose levels of active treatment and a placebo control, we would test the main outcome at two-sided alpha= 0.05/2. From preliminary studies of the MATRICS battery, the ICC for the individual tests is somewhere between 0.6 and 0.8, suggesting that \( R \) for the overall z-score will fall within this range. If \( R=0.6 \), the detectable effect size will be 0.71; with \( R=0.8 \), the detectable effect size will be 0.50. Thus the planned study should have power to detect a large effect on neurocognitive outcomes, as well as providing preliminary effect size estimates for future, large N studies.

3. **Secondary and Tertiary Analyses**: Secondary and tertiary analyses will evaluate effects of AL-108 on clinical and functional outcome measures (secondary analyses), and safety measures (tertiary analyses).

   Secondary analyses of other outcome measures will include:

   i. Proxy measures of functional capacity (UPSA)
   
   ii. Interview-based measures of cognition (SCoRS)
   
   iii. Symptom rating scales: BPRS total score and BPRS positive symptom score (items for Conceptual disorganization; Unusual thought content; Hallucinatory
behavior; Suspiciousness), SANS total score and subscale scores for Affect; Alogia, asociality/Anhedonia; Avolition; Calgary Depression Scale total score, and CGI score.

iv. Ratings of potential side effects of treatment include: Simpson-Angus Scale (SAS) total score; Barnes Akathisia Scale (BAS) total score; Abnormal Involuntary Movement Scale (AIMS) total score, and a side effect checklist.

All exploratory tests for treatment effects on these other outcome measures will be conducted at two-sided alpha=0.05, using the Holm-Bonferroni procedure (27) to control for multiple comparisons between groups, but not adjusting for multiplicity of outcome measures. Ratings on the first three categories of outcome measure will be analyzed using a mixed model for repeated measures to look for difference in (linear) trend in symptom rating scores versus time, using the model score = intercept + treatment + time + treatment x time, where the treatment x time interaction tests for differences between placebo and the two doses of active treatment time trends in symptom scores.

Extrapyramidal side effect (EPS) ratings on the SAS, BAS and AIMS total scores will be analyzed using pairwise comparisons of dose groups to placebo, using a rank test for differences in the correlation between EPS score and study visit, which is suitable for detecting presence of adverse trends in vulnerable subgroups, and more suited to non-normally distributed EPS data (28). The twenty-two side effects specified on a side effects checklist are rated biweekly during double blind treatment as 1=none, 2=mild, 3=moderate or 4=severe. To summarize this rating data, the most severe rating of each side effect for each subject during follow-up will be determined. The treatments will then be compared on worst ranking using a Mantel-Haenszel chi-square statistic for ordinal data. It should be noted that the eligibility criteria restrict study participation to subjects with limited positive symptoms and extrapyramidal symptoms, so that it may be unlikely that such a large response in these areas will be observed such a response in this trial. Analysis of effects on these symptoms will be conducted mainly with the goal of identifying any potential for symptom worsening, or of unexpectedly strong symptom remission, which might confound interpretation of cognitive changes. Tertiary analyses will assess alterations in general, laboratory and medication specific safety measures during treatment. Frequency of adverse events and out-of-range values will be tabulated. Statistical analyses will be performed using categorical or continuous statistical approaches, as appropriate.

Tertiary measures will include physical and laboratory safety parameters, including blood chemistries, CBC, EKG and SIT, as well as rates of AEs and SAEs. Data will be tabulated per treatment arm, and analyzed using categorical or continuous statistical approaches, as appropriate.

**Risk/benefit considerations**

**Potential Risks/Discomforts:**

The doses of AL-108 used in this study and the intranasal route of administration are not anticipated to produce any significant deleterious effect on the intranasal epithelium or
the subject’s olfactory function. To further determine the potential for decline in olfactory function, each subject will be administered a smell identification test (SIT) at baseline, week 6, and at the final study visit (week 12).

In study AL-108-001A in which subjects received a single dose of AL-108 the most frequently reported AE was headache; 9 events were reported by 20% of subjects (5/25) in the AL-108 group and 60% of subjects (3/5) in the placebo group. Six occurrences of headache were considered by the investigator to have a suspected relationship to study drug.

In the phase 1b study (AL-108-102) of healthy elderly adults (50 to 85 years of age), subjects received either AL-108 10 mg/day or placebo (cohort 1); AL-108 15 mg/day or placebo (cohort 2); AL-108 10 mg BID or placebo (cohort 3); or AL-108 15 mg BID or placebo (cohort 4).

Among the subjects in cohort 1 (AL-108 10 mg/day or placebo), the most frequently reported AE was leg pain reported 3/8 (38%) subjects with all occurrences suspected by the investigator to be related to study drug. Similarly, 2 of these 3 subjects also experienced muscle soreness/generalized body aches, also suspected to be related to study drug. Other events reported by >1 subject with a suspected relationship to study drug were nausea, sleepiness, and dizziness (2/8 subjects each, 25%). Suspected drug related AEs reported by 1/8 (13%) subjects each included: headache, chills, and sweats.

Among the subjects in cohort 2 (AL-108 15 mg QD or placebo), the most frequently reported AE was bilateral leg cramps reported by 2/8 (25%) subjects, with both occurrences suspected by the investigator to be related to study drug. One subject (13%) also reported headache, which the investigator suspected was related to study drug.

In cohort 3 (AL-108 5 mg BID or placebo) 7 different events were reported by 4/8 subjects. Of the 3 subjects who reported 2 events, 1 subject reported events related to an upper respiratory tract infection (cough and runny nose), 1 reported 2 gastrointestinal events (constipation and heartburn), and 1 reported neck and low back pain. None of the 7 events had a suspected relationship to study treatment.

In cohort 4 (AL-108 15 mg BID or placebo) 7 events were reported by 3/8 subjects.

General clinical procedures utilized to ensure against or minimize potential risks associated with AL 108 treatment include a pre-investigation medical history, physical examination, EKG, and clinical laboratory tests. Side effect and vital sign assessments will be conducted biweekly throughout the course of the study. A pregnancy test will be performed prior to study entrance and pregnant women will be excluded from the study. In addition, we provide 24-hour/7-day on call coverage for all outpatient subjects.

The clinical and neuropsychological tests are non-invasive clinical assessments with minimal risk. To minimize risks associated with clinical assessments, research assistants and therapists, trained to detect any untoward effects, will administer the clinical assessments and neuropsychological tests. There is the possibility that the subject will
find the evaluations stressful. The likelihood of this occurring is not very significant. However, frequent breaks in the evaluations will be allowed to insure that the subject does not become overly stressed.

The NIMH Data and Safety Monitoring Board will monitor the occurrence of side effects and adverse events. A Serious Adverse Event (SAE) is any untoward medical outcome occurring in the context of the conduct of this project, and includes any event that: requires hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, results in the death of the subject, or results in a congenital anomaly/birth defect.

In addition to the monitoring and reporting of the above adverse events, study participants will be withdrawn from the protocol if any of the following criteria are met.

1) Objective Evidence for Clinical worsening, as indicated by one of the following:
   a) The subject is judged to be entering an exacerbation of his/her illness by the treating clinician;
   b) Either of the following:
      i. relative to the baseline BPRS; an increase of 3 points or more on any one of the following BPRS items: somatic concern, conceptual disorganization, hostility, suspiciousness, hallucinatory behavior, and unusual thought content; or
      ii. an increase of 2 or more on the CGI global severity item
   c) Complete cessation of eating and drinking for period exceeding 24 hours.

2) Pregnancy

Subjects withdrawn from the study and remaining within the study site will continue to be followed naturalistically until their originally scheduled termination date.

From the standpoint of privacy and confidentiality, the subject's welfare will be safeguarded by responsible, systematically controlled procedures for the collection of information for both clinical and research purposes. NKI and TURNS investigators, the NKI IRB and other TURNS IRBs, the NIH Data and Safety Monitoring Board, the US Food and Drug Administration and the IND sponsor (Allon pharmaceuticals) will have access to the research files. Also, the subject's agreement to release information will be required for requests for information to and from other agencies. Recorded information for research purposes, including computer input data, will be identified by code number rather than by name, and subsequent published or presented material related to the project will not be traceable to specific individuals. Following completion of data collection, patient files will be maintained in a secure area in retrievable form for future patient-requested clinical use.

**Potential Benefits:**

There is no guarantee that a participant will receive direct benefit from this study. However, possible benefits of participating in this research may include improvement of one's condition and relief of symptoms. Also, a subject's condition may be monitored more closely than usual. In addition, new information may be learned that would be helpful in developing new therapies for other people with similar illnesses. It is possible
however, that no therapeutic or other direct benefits will result during or following completion of this study.

**Risk/Benefit Ratio:**

Because the study drug AL-108 is early in development, there is limited information about the safety profile of this product in the target population of subjects, although the product was well tolerated in healthy, older adult subjects in the phase 1b study.

While doctors hope that AL-108 will be a useful treatment for cognitive impairment associated with schizophrenia, there is no proof of this yet. Taking part in this study may or may not improve your symptoms of mild cognitive impairment. We do know the information from this study will help doctors learn more about AL-108 as a treatment for mild cognitive impairment. This information could help future patients.

The symptom ratings, cognitive and neuropsychological assessments, and blood draws are associated with minor risks only. In phase I studies involving 62 subjects to date, no clinically significant laboratory values or clinical laboratory AEs have been reported, and all doses of study drug administered have been safe and well tolerated. The potential for developing a drug that treats abnormalities in cognition in schizophrenia outweighs the potential risks of the experimental compound, given the observed lack of appreciable side effects to date.

**Consent Procedures:**

All subjects will be provided a complete description of the proposed study, including the purpose of the study, procedures, risks, and alternatives to participation. Should the subject express interest in participation, personnel trained in the informed consent process will review the Informed Consent document with the subject, and the subject will be given a copy of the form for further study. A non-investigator clinician member of the treatment team will assess whether the prospective participant is able to participate in the informed consent process. If the subject is able to participate in the informed consent process and agrees to participate in the study, then agreement to participate will be documented on the Informed Consent form. If the subject is not able to participate in the informed consent process, then they will not be entered into the study.

**Access to Research Data:**

TURNS research staff and data management team will have access to this data. Allon Therapeutics will have access to coded data.

**Subject Payment:**

Participants will receive payment for their study participation. Payment rates and schedules are site specific.

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**DATA MANAGEMENT AND PROCEDURES**
Design and Development

This protocol will utilize a centralized data management center (CDMC), the Nathan Kline Institute for Psychiatric Research. The CDMC will be responsible for development of the case report forms (CRFs), development and validation of the clinical study database, ensuring data integrity, and training clinical site and other protocol staff on applicable data management procedures and computerized systems. A web-based distributed data entry model will be implemented. This system will be developed to ensure that applicable guidelines and regulations surrounding the use of computerized systems used in clinical trials are upheld.

Data Collection Forms

Data will be collected at the study sites on paper CRFs. The CDMC will provide sites with a final set of standardized CRFs and CRF completion instructions. The CRFs will be distributed to the clinical sites by the CDMC. These forms are to be completed on an ongoing basis during the study. Forms should be completed according to the instructions provided and as discussed during training. The investigator is responsible for maintaining accurate, complete and up-to-date records, and for tracking the completion of CRFs for each research participant. The investigator is also responsible for maintaining any source documentation related to the study, including any films, tracings, computer discs or tapes.

Data Acquisition and Entry

For paper CRFs, all CRFs must be completed legibly with black ballpoint pen. A correction should be made by striking through the incorrect entry with a single line and entering the correct information adjacent to the incorrect entry. Corrections to paper CRFs must be initialed and dated by the person making the correction. Data entered into electronic CRFs shall only be performed by authorized individuals. Corrections to electronic CRFs shall be tracked electronically with time, date, individual making the change, and what was changed. Selected CRFs also require the investigators written signature or electronic signature, as appropriate. CRFs will be monitored for completeness, accuracy, legibility and attention to detail during the study. The investigator must retain a copy of all CRFs.

Data Center Responsibilities

The CDMC will 1) develop a data management plan and will conduct data management activities, 2) provide final CRFs for the collection of all data required by the study, 3) develop data dictionaries for each CRF that will comprehensively define each data element, 4) conduct ongoing data monitoring activities on study data from all participating clinical sites, 5) monitor any preliminary analysis data clean up activities, 6) rigorously monitor final study data clean up activities, (7) lock the study database and (8) provide the final, locked study database to designated organizations and personnel.
Data Editing

Completed forms/electronic data will be entered into the CDMC automated data acquisition and management system. On-line, real time data editing will be conducted. In addition, data will be edited each night for completeness and accuracy. If incomplete or inaccurate data are found, a data clarification request will be forwarded to the clinical sites for a response. The clinical sites will resolve data inconsistencies and errors and enter all corrections and changes into the CDMC automated data acquisition and management system.

Data Transfer

The CDMC will transfer the final database to NIMH and other parties designated by NIMH, as requested, for storage and archive.

Documentation

Study documentation includes all case report forms, data correction forms, electronic data files, workbooks, source documents, monitoring logs, appointment schedules, sponsor-investigator correspondence and regulatory documents (e.g., signed protocol and amendments, IRB correspondence and approved consent form and signed patient consent forms, Statement of Investigator form, and clinical supplies receipt and distribution records).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study. Source documents include, but are not limited to, laboratory reports, ECG tracings, X-rays, radiologist reports, patient diaries, ultrasound photographs, patient progress notes, hospital charts, pharmacy records and any other similar reports or records of any procedure performed in accordance with the protocol.

Whenever possible, the original recording of an observation should be retained as the source document; however, a photocopy is acceptable provided that it is a clear, legible, and exact duplication of the original document.

References:
### TURNS -- AL-108 SCHEDULE OF ASSESSMENTS

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<td><strong>REG.</strong></td>
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<td>Inclusion/Exclusion Checklist</td>
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<td><strong>MHX</strong></td>
<td>Medical History</td>
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<td>Physical Exam</td>
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<td>SCID Interview</td>
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<tr>
<td><strong>UTX</strong></td>
<td>Urine Toxicology</td>
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<td>Urinalysis</td>
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<tr>
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<td>Randomization</td>
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<td>Medication Training</td>
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<td><strong>DSP</strong></td>
<td>Medication Dispensation</td>
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<td><strong>ACT</strong></td>
<td>Medication Accountability</td>
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<td><strong>BPRS</strong></td>
<td>Brief Psychiatric Rating Scale</td>
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<tr>
<td><strong>SANS</strong></td>
<td>Scale of the Assessment of Negative</td>
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<tr>
<td><strong>CDRS</strong></td>
<td>Calgary Depression Rating Scale</td>
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<td><strong>CGI</strong></td>
<td>Clinical Global Impressions</td>
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<td><strong>SAS</strong></td>
<td>Simpson-Angus Scale</td>
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<td><strong>AIM</strong></td>
<td>AIMS</td>
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<tr>
<td><strong>SEC</strong></td>
<td>Side Effects Checklist</td>
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<tr>
<td><strong>AEF</strong></td>
<td>Adverse Event Form</td>
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<td><strong>STT</strong></td>
<td>Study Treatment Termination</td>
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<tr>
<td><strong>ADL</strong></td>
<td>Antipsychotic Drug Levels</td>
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<td></td>
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</tbody>
</table>

**Legend:**
- **X** indicates the assessment is performed at the specified visit.

**Notes:**
- **VIS** Visit Form
- **REG.** Subject Registration
- **DEM** Subject Demographics
- **IEC** Inclusion/Exclusion Checklist
- **MHX** Medical History
- **PEX** Physical Exam
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- **AIM** AIMS
- **SEC** Side Effects Checklist
- **AEF** Adverse Event Form
- **STT** Study Treatment Termination
- **ADL** Antipsychotic Drug Levels
Appendix I: Physical and safety assessment procedures

Vital Signs
Vital signs including blood pressure, heart rate, and respiration will be monitored at every visit.

Electrocardiograms
Twelve-lead ECGs will be performed as safety assessments at screening and endpoint. The investigator may perform repeat ECGs for safety at any scheduled time or additional ECGs for safety at other times if deemed necessary. Relevant data will be captured in CRF.

Physical Assessments
A physician or other qualified personnel will examine each subject at screening. Additional targeted physical assessments may be performed at other times if deemed necessary by the investigator (e.g., to confirm resolution of an AE). Treatment-emergent clinically significant abnormalities observed during the physical assessments will be recorded in the CRF as AEs.

Laboratory Parameters
Laboratory parameters will consist of the following tests:

<table>
<thead>
<tr>
<th>Clinical Chemistry</th>
<th>Hematology</th>
<th>Other</th>
</tr>
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<tbody>
<tr>
<td>Sodium</td>
<td>Hemoglobin</td>
<td>Routine urinalysis</td>
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<tr>
<td>Potassium</td>
<td>Hematocrit</td>
<td>Thyroid T3/T4</td>
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<tr>
<td>Glucose (random)</td>
<td>Platelets</td>
<td>Urine toxicology</td>
</tr>
<tr>
<td>ALT</td>
<td>White blood cell count</td>
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</tr>
<tr>
<td>AST</td>
<td>automated differential</td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>Red blood cell count</td>
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<tr>
<td>Serum calcium</td>
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<tr>
<td>Chloride</td>
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<tr>
<td>Bicarbonate</td>
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<td>Uric acid</td>
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<td>Blood urea nitrogen</td>
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<tr>
<td>Alkaline phosphatase</td>
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<td>Total bilirubin</td>
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<tr>
<td>Albumin</td>
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<td></td>
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<tr>
<td>Total protein</td>
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<tr>
<td>Lactic dehydrogenase</td>
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</table>
## Appendix II: Mock drug label

<table>
<thead>
<tr>
<th>Study Medication, Unit #XXXX</th>
<th>Protocol: TURNS-Allon</th>
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</thead>
<tbody>
<tr>
<td>AL-108 Nasal Spray, 2.5 mg per 0.1 mL</td>
<td>Unit #XXXX</td>
</tr>
<tr>
<td>or Placebo to Match</td>
<td>Site # ____________</td>
</tr>
<tr>
<td>Lot: CXXXXX or CXXXXX</td>
<td>Subject #: ____________</td>
</tr>
<tr>
<td>Retest Date: mm-yyyy</td>
<td>Date Dispensed: ________</td>
</tr>
<tr>
<td>Store upright at 2-8°C</td>
<td>Allon Therapeutics Inc., 1168 Hamilton Street, Suite 506</td>
</tr>
<tr>
<td>CAUTION: New Drug—Limited by Federal Law to Investigational Use Only</td>
<td>Vancouver, British Columbia, Canada V6B 2S2</td>
</tr>
<tr>
<td>Allon Therapeutics Inc., 1168 Hamilton Street, Suite 506</td>
<td>(604) 736-0634</td>
</tr>
</tbody>
</table>