Journal of Alzheimer's Disease 44 (2015) 549–560
DOI 10.3233/JAD-141626
IOS Press

A Clinical Study of Lupron Depot in the Treatment of Women with Alzheimer's Disease: Preservation of Cognitive Function in Patients Taking an Acetylcholinesterase Inhibitor and Treated with High Dose Lupron Over 48 Weeks

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Handling Editor: Massimo Tabaton

Accepted 8 September 2014

Abstract. To test the efficacy and safety of leuprolide acetate (Lupron Depot®) in the treatment of Alzheimer's disease (AD), we conducted a 48-week, double-blind, placebo-controlled, dose-ranging study in women aged 65 years or older with mild to moderate AD. A total of 109 women with mild to moderate AD and a Mini-Mental State Examination score between 12 and 24 inclusive were randomized to low dose Lupron Depot® (11.25 mg leuprolide acetate), high dose Lupron Depot® (22.5 mg leuprolide acetate), or placebo injections every 12 weeks. There were no statistically significant differences in primary efficacy parameters (ADAS-Cog and ADCS-CGIC), although there was a non-statistically significant trend in favor of the high dose Lupron group on the ADAS-Cog. There were no statistically significant differences in secondary efficacy parameters (NPI, ADCS-ADL, BI, and ADCS-Severity Rating). However, in the *a priori* designated subgroup analysis of patients taking an

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ISSN 1387-2877/15/$27.50

acetylcholinesterase inhibitor (AChEI), there was a statistically significant benefit in the high dose group compared to both the low dose and placebo groups as determined by ADAS-Cog (mean decline: 0.18, 4.21, and 3.30), ADCS-CGIC (% subjects experiencing decline: 38, 82, and 63), and ADCS-ADL (mean decline: −0.54, −8.00, and −6.85), respectively. No differences between treatment groups were seen on the NPI, ADCS-CGI Severity Rating, or the BI in the subgroup analysis. These data indicate that cognitive function is preserved in patients treated with high dose Lupron who were already using AChEIs. The positive interaction between Lupron and AChEIs warrants further investigation for the treatment of AD.

Keywords: 17β-estradiol, acetylcholinesterase inhibitor, Alzheimer's disease, apolipoprotein E, clinical trial, cognitive testing, gonadotropin-releasing hormone, Lupron, luteinizing hormone, women

INTRODUCTION

Age-related changes in hormones of the hypothalamic-pituitary-gonadal axis have been suggested as a major etiological factor in Alzheimer's disease (AD) [1–4]. In addition to the age-related decline in circulating sex steroids, there is evidence to suggest that simultaneous elevations in the circulating concentrations of gonadotropins and gonadotropin-releasing hormone (GnRH) at this time play a role in AD [5–8]. Evidence for suppressing GnRH and gonadotropin signaling in the treatment of AD comes from a growing number of epidemiological, preclinical and biological studies.

Compelling epidemiological data suggest that Lupron Depot® (otherwise referred to as Lupron) treatment decreases the risk for AD in men. The most frequent use of Lupron is for the treatment of prostate cancer. A study, utilizing the Medicare inpatient database, of men who underwent prostatectomy for prostate cancer ($n = 115{,}789$) found that the incidence of dementia within 5 years of the procedure date was ∼ 34–55% that of age-matched men undergoing a similar surgical procedure ($n = 433{,}736$) ([9] and Beaird, Bowen, Perry, Atwood et al., unpublished data). That GnRH agonist treatment was the cause of this dramatic decrease in AD incidence was verified by D'Amico and colleagues [10] who demonstrated a significant 55% reduction in the risk of death from AD in men with prostate cancer treated with a GnRH agonist compared with untreated patients.

Preclinical evidence for the use of Lupron in the treatment of AD comes from studies of both normal and amyloid-β protein precursor (AβPP)-transgenic models of AD. Suppression of gonadotropins with Lupron improves cognitive performance in aged AβPP-transgenic mice [2] while increases in luteinizing hormone (LH)/human chorionic gonadotropin (hCG) have been attributed to cognitive decline in ovariectomized rats [11], LHβ-transgenic mice [12], and ovariectomized C57/Bl6 mice [13]. Moreover, Lupron treatment has been shown to decrease amyloid-β (Aβ) production in C57/Bl6 mice [8] and Aβ load in aged AβPP-transgenic mice [2]. The role of LH in mediating AβPP processing was confirmed in a bigenic mouse model that expresses $A\beta PPsw^{+}$ in the background of a LH receptor (*Lhr*) knockout ($A\beta PPsw^{+}/Lhr^{-/-}$; [14]). Despite the ∼10-fold elevation in AβPP/Aβ production by $A\beta PPsw^{+}$ mice [15], genetic ablation of *Lhr* significantly reduced amyloid load and the total number of Aβ plaques in the hippocampus and cerebral cortex of male and female mice. Genetic ablation of *Lhr* in $A\beta PPsw^{+}$ mice also decreases tau phosphorylation by ∼50% that induced by AβPP overexpression in these mice [14].

Pathological and biochemical studies support the role of gonadotropins in amyloidosis and neurofibrillary tangle formation. LH/hCG promotes the processing of AβPP toward the amyloidogenic pathway *in vitro* [16]. LH induced an increase in the generation and secretion of Aβ, coupled with decreased secretion of AβPP and increased AβPPCT100 production in human neuroblastoma cells [8].

This clinical study was conducted as a dose-ranging study designed to investigate the efficacy and safety of Lupron in the treatment of individuals with AD. In order to minimize any effects due to the loss of sex steroids, it was decided to make this a woman only study since women in this age group are postmenopausal and have little if any endogenous sex steroid production. The study design, patient selection criteria, and outcome measures were guided by regulatory standards in clinical studies. We find that Lupron treatment in combination with acetylcholinesterase inhibitor (AChEI) halts or slows the progression of cognitive decline in women with mild-moderate AD.

METHODS

The study was conducted from April 16, 2003 through December 16, 2004. Participants were

recruited from five U.S. sites. The institutional review board at each site (Baumel-Eisner Neuromedical Institute – three sites; Sun Health Research Institute; Meridien Research) reviewed and approved the study protocol. 109 patients were enrolled who met all of the following criteria: had given their consent by signing the Informed Consent Form and the responsible caregiver also had signed the consent form; or, if the patient was judged by the investigator to be unable to give consent, the legally authorized representative gave consent by signing the consent form and the patient gave assent, in accord with local regulations; were female; were 65 years of age or older; had a diagnosis of probable AD according to the National Institute of Neurological and Communicative Disorders-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria, and the investigator ascertained that the condition had been present at least 6 months prior to screening; were either presently taking a AChEI, and had begun taking it at least 90 days prior to baseline and, in the investigator's opinion, the dosage would likely remain stable throughout the study or they had never taken AChEIs or stopped taking such medication at least 90 days prior to baseline and would likely remain off AChEIs throughout the study; if they were taking other drugs or substances that have purported cognition enhancing properties such as *Ginkgo biloba* and vitamin E, they had begun taking it at least 60 days prior to baseline and, in the investigator's opinion, the dosage would remain stable throughout the study; had scored no lower than 12 or higher than 24 on the Mini-Mental State Examation (MMSE) administered at the screening visit; had a brain imaging study (CT scan, MRI, or PET) performed at the time of their initial diagnosis of AD or after that time, and the findings had been consistent with a diagnosis of probable AD (if a brain imaging study had not been performed, one was performed during the screening process); had a Rosen Modified Hachinski score of 4 or lower at the screening visit, supporting the investigator's clinical judgment that the patient's dementia was "probable AD" and not a dementia of vascular origin; were fluent in English or Spanish and had completed at least 6 years of education; lived at home or in a congregate living facility for requirements other than skilled nursing care, and had a caregiver who saw the patient at least three times a week for a total of at least 10 hours and could sign the consent form, provide information pertinent to the patient's cognitive status, accompany the patient on clinic visits, and participate in the evaluations; hormone replacement therapy, if any, had been stable for at least 60 days prior to baseline, and was not expected to change during the course of the study; scored less than 15 on the Hamilton Depression Scale (17-item version) administered as part of the screening evaluation; values on their screening laboratory tests did not indicate significant medical conditions that would have interfered with their participation in, and completion of, the study.

Exclusion criteria were: The presence of a significant neurological disease affecting the brain, or psychiatric disease other than AD, such as major depression, schizophrenia, epilepsy, Parkinson's disease, or stroke; current significant systemic illness or symptoms of organ failure; a screening electrocardiogram (ECG) that showed evidence of a serious and/or unstable condition or a recent (within 6 months) myocardial infarction; a history of cancer within the last 5 years, except for basal cell or squamous cell cancer, or cervical carcinoma *in situ*; receiving Coumadin or anti-Parkinsonian medications; receiving other investigational drugs within 30 days or 5 half-lives prior to randomization, whichever was longer; taking other medications known to affect serum gonadotropin concentrations, such as gosorelin or danazol, except for estrogen and/or progesterone; had a history of bone fracture secondary to low bone mineral density; had a history of osteoporosis/osteopenia, unless they were receiving therapy for osteoporosis/osteopenia for at least 3 weeks prior to baseline, and the treatment regimen was expected to remain stable; abuse or dependence on alcohol or other substances satisfied criteria for DSM-IV categories 303.9 or 305; had donated blood within 30 days of baseline or were likely to do so during the course of the study.

Intervention

The study was a 48-week, double-blind, placebo-controlled, stratified, parallel-group study conducted in a group of women aged 65 years or older with mild to moderate AD at five sites in the United States. Those whose screening assessments showed that they were eligible to enter the study were assigned to receive either: An 11.25 mg formulation (marketed by TAP Pharmaceuticals Inc. of Lake Forest, Illinois, as Lupron Depot®-3 Month 11.25 mg) given as intramuscular injections; a 22.5 mg formulation (marketed by TAP Pharmaceuticals Inc. of Lake Forest, Illinois, as Lupron Depot® -3 Month 22.5 mg) given as intramuscular injections; or a placebo (physiologic saline) injection. Patients received intramuscular injections of study drug at Day 0 (baseline visit), week 12 (visit 5), week 24 (visit 7), and week 36 (visit 10) (see Table 1

Table 1
Schedule of assessments

	Screening	Baseline	Post-baseline Visits									
Visit Number	1	2	3[1]	4	5	6[1]	7	8	9[1]	10	11	12
Weeks Post-baseline	≤ -6	0	1	4	12	18	24	26	30	36	42	48
Informed Consent	X											
Medical & Social History	X											
Diagnosis of Probable AD[2,3]	X											
MMSE	X											
Rosen Modified HIS	X											
ECG	X											
Ham-D (17 item version)	X	X						X				X
Review Inclusion/Exclusion Criteria	X	X										
AE Assessment		X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination	X											
Height	X											
Hematology & Chemistry	X	X		X	X		X	X		X	X	X
Urinalysis	X											
DEXA scan	X											X
APOE Assay		X										
TSH	X											
FSH, LH, Estradiol Assays[4]		X		X	X		X	X		X	X	X
Weight & Vital Signs	X	X		X	X		X	X		X	X	X
Randomization		X										
Administer Study Drug		X			X		X			X		
Phone Contact			X			X			X			
ADAS-Cog		X		X	X		X	X		X	X	X
ADCS-CGIC				X	X		X	X		X	X	X
NPI		X						X				X
BI		X		X	X		X	X		X	X	X
ADCS-ADL		X		X	X		X	X		X	X	X
ADCS-CGI Severity Rating		X										X

[1]Patients and caregivers were contacted by phone for assessments of safety and concomitant medications. [2]Defined in the NINCDS-ADRDA, including neuro imaging, history or cognitive and memory loss, and examinations to exclude other causes of dementia. [3]Brain imaging was obtained during screening period if not previously obtained after onset of symptoms of AD. [4]Optional blood samples were to be collected only if patients had consented to them in the Informed Consent Form. AD, Alzheimer's disease; MMSE, Mini-Mental State Examination; HIS, Hachinski Ischemic Score; ECG, electrocardiography; Ham-D, Hamilton Depression Rating Scale; AE, adverse event; DEXA, dual-energy x-ray absorptiometry; *APOE*, apolipoprotein E; TSH, thyroid-stimulating hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; ADAS-Cog, Alzheimer's Disease Assessment Scale - Cognitive Subscale; ADCS-CGIC, Alzheimer's Disease Cooperative Study-Clinical Global Impression of Change; NPI, neuropsychiatric inventory; BI, burden interview; ADCS-ADL, Alzheimer's Disease Cooperative Study-Activities of Daily Living Inventory.

for Schedule of Assessment). The study randomization was stratified so that the number of patients with and without evidence of osteoporosis or osteopenia, based on dual-energy x-ray absorptiometry (DEXA) scan findings, was balanced among the three treatment groups. Lupron Depot® is composed of leuprolide acetate, an analogue of the endogenous decapeptide GnRH. It has a substitution of a D-amino acid for glycine at position 6 and deletion of glycine at position 10 with the insertion of ethylamide, causing it to have a longer half-life and much higher affinity for the GnRH receptor than endogenous GnRH [17]. Once administered, it elicits an initial surge in LH and subsequently sex steroids, but within 2 weeks, GnRH receptors are down regulated resulting in very low levels of LH and follicle-stimulating hormone (FSH) [18] (Table 2).

Outcome measures

Outcome and safety measures were evaluated at baseline and weeks 4, 12, 24, 26, 36, 42, and 48. Additional telephone assessments were performed at weeks 1, 18, and 30. The primary efficacy parameters were the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) and the Alzheimer's Disease Cooperative Study Clinical Global Impression of Change (ADCS-CGIC). Secondary efficacy parameters were the Neuropsychiatric Inventory (NPI),

Table 2
Serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) concentrations is per protocol patients

Serum LH in patients treated with placebo or lupron (mean ± SD; mIU/mL)			
Study week	11.25 mg $n=24$	22.5 mg $n=21$	Placebo $n=24$
Baseline	27.7 ± 11.2*	30.9 ± 19.1*	24.1 ± 14.5
Week 4	2.3 ± 0.9	2.6 ± 1.8	25.7 ± 14.7
Week 24	0.7 ± 0.3	0.3 ± 0.2	26.5 ± 15.4
Week 26	0.4 ± 0.2	0.3 ± 0.2	25.8 ± 15.1
Week 48	0.8 ± 0.4	0.4 ± 0.2	25.9 ± 15.3
Serum FSH in patients treated with placebo or lupron (mean ± SD; mIU/mL)			
Study week	11.25 mg $n=24$	22.5 mg $n=21$	Placebo $n=24$
Baseline	52.3 ± 20.9*	48.5 ± 31.6*	46.3 ± 19.9
Week 4	5.0 ± 1.9	3.8 ± 2.4	47.4 ± 20.1
Week 24	7.3 ± 2.6	5.1 ± 3.3	50.8 ± 20.7
Week 26	4.9 ± 1.9	4.7 ± 3.1	50.5 ± 20.9
Week 48	6.8 ± 2.9	5.3 ± 3.5	50.1 ± 20.8

*Statistical difference ($p<0.001$) between Baseline and Weeks 4, 24, 26, and 48 for low and high dose Lupron for serum LH and FSH.

Alzheimer's Disease Cooperative Study-Activities of Daily Living Inventory (ADCS-ADL), Burden Interview (BI), and ADCS-Severity Rating.

Safety was assessed by reviews of treatment-emergent adverse events and post-baseline changes in vital signs, physical examinations, clinical laboratory measures, and bone mineral density.

Bone mineral density

Bone mineral density was measured by means of DEXA scans of the lumbar vertebrae and a hip (including femoral neck). A DEXA scan was performed at screening and the end of study (week 48). The final DEXA scan was performed within 2 weeks before or after the final visit.

APOE genotyping

Direct sequencing of *APOE* genotype was performed by the Michigan State University DNA Diagnostic Program, East Lansing, MI.

Hormonal analyses

Serum LH, FSH and 17β-estradiol concentrations were measured at Quest Diagnostics, Miramar, FL.

Statistical analyses

All groups were analyzed for primary and secondary efficacy endpoints. In addition, pre-defined subgroup analyses included AChEI use and *APOE* status.

Primary efficacy analyses

The primary efficacy analyses were defined as comparisons between treatment groups for scores on the ADAS-Cog and ADCS-CGIC and were performed on the Intent-to-Treat population. The Intent-to-Treat population was defined as patients who received at least one dose of randomized drug and who had at least one post-baseline assessment of at least one primary efficacy variable.

ADAS-Cog: The efficacy analysis of the ADAS-Cog score for both treatment groups (low and high doses of Lupron Depot®) and the placebo group were analyzed by the method of analysis of variance and analysis of covariance. The primary analysis was the two-way analysis of variance model containing the main effects for both the treatment groups and the study sites along with their possible interaction. The final analysis was carried out on the 48-week endpoint by using the change in ADAS-Cog score from baseline.

ADCS-CGIC: The primary efficacy comparisons of the ADCS-CGIC score for both active treatment groups and the placebo group were analyzed by the Cochran-Mantel-Haenszel test which treats study sites as strata. In order to adjust for the other covariate effects, similar tests were based on the strata according to the levels of covariates. These covariates included the baseline osteoporosis/osteopenia status, the *APOE* genotype status, and the education level. If there was a significant association between the treatment groups and the ADCS-CGIC score, the common odds ratios were estimated by the Mantel-Haenszel estimator and the corresponding confidence interval determined across strata that ADCS-CGIC improved (or at least stabilized) over time between each active treatment group and the placebo group. The final analysis was carried out on the 48-week endpoint for ADCS-CGIC score.

Secondary efficacy analyses

The secondary efficacy analyses were the comparisons between treatment groups in scores on the ADCS-ADL, NPI (degree of behavioral disturbances associated with AD), BI (the impact of the patient's illness on the caregiver), and ADCS-CGI Severity Rating. Methods of statistical analysis similar to those

used for ADAS-Cog score were used to analyze the change from baseline for the ADCS-ADL, NPI, and BI. The change from baseline in ADCS-ADL, NPI, and BI were analyzed by ANOVA and ANCOVA with the incorporation of important covariates such as the baseline age, the baseline osteoporosis/osteopenia status, the *APOE* status, and the education level. The effects of treatment on the change in ADCS-ADL, NPI, and BI were assessed using the appropriate hypotheses tests and confidence interval estimations.

In addition, the ADCS-CGI severity rating was summarized descriptively using frequency and percentage for each level of the rating at baseline, and using continuous statistics in the change from baseline at week 48.

In the use of all of these techniques in efficacy analysis, a variety of technical assumptions were required for each type of analysis. In order to assure that the reported results were not simply artifacts of the particular method of analysis, different analyses with a variety of analytic techniques that have slightly differing theoretical assumptions were carried out and compared. In order to control the Type I error rate for the final analysis, Bonferroni's method was used to adjust for the multiple comparisons made between each of the two active treatment groups and the placebo group. However, no adjustment was made for multiple analyses in the *a priori* subgroup analysis of patients taking AChEIs.

RESULTS

Demographic and clinical characteristics

The demographics and baseline characteristics of each treatment group are listed in Table 3. Each group was comparable for all demographic and clinical characteristics ($p > 0.05$) which included: age, race, height, weight, education level, *APOE* genotype, AChEI usage, MMSE score, Rosen Modified Hachinski Ischemic Score, Hamilton Psychiatric Rating Scale for Depression, abnormal physical exam findings at screening, abnormal ECG findings at screening, and 17β-estradiol, LH and FSH concentrations.

Of the 109 patients who entered the study, 37 were assigned to low dose, 36 to high dose, and 36 to placebo. There was no significant difference in completion rates between the groups: 72 patients (66%) completed the study; 25 (68%) in the low dose group, 22 (61%) in the high dose group, and 25 (69%) in the placebo group (Supplementary Table 1).

Primary outcomes

In the primary analysis there was a trend, although not statistically significant, in favor of the high dose Lupron group on the ADAS-Cog. The mean decline in the ADAS-Cog scores after 48 weeks of treatment was 1.7 points in the high dose group compared to 2.4 points in the placebo group and 4.9 points in the low dose group (Fig. 1A). A similar, although not as pronounced trend, was observed for ADCS-CGIC scores with 39% of patients in the high dose group exhibiting decline compared to 54% in the placebo group and 72% in the low dose group (Fig. 1B).

However, in the *a priori* designated subgroup analysis of patients taking AChEIs, there was a statistically significant benefit to subjects as determined by the ADAS-Cog and the ADCS-CGIC in the high dose Lupron group compared to both the placebo and low dose groups (Fig. 2). The mean decline in the ADAS-Cog scores after 48 weeks of treatment was 0.18 points in the high dose group compared to 3.30 points in the placebo group and 4.21 points in the low dose groups (Fig. 2A). Similarly, 9% of patients in the high dose group exhibited decline on ADCS-CGIC scores after 48 weeks of treatment compared to 63% in the placebo group and 82% in the low dose group (Fig. 2B). In patients not taking AChEIs, there was no significant difference by the ADAS-Cog and the ADCS-CGIC between individuals in the high dose Lupron, low dose Lupron, or placebo groups (see Supplementary Figure 1).

Secondary outcomes

In the primary analysis, there was no statistically significant difference on any of the secondary outcome measures, which included the ADCS-ADL (Fig. 3), the NPI, the ADCS-CGI Severity Rating, and the BI.

However, in the *a priori* subgroup analysis, patients taking high dose Lupron showed a statistically significant benefit seen on the ADCS-ADL. The mean decline in the high dose group was 0.54 points compared to 6.9 points in the placebo group and 8.0 points in the low dose group (Fig. 3). No differences between treatment groups were seen on the NPI, ADCS-CGI Severity Rating, or the BI in the subgroup analysis. In patients not taking AChEIs, there was no significant difference by the ADCS-ADL between individuals in the high dose Lupron, low dose Lupron, or placebo groups (see Supplementary Material).

It is known that patients who are homozygous for *APOE* ε4 allele have an increased risk of AD.

Table 3
Demographics and baseline characteristics of each treatment group

Characteristic	Category	Lupron 11.25 mg ($n=36$)	Lupron 22.5 mg ($n=36$)	Placebo ($n=36$)	*p-value*
Age (years)	Mean ± Std	78.75 ± 6.25	78.25 ± 6.01	76.97 ± 5.54	0.461[1]
	Median	80.0	80.0	77.5	
	Interquartile Range	73.5 – 83.0	73.5 – 83.0	74.0 – 80.0	
	Min-Max	67–93	67–88	65–88	
Race	Caucasian	30 (83.3%)	26 (72.2%)	27 (75%)	0.514[2]
	African-American	1 (2.8%)	2 (5.6%)	0	
	Hispanic	5 (13.9%)	8 (22.2%)	9 (25.0%)	
Height (inches)	Mean ± Std	60.95 ± 1.94	60.97 ± 2.88	61.58 ± 2.53	0.508[1]
	Median	61	61.5	61.3	
	Interquartile Range	59.0 – 62.0	59.0 – 62.8	60.0 – 64.0	
	Min-Max	57.0 – 64.5	55.0 – 67.0	56.0 – 67.0	
Weight (pounds)	Mean ± Std	131.9 ± 27.3	139.4 ± 20.9	140.4 ± 25.1	0.373[1]
	Median	132.0	134.8	136.5	
	Interquartile Range	113.0 – 147.0	127.5 – 146.0	123.0 – 152.3	
	Min-Max	92.0 – 220.0	108.0 – 193.0	95.0 – 223.0	0.817[2]
Education	Grade 6	6 (16.7%)	6 (16.7%)	6 (16.7%)	
	High school Grad	20 (55.6%)	21 (58.3%)	23 (63.9%)	
	Some College	5 (13.9%)	4 (11.1%)	3 (8.3%)	
	College Grad	5 (13.9%)	4 (11.1%)	2 (5.6%)	
	Post-Grad	0	1 (2.8%)	2 (5.6%)	
APOE Genotype	2/3	2 (5.6%)	2 (5.6%)	0	0.484[2]
	2/4	0	3 (8.8%)	1 (2.8%)	
	3/3	15 (41.7%)	16 (44.4%)	12 (33.3%)	
	3/4	16 (44.4%)	12 (33.3%)	18 (50.0%)	
	4/4	1 (2.8%)	2 (5.6%)	2 (5.6%)	
AChEI	Yes	28 (77.8%)	23 (63.9%)	26 (72.2%)	0.462[2]
	No	8 (22.2%)	13 (36.1%)	10 (27.8%)	
Estrogen supplementation	Yes	4	1	3	
Serum 17β-estradiol (pg/mL)	Mean±Std	73.8 ± 214.9 ($n=36$)	21.1 ± 25.0 ($n=35$)	32.3 ± 48.3 ($n=35$)	>0.05[3]
	Median	15.5	15.0	17.0	
	Interquartile Range	11.0 – 25.0	11.0 – 20.0	10.0 – 22.0	
	Min-Max	10.0 – 1170.0	10.0 – 155.0	10.0 – 214.0	
FSH (mIU/mL)	Mean±Std	48.2 ± 22.2	52.4 ± 28.8	48.8 ± 21.8	
	Median	45.1	48.0	43.8	>0.05[3]
	Interquartile Range	32.5 – 63.7	30.9 – 70.8	32.7 – 65.4	
	Min-Max	7.4 – 105.0	13.3 – 145.0	15.7 – 106.0	
LH (mIU/mL)	Mean±Std	27.7 ± 15.0	33.6 ± 22.6	27.6 ± 14.8	>0.05[3]
	Median	26.3	30.5	23.5	
	Interquartile Range	22.0 – 32.3	18.9 – 40.9	17.6 – 36.1	
	Min-Max	4.3 – 84.5	3.9 – 130.1	3.3 – 71.9	
ADAS-Cog	Overall	19.73 ± 6.41	20.14 ± 9.36	21.90 ± 9.50	>0.29[3]
	Sub-group analysis (AChEI users)	20.73 ± 5.94	20.31 ± 9.03	24.29 ± 9.93	>0.06[4]
ADCS-ADL	Sub-group analysis (AChEI users)	59.2 ± 7.8	55.8 ± 12.7	55.6 ± 13.9	
NPI	Overall	8.9 ± 11.8	8.8 ± 9.6	9.1 ± 8.5	1.00[5]
MMSE	Overall	18.2 ± 3.3	18.6 ± 3.5	17.9 ± 3.3	0.33[6]
Rosen Modified HIS	Overall	0.72 ± 0.74	0.50 ± 0.56	0.72 ± 0.88	0.82[6]
Ham-D	Overall	3.3 ± 3.0	4.1 ± 3.6	4.6 ± 3.2	0.12[6]
Abnormal physical findings	Overall	29 (81%)	32 (89%)	26 (72)	0.13[7]
Abnormal ECG findings	Overall	20 (56%)	30 (83%)	27 (75)	0.008[7]

[1] *p-values* for treatment comparisons using a two-way analysis of variance test with factors of treatment and site (if the assumptions of ANOVA are satisfied) or using Friedman's test if these assumptions are not satisfied. [2] *p-values* for treatment comparisons using the Cochran-Mantel-Haenszel test for general association, adjusted for site. [3] *p-values* for baseline serum hormone concentrations. [4] *p-value* for placebo versus high dose group. [5] *p-values* and confidence intervals for treatment comparisons from analysis of variance with treatment and site as factors. [6] *p-values* for treatment comparisons using Friedman's test with factors of treatment and site. [7] *p-values* for treatment comparisons using Cochran-Mantel-Haenszel test for general association, adjusted for site. MMSE, Mini-Mental State Examination; HIS, Hachinski Ischemic Score; ECG, electrocardiography; Ham-D, Hamilton Depression Rating Scale; *APOE*, apolipoprotein E; FSH, follicle-stimulating hormone; LH, luteinizing hormone; ADAS-Cog, Alzheimer's Disease Assessment Scale - Cognitive Subscale; NPI, neuropsychiatric inventory; ADCS-ADL, Alzheimer's Disease Cooperative Study-Activities of Daily Living Inventory; AChEI, acetylcholinesterase inhibitor.

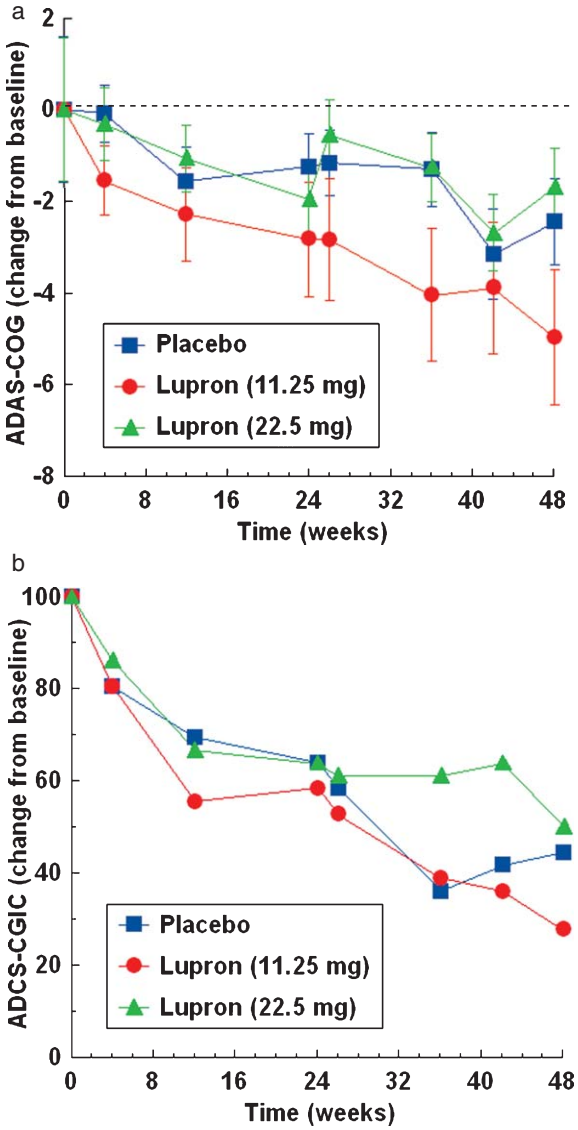

Fig. 1. Changes in cognitive performance over 48 weeks in individuals treated with placebo, low dose Lupron, or high dose Lupron as determined with (A) ADAS-Cog and (B) ADCS-CGIC ($n = 36$ in each group). For ADAS-Cog and ADCS-CGIC, there were no significant differences for placebo versus high dose, placebo versus low dose, and low dose versus high dose at 26 and 48 weeks.

Sub-analyses were performed for efficacy endpoints based upon patients' *APOE* status. No statistical differences were found.

Safety

The safety profile of Lupron at doses similar to those used in this study has been established in other indications such as the treatment of advanced prostate cancer,

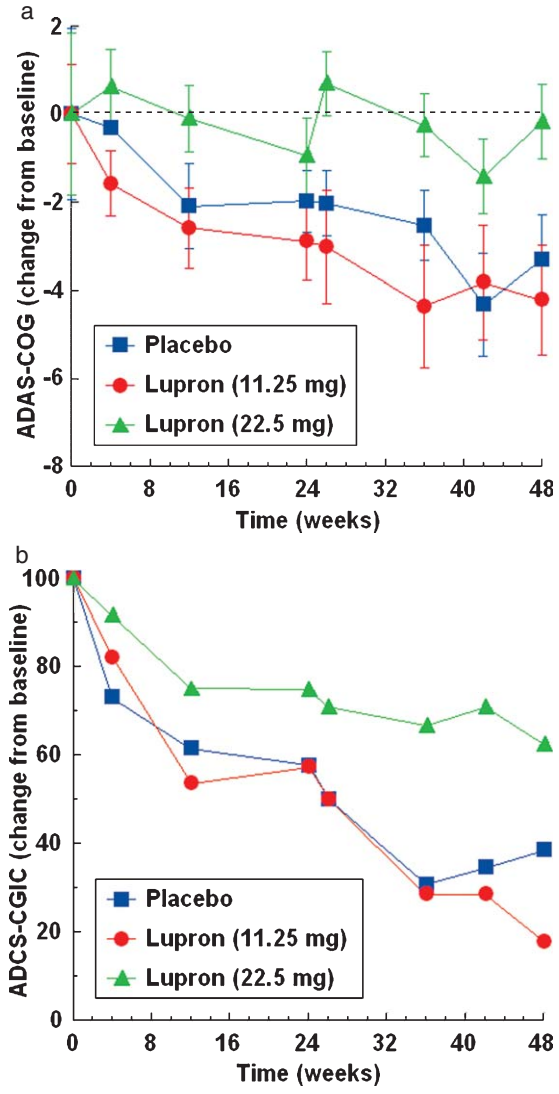

Fig. 2. Changes in cognitive performance over 48 weeks in individuals taking AChEIs and treated with placebo ($n = 26$), low dose ($n = 28$), or high dose ($n = 24$) Lupron as determined with (A) ADAS-Cog and (B) ADCS-CGIC. A) Statistical differences, ADAS-Cog - Adjusted for multiple comparisons: *p-value* and 95% confidence interval for the high dose group versus placebo with treatment and site as factors using ANOVA = 0.037 (−5.78, −0.18) and with treatment and site as factors and baseline ADAS-Cog score as covariate using ANCOVA = 0.057 (−5.67, 0.08) at week 26. *p-value* and 95% confidence interval for the high dose group versus placebo with treatment and site as factors using ANOVA = 0.042 (−6.14, −0.11) and with treatment and site as factors and baseline ADAS-Cog score as covariate using ANCOVA = 0.060 (−6.08, 0.12) at week 48. ADAS-Cog - Unadjusted for multiple comparisons: The p values, unadjusted for multiple analyses for high dose and placebo were = 0.0009 and 0.026 at weeks 26 and 48, respectively. B) Statistical differences, ADCS-CGIC - Adjusted for multiple comparisons: There were no statistical differences between any treatment groups. ADCS-CGIC - Unadjusted for multiple comparisons: The *p-values*, unadjusted for multiple analyses for high dose and placebo, were 0.223 and 0.031 at weeks 26 and 48, respectively.

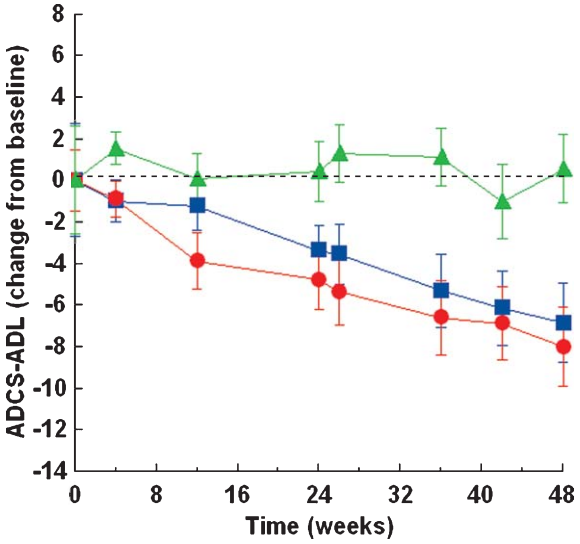

Fig. 3. Changes in cognitive performance as determined by ADCS-ADLs over 48-weeks in individuals treated with placebo ($n=26$), low dose ($n=28$), or high dose ($n=24$) with AChEIs. The *p-values*, unadjusted for multiple analyses for high dose and placebo, were = 0.016 and 0.015 at weeks 26 and 48, respectively.

children with central precocious puberty, endometriosis, and uterine fibroids. However, the safety of Lupron treatment in patients with AD has not previously been described. The majority of patients (77 of 109 patients or 71%) experienced at least one adverse event (AE) (Table 4 and Supplementary Table 2). These were mostly mild or moderate in severity and the ADCS safety monitoring committee regarded these as mainly unrelated to study drug. The most common AEs reported were consistent with the known safety profile of Lupron (Supplementary Table 3). There were 8 patient discontinuations due to AEs.

Twenty serious AEs were reported in 18 patients including two deaths. One death was attributed to respiratory failure in the high dose group, and one death was attributed to cerebral hemorrhage in the placebo group. The ADCS safety monitoring committee categorized three AEs as possibly related to study drug: Two cases (upper gastrointestinal hemorrhage and a syncopal episode) occurred in the low dose group and one case (deep vein thrombosis) occurred in the placebo group. Therefore, based on this data, there were no unexpected safety concerns in this patient population. This is consistent with the well-established safety profile of Lupron in the treatment of other conditions.

The extended use of Lupron is known to cause the loss of bone mineral density in men [19–22] and pre-menopausal women [23, 24]. Whether any similar effect occurs in postmenopausal women has not been studied. In our study, there were no significant differences in bone mineral density between any of the treatment groups over 48 weeks of treatment.

DISCUSSION

This Phase II dose-ranging study demonstrated that high dose Lupron in combination with AChEIs halted the progression of cognitive decline in women with mild to moderate AD over a 48-week period (Figs. 2 and 3). A similar effect was not observed in the low dose Lupron group taking AChEIs or in the placebo group taking AChEIs (Figs. 2 and 3). This combination treatment was safe and well tolerated at both dose levels (Table 4) and AEs were consistent with the current product labels for other indications.

In the primary analysis there was a trend, although not statistically significant, in favor of the high dose Lupron group on the ADAS-Cog (Fig. 1). In the *a priori* designated subgroup analysis of patients already taking AChEIs, there was a clear statistically significant benefit demonstrated on the ADAS-cog, ADCS-CGIC, and the ADCS-ADL in the high dose Lupron group compared to the low dose and placebo groups (Figs. 2 and 3).

All drugs currently approved for the treatment of AD confer an initial improvement in cognitive function followed by a decline whose rate is similar to placebo [25]. In contrast to these treatments, there was no initial improvement in cognitive function following initiation of Lupron treatment but most importantly, there was no decline in cognitive performance in the high dose/AChEI group. These findings together with biological and epidemiological evidence suggest that the effects seen with high dose Lupron are one of potential disease modification rather than symptomatic improvement [1–9, 11–14, 26].

The mechanism by which Lupron acts with AChEI to improve cognitive performance is unclear. It is known that the AChEI rivastigmine can reduce the lipopolysaccharide-induced decreases in GnRH and LH, and perhaps stimulate GnRH/LH secretion [27]. In this connection, the modulation of GnRH release has been suggested to be mediated via cholinergic (and GABAergic) neurotransmission [28]. Thus, one possible additive mechanism of action might involve the further downregulation of GnRH receptor signaling and LH expression/signaling. Alternatively, since GnRH mediates neurotransmission itself [29, 30], Lupron might act directly to improve cognitive per-

Table 4
Summary of adverse events (AEs)

	Treatment			*p-value*[1]		
	Leuprolide 11.25 mg ($n = 37$)	Leuprolide 22.5 mg ($n = 36$)	Placebo ($n = 36$)	11.25 versus placebo	22.5 versus placebo	11.25 versus 22.5
Patients with at least 1 AE	27 (72.9%)	27 (75.0%)	23 (63.9%)	0.40	0.31	0.84
Not related	13 (35.1%)	12 (33.3%)	8 (22.2%)			
Probably not related	6 (16.2%)	10 (27.7%)	6 (16.7%)			
Possibly related	7 (18.9%)	3 (8.3%)	8 (22.2%)			
Probably related	1 (2.7%)	2 (5.5%)	1 (2.8%)			
Related	0	0	0			
Patients with serious AE	10 (27.0%)	4 (11.1%)	5 (13.8%)	0.17	1.0	0.08
Not related	7 (18.9%)	1 (2.8%)	2 (5.6%)			
Probably not related	1 (2.7%)	3 (8.3%)	2 (5.6%)			
Possibly related	2 (5.4%)	0	1 (2.8%)			
Probably related	0	0	0			
Related	0	0	0			
Patients with AEs that led to discontinuation	4 (10.8%)	2 (5.5%)	0	0.12	0.49	0.67
Patients with AEs resulting in death	0	1 (2.8%)	1 (2.8%)	0.49	1.0	0.49

At each level of summarization each patient is only counted once. [1] *p-values* for treatment comparisons from Pearson's Chi-square test or Fisher's exact test if appropriate.

formance. Another possibility is that Lupron acts to halt any further neurodegeneration thereby allowing AChEIs to act on remaining neurons to maintain cholinergic function.

The dose effect seen in this study suggests that Lupron's action is not solely due to its suppression of peripheral circulating concentrations of gonadotropins, which were similarly suppressed in low dose and high dose groups (Table 2). Therefore, Lupron's actions might also be due to 9 direct effect on GnRH receptor signaling within the brain [31]. GnRH receptors are expressed throughout the brain and their expression correlates to those areas with AD neuropathology [31]. In this connection, we recently identified the existence of autocrine/paracrine feedback loops within the brain, in essence a feedback loop similar to the hypothalamic-pituitary-gonadal axis that regulates neurohormone production [32]. Since GnRH receptor mediates neuronal LH expression and LH receptor signaling, high doses of Lupron might suppress the neuroautocrine production of LH, which we have previously demonstrated is elevated in expression and colocalizes with AD neuropathology [33], while low doses might stimulate LH production.

This dose effect might also explain some conflicting preclinical results. Most researchers have found that lowering LH signaling with the GnRH agonist Lupron decreases Aβ levels and improves cognitive performance in wild-type mice [8, 34] and AβPP-transgenic mice [2]. However, a decrease in brain Aβ and improvement in cognition following leuprolide acetate treatment was not observed in the overexpressing AβPP(Swt), PS1(M146 V), and tau(P301L) (triple) transgenic mice [35]. Whether this is a dose effect (or an artifact of the 3xTg mice) is not clear since multiple doses have not been evaluated. Future animal studies are warranted to help understand the dose effect and the synergism with AChEIs.

In conclusion, our data demonstrate that cognitive function was preserved in patients treated with high dose Lupron who were already using AChEIs. Caution should be used in the interpretation of the results due to: The small sample size, which did not allow determination of whether this treatment is best suited to early or later phases of the disease; the fact that baseline demographics were not compared for the subgroup; and non-adjustment for multiple analyses. The results of this study should however encourage further investigation of GnRH agonist therapy for the treatment of AD. Future clinical studies should be conducted with Lupron at doses providing systemic exposure at least equivalent to those provided by Lupron 22.5 mg every 12 weeks. Such studies could be expanded to include the use of GnRH antagonists.

ACKNOWLEDGMENTS

The authors thank John Stone for his unwavering support and encouragement. The opinions expressed herein are those of the authors. The contents do not represent the views of the Department of Veterans Affairs or the US Government.

Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=2545).

SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD141626.

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