HHS Public Access

Author manuscript

Contemp Clin Trials. Author manuscript; available in PMC 2022 August 01.

Published in final edited form as:

Contemp Clin Trials. 2021 August ; 107: 106488. doi:10.1016/j.cct.2021.106488.

Rationale, study design and implementation of the LUCINDA Trial: Leuprolide plus cholinesterase inhibition to reduce neurologic decline in Alzheimer's.

Tracy Butler[1], **Judith D. Goldberg, ScD**[2], **James E Galvin, MD, MPH**[3], **Thomas Maloney, PhD**[1], **Lisa Ravdin, PhD**[4], **Lidia Glodzik, MD, PhD**[1], **Mony J. de Leon, EdD**[1], **Tsivia Hochman, MsC**[2], **Richard L Bowen, MD**[5], **Craig S. Atwood, PhD**[6]

[1.]Brain Health Imaging Institute, Department of Radiology, Weill Cornell Medicine, New York, NY 10021, USA

[2.]Departments of Population Health and Environmental Medicine, New York University School of Medicine, New York, NY 10016 USA

[3.]Comprehensive Center for Brain Health, Departments of Neurology and Psychiatry, University of Miami, Miller School of Medicine, Boca Raton, FL 33433, USA

[4.]Department of Neurology, Weill Cornell Medicine, New York, NY 10021, USA

[5.]OTB Research, Charleston, SC, USA.

[6.]Division of Geriatrics and Gerontology, Department of Medicine, University of Wisconsin School of Medicine and Public Health, and Geriatric Research, Education and Clinical Center, Veterans Administration Hospital, Madison, WI 53705 USA

Abstract

The LUCINDA Trial (Leuprolide plus Cholinesterase Inhibition to reduce Neurologic Decline in Alzheimer's) is a 52 week, randomized, placebo-controlled trial of leuprolide acetate (Eligard) in women with Alzheimer's disease (AD). Leuprolide acetate is a gonadotropin analogue commonly used for hormone-sensitive conditions such as prostate cancer and endometriosis. This repurposed drug demonstrated efficacy in a previous Phase II clinical trial in those women with AD who also received a stable dose of the acetylcholinesterase inhibitor donepezil (Bowen et al., 2015). Basic biological, epidemiological and clinical trial data suggest leuprolide acetate mediates improvement and stabilization of neuropathology and cognitive performance via the modulation of gonadotropin and/or gonadotropin-releasing hormone signaling. LUCINDA will enroll 150 women with mild-moderate AD who are receiving a stable dose of donepezil from three study sites in the United

Corresponding Author: Tracy Butler, MD, Weill Cornell Medicine Department of Radiology, 420 East 70th Street NY, NY 10021, 646-248-6734, Tab2006@med.cornell.edu.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

States. Cognition and function are the primary outcome measures as assessed by the Alzheimer's Disease Assessment Scale-Cognitive Subscale. Blood and MRI biomarkers are also measured to assess hormonal, inflammatory and AD biomarker changes. We present the protocol for LUCINDA and discuss trial innovations and challenges including changes necessitated by the covid-19 pandemic and study drug procurement issues.

1 Introduction

Alzheimer's Disease (AD) has a significant economic, social and emotional impact on virtually every stratum of our society. In the United States alone, 5.4 million, or 1 of every 9 people over age over age 65, are currently suffering from AD (www.alz.org). Currently approved medications for AD have limited effects in slowing disease progression.[1] Recent failures of anti-amyloid medication trials[2] highlight the critical need for new models of understanding and treating AD. Here, we describe the rationale, methods and implementation of the LUCINDA (Leuprolide plus Cholinesterase Inhibition to reduce Neurologic Decline in Alzheimer's) Trial. Premised on the Cell Cycle Theory of aging and AD[3, 4] and supported by robust preclinical evidence and prior human trials,[5, 6] LUCINDA trial aims to repurpose the injectable medication leuprolide acetate for AD.

1.1 Study Rationale

Leuprolide is an analogue of gonadotrophin-releasing hormone (GnRH), a ten amino acid peptide that is synthesized and secreted from neurons in the anterior hypothalamus. GnRH is secreted in a pulsatile fashion into the hypophysial portal bloodstream at the median eminence and is carried to the pituitary gland where it binds to GnRH receptors on gonadotrope cells and signals the synthesis of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). When given chronically, GnRH analogues like leuprolide disrupt the pulsatile release of GnRH, thereby resulting in the downregulation of FSH and LH synthesis and secretion, and the suppression of gonadal sex hormone (estrogen and testosterone) production in men and pre-menopausal women. Leuprolide is commonly used to treat prostate cancer, in fertility regimens, to treat severe endometriosis, to shrink uterine fibroids and to treat precocious puberty in children.[7] For these conditions, leuprolide's suppression of circulating sex hormones is considered the primary mechanism of action. In post-menopausal women, leuprolide decreases LH and FSH but has no measurable effect on the already low concentration of ovarian hormones.[8]

While LH is important for normal brain structure and function when the HPG axis is in balance, the endocrine dyscrasia that results with aging (marked elevations in LH signaling but loss of sex steroid signaling) is a driver of neurodegeneration [4, 5] According to the Cell Cycle Theory of Aging and AD, elevated levels of LH, a powerful mitogen,[9–11] in the absence of normal circulating sex steroids, are a signal that initiates the abortive re-entry of post-mitotic, terminally differentiated, pyramidal neurons in the hippocampus and neocortex into an abortive mitotic cell cycle[3, 4] as shown in Figure 1. Extensive preclinical validation work has demonstrated that decreasing LH improves cognitive performance and decreases amyloid deposition and tau phosphorylation in multiple animal models of AD.[4, 5, 12–25]

In addition to its LH-lowering effect, leuprolide may also have anti-inflammatory effects. Although the role of inflammation in AD is complex, there is strong evidence that peripheral inflammation is associated with AD progression [26, 27] and inflammation remains an active therapeutic target in AD.[28, 29] Anti-inflammatory effects of GnRH analogues,[30–35] noted in animal studies and human studies of other diseases will be evaluated in this trial.

1.1.1 Human evidence for leuprolide as a treatment for AD—A clinical trial of leuprolide for AD was initially motivated by anecdotal experience of patients with advanced AD who improved dramatically after receiving leuprolide as treatment for prostate cancer.[36–38] A small European clinical trial showed cognitive improvement in male AD patients treated with leuprolide for prostate cancer.[6] In a second trial, a phase II, 48-week, double-blind, placebo-controlled, dose-ranging study,[4] a total of 109 women with mild to moderate AD were randomized to low dose leuprolide (11.25 mg), high dose leuprolide (22.5 mg) or placebo injections every 12 weeks. At the 48 week endpoint, there were no statistically significant differences in primary efficacy parameters: the Alzheimer's Disease Assessment Scale–Cognitive Subscale[39] (ADAS-Cog) and Alzheimer's Disease Cooperative Study - Clinical Global Impression of Change[40] (ADCS-CGIC) although there was a trend in favor of the high dose leuprolide group. However, in a pre-specified subgroup analysis of patients taking the AChEI donepezil (72% of patients) there was a statistically significant benefit in the high dose leuprolide group compared to both the low dose and placebo groups. While this trial did not succeed in showing efficacy in the primary analysis of the total study population, results of the planned secondary subgroup analyses showed cognitive and clinical improvement in women treated with high dose leuprolide who were already using an AChEI when compared to women treated with AChEI alone. The purpose of the LUCINDA trial is to further investigate this positive interaction between leuprolide and AChEIs in the treatment of AD. All LUCINDA participants are required to be taking a stable dose of the AChEI donepezil.

1.1.2 Leuprolide and donepezil synergy—While the mechanism of synergy between leuprolide and donepezil remains uncertain, we posit three possibilities: a general role of cholinergic mechanisms in neuroplasticity, with adequate cholinergic tone required for neural repair and reorganization,[41] an effect on inflammation[42] and/or an effect on kisspeptin, a hypothalamic peptide that is the key regulator of GnRH pulsatile secretion[43] and which also affects cholinergic transmission.[44] The latter two of these possibilities will be evaluated in exploratory analyses in LUCINDA.

2 Methods

2.1 Study Design

The LUCINDA trial is a three site, randomized, placebo-controlled double-blind study to assess the effect of a 48-week regimen of leuprolide acetate (Eligard, 22.5 mg subcutaneous injection every 12 weeks) compared to placebo on cognition, and blood and neuroimaging biomarkers in women (n=150) with AD or Mild Cognitive Impairment (MCI) due to AD as defined by current research criteria[45, 46] who are also taking the AChEI donepezil. The three sites are: Weill Cornell Medicine, University of Miami, and University of Wisconsin

– Madison. Each site Principal Investigator (PI) is also multiple PI for the NIH grant supporting this project. This project is approved by the BRANY (Biomedical Research Alliance of New York) single IRB.

2.2 Study Endpoints

The **Primary Study Endpoint** is change in cognition from baseline to post-treatment as measured by the ADAS-Cog.[39] The ADAS-Cog is a standard measure of cognition commonly used in AD therapeutic trials.

Secondary efficacy / covariate endpoints are Alzheimer's Disease Cooperative Study – Activities of Daily Living[47] (ADCS-ADL; a caregiver interview to assess functioning), ADCS-CGIC[40] (a clinician-administered semi-structured interview with both the subject and caregiver [separately] to assess clinically meaningful global change), NPI-Q[48] (a caregiver questionnaire assessing psychiatric symptoms) and Burden Inventory (BI; a caregiver questionnaire assessing the burden of caring for the subject.)[49] These tests were used in the prior trial of leuprolide for AD,[4] which LUCINDA aims to replicate and extend. In addition, LUCINDA includes the Brief Pain Inventory,[50] and the Repeatable Battery for the Assessment of Neuropsychological Status[51] (RBANS) to more carefully assess cognition. Pain is being measured because GnRH analogues improve pain and mobility in elderly patients with rheumatoid arthritis,[32] and decreased pain could be associated with improved function independent of cognitive benefit.

Secondary Neuroimaging Biomarker Endpoints are pre-to-post treatment change in MRI-measured brain volumes (bilateral hippocampus, bilateral precuneus, bilateral posterior cingulate, total gray matter, total ventricular volume) and Arterial Spin Labeling Magnetic Resonance Imaging (ASL MRI)-measured hippocampal perfusion.

Secondary Blood Biomarker Endpoints are pre-to-post treatment change in C-Reactive Protein (CRP), Erythrocyte Sedimentation Rate (ESR) and cytokines, in particular IL1-β, TNF-α and IL6 which are elevated in AD[26] and have been shown to decrease in response to GnRH analogues.[31, 32]

2.3 Participants

LUCINDA will enroll 150 women with mild-moderate AD or MCI due to AD. In accord with current research criteria, subjects are diagnosed with MCI due to AD when in addition to demonstrable cognitive decline, they have biomarker evidence of AD pathology, in this case, cortical amyloid detected with Neuraceq PET.[45, 46] Subjects must have a Clinical Dementia Rating (CDR) global score between .5 and 2. They must be taking a stable dose of donepezil for at least 90 days prior to enrollment. There must amyloid present in cortex based on interpretation of Neuraceq PET by a board-certified radiologist in accord with standard criteria.[52] Approximately equal numbers of subjects will be enrolled at each of the three sites. All subjects and/or their study partner / caregiver will provide informed consent to participate. Full inclusion and exclusion criteria are presented in Table 1.

2.3.1 Amyloid negative sub-study—

In addition, up to thirty subjects diagnosed clinically with AD and meeting all study inclusion criteria except found to be amyloid

negative at Neuraceq PET scanning will be randomized into a separate sub-study with procedures identical to the main study. Results from these amyloid negative subjects will be examined separately.

2.4 Recruitment and Enrollment

Patients are recruited from investigators' clinical practices or research cohorts, letters/emails to and referrals from other clinicians, via outreach efforts in the community (e.g. health fairs, religious or social groups, assisted living or retirement communities, AD patient advocacy and caregiver support groups), posted flyers and brochures, newspaper or radio advertisements, the lucinda.weill.cornell.edu website, digital marketing (e.g. paid google search advertisements), and social media campaigns (e.g. facebook, Instagram, twitter).

2.5 Randomization

There will be a random (1:1) assignment of patients to leuprolide acetate or placebo. Randomization will be performed separately for amyloid positive (main study) and amyloid negative (sub-study) participants. Randomization for the main study and amyloid-negative sub-study will be stratified by site.

2.6 Study Drug

2.6.1 Eligard—LUCINDA uses Eligard, a polymeric matrix formulation of leuprolide acetate for subcutaneous injection designed to deliver drug at a controlled rate over a 1–6 month period. It is supplied as two separate syringes which must be mixed immediately (<30 minutes) prior to injection. Eligard is FDA approved to treat prostate cancer. As discussed in section 2.1 above, while leuprolide is used clinically to reduce sex hormone levels to treat disease such as prostate cancer and endometriosis, reduction of LH is considered the primary mechanism of leuprolide's anti-AD effect. Eligard 22.5mg for subcutaneous administration every 3 months is supplied by the manufacturer, Tolmar Pharmaceuticals.

2.6.2 Placebo—Placebo injections consist of 0.375 cc of 0.9% sterile sodium chloride Injection administered with a 5/8 inch long 20-gauge needle (identical to the Eligard needle) on a single-use syringe.

2.6.3 Blinding Procedures—Because it was cost prohibitive to create placebo identical to Eligard, LUCINDA procedures require that study drug be obtained from the pharmacy, prepared and injected by an unblinded study staff member who plays no other role in the study. Study drug is injected in the buttock, out of view of the subject, with the study partner in a separate room.

2.7 Study procedures / evaluations

A schedule of study procedures is presented in Appendix 1. Key study procedures are described in greater detail:

2.7.1 Amyloid Positron Emission Tomography—PET scanning uses the FDA-approved tracer florbetaben (Neuraceq) which is supplied by the manufacturer, Life Molecular Imaging, to each site. PET scans are interpreted as positive or negative by

a board-certified radiologist in accordance with standard guidelines.[52] Amyloid positive subjects are randomized in the main study while amyloid negative subjects (stratified by site; maximum of 30 total) are randomized into an amyloid-negative sub-study with identical procedures.

2.7.2 MRI: structural and ASL sequences—MRI examinations are performed on a 3T scanner at each site using acquisition parameters based on those used for ADNI-GO 3T imaging (http://adni.loni.usc.edu/methods/documents/mri-protocols/) Regional volume measurements use Freesurfer's longitudinal processing stream[56] and mixed linear effect model[57] to optimize reliability and statistical power for measuring within-subject volumetric changes over time. We will focus on the following structural MRI biomarkers associated with AD: hippocampal, posterior cingulate, precuneus, total gray matter and total ventricular volume. To image hippocampal perfusion, a functional measure similar to that obtainable using FDG PET, a pulsed ASL sequence is used.[58] Average bi-hippocampal perfusion will be quantified as described previously,[59, 60] and pre- to post-treatment change will be compared between treatment groups. Appropriate methods will be taken to reduce site-related differences in image acquisitions and derived volumetric and perfusion estimates.[61]

2.7.3 Cognitive and other assessments and modifications for remote use—Assessments used for inclusion criteria and outcome measurement include standardized scales for cognition, function, behavior/mood and other domains. Assessments and their mode of administration are listed in Table 2.

To minimize the risk of exposing our vulnerable subject population to Covid-19 during study visits, we revised our protocol to allow as many assessments as possible to be administered remotely. To ensure comparability within subject across timepoints, and across subjects, we chose modes of administration that would be accurate and appropriate even when/if risk of Covid-19 exposure diminishes. For some assessments such as the Montreal Cognitive Assessment (MOCA), a version without visual elements – the MOCA-blind[62] - had already been validated and was in use as part of the National Alzheimer's Coordinating Center Unified Data Set Telephone Cognitive Battery (naccdata.org.)

For the ADAS-cog,[39] our primary outcome measure, we designed what we have termed a hybrid ADAS-cog administration involving both an on-site study coordinator and a remote tester in a separate location. According to this approach, the subject is tested in a private room in the medical center but the majority of test items are administered via teleconference by a remote tester. The on-site coordinator sets up the videoconference system (we are using Zoom) which includes an additional overhead camera so the remote tester can observe the subject's face, hands and body. While the majority of communication is between the subject and the remote tester, the on-site coordinator presents study materials such as naming objects and paper for copying figures. Importantly, neither the subject nor the remote tester wear face masks during administration; communication between subject and tester through face masks can be challenging, especially when a subject is cognitively impaired.

Modifications to assessments motivated by Covid-19 will continue throughout the trial at all sites for all subjects, regardless of pandemic conditions.

2.7.4 Laboratory Evaluations—Routine clinical laboratory tests are be performed at each site's clinical laboratory at baseline to exclude significant medical conditions and at each study visit to monitor subject health and safety. Other laboratory tests are be performed at the Weill Cornell Clinical and Translational Science Center Core Laboratory. Apolipoprotein E (APOE) genotyping is ascertained for all subjects. Subgroup analyses will consider APOE4 status. CRP, cytokine panel and ESR are inflammation biomarker endpoints. Determinations of serum concentrations of LH, FSH, fractionated estradiols, free and total testosterone, progesterone, sex hormone binding globulin, DHEAS, inhibin-B, kisspeptin leuprolide and donepezil will be used for covariate analyses.

2.8 Statistical Plan

2.8.1 Sample Size—Based on the prior trial of leuprolide for AD[8] showing a point difference in ADAS-Cog[39] of 3.12 (mean decline for leuprolide 22.5mg + AChEI = 0.18; placebo + AChEI = 3.30; effect size of .56), 150 subjects (75 per arm; 1:1 randomization) will be randomized to detect a difference in change from baseline between the two treatment groups of +/−0.46 standard deviations in this primary endpoint. Accounting for a dropout rate of approximately 20%, we would have 60 subjects per group and a detectable difference of +/−0.52 standard deviations. The study will have 80% power to demonstrate a treatment effect on ADAS-Cog[39] with alpha of .05 (2-sided) based on a t-test with transformation of the data if required to meet the assumptions of the t-test.

2.8.2 Measures to Minimize Bias—All analyses will be based on all randomized patients (intent to treat). Changes from baseline over time will be compared using mixed effects regression models that include a random effect for each subject and fixed effects of treatment group, center, and time, and include the repeated observations on each patient over time taking into account that the observations are not independent within a patient. Interactions between treatment group, center, and time will also be considered included in the models. The effects of potential confounding factors such as age, education, LH and other hormone levels, leuprolide levels, donepezil level and/or other measure of donepezil compliance (study drug compliance is ensure by injection at study visits) and baseline cognitive scores on outcomes will be explored. All variables will be transformed as necessary to meet the assumptions of the proposed analyses.

2.8.3 Primary Analysis—The primary efficacy analysis will be based on the difference between the leuprolide treated and placebo-treated group groups with respect to changes from baseline at 48 weeks in ADAS-Cog[39] score. Mixed effects regression models will be used, and the primary efficacy assessment will be based on the 48-week ADAS-Cog measurement. Additional analyses will consider changes from baseline at each time point up to and including 48 weeks and sensitivity analysis based on examination of results in dropouts vs those who complete the study by treatment group among other analyses.

2.8.4 Secondary analyses—Secondary analyses will be based on the difference between the two treatment groups with respect to changes from baseline in laboratory measurements and scoring instruments (ADCS-ADL,[47] ADCS-CGIC+,[40] NPI-Q;[48] BI,[49] Brief Pain Inventory[50] and the RBANS[51]) at 48 weeks.

2.8.5 Planned subgroup analyses—Results will be presented for specified subgroups of subjects based on relevant factors including APOE4 genotype status (APOE4 allele present or absent) and initial MOCA (high = 21–30; low = 11–20) or Blind MOCA (high = 16–22; low = 8–15) score for each of the primary and secondary outcomes. No statistical adjustments will be made for these multiple subgroup analyses.

All primary and secondary outcomes will be summarized descriptively in the amyloid negative sub-study consisting of subjects diagnosed clinically with AD but found to be amyloid negative via Neuraceq PET scanning.

2.8.6 Interim Analyses—One interim analysis for efficacy and futility will be conducted when 50% of the randomized patients could have completed 12 months of follow up based on the comparison of changes from baseline in ADAS-COG at 12 months estimated from the mixed effects regression models. This interim analysis is expected to occur at approximately 30 months after the study starts with approximately 75 patients who could have completed 12 months of follow-up.

2.8.7 Biomarker Analyses—Pre- to post-treatment change will be compared between patients treated with leuprolide versus placebo for plasma inflammatory markers (IL1-β, TNF-α, IL6, ESR, CRP) with appropriate correction for multiple comparisons. For structural MRI measures, pre- to post-treatment change in Freesurfer-measured bilateral regional or total gray matter volume, normalized to total intracranial volume, and including age and site as covariates, will be compared between treatment groups. For ASL MRI, average bi-hippocampal perfusion will be quantified as described previously,[59, 60] and pre- to post-treatment change will be compared between treatment groups. Methods are similar to those of the primary analysis. Further, these results for each subject will be integrated with the primary study results and the contributions of these markers to outcome assessed.

2.8.8 Exploratory Analyses—To identify whether leuprolide has a disease-modifying effect in AD, correlations between neuroimaging biomarkers and cognitive and functional scores will be assessed. Exploratory analyses will assess pre-treatment plasma inflammatory markers as predictors of leuprolide efficacy and MRI changes, and whether plasma kisspeptin levels (which could plausibly mediate the synergy between leuprolide and donepezil[44]) change with treatment (vs placebo) and/or predict leuprolide efficacy.

3 Discussion

Effective therapies for treating AD are desperately needed. Treatments aimed at reducing or removing amyloid have not been successful to date.[2] The LUCINDA trial is one of the first trials premised on the promising Cell Cycle Theory of Aging and AD.[3, 4] It uses a safe, repurposed agent (leuprolide) which targets multiple pathophysiological mechanisms in AD. By repurposing an existing medication, in combination with a current AD treatment (donepezil), we will be able to build upon extensive previous research and development efforts, reducing the time frame and costs of making a promising therapy available to patients with AD. The LUCINDA trial protocol required solving a number of logistical and

other problems. We highlight these issues and innovations with the hope that this will benefit other clinical investigators.

3.1 Amyloid negative sub-study

Thirty subjects diagnosed clinically with AD and meeting all study inclusion criteria except found to be amyloid negative at Neuraceq PET scanning will be randomized into a separate sub-study with procedures identical to the main study. The rationale for enrolling these subjects is that (1) the prior trial of leuprolide for AD,[8] which met its endpoint in a prespecified subgroup, would have likely included approximately 15% amyloid negative subjects[64] since amyloid PET was not performed, (2) there is preclinical evidence indicating that amyloid-negative older rodents benefit cognitively from leuprolide,[35] (3) there is increasing realization that AD is highly heterogeneous, with the majority of patients diagnosed in life with AD actually having mixed pathology at autopsy[65] and (4) the cost of maintaining study subjects who have already undergone MRI, PET and other screening procedures is relatively low. The rationale for not including amyloid-negative subjects in the primary study analysis is that they do not actually have AD according to current research criteria.[64, 66] Enrolling these subjects in a separate sub-study was considered an appropriate strategy for addressing these issues. We suggest this could be considered for other trials of AD therapies not focused exclusively on amyloid removal.

3.2 Study Drug Procurement Issues:

While repurposing an existing medication was expected to be quicker, simpler and less expensive than developing an entirely new drug for AD, we encountered significant difficulties procuring the leuprolide formulation we had originally planned to use in this trial. This leuprolide formulation, though off-patent, was not available in generic form because of technical barriers to manufacturing. The sole manufacturer of this leuprolide formulation, which has its own AD therapies under development, was not willing to provide study drug for LUCINDA at any price. Attempting to purchase this leuprolide formulation from retail pharmacies revealed remarkable differences in price across the three study sites ranging from $300 to $6000 per dose, with significant price fluctuations over time. Realizing use of the original formulation of leuprolide was logistically and financially not feasible, we approached a different company, Tolmar Pharmaceuticals, which manufactures Eligard, a newer version of leuprolide. Tolmar agreed to provide Eligard at no cost for this trial. However, trial enrollment was delayed substantially because of the need to switch study drug formulation. This is an example of a major barrier to drug repurposing to treat AD and other human disorders, and highlights the need to incentivize cooperation from pharmaceutical companies.[67]

3.3 Trial modifications due to the Covid-19 pandemic

The Covid-19 pandemic shut down clinical research for months. Before enrolling the first subject, we modified LUCINDA procedures significantly to ensure that we could safely enroll subjects while minimizing infectious risk. The first LUCINDA subject was enrolled in November 2020, in the midst of the pandemic. The study visit schedule minimized the number of in-person visits to those required for PET, MRI scanning and study drug injection. We are conducting informed consent remotely in accord with the FDA's 2016

guidance document “Use of Electronic Informed Consent: Questions and Answers Guidance for Institutional Review Boards, Investigators, and Sponsors” (81 FR 90855.) We have IRB approval to obtain remote informed consent via several methods depending on the communication technology available to the subject. Our consent form mentions specifically the risk of Covid-19 exposure during study visits, which arguably constitutes greater health risk to participants than any other trial procedures including study drug injection.

As detailed in Section 2.69, we modified study assessments for remote use whenever possible. For assessments which involve only verbal communication or filling out surveys, this was relatively simple, e.g. we converted the paper BI[49] and NPI-Q,[48] designed for caregiver self-administration, into redcap surveys which can be administered via any electronic device in any location. In some cases, we were able to use existing, validated instruments such as the MOCA-Blind.

For the ADAS-cog,[39] our primary outcome measure, we were wary of relying upon fully remote administration. Cognitive testing via teleconference is limited by test requirements for paper or other physical test materials and by the subject/caregiver’s technological capabilities and equipment, with significant variations in testing conditions (technological and environmental) across subjects and potentially within subjects across time periods. While prior studies have shown acceptable correlation between the results of in-person and remote video teleconference ADAS-cog administration,[68, 69] the correlation was less good in subjects with greater cognitive impairment and was particularly poor in the domain of language.[69] This finding may reflect the ADAS-cog’s use of real objects to assess naming ability, and the fact that physical, graspable objects are processed differently by the brain than 2-D depictions of those objects.[70, 71] Children perform better when naming real objects as compared to pictures of those objects,[72] and it is plausible that this may also be the case for cognitively impaired subjects, though we are unaware of specific studies demonstrating this. We therefore designed a hybrid ADAS-cog administration involving both an on-site study coordinator and a remote tester in a separate location. This hybrid administration was designed to be appropriate for use even when Covid-19 is no longer a significant risk. An additional benefit of this mode of administration in multi-site studies is that it allows trained testers at one site to serve as back-up or even primary testers for other sites, regardless of physical distance.

3.4 Conclusion

We hope presenting the LUCINDA trial protocol and discussing implementation challenges and solutions will prove useful to other clinical researchers.

Acknowledgments

Funding

This work was supported by the National Institute of Aging of the National Institutes of Health, grant number R01AG057681 and by the Weill Cornell Clinical and Translational Science Center (grant UL1RR 024996.) Partial support for design (JDG) provided by UL1RR029893 from NIH/NCRR NYU+HHC Clinical Translational Science Award

Appendix

Appendix 1.

Schedule of Study Visits and Procedures

		SCREENING	SCREENING PET & MRI	BASELINE 1st injection	2nd injection	3rd injection	4th injection	f/u MRI	PK/PD
VISIT		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
PROCEDURE	LOCATION	≤31 days before baseline	≤31 days before baseline	WEEK 0	WEEK 12	WEEK 24	WEEK 36	WEEK 48	WEEK 52
Informed Consent	remote	X							
Medical & Social History	remote	X							
Review of concomitant medications	Remote or in person	X		X	X	X	X	X	
CDR	Remote	X							
MOCA-Blind	remote	X							
Geriatric Depression Scale – Short Form[63]	remote	X							
Hachinski Ischemia Score	remote	X							
Physical/ Neurologic Examination	In person		X					X	
Weight & vital signs	In person		X	X	X	X	X	X	
ECG	In person		X						
Blood for B12, homocysteine, RPR, thyroid panel, HA1C, lipid panel	In person		X						
Blood for CBC, metabolic panel	In person		X	X	X	X	X	X	
Review Inclusion/ Exclusion Criteria	remote	X	X						
Multisequence MRI	In person		X					X	
Neuraceq PET	In person		X						
Randomization	remote			X					
ADAS-Cog[39]	hybrid			X	X	X	X	X	
ADCS-CGIC+	remote			X	X	X	X	X	
Burden Inventory, ADCS-ADL, Neuropsychiatric Inventory	Remote or in person			X	X	X	X	X	

		SCREENING	SCREENING PET & MRI	BASELINE 1st injection	2nd injection	3rd injection	4th injection	f/u MRI	PK/PD
VISIT		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Brief Pain Inventory[50]	In person			X	X	X	X	X	
RBANS except coding	remote			X		X		X	
RBANS coding	In person			X		X		X	
Blood for ApoE genotyping	In person			X					
Blood for cytokines, CRP	In person			X		X		X	
Blood for covariate analyses: estrogens, testosterone, progesterone, SHBG, inhibin, kisspeptin, donepezil	In person			X		X		X	
Blood for covariate and PK/PD analyses: LH, FSH and leuprolide	In person			X	X	X	X	X	X
STUDY DRUG INJECTION (must occur after any blood tests or assessments scheduled for that visit)	In person			X	X	X	X		
AE assessment	Remote or in person				X	X	X	X	X
Unblinding questionnaire	Remote or in person							X	

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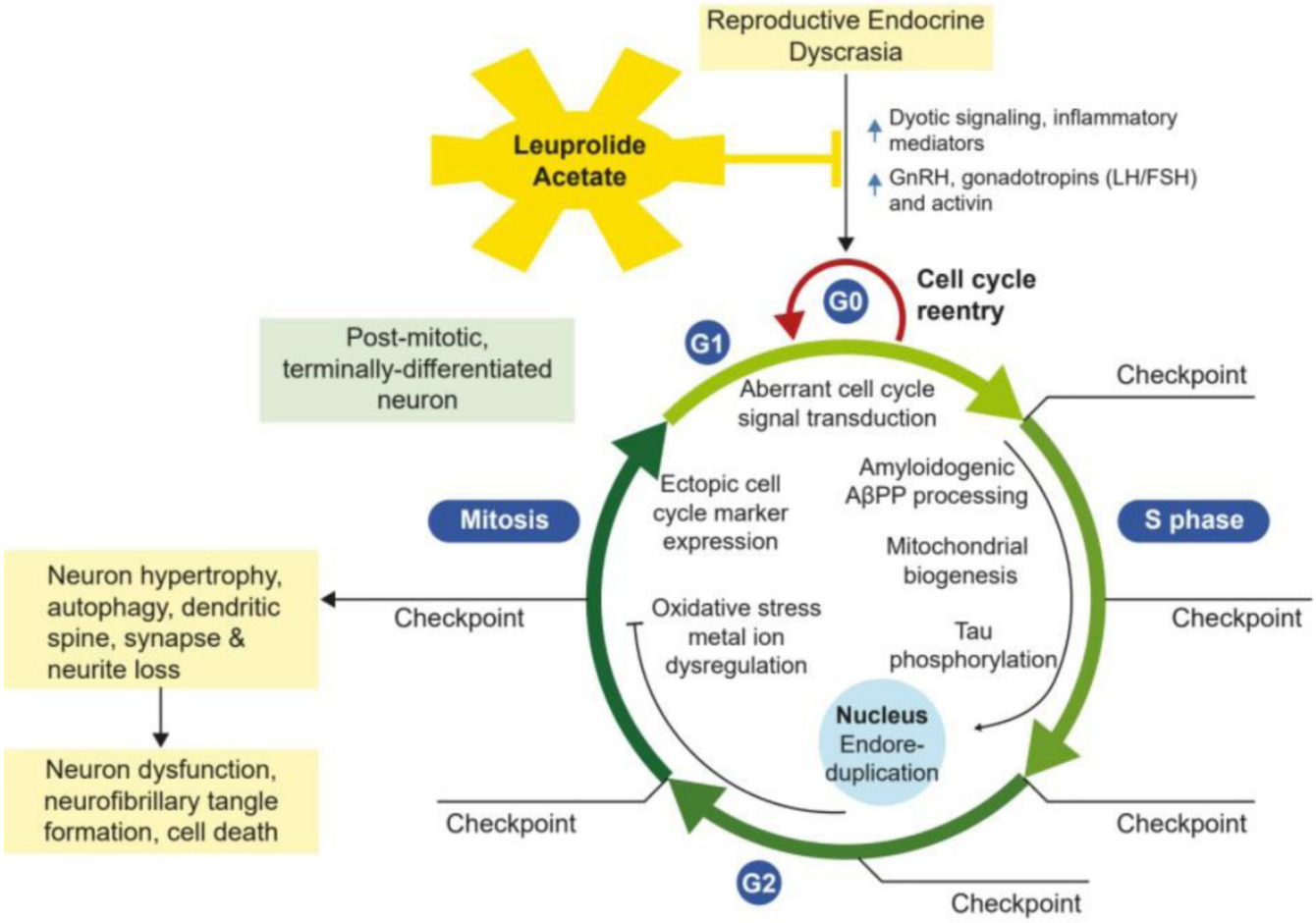

Figure 1. Leuprolide Acetate Action on Neuronal Cell Cycle.

Model of aberrant cell cycle re-entry initiated by endocrine dyscrasia (low sex hormones but elevated GnRH, LH/FSH and activin signaling) following menopause/andropause. The abortive reactivation of the cell cycle in a post-mitotic, terminally differentiated neuron drives endoreduplication, neuron hypertrophy, amyloid deposition, neurofibrillary tangle formation, autophagy, dendritic spine, synapse and neuron loss, neuron dysfunction, and ultimately cell death. Leuprolide acetate suppresses post-reproductive elevations in GnRH and LH/FSH, and thereby blocks the re-entry of neurons into the cell cycle, preventing neurodegeneration.

Table 1.

Inclusion and exclusion criteria.

Key Inclusion Criteria for the main study are:

- Age >65
- Female, post-menopausal
- Probable AD or MCI due to AD according to NIA-AA criteria[45, 53]
- Clinical Dementia Rating (CDR) between 0.5 and 2
- Amyloid present in brain based on interpretation of Neuraceq PET by a board-certified radiologist
- Taking a stable dose of the AChEI donepezil for at least 90 days prior to baseline, and dosage likely to remain stable throughout the trial
- Stable doses of any other medication, supplement or medical food that may affect brain function
- MOCA >11 (out of 30) or MOCA-BLIND > 8 (out of 22) at screening visit
- Hachinski score[54] <5 supporting clinical judgment that dementia is not of vascular origin
- Fluent in English
- Living at home or in a facility other than a nursing home with a caregiver who sees the patient at least three times a week for a total of at least 10 hours and can sign the consent form, accompany the patient on clinic visits, and participate in evaluations

Key Exclusion Criteria for the main study are:

- Presence based on exam, history or MRI of significant brain disease other than AD such as schizophrenia, epilepsy, Parkinson's disease or large territory stroke
- Current substance abuse in accord with DSM V criteria
- Significantly depressed (Geriatric Depression Scale[55] < 10)
- Physical or psychological MRI contraindications, or likely unable to tolerate neuroimaging
- Taking memantine within 90 days of baseline
- Taking medications known to affect serum sex hormones such as GnRH agonists or estrogen and/or progesterone for hormone replacement therapy
- Presence of significant systemic illness likely to interfere with participation in or completion of the study or to affect study results such as cancer within 5 years (other than non-melanoma skin cancer), autoimmune disease, recent myocardial infarction, signs/symptoms of organ failure based on history, ECG, screening laboratory and/or physical exams
- Receiving other investigational drugs within 30 days or 5 half-lives prior to randomization
- Ever treated with active or passive immunization as part of a different clinical trial for AD due to unknown alterations in systemic and brain inflammation, which may confound results
- Known hypersensitivity to GnRH, GnRH analogs or any of the components of Eligard

Criteria for amyloid-negative sub-study:

- same as above except amyloid absent in brain based on interpretation of Neuraceq PET

Table 2.

LUCINDA assessments and mode of administration.

TEST	USE	INCLUSION CRITERIA	ADMINISTRATION
MOCA-Blind	Screening	>11/22	remote
CDR	Screening	.5–2 (global)	remote
Hachinski	Screening	<5	remote
Geriatric Depression Scale[63]	Screening	<11	remote
ADAS-cog[39]	Primary Outcome		Hybrid administration
RBANS	Outcome		Remote except for coding
CGIC	Outcome		Remote
ADCS-ADL	Outcome		Remote or in person – electronic caregiver survey
NPI-Q[48]	Outcome / covariate		Remote or in person – electronic caregiver survey
Burden Inventory	Outcome		Remote or in person – electronic caregiver survey
Brief Pain Inventory[50]	Outcome / covariate		In-person
Unblinding questionnaire	Covariate		Remote or in-person