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Featured Article

A 24-week study to evaluate the effect of rilapladib on cognition and cerebrospinal fluid biomarkers of Alzheimer's disease

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Abstract

Background: The lipoprotein-associated phospholipase A_2 inhibitor (Lp-PLA_2), rilapladib (SB659032), is being evaluated as a potential treatment to slow the progression of Alzheimer's disease (AD).

Methods: One hundred twenty-four subjects with possible mild AD and with neuroimaging evidence of cerebrovascular disease were randomized to placebo or 250-mg rilapladib once daily, for 24 weeks, in addition to stable background acetylcholinesterase inhibitor and/or memantine. The study assessed the safety and tolerability of rilapladib and its effects on cognition, mechanistic, and disease-related biomarkers. Although the overall intent behind the study was to take a broad exploratory view of the data, two primary end points of interest (cerebrospinal fluid [CSF] amyloid beta peptide 1–42 [$A\beta_{1-42}$] and CogState executive function/working memory [EF/WM] composite score at week 24) were prespecified in the analysis plan for inferential statistical analysis.

Results: Rilapladib was well tolerated with no significant safety concerns. A significant difference from placebo was observed for rilapladib on change from baseline in EF/WM (effect size, 0.45; $P = .026$). There was no significant difference between groups on the change from baseline in CSF $A\beta_{1-42}$ ($P = .133$). Preliminary evidence of effects was detected on other mechanistic (albumin quotient) and disease-related biomarkers (tau/P-tau and neurofilament light chain).

Conclusion: These data provide initial evidence supporting Lp-PLA_2 inhibition as a novel treatment for dementia.

Clinical Trial Registration: Clinicaltrials.gov identifier: NCT01428453.

Keywords: Lp-PLA_2; Alzheimer's disease; Cerebrovascular disease; Cognition; Rilapladib; SB659032; Tau; Amyloid-beta peptide; Albumin quotient; Neurofilament light chain; Cerebrospinal fluid; Biomarkers; Small vessel disease

1. Introduction

Rilapladib (SB659032) is a potent and selective inhibitor of the enzyme lipoprotein-associated phospholipase A_2 (Lp-PLA_2). Lp-PLA_2 is a calcium-independent phospholipase A_2 that is actively secreted by monocyte-derived macrophages, T lymphocytes, and mast cells and circulates in plasma as a complex with low-density lipoprotein (LDL) and, to a lesser extent, high-density lipoprotein [1]. A range of studies demonstrate that inhibition of Lp-PLA_2 can reduce peripheral measures of inflammation in nonclinical [2] and clinical studies [3–5]. Based on nonclinical data, rilapladib is not believed to be brain penetrant and has been evaluated previously in subjects with stable atherosclerosis [6].

Lp-PLA_2 has substrate specificity toward oxidized phospholipids, in particular, those containing a polar fatty acid moiety that are generated during the oxidation of LDL

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(oxLDL) and apoptosis [1]. Lp-PLA_2 rapidly cleaves oxidized phosphatidylcholine in tissue, generating proinflammatory lysophosphatidylcholine (lysoPC) and oxidized nonesterified fatty acids. LysoPC has also been demonstrated to be a mediator of inflammatory stress on brain microvascular endothelial cells [7] and to increase the permeability of endothelial cells [8]. Literature supports that oxLDL can be detected in the central nervous system (CNS) after blood-brain barrier (BBB) disruption [9].

In a diabetic mellitus (DM) and hypercholesterolaemic (HC) pig model, treatment with darapladib (another Lp-PLA_2 inhibitor) numerically reduced the extent of immunoglobulin G brain parenchyma penetration suggesting a reduction in BBB leakage and significantly lowered the total amount of brain amyloid beta peptide 1–42 ($A\beta_{1–42}$) deposition compared with untreated DM/HC pigs [10]. Both findings are relevant and potentially linked, through brain Aβ efflux mechanisms at the BBB, to the pathogenesis and progression of Alzheimer's disease (AD) [11,12].

Age-related cerebrovascular dysfunction, and associated cerebrovascular disease (CVD), plays an important role in the initiation and progression of AD [13–15]. Cerebral small vessel disease (SVD) is a CVD subtype that is associated with a high proportion of AD cases [16–18]. The associated pathologic changes in the parenchymal small arteries and arterioles (e.g., arteriolosclerotic changes such as fibrinoid necrosis, lipohylinosis, microatheroma, and microaneurysms) extend to the endothelial barriers of the small vessels and capillaries (i.e., the BBB) resulting in permeability changes and extravasation of plasma components into the vessel walls and brain parenchyma [19]. Postmortem analyses of AD brain tissue have demonstrated changes to the microvasculature through the presence of extravasated serum proteins, such as albumin and immunoglobulin [20–23], as well as white matter lesions and the widespread deposition of cerebral amyloid angiopathy, with associated microbleeds; all of which may contribute to decline in vascular integrity and function [19]. These observations, together with the findings from the nonclinical models, informed on the choice of AD subjects with neuroimaging evidence of CVD (e.g., white matter lesions and/or lacunes, typical of SVD) in the present study.

In summary, it is hypothesized that rilapladib will peripherally reduce the production of proinflammatory and toxic mediators, thereby restoring BBB integrity and reducing its permeability. Resultant, or downstream, effects may include reduced levels of neuroinflammation/toxicity and reductions in CNS Aβ, either through a reduction in influx or a restoration of efflux mechanisms.

The present study was designed to investigate the extent to which the mechanisms observed in preclinical models are present in subjects with AD and CVD and whether any downstream impact on neurodegenerative biomarkers or cognition could be detected over a 24-week treatment period.

2. Methods

2.1. Study design

This exploratory study was a randomized, double-blind, placebo-controlled, parallel group, repeat dose study to evaluate the effect of rilapladib on biomarkers related to the pathogenesis and progression of AD and cognitive function. Subjects were randomized to either 250 mg of rilapladib or placebo once daily for 24 weeks in addition to their stable background therapy (i.e., acetylcholinesterase inhibitor [AChEI] and/or memantine). Study duration was 30 weeks comprising 4-week screening, 24-week treatment period, and 2-week follow-up. The study was conducted at 24 sites in Germany, Spain, Italy, Sweden, Bulgaria, and Canada.

The study was conducted in accordance with the *International Conference on Harmonization Good Clinical Practice guidelines* and the ethical principles that are outlined in the *Declaration of Helsinki 2008*. The protocol was reviewed and approved by ethics committees or institutional review boards at each institution.

2.2. Subjects

Eligible subjects were 50–80 years inclusive and met National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria for possible AD [24]. Subjects had radiological (magnetic resonance imaging [MRI] or computed tomography [CT]) evidence of significant CVD, assessed within the last 12 months, by meeting at least one of the criteria in the following:

MRI evidence: White matter lesions: extending caps, irregular halo, diffusely confluent hyperintensities, or extensive white matter changes.
CT evidence: Extensive periventricular and deep white matter lesions: patchy or diffuse symmetrical areas of low attenuation (intermediate density between normal white matter and cerebrospinal fluid [CSF]), with ill-defined margins extending to the centrum semiovale, and at least one lacunar infarct.
MRI or CT evidence: Lacunar cases: multiple lacunes (e.g., >5) in the deep gray matter.

Subjects were required to have a Mini-Mental Status Examination score of 20–26 at screening, a Clinical Dementia Rating of 0.5 or 1.0, and a documented history of $\geq$6-month AChEI therapy, with two months at a stable dose.

Exclusion criteria included significant psychiatric illness; history/evidence of another cause of dementia; history of seizures; abnormal findings that would preclude participation; treatment with monoamine oxidase inhibitors, conventional antipsychotics, an investigational drug or treatment with a potential for interaction with rilapladib. See Supplementary Materials for further details.

2.3. Study procedures, outcomes, and assessments

CSF was collected at baseline and week 24 (or early withdrawal) for the assessment of $A\beta_{1-42}$ (primary end point of interest), $A\beta_{1-40}$, total tau (T-tau), 181 phosphorylated tau (P-tau), Lp-PLA_2 activity, and as an exploratory marker of axonal degeneration and white matter damage, neurofilament light chain (NF-L). Albumin quotient (AlbQ) was also determined as a marker of BBB permeability. Plasma samples were collected at baseline, week 1, week 12, and week 24 for the assessment of $A\beta_{1-40}$, $A\beta_{1-42}$, and Lp-PLA_2 activity. Pharmacokinetics (PKs) of rilapladib were assessed throughout the study from baseline to week 24. Further details of assays used to measure these end points are provided in the Supplementary Materials.

Cognitive assessment was performed at screening, baseline, week 12, and week 24 (or early withdrawal) using a battery of computerized (CogState [25]) as well as pen and paper neuropsychological assessments, with executive function/working memory composite score (EF/WM) as the primary end point of interest. The individual tests were selected on the basis of being relatively free of floor and ceiling effects and their ability to probe cognitive functions that were likely to demonstrate decline over 24 weeks in the selected population. The individual tests and composite scores are described in the following and in more detail in the Supplementary Materials.

EF/WM composite: Controlled Oral Word Association Test, category naming, one back, Trails B, and Go-NoGo.
Attention composite: Identification (a choice reaction time task) and Trails A.
Episodic memory composite: International Shopping List Task (ISLT) immediate recall, ISLT delayed recall. Note: the reporting and analysis plan prespecified a change to the protocol before unbinding that the episodic memory composite would only include immediate recall.
Overall composite: all nine subtests.

Safety was assessed throughout the study through collection of adverse events (AEs), assessment of vital signs, electrocardiograms, and routine laboratory assessments. In addition, eye examinations (all sites) and electron microscopy of peripheral blood lymphocytes (in sites with appropriate facilities) were performed at screening and week 24 to identify any signs of potential phospholipidosis.

2.4. Randomization and masking

After the run-in period, subjects were randomized in random permuted blocks of four in a 1:1 ratio to double-blind treatment with rilapladib of 250 mg or matching placebo which were provided by GlaxoSmithKline (GSK) as tablets to be taken once daily after breakfast. The randomization schedule was generated by GSK and implemented using an interactive voice-response system. With the exception of two subjects for whom the investigator was unblinded (but not the sponsor) after a code break, all investigator and sponsor staff remained blinded to treatment until the database was finalized.

2.5. Statistical analysis

Although the overall intent behind the study was to take a broad exploratory view of the data, two primary end points of interest were prespecified in the analysis plan for inferential statistical analysis: end of study/week 24 treatment differences between placebo and rilapladib of 250 mg for the ITT population for CSF $A\beta_{1-42}$ and CogState EF/WM. Assuming a post randomization dropout rate of 15%, approximately 120 subjects were randomized to ensure a total of 102 evaluable subjects (51 per group). A sample size of 51 evaluable subjects per arm allowed a difference of 70 pg/mL in CSF $A\beta_{1-42}$ between placebo and rilapladib to be detected with 80% power at a 5% (two sided) significance level assuming an underlying standard deviation (SD) of 120.

Three populations were defined for efficacy and safety analyses: safety (subjects randomized who took at least one dose of study medication), intent-to treat (ITT; subjects in the safety population who also had at least one post-baseline efficacy assessment), and per protocol (PP; subjects in the ITT population who were not major protocol deviators).

Changes from baseline in CSF parameters were analyzed using an analysis of covariance, adjusting for baseline CSF parameter, age, and treatment. Change from baseline in plasma biomarker parameters and CogState end points were analyzed using a mixed model for repeated measures, assuming an unstructured covariance matrix, with the following terms included in the model: treatment, visit, baseline, treatment by visit, and baseline by visit.

Statistical significance was interpreted using a two-sided test at the 5% significance level for the two primary comparisons of interest only. No adjustments were made for multiplicity of these two coprimary end points. CSF $A\beta_{1-42}$ and CogState EF/WM were also analyzed using the PP population.

Results for the analysis of the cognitive end points are presented as differences in standardized scores (Z scores, using the mean and SD for the ITT population at baseline) and effect sizes (treatment difference divided by the standard error).

Results of primary and some secondary end points are also presented as posterior probabilities (using a noninformative prior) for the treatment differences/effect sizes being above relevant thresholds. See Supplementary Materials for more details.

Safety data were summarized using descriptive statistics.

3. Results

3.1. Patient disposition and demographics

Of the 170 subjects screened for the study, 124 were randomized to treatment with rilapladib or placebo. Early withdrawal rates were 9/61 subjects (15%) for rilapladib and 6/62 subjects (10%) for placebo. The most common reasons for withdrawal were AEs (seven subjects [11%] for rilapladib and two subjects [3%] for placebo) and withdrawn consent (one subject [2%] for rilapladib and three subjects [5%] for placebo). Most subjects (93%) in the ITT population were compliant with treatment.

Demographic and baseline characteristics were similar across treatment groups (Table 1). All subjects were Caucasian. Most subjects were receiving treatment with an AChEI with only 12%–13% receiving memantine (Supplementary Table 1). Examination of baseline CSF profiles of $A\beta_{1–42}$, tau, and P-tau indicated that approximately 50%–60% of subjects met the biomarker thresholds for amyloid positivity and tau levels indicative of AD (Table 1).

3.2. PKs and pharmacodynamics

Rilapladib plasma concentrations were within the range of observation from previous studies [6]. The mean plasma exposure to SB-664601, one of the major metabolites of rilapladib, was about 16% of rilapladib.

Target engagement in the plasma was confirmed by approximately 80% reduction in plasma Lp-PLA_2 activity in the rilapladib group. See Supplementary Table 2 for further details.

CSF Lp-PLA_2 activity was an exploratory measure. Mean change from baseline at the end of study was −0.464 μmol/min/L in the rilapladib group and 0.026 μmol/min/L in the placebo group, although the data were variable (SD of 5.4263 and 7.2657 for rilapladib and placebo, respectively). See Supplementary Table 3 for further details.

3.3. Efficacy assessments

3.3.1. Aβ biomarkers

No statistically significant differences were observed for the change in baseline at week 24 for CSF $A\beta_{1–42}$ ($P = .133$; ITT Population, Table 2). PP analysis of CSF $A\beta_{1–42}$ was consistent with the ITT analysis, as was a sensitivity analysis excluding subjects who provided CSF at the early withdrawal visit or follow-up visit (See Supplementary Table 4).

Based on model checking a number of outliers for CSF $A\beta_{1–40}$ and $A\beta_{1–42}$ were identified. A sensitivity analysis excluding these outliers from the ITT population was also conducted and found to reduce the observed differences between treatments for both CSF $A\beta_{1–42}$ and $A\beta_{1–40}$ but still supported the overall interpretation of the ITT analysis (Supplementary Table 4).

Changes in plasma Aβ were small with no differences detected between treatment groups (Table 2).

3.3.2. Albumin quotient

AlbQ was assessed as a mechanistic biomarker of BBB integrity. Bayesian posterior probability that there was a true treatment difference in favor of rilapladib for AlbQ, given the observed data, was approximately 83%. See Table 2.

3.3.3. Other disease-related biomarkers

T-tau and P-tau were assessed as markers of neurodegeneration and Alzheimer-specific neurodegeneration,

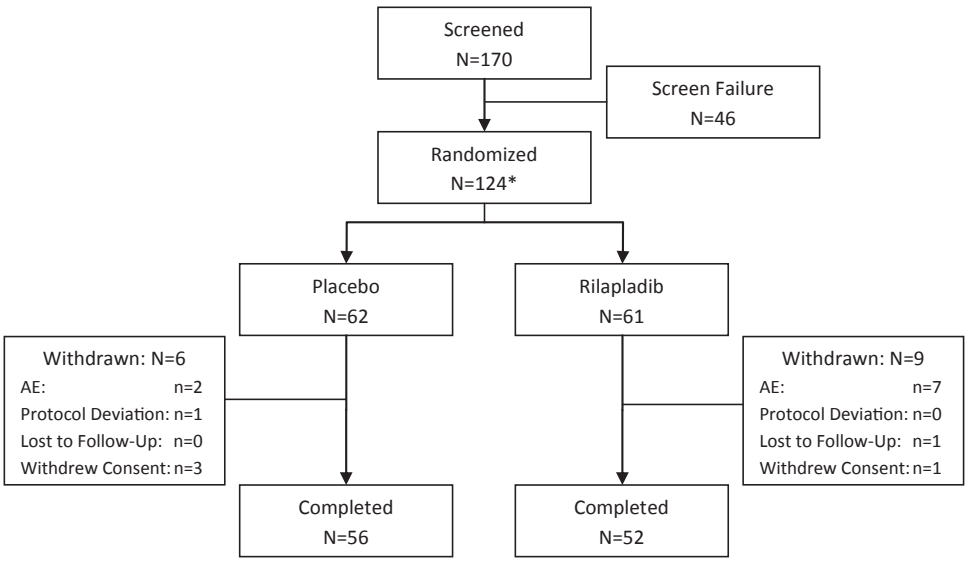

Fig. 1. Subject disposition (safety population). *One subject was randomized in error and did not receive any study treatment. Two further subjects (one in each treatment group) were randomized and received treatment but withdrew before any postbaseline measurements and are therefore excluded from the ITT population. Abbreviations: AE, adverse event; ITT, intent-to-treat.

respectively. NF-L was assessed as a marker of white matter damage. Bayesian posterior probabilities that there was a true treatment difference in favor of rilapladib, given the observed data, were approximately 90% for T-tau and P-tau and approximately 80% for NF-L. See Table 2.

3.3.4. Cognition

Inferential testing on the EF/WM composite score showed that the treatment difference at week 24 was statistically significant (P = .026). Consistent findings were observed for the overall composite score with both end points showing an effect size of >0.4 and a Bayesian posterior probability of >98% that given the observed data, the true effect size was >0. Effect sizes for attention and episodic memory were smaller (Table 3).

The time profile for the EF/WM composite is shown in Fig. 2. The profile for other composite scores was similar.

PP analysis of CogState EF/WM was consistent with the ITT analysis. A number of ad hoc sensitivity analyses were performed on the cognitive data to explore the impact of missing data; these analyses supported the interpretation of the ITT analysis (see Supplementary Table 5 for further details). In addition to the treatment by baseline covariate interaction testing performed for the two primary end points, interaction graphics were produced to explore the relationship between baseline CSF $A\beta_{1–42}$ and the change from baseline in CogState executive function/working memory. None of these interactions were significant; further visual inspection of the interaction graphs did not suggest that baseline $A\beta_{1–42}$ impacted the level of response to rilapladib.

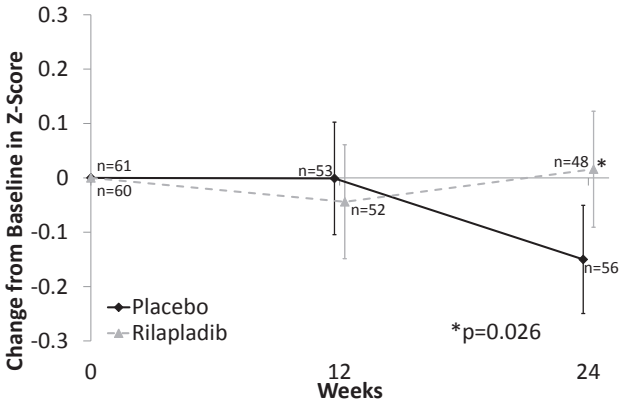

Fig. 2. Time profile of executive function/working memory composite score (adjusted mean change from baseline in Z score ± 95% confidence intervals [MMRM analysis]). Abbreviation: MMRM, mixed model repeated measures.

Table 1
Populations and demographic and baseline characteristics (ITT population)

Population/Characteristic	Placebo	Rilapladib
Randomized population, N	62	62
Safety population, N	62	61
ITT population, N	61	60
PP population, N	54	50
Demographics and baseline characteristics of ITT population		
Gender, % male:female	46:54	53:47
Age, y	73.1 (5.40)	72.9 (5.15)
BMI, kg/m^2	26.5 (4.83)	26.2 (3.28)
Median time since first diagnosis, y (range)	1.3 (1-14)	1.4 (1-5)
MMSE	22.9 (1.98)	22.8 (2.12)
CDR sum of boxes	3.8 (1.66)	3.8 (1.58)
CDR global score, % 0.5:1.0	54:46	53:47
CSF $A\beta_{1–42}$ <600 ng/L (%)	62	60
CSF T-tau >400 ng/L (%)	59	58
CSF P-tau >70 ng/L (%)	38	30
CSF $A\beta_{1–42}$ <600 ng/L and CSF T-tau >400 ng/L or CSF P-tau >70 ng/L (%)	48	50
White matter lesions* (%)	84	77
Multiple (>5) lacunes in the deep gray matter (%)	3	10
Both white matter lesions and multiple (>5) lacunes in the deep gray matter (%)	13	13
Hachinski score†	2.6 (1.78)	3.2 (2.18)

Abbreviations: ITT, intent-to-treat; PP, per protocol; BMI, body mass index; MMSE, Mini-Mental Status Examination; CDR, Clinical Dementia Rating; CSF, cerebrospinal fluid; $A\beta_{1–42}$, amyloid beta peptide 1–42; T-tau, total tau; P-tau, 181 phopshorylated tau.

NOTE. Data presented as mean (SD) unless otherwise stated. Percentages are calculated as percent of subjects in the ITT population.

*White matter lesions were defined as computed tomographic evidence of extensive periventricular and deep white matter lesions: patchy or diffuse symmetrical areas of low attenuation (intermediate density between normal white matter and cerebrospinal fluid), with ill-defined margins extending to the centrum semiovale, and at least one lacunar infarct and/or magnetic resonance imaging evidence of white matter lesions: extending caps, irregular halo, diffusely confluent hyperintensities, or extensive white matter changes.

†Hachinski scores were collected retrospectively after enrollment from review of historical medical records.

Table 2
Statistical analysis of change from baseline in CSF and plasma biomarkers at week 24/end of study (ITT population)

Biomarker*	Treatment	N	Adjusted mean (SE)	Difference vs. placebo†, Δ (95% CI)	Posterior probability Δ < 0
CSF $A\beta_{1–42}$ (ng/L)	Placebo	53	−6.3 (18.10)	39.8 (−12.4, 92.0)	0.934 (Δ > 0)
	Rilapladib 250 mg	48	33.6 (19.02)	$P = .133$‡	0.573 (Δ > 35)
					0.127 (Δ > 70)
CSF $A\beta_{1–40}$ (ng/L)	Placebo	53	−77.4 (181.33)	−250.3 (−772.9, 272.2)	0.829
	Rilapladib 250 mg	48	−327.7 (190.56)		
CSF $A\beta_{1–42}$/CSF $A\beta_{1–40}$ ratio	Placebo	53	0.002 (0.0068)	0.016 (−0.003, 0.036)	Not calculated
	Rilapladib 250 mg	48	0.018 (0.0071)		
AlbQ	Placebo	46	0.11 (0.172)	−0.24 (−0.74, 0.26)	0.828
	Rilapladib 250 mg	40	−0.13 (0.184)		
T-tau (ng/L)	Placebo	52	38.2 (29.98)	−57.1 (−144.5, 30.3)	0.902§
	Rilapladib 250 mg	46	−18.8 (31.90)		
P-tau (ng/L)	Placebo	52	1.3 (1.67)	−3.0 (−7.9, 1.8)	0.892§
	Rilapladib 250 mg	47	−1.7 (1.76)		
NF-L (ng/L)	Placebo	52	191.2 (215.45)	−256.6 (−878.6, 365.4)	0.792§
	Rilapladib 250 mg	48	−65.4 (224.38)		
Plasma $A\beta_{1–42}$ (ng/L)	Placebo	51	1.5 (0.90)	−1.3 (−3.9, 1.2)	Not calculated
	Rilapladib 250 mg	47	0.2 (0.94)		
Plasma $A\beta_{1–40}$ (ng/L)	Placebo	51	8.8 (3.91)	1.0 (−10.2, 12.2)	Not calculated
	Rilapladib 250 mg	47	9.7 (4.06)		
Plasma $A\beta_{1–42}$/Plasma $A\beta_{1–40}$ ratio	Placebo	51	−0.010 (0.0045)	−0.003 (−0.016, 0.010)	Not calculated
	Rilapladib 250 mg	47	−0.012 (0.0047)		

Abbreviations: CSF, cerebrospinal fluid; ITT, intent-to-treat; SE, standard error; CI, confidence interval; $A\beta_{1–42}$, amyloid beta peptide 1–42; $A\beta_{1–40}$, amyloid beta peptide 1–40; AlbQ, albumin quotient; T-tau, total tau; P-tau, 181 phopshorylated tau, NF-L, neurofilament light chain.

*CSF parameters are using end of study samples, plasma parameters use week 24 samples.

†Difference in adjusted least square means is shown (rilapladib minus placebo).

‡Hypothesis testing only performed on CSF $A\beta_{1–42}$.

§Posterior probabilities for these end points were calculated as post hoc analysis.

3.4. Safety and tolerability

On-treatment AEs were reported by 63% of placebo subjects and 64% of rilapladib subjects. Most AEs were of mild-to-moderate intensity. Most common AEs are summarized in Table 4. The incidence of AEs where investigators determined there was a reasonable possibility of a relationship to study drug was 15% in each treatment group.

More rilapladib subjects reported a serious adverse event (SAE; eight subjects [13%]) than placebo (five subjects [8%]). No individual SAE was reported by more than one subject. Two deaths occurred. One subject in the rilapladib group died of a cerebral hemorrhage, 125 days after starting drug and 8 days after their last dose. One subject in the placebo group completed the 24-week treatment period and died of pulmonary embolism 23 days later. See Supplementary Table 6 for further details.

More rilapladib subjects had an AE leading to withdrawal (seven subjects [11%]) than placebo (two subjects [3%]; Table 5).

Mean changes in hematology, clinical chemistry, urinalysis, vital signs, and ECG parameters were generally small and comparable across treatment groups with no excess of clinically significant changes on rilapladib compared to placebo. There was no evidence of phospholipidosis as evaluated by eye examinations and electron microscopy of peripheral blood lymphocytes.

4. Discussion

The aim of this exploratory study was to assess whether evidence of the findings from nonclinical studies could be detected in a relevant AD population and to test the hypothesis that rilapladib, through restoration of BBB integrity (i.e., a reduced AlbQ), would affect downstream markers of Aβ metabolism (i.e., CSF and plasma Aβ), neurodegeneration (i.e., tau, P-tau, and NF-L) and cognition.

The study demonstrated an improved outcome on cognition with rilapladib. There was no biomarker evidence supporting an effect of Lp-PLA_2 inhibition on Aβ. The study also provided preliminary evidence of directionally consistent, effects on AlbQ and all the measured neurodegenerative biomarkers, although the confidence intervals of differences between treatments encompassed zero. The study, therefore, provides partial translational support for the nonclinical observations [10]. However, given the atypical nature of the preclinical model, the findings on cognition and neurodegenerative markers in this present study provide a rationale for the further evaluation of this novel mechanism.

The plasma PKs and pharmacodynamics of rilapladib were similar to that observed in a previous clinical study in atherosclerosis [6] with plasma Lp-PLA_2 activity reduced by approximately 80% and to a level consistent with the effects observed in the nonclinical model [1,10].

Table 3
Statistical analysis of change from baseline in cognitive data at week 24 (ITT population)

Composite score	Treatment	n	Adjusted mean (SE)	Difference vs. placebo*, Δ (95% CI)	ES (95% CI)	Posterior probability ES >0, 0.15, 0.3
Working memory/ executive function[†]	Placebo	56	−0.150 (0.0501)	0.167	0.446	0.987 (Δ > 0)
	Rilapladib 250 mg	48	0.016 (0.0538)	(0.021, 0.313)	(0.055, 0.836)	0.930 (Δ > 0.15)
				$P = .026$[‡]		0.764 (Δ > 0.3)
Overall composite[§]	Placebo	53	−0.121 (0.0445)	0.138	0.428	0.982 (Δ > 0)
	Rilapladib 250 mg	48	0.017 (0.0466)	(0.010, 0.267)	(0.032, 0.824)	0.912 (Δ > 0.15)
						0.729 (Δ > 0.3)
Episodic memory[‖]	Placebo	53	−0.144 (0.0989)	0.197	0.274	0.915 (Δ > 0)
	Rilapladib 250 mg	50	0.053 (0.1015)	(−0.085, 0.479)	(−0.119, 0.667)	0.731 (Δ > 0.15)
						0.443 (Δ > 0.3)
Attention[¶]	Placebo	55	−0.089 (0.0686)	0.070	0.137	Not calculated
	Rilapladib 250 mg	48	−0.019 (0.0729)	(−0.130, 0.269)	(−0.256, 0.530)	

Abbreviations: ITT, intent-to-treat; SE, standard error; Δ, difference between treatments; CI, confidence interval; ES, effect size.
*Difference in adjusted least square means is shown (rilapladib minus placebo).
[†]Working memory/executive function composite score included Controlled Oral Word Association Test, category naming, one back, Trails B, and Go/NoGo.
[‡]Hypothesis testing only performed on working memory/executive function composite score.
[§]Overall composite score included all nine subtests: International Shopping List Task (ISLT) immediate recall, ISLT delayed recall, Controlled Oral Word Association Test, category naming, one back, Identification, Trails A, Trails B, and Go/NoGo.
[‖]Episodic memory included ISLT immediate recall only. This change to the protocol was prespecified in the reporting and analysis plan before unblinding.
[¶]Attention composite included Identification and Trails A.

Two germane features of the study population are the inclusion of subjects with a clinical diagnosis of possible AD and the requirement for neuroimaging evidence of significant CVD. Examination of baseline CSF profiles of $A\beta_{1-42}$, tau, and P-tau indicated that approximately 50% of these subjects met the assay thresholds for AD. This may reflect the early stage of the disease and/or an impact of cerebrovascular lesions on the presenting dementia. Although amyloid scans were not performed, the baseline CSF $A\beta_{1-42}$ profile indicates that approximately 60% of the study population may have been amyloid positive. Exploratory interaction testing, for the two primary end points, did not suggest that baseline $A\beta_{1-42}$ impacts the level of treatment response. This may indicate that the effect of rilapladib on cognition is not dependent on the presence of cerebral amyloidosis, although this will require further review after larger studies in a similar dementia population.

The neuroimaging evidence (i.e., white matter abnormalities and lacunes) required for the study is typical of those associated with SVD, as it was hypothesized that this may have increased the potential to detect treatment effects because of the pathologic changes associated with these lesions (e.g., increased BBB permeability and extravasation of plasma components into the brain) reflecting the changes in the nonclinical model [10]. The neuroimaging evidence associated with the study population was largely related to white matter changes (approximately 90%). No further information was collected regarding the nature of the cerebrovascular disease. Consequently, it is a recognised weakness of the study that it is not possible to further examine the data to understand the relationship of the underlying CVD pathology of the dementia to any effect of rilapladib. Future studies should seek to address these questions.

A bespoke cognitive battery targeting the domains of executive function, working memory, attention, and episodic memory was used to maximize the possibility of identifying a placebo decline and a treatment response in this small, short duration study. An EF/WM composite was the primary comparison of interest within the cognitive battery based on its relevance to the study population [26]. By week 24, decline in cognition was evident across the composite scores assessed for placebo but not for rilapladib. This effect on cognition is noteworthy as it was achieved in addition to stable symptomatic therapy, and it was the maximum achievable under the constraints of the study design for a mechanism that may slow disease progression and not anticipated to be procognitive. This profile was supported by the absence of procognitive effects at 12 weeks.

Table 4
Summary of on treatment AEs occurring in ≥5%* of subjects (safety population)

Preferred term*	Placebo (N = 62)	Rilapladib 250 mg (N = 61)
Any event, n (%)	39 (63)	39 (64)
Headache	10 (16)	3 (5)
Dizziness	4 (6)	3 (5)
Nausea	5 (8)	2 (3)
Urinary tract infection	6 (10)	1 (2)
Diarrhea	2 (3)	4 (7)
Cystitis	0	4 (7)
Fatigue	3 (5)	2 (3)

Abbreviation: AE, adverse event.
*Individual adverse events presented in the table are only those preferred terms with an incidence ≥5% in any treatment group.

Table 5
Summary of SAEs and AEs leading to withdrawal (safety population)

Event	Placebo (N = 62)	Rilapladib 250 mg (N = 61)
Any SAE*	5 (8)	8 (13)
Any AE leading to withdrawal, n (%)	2 (3)	7 (11)
Cerebral hemorrhage	0	1 (2)
Dementia Alzheimer's type	0	1 (2)
Dizziness	1 (2)	0
Hypoaesthesia	1 (2)	0
Agitation	0	1 (2)
Anxiety	0	1 (2)
Confusional state	0	1 (2)
Disorientation	0	1 (2)
Nausea	1 (2)	1 (2)
Femoral neck fracture	0	1 (2)
Pain in extremity	1 (2)	0

Abbreviations: SAE, serious adverse event; AE, adverse event.
*Further details of SAEs are provided in Supplementary Table 6.

The effects on CSF neurodegenerative markers are encouraging with 80%–90% Bayesian posterior probabilities that, given the observed data, at this early time point there was a true difference between treatments. Tau and P-tau are considered measures of neuronal damage and neurodegeneration, and both biomarkers are increased early in the disease and remain elevated during the course of the disease [27]. NF-L was assessed as an exploratory measure of white matter (axonal) damage, as it has been reported to be increased in some forms of dementia with subcortical involvement [28] and in demyelinating diseases, such as multiple sclerosis where treatment-related reductions in this marker have been noted [29]. The consistent directional pattern of changes in the disease-related biomarkers in the present study is supportive of the cognitive findings and suggests the potential for an underlying slowing of the progression of the disease [27].

The underlying mechanism behind these changes is not certain as the study provided only partial support to the nonclinical observations in the pig model [10]. The small decrease in AlbQ is supportive of a reduction in BBB permeability but it is unclear whether the magnitude of the change is clinically significant. Although a number of studies have reported increases in BBB permeability in AD, vascular forms of dementia and SVD, there are limited data on longitudinal changes and their impact on disease progression [30]. Additional supporting evidence of an effect of $LpPLA_2$ inhibition on reducing the permeability of CNS barriers has been provided from the investigation of the structurally related compound darapladib (SB480848) in a phase 2a study of diabetic macular edema patients (Clinicaltrials.gov identifier: NCT01506895), which demonstrated treatment-related reductions in macular edema [31].

The observed effects on CSF and plasma Aβ are not supportive of an effect of rilapladib on Aβ metabolism. This may reflect the insensitivity of the Aβ-related measures used in the present study, a lack of translation from the nonclinical model or a more dominant role of the cerebrovascular mechanisms on the observed treatment responses.

Rilapladib was generally well tolerated in this study although there were a greater number of subjects with SAEs and a greater number of subjects who withdrew due to AEs. One subject died in each treatment group. No SAE was experienced by more than one subject, and there was no obvious pattern associated with the SAEs. A number of the AEs leading to withdrawal in the rilapladib group were psychiatric events (agitation, anxiety, confusion, and disorientation). These events did not individually lead to withdrawal in more than one subject in the rilapladib group, and they were experienced as frequently by subjects in the placebo group but did not lead to withdrawal. Given the small size of the study and the correspondingly small number of events, it is not possible to draw wider conclusions on these findings and this will need to be monitored in future studies.

Although these findings are encouraging, a note of caution is required. This is the first study to investigate Lp-PLA_2 inhibition in AD. Replication of data in AD has been notoriously difficult in recent years particularly for disease-modifying compounds and particularly when moving from small, experimental phase 2 studies to longer term clinical studies. The study was a small study of short duration and was not intended to definitively determine the likelihood of long-term benefit. Important next steps to build on the findings here will be to more fully understand the mechanism and its relationship to different segments of the dementia population; to further evaluate the cognitive profile over a longer time course and across a wider range of domains; and to assess the impact on other clinically established outcomes such as function and quality of life.

As a whole, the findings provide initial evidence supportive that rilapladib and inhibition of Lp-PLA_2 may have the potential to slow the progression of AD and alter the underlying pathology in a subpopulation of AD patients with neuroimaging evidence of CVD.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.trci.2015.06.003.

RESEARCH IN CONTEXT

1. Systematic review: Changes to the neurovascular unit are an increasingly recognised pathologic feature of Alzheimer's disease, which may be linked to changes in amyloid beta peptide (Aβ) metabolism in the brain. Findings from a nonclinical model suggested that inhibition of lipoprotein phospholipase A2 (Lp-PLA_2) might provide therapeutic benefit through effects on the blood-brain barrier (BBB) and (Aβ). An exploratory study was designed to examine whether these nonclinical findings were translatable using a subgroup of AD patients with neuroimaging evidence of cerebrovascular disease through the use of a targeted battery of mechanism based and disease-related biomarkers and cognitive tests.
2. Interpretation: A consistent pattern of improved cognitive outcomes was observed across all assessed domains, which was statistically significant on the Executive Function/Working Memory composite score compared to placebo. Although there was no biomarker evidence supporting an effect of Lp-PLA_2 inhibition on any of the Aβ-related mechanism-based biomarkers, there was preliminary evidence of directionally consistent effects on BBB permeability (i.e., albumin quotient) and neurodegenerative biomarkers (i.e., tau, P-tau, and neurofilament light chain). Together these findings provide preliminary evidence that Lp-PLA_2 inhibition may play a role in reducing BBB permeability, which may lead to an effect on the underlying disease process. It is uncertain at this point whether Aβ plays a role in this mechanism.
3. Future directions: Although the study provides preliminary evidence that rilapladib and inhibition of Lp-PLA_2 may have the potential to slow the progression of AD, these findings require replication and extension in longer term clinical trials to fully evaluate the safety and therapeutic potential of Lp-PLA_2 inhibition as a treatment approach for AD.

References

[1] Wilensky RL, Macphee CH. Lipoprotein-associated phospholipase A(2) and atherosclerosis. Curr Opin Lipidol 2009;20:415–20.
[2] Zhang H, Zhang JY, Sun TW, Shen DL, He F, Dang YH, et al. Amelioration of atherosclerosis in apolipoprotein E-deficient mice by inhibition of lipoprotein-associated phospholipase A2. Clin Invest Med 2013;36:E32–41.
[3] Johnson JL, Shi Y, Snipes R, Janmohamed S, Rolfe TE, Davis B, et al. Effect of darapladib treatment on endarterectomy carotid plaque lipoprotein-associated phospholipase A_2 activity: A randomized, controlled trial. PLoS One 2014;9:e89034.
[4] Mohler ER III, Ballantyne CM, Davidson MH, Hanefeld M, Ruilope LM, Johnson JL, et al. The effect of darapladib on plasma lipoprotein-associated phospholipase A2 activity and cardiovascular biomarkers in patients with stable coronary heart disease or coronary heart disease risk equivalent: The results of a multicenter, randomized, double-blind, placebo-controlled study. J Am Coll Cardiol 2008; 51:1632–41.
[5] Berger JS, Ballantyne CM, Davidson MH, Johnson JL, Tarka EA, Lawrence D, et al. Peripheral artery disease, biomarkers, and darapladib. Am Heart J 2011;161:972–8.
[6] Tawakol A, Singh P, Rudd JH, Cai G, Nucic E, Brannan SP, et al. Effect of treatment for 12 weeks with rilapladib, a lipoprotein-associated phospholipase A2 inhibitor, on arterial inflammation as assessed with 18F-fluorodeoxyglucose-positron emission tomography imaging. J Am Coll Cardiol 2014;63:86–8.
[7] Lum H, Qiao J, Walter RJ, Huang F, Subbaiah PV, Kim KS, et al. Inflammatory stress increases receptor for lysophosphatidylcholine in human microvascular endothelial cells. Am J Physiol Heart Circ Physiol 2013;285:H1786–9.
[8] Huang F, Subbaiah PV, Holian O, Zhang J, Johnson A, Gertzberg N, et al. Lysophosphatidylcholine increases endothelial permeability: Role of PKCalpha and RhoA cross talk. Am J Physiol Lung Cell Mol Physiol 2005;289:L176–85.
[9] Shie FS, Neely MD, Maezawa I, Wu H, Olson SJ, Jurgens G, et al. Oxidized low-density lipoprotein is present in astrocytes surrounding cerebral infarcts and stimulates astrocyte interleukin-6 secretion. Am J Pathol 2004;164:1173–81.
[10] Acharya NK, Levin EC, Clifford PM, Han M, Tourtellotte R, Chamberlain D, et al. Diabetes and hypercholesterolemia increase blood-brain barrier permeability and brain amyloid deposition: Beneficial effects of the Lp-PLA_2 inhibitor darapladib. J Alzheimers Dis 2013;35:179–98.
[11] Zlokovic BV. The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron 2008;57:178–201.
[12] Deane R, Bell RD, Sagare A, Zlokovic BV. Clearance of amyloid-beta peptide across the blood-brain barrier: Implication for therapies in Alzheimer's disease. CNS Neurol Disord Drug Targets 2009;8:16–30.
[13] Jagust W. Vulnerable neural systems and the borderland of brain aging and neurodegeneration. Neuron 2013;77:219–34.
[14] Brickman AM. Contemplating Alzheimer's disease and the contribution of white matter hyperintensities. Curr Neurol Neurosci Rep 2013;13:415.
[15] Iadecola C. The overlap between neurodegenerative and vascular factors in the pathogenesis of dementia. Acta Neuropathol 2010; 120:287–96.
[16] MRC CFAS. Pathological correlates of late-onset dementia in a multicentre, community-based population in England and Wales. Lancet 2001;357:169–75.
[17] Esiri MM, Joachim C, Sloan C, Christie S, Agacinski G, Bridges LR, et al. Cerebral subcortical small vessel disease in subjects with pathologically confirmed Alzheimer disease: A clinicopathologic study in the Oxford Project to Investigate Memory and Ageing (OPTIMA). Alzheimer Dis Assoc Disord 2014;28:30–5.

[18] Richardson K, Stephan BC, Ince PG, Brayne C, Matthews FE, Esiri MM. The neuropathology of vascular disease in the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS). Curr Alzheimer Res 2012;9:687–96.

[19] Grinberg LT, Thal DR. Vascular pathology in the aged human brain. Acta Neuropathol 2010;119:277–90.

[20] Licandro A, Ferla S, Tavolato B. Alzheimer's disease and senile brains: An immunofluorescence study. Riv Patol Nerv Ment 1983; 104:75–87.

[21] Alafuzoff I, Adolfson R, Bucht G, Winblad B. Albumin and immunoglobulin in plasma and cerebrospinal fluid, and blood-cerebrospinal fluid barrier function in patients with dementia of the Alzheimer type and multi-infarct dementia. J Neurol Sci 1983;60:465–72.

[22] Clifford PM, Zarrabi S, Siu G, Kinsler KJ, Kosciuk MC, Venkataraman V, et al. Abeta peptides can enter the brain through a defective blood-brain barrier and bind selectively to neurons. Brain Res 2007;1142:223–36.

[23] Wisniewski HM, Kozlowski PB. Evidence for blood-brain barrier changes in senile dementia of the Alzheimer type (SDAT). Ann N Y Acad Sci 1982;396:119–29.

[24] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984;34:939–44.

[25] Cogstate. Cognitive Tasks. Available at: http://cogstate.com/computerized-tests/cognitive-tasks/. Accessed June 30, 2015.

[26] Weintraub S, Wicklund AH, Salmon DP. The neuropsychological profile of Alzheimer disease. Cold Spring Harb Perspect Med 2012; 2a:a006171.

[27] Hampel H, Blennow K, Shaw LM, Hoessler YC, Zetterberg H, Trojanowski JQ. Total and phosphorylated tau protein as biological markers of Alzheimer's disease. Exp Gerontol 2010;45:30–40.

[28] Bjerke M, Zetterberg H, Edman A, Blennow K, Wallin A, Andreasson U. Cerebrospinal fluid matrix metalloproteinases and tissue inhibitor of metalloproteinases in combination with subcortical and cortical biomarkers in vascular dementia and Alzheimer's disease. J Alzheimers Dis 2011;27:665–76.

[29] Gunnarsson M, Malmeström C, Axelsson M, Sundström P, Dahle C, Vrethem M, et al. Axonal damage in relapsing multiple sclerosis is markedly reduced by natalizumab. Ann Neurol 2011; 69:83–9.

[30] Farrall A, Wardlaw JM. Blood-brian barrier: Ageing and microvascular disease—systematic review and meta-analysis. Neurobiol Aging 2009;30:337–52.

[31] Staurenghi G, Li Y, Magee MH, Danis RP, Wurzelmann J, Adamson P, et al., Darapladib DME Study Group. Darapladib, a lipoprotein-associated phospholipase A2 inhibitor, in diabetic macular edema: A 3-month placebo-vontrolled study. Ophthalmology 2015;122:990–6.