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Phenserine Efficacy in Alzheimer's disease

Bengt Winblad[a], **Ezio Giacobini**[b], **Lutz Frölich**[c], **Lawrence T. Friedhoff**[d], **Gosse Bruinsma**[e], **Robert E. Becker**[f,g], and **Nigel H. Greig**[g]

[a]Alzheimer's Disease Research Center, Department of Neurobiology, Care Sciences and Society, Karolinska Institutet, Stockholm, Sweden [b]Department of Rehabilitation and Geriatrics, University of Geneva, Medical School, University Hospitals of Geneva, CH-1226 Thonex-Geneva, Switzerland [c]Department of Geriatric Psychiatry, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Germany [d]Pharmaceutical Special Projects Group, LLC, River Vale, NJ, USA [e]Ciurem Pharma BV, Leiden, Netherlands [f]Aristea Translational Medicine Corporation, Freeport, ME, USA [g]Drug Design & Development Section, Laboratory of Neurosciences, Intramural Research Program, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA

Abstract

To gather preliminary evidence in Alzheimer's disease (AD) for the efficacy of phenserine, a non-competitive acetylcholinesterase inhibitor that has independent modulatory effects on Aβ generation, a 12 week comparison of patients receiving phenserine (10 and 15 mg BID) or placebo was conducted under double-blind conditions. Patients who completed 12 weeks of the double-blind before others were continued in the double-blind to determine longer-term treatment effects. At 12 weeks, mean ADAS-cog (AD assessment scale-cognitive) changes from baseline were −2.5 and −1.9 for high-dose phenserine (N=83) and placebo (N=81) groups, respectively, a non-statistically significant improvement for the high-dose phenserine group relative to placebo. CIBIC + (clinician's interview based impression of change + caregiver's input) values for the high-dose and placebo groups were similar at 12 weeks. For patients who received more than 12 weeks of therapy, the ADAS-cog changes were −3.18 and −0.66 for the high-dose phenserine (N=52) and placebo (N=63) groups, respectively, a difference achieving statistical significance (p=0.0286). After 12 weeks, CIBIC+ values were 3.59 and 3.95 for the high-dose (N=54) and placebo (N=66) groups respectively (p=0.0568).

These results from this short-term study are consistent with phenserine potentially benefiting mild to moderate Alzheimer's disease symptomatically but do not address possible amyloid metabolic mediated effects on disease processes in AD.

Corresponding Author: Nigel H. Greig Greign@mail.nih.gov.

CONFLICT OF INTEREST

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Keywords

Alzheimer's disease; phenserine; Alzheimer clinical trial; cholinesterase inhibitor; amyloid-β peptide; amyloid-β precursor protein; acetylcholinesterase

INTRODUCTION

Recent evidence supports the view that some of the numerous Alzheimer's disease (AD) clinical trial (CT) failures that have occurred during recent years can be ascribed to procedural errors in drug developments rather than a lack of drug efficacy. A wide range of errors can interfere with and threaten the validity of neuropsychiatric CTs [1,2] resulting in CT failure that is generally not distinguished from a lack of efficacy. Given the current emphasis on translational medicine, we undertook an analysis of a recent CT of the AD experimental drug phenserine to determine whether or not it deserved further study to realize its potential efficacy. This paper provides previously unreported data that supports the view that the abandonment of phenserine development cannot be justified based on lack of evidence of potential efficacy in AD.

Drug discovery

The discovery of cholinergic deficits in AD [3], a chronic, progressive neurodegenerative disorder characterized by deterioration of memory and cognitive functions, progressive impairments of daily activities, together with episodic neuropsychiatric symptoms [4,5], spurred a drug discovery program within the Intramural Research Program of the National Institute of Aging (NIA) aimed at identifying a therapeutic agent. Cholinergic deficits in AD guided the initial drug discovery program. Limitations in the effectiveness of cholinergic therapies and subsequent neurochemical discoveries, such as progressive neuronal and synaptic dysfunctions, and cell death with hallmark neuropathological lesions [6–10], opened to investigators a wide range of additional potential targets for AD therapies. The most frequently addressed targets are neuritic plaques, primarily composed of aggregated amyloid-β peptide (Aβ), a 40- to 43-amino acid peptide derived by cleavage of the amyloid-β precursor protein (AβPP), and oligomeric Aβ forms reported to induce toxicity and able to account for cellular dysfunction, pathology and death [6–11]. These amyloid targets, together with neurofibrillary tangles that comprise phosphorylated tau, occur chiefly in the neocortex, entorhinal cortex, and hippocampus of AD brains [6–11].

Mutations in one of several genes associated with AβPP and presenilins (PS1, PS2) elevate generation of Aβ and, in particular, the longer more hydrophobic Aβ forms [6–8,11]. Recent studies suggest that soluble aggregates of Aβ target synapses and impair memory [12,13]. Drug discovery strategies currently aim to lower oligomeric forms of Aβ by altering the activities of secretases (β and γ) able to cleave APP and generate Aβ [6,7,10,14] and other toxic APP products, such as C31 and N-APP [15,16]. Other approaches to AD therapies aim to remove amyloid [6–10] from brain and to overcome consequences from tau hyperphosphorylation, apolipoprotein E, and neuroinflammation known to impact the progression of AD [16–19].

Pharmacology and toxicology

In 1995 NIA investigators chose phenserine as a lead clinical candidate based on its favourable pharmacological profile as an acetyl-selective cholinesterase inhibitor [20,21]. A potent, pseudo-irreversible non-competitive inhibitor of acetylcholinesterase (human erythrocyte AChE IC_{50} 22 nM) with low butyrylcholinesterase (human plasma BChE IC_{50} 1560 nM) action, and a high brain:plasma ratio (10:1); phenserine was shown to be well tolerated, elevate brain acetylcholine (ACh) levels and to improve cognition in both rodent and canine models using multiple paradigms [21–25]. Whereas the pharmacokinetic half-life of phenserine proved to be short in rodents (12.6 and 8.5 min in plasma and brain), its duration of AChE inhibition proved to be longer (8.25 hr) due to a slow rate of drug/enzyme dissociation [21,23]. We and others regarded these properties, rapid clearance of drug and prolonged steady-state AChE inhibition as desirable features because of lowered risks of drug accumulation and more physiological patterns of support for brain cholinergic functioning [26].

Cholinesterase inhibitors, the primary treatment for mild to moderate AD subjects [27], provide time-limited symptomatic relief by augmenting ACh neurotransmitter levels known to be impaired in AD brain, but no verifiable impact on disease course [28]. In response to the emergence of the Aβ molecule as a target for AD therapy, we determined that phenserine reduced APP synthesis and Aβ concentrations in neuronal cultures independent of its cholinergic activities (EC_{50} 670 nM) [29–31]. Phenserine acts post-transcriptionally at the level of the 5′-untranslated region of AβPP mRNA [29] to reduce AβPP translational efficiency and, thereby, Aβ in rodent brain [30,32]. These studies raised the possibility that phenserine may independently afford symptomatic relief in AD by augmenting cholinergic function and exert disease-modifying activity by lowering AβPP/Aβ generation, a mechanism implicated by the amyloid hypothesis in AD as a potential pathological process leading to disease progression. Given these joint properties, phenserine was licensed from the National Institute on Aging by Axonyx Corporation in 1997 for commercial development.

Early clinical studies

Phase I trials of phenserine in healthy elderly volunteers, initiated by Axonyx in late 1999, identified a safe therapeutic range (5 to 20 mg) for single and multiple oral doses, and demonstrated a non-linear dose-response that achieved a maximal mean inhibition of erythrocyte AChE of 26.3% and 47% at 10 and 20 mg, respectively, with an inhibition half-life that varied between 11.1 hr and 5.2 hr for these doses, respectively [32]. Dose-limiting nausea and vomiting, but not other toxicity, was reported in the 20 mg dose group [33]. A double-blind, placebo-controlled, randomized, phase II study of phenserine (placebo (N=24), phenserine (N=48): 5 mg BID for 2 weeks escalated to 10 mg BID for 10 weeks, total 12 week study) was initiated in late 2000 in 72 mild to moderate AD patients [24,25]. The study assessed clinical efficacy on the primary variables: AD Assessment Scale, Cognitive subscale (ADAS-Cog), and Clinical Global Impression of Change (CGIC), and the secondary variables: Mini Mental State Examination (MMSE), AD Cooperative Study, Activities of Daily Living (ADCS-ADL), Neuropsychiatric Inventory (NPI) and Cambridge Neuropsychological Test Automated Battery Paired Associates Learning (CANTAB-PAL)

assessments. Phenserine proved to be safe and well tolerated, with no serious adverse effects. As detailed in the discussion, several variables showed positive trends, including CANTAB ($p = 0.045$), revealing statistically significant benefits from phenserine treatment [25].

In mid 2003, a randomized, placebo-controlled, double-blind phase IIb trial of phenserine was initiated to assess the effects of phenserine (10 and 15 mg BID) on AβPP and Aβ in plasma and CSF in 75 mild to moderate AD patients over 6 months [25]. Analysis of plasma and CSF samples from patients treated with phenserine showed convincing trends in reducing levels of Aβ that failed to reach statistical significance in available samples (Unpublished data) [25]. Additionally in 2003, a randomized, placebo-controlled, double-blind phase III trial was initiated prior to completing all phase II analyses. This enrolled 375 patients with mild to moderate AD in which phenserine (10 mg BID (N=150) or 15 mg BID (N= 149)) was administered orally for 6 months and compared to placebo (N=76). The primary efficacy endpoints were ADAS-Cog and the CIBIC+ assessments, and secondary outcomes included the ADCS-ADL and NPI assessments [25].

In early 2005, data analyses indicated that this Axonyx multicenter phase III CT had not reached statistical significance for primary outcome variables [25]. Having reached a decision that this failure was due to design and other problems (methodological failures that were later independently verified [34,35]) that had the potential of compromising their ongoing phase III CTs, Axonyx terminated their two ongoing multicenter CTs that are the focus of this article, albeit that these CTs had been initiated by separate investigators.

MATERIALS AND METHODS

At the time of the Axonyx decision to terminate its two multicenter CTs, 255 patients were under randomized assignment to double-blind, placebo-controlled treatment with phenserine at two dosing levels (10 and 15 mg BID) or placebo. To use these subjects for an evaluation of phenserine treatment in relation to placebo, a protocol was developed to continue each patient in the double-blind under his or her current treatment condition until all enrolled patients had received a minimum of 12 weeks under double-blind, placebo controlled conditions. Under this protocol, reported herein, two comparisons were planned: (i) for all patients at 12 weeks and (ii) for all patients who had greater than 12 weeks of double blind treatment.

Study population

All patient subjects entered into this new 12 week study protocol met the following conditions. Men and women aged at least 50 years or more with probable AD recruited at 72 sites in 12 countries. For the diagnosis of AD, patients satisfied both the criteria defined by the guidelines of the National Institute of Neurological and Communicative Disorders and Stroke and AD and Related Disorders Association (NINCDS-ADRDA) for probable AD [36]. Patients were also required to have a Modified Hachinski Ischemic Scale Score [37] of less than or equal to 4, general health consistent with participation in a 6-month clinical trial and no risk factors for treatment with a cholinesterase inhibitor.

Patients were required to have mild to moderately severe AD at screening, as defined by Mini-Mental State examination [38] scores between 12 and 24 inclusive. All patients had a computerized tomograpy or magnetic resonance imaging scan within the preceding 12 months, the results of which were compatible with AD.

This study was conducted in accordance with the principles of the Declaration of Helsinki [39] and the European Community Good Clinical Practices Guidelines. All centers had local ethics committee approval. Prior to screening, the nature and purpose of the investigation was explained to the patient and caregiver and written informed consent was obtained as required by local laws and regulations.

Study design

This was a randomized, multinational, multicenter placebo-controlled parallel-group study (accruing subjects primarily from Croatia and Austria, with some from Spain and England). Since the original multicenter CT protocols that entered subjects into the placebo-controlled double-blind were terminated, under the protocol for this CT all entered patients completed at least 12 weeks of therapy but all did not reach the originally scheduled 26-week end of treatment. To be included under the protocol we report, patients had to have been screened within 21 days of entry and randomly assigned to receive 10 or 15 mg of phenserine twice daily or placebo. Medications were administered with food. A blinded titration schedule was used so that patients randomized to active treatment received 5-mg twice daily for the first 4 weeks of the study followed by 10-mg twice daily for the next 4 weeks. Patients randomized to 15-mg twice daily received this dose starting on Week 9. Treatment at the assigned dose was continued for up to 26 weeks. Efficacy evaluations were conducted at weeks 4, 8, 12 and termination of the study. By the time of the 12-week visit, patients randomized to 10-mg twice daily had received this dose for approximately 8 weeks. Patients randomized to receive 15-mg twice daily had received this dose for approximately 4 weeks.

Outcome measures

A. Efficacy—The Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-cog) [40,41] and the Clinician Interview Based Impression of Change with Caregiver Input (CIBIC+) [42,43] were the primary outcome measures reflecting cognition and global function respectively.

B. Safety—Adverse events were monitored at each visit by questioning both the patient and the caregiver, as well as through direct observation. All adverse events, whether reported or observed, were recorded together with the date of onset and cessation, severity of condition and whether, in the opinion of the investigator, the event was related to active treatment.

Blood pressure and pulse were recorded at all visits and standard laboratory assessments (hematology, clinical chemistry and urinalysis) were measured prior to randomization and at all post randomization visits. A standard 12-lead ECG was performed at the screening and final study visits.

Statistical assessments

To estimate power requirements a sample size of 150 patients/treatment group was estimated, based on the results obtained from an earlier phase II study [44]. This sample size was estimated to have a 93% power to detect a 2-point reduction in ADAS-cog scores. The assumed patient completion rate was 80%. The 12-week evaluation was selected as the primary analysis. A secondary analysis included data from all patients who completed more than 12 weeks of treatment and the last observed evaluation for each patient regardless of when it occurred. Each of these analyses was conducted using an a priori assigned last observation carried forward and intention-to-treat database. A safety population was also defined. This included all patients who received treatment medication. This population was used to assess the prevalence of adverse events.

The ADAS-cog was assessed using analysis of covariance (ANCOVA) to compare the treatment groups. When the model assumptions of non-normality were violated a non-parametric assessment involving a similar ANCOVA model but with ranks assigned to the original data was used to confirm the results obtained from the ANCOVA. The CIBIC+ was assessed using Cochran-Mantel-Haenszel statistics with modified ridit scoring.

Demographic variables were visually assessed for comparability but these were not formally analyzed.

Effects on vital signs were assessed using inspection of mean changes from baseline.

Adverse events were coded using the MedDRA MSSO Medical Regulatory Terminology Version 6.0 (Northrop Grumman, Chantilly, VA) prior to breaking the treatment codes.

RESULTS

In total, 255 patients were randomized to treatment in the trial. Patient demographics at baseline are summarized in Table 1. No statistically significant differences occurred among groups after patient randomization. The 12 week completion rates for the study were 89, 78 and 85% for the placebo, 10 and 15-mg/day groups respectively.

Assessment of cognition and global function

ADAS-cog results are summarized in Table 2. ADAS-cog changes of the 15-mg twice daily dose were numerically superior to placebo at all evaluations, and reached statistical significance at the post 12-week assessment (p=0.0286). When interpreting these results, it should be remembered that patients randomized to Phenserine treatment were titrated to their target dose in 5 mg incremental escalations at 4-week intervals. Thus, both active groups received 5 mg twice daily through the Week 4 visit and 10 mg twice daily through the Week 8 visit. The 15-mg twice daily group was increased to this dose following the Week 8 visit.

Effects on global function were assessed using the CIBIC+ (See Table 3)

Assessment of safety

A total of 77% of patients completed the study protocol. The overall discontinuation rate for the 15 mg twice daily group (23.6%) was only slightly higher than for the placebo group (17.1%) (Table 4). This difference was mainly due to a higher percentage who withdrew consent in the 15 mg twice daily group. Withdrawals due to adverse events were slightly lower in the 15 mg twice daily group (5.9%) than in the placebo group (6.1%). Nausea, vomiting, dizziness and headache were more frequent in active drug treated patient groups but did not contribute to dropouts (Table 5).

DISCUSSION

ADAS-cog score reductions indicating improvement for the phenserine 15-mg twice daily dose group were superior to placebo scores at 12 weeks, termination, and for those subjects who completed the originally planned 26 weeks. The post 12-week termination difference between phenserine 15-mg twice daily and placebo reached statistical significance (p=0.0286). Analysis of CIBIC+ scores across treatment groups and times (Table 3) showed a similar pattern although the post 12-week difference between 15-mg twice daily and placebo did not achieve statistical significance. The overall tolerability of phenserine was reflected by the patient completion rate of 77%, which is similar to the value found in a multinational study of donepezil hydrochloride [45].

The current study, within itself and consistent with a recent small (N=20) randomized, double-blind, placebo-controlled clinical trial on phenserine in mild AD [46] provides evidence consistent with clinical cognitive benefits and a strong trend for global benefits from 15-mg twice daily phenserine used in patients with mild to moderate AD. The earlier phase III study [25], criticized for methodological weaknesses able to account for failure due to excessive variance [1,34,35,47], found a treatment difference of −0.67 (95% CI: −2.93 to 1.58) ADAS-cog points favoring phenserine 15-mg twice daily treated patients over placebo treated. Given the evidence that methodological errors can reduce the effect size of CTs [48], introduce possible Type II errors [1,34,47], and the evidence that excessive variance [35,49] can account for this earlier CTs failing we regard the direction of difference as additional support for the view that phenserine development may have been prematurely interrupted.

Unlike the present study, this earlier one utilized a 2:2:1 randomization between the two active treatments and the placebo, which has been criticized by Schneider [50] to contribute to measurement bias and, thereby, reduce the likelihood for the placebo group to deteriorate and consequently the chances for a significant drug-placebo difference to be observed [51]. The earlier study had placebo standard deviations for the ADAS-cog change from baseline of 6.5 and 8.8 at 12 and 26 weeks respectively, which can be compared with standard deviations of 5.44 and 5.70 in the current study. The known high variability of the ADAS-cog when combined with a small placebo sample size has been reported to greatly minimize the chances for a placebo group to deteriorate and consequently the chances for a significant drug-placebo difference to be observed [50,51]. The lower SD values and more statistically powerful design in the study reported herein suggest that the current study was more likely to be an accurate estimate of the effects of phenserine on the signs and symptoms of AD than the described earlier one. This would be particularly pertinent for those receiving

phenserine 15 mg twice daily for more than 12 weeks, as an 8 week dose titration was required to achieve this dose. Unfortunately, the number of subjects completing the full 26 weeks of treatment prior to study closure was small, thereby making statistical comparisons between phenserine and placebo difficult at this time point. In an attempt to counter this, statistical comparison of the combined phenserine treatment groups (15 and 10 mg BID) versus placebo was assessed at 26 weeks, and approached ($p = 0.08$) but did not achieve significance.

The results we report build on the earlier completed (N=72) double-blind, placebo-controlled, randomized, phase II study of phenserine in mild-to-moderate AD [52], in which two thirds of patients (N=48) received phenserine (5 mg BID for 2 weeks, followed by 10 mg BID for 10 weeks) and the remaining third (N=24) received placebo [25]. In this study the ADAS-Cog (which included the numbers cancellation test, the maze test and the delayed word list recall) in a per protocol analysis (N=54), documented a phenserine associated improvement of 2.6 points from baseline, versus a 0.7-point placebo improvement. The maze test of the extended ADAS-Cog (an evaluation of executive function) and CANTAB demonstrated significance of phenserine over placebo-treated patients ($p = 0.034$ and 0.045, respectively), as did an intention-to-treat analysis of the CANTAB-PAL ($p < 0.05$) [25,52].

A more recent study demonstrated that phenserine (15 mg BID) treatment was associated with an improvement in cognition compared to both placebo at three months as well as when these placebo patients were administered donepezil over the following 3 months [46]. Phenserine-induced cognitive improvement was associated with an elevation in the cortical metabolic rate for glucose (rCMRgl), a marker of functional activity, as assessed by FDG-PET and, an increase in $A\beta_{1\text{-}40}$ in cerebrospinal fluid, which inversely correlates to cortical ^{11}C-PIB retention in brain, a marker of brain Aβ. This represented the first finding of a direct effect of anti-amyloid therapy using the ^{11}C-PIB PET technique [46,53], and is in line with prior actions of phenserine on Aβ generation in humans [54] and animal models [21,24,31,32].

In the present study, consistent with other evidence, phenserine at 15-mg twice daily doses shows statistically suggestive evidence for symptomtic benefits in AD. In a more recent report, Kadir et al. [46] found changes in ^{11}C-PIB retention that they interpreted as consistent with possible phenserine effects on disease course. In a recent review, it was concluded that drugs like phenserine, with multiple modes of action, might provide a better approach to AD therapy [55]. Based on the evidence available to us, we view phenserine, that has been administered to in excess of 500 humans for periods up to 12 months and up to 18 months in animal models, as a compound with probable safety in use in AD and possible potential to benefit AD patients through independent but co-present disease symptomatic and course modifying effects.

In 2008 we reviewed the literature and determined that a number of AD drugs may have failed due to methodological shortcomings in their development schemes [1,47], we regard phenserine as a drug that has not yet been fully evaluated consequent to such problems – where drug development may have been abandoned too quickly consequent to methodological problems identified by the developers and others in an earlier phase III trial.

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Table 1

Baseline Demographic Information, Mean (SD) – ITT Population

Variables	Placebo	Phenserine	
		15-mg BID	10-mg BID
Age (yr)	71.2 (8.2)	70.0 (8.5)	71.2 (9.8)
Sex (% Female)	63.0	49.4	59.0
Duration of AD (months)	7.2 (12.6)	9.6 (18.6)	8.9 (18.6)
MMSE	19.6 (3.1)	19.3 (3.3)	19.0 (3.6)

MMSE: Mini-Mental Status Exam

Table 2

ADAS-cog Changes from Baseline, Mean ± SD (N)

Dose Group	4 Weeks	8 Weeks	12 Weeks-LOCF	Post 12 Weeks	26 Weeks
Placebo	−0.9 ± 5.31 (80)	−1.7 ± 5.7 (79)	−1.9 ± 5.84 (81)	−0.66 ± 5.44 (63)	−2.9 ± 5.70 (12)
10-mg BID	−1.2 ± 5.10 (82)	−1.0 ± 5.28 (75)	−1.3 ± 6.30 (83)	−1.31 ± 6.04 (57)	−3.5 ± 4.82 (15)
15-mg BID	−1.4 ± 4.45 (83)	−2.2 ± 5.09 (81)	−2.5 ± 6.21 (83)	−3.18 * ±9.50 (52)	−4.8 ± 8.99 (11)

* Statistically significantly different from placebo p=0.0286

Table 3

Mean CIBIC+ Scores, Mean ± SD (N)

Dose Group	4 Weeks	8 Weeks	12 Weeks-LOCF	Post 12 Weeks	26 Weeks
Placebo	3.66 ± 0.67 (81)	3.60± 0.96 (78)	3.72 ± 1.12 (81)	3.95 ± 1.25 (66)	4.36 ± 1.12 (11)
10-mg BID	3.91 ± 0.71 (81)	3.79 ± 0.74 (75)	3.89 ± 0.77 (82)	4.02 ± 1.03 (62)	3.73 ± 0.8 (15)
15-mg BID	3.74 ± 0.65 (80)	3.75 ± 0.93 (81)	3.80 ± 1.03 (83)	3.59[a] ± 1.16 (54)	3.55 ± 1.44 (11)

[a] p= 0.0568 relative to placebo

Table 4

Percentage of Discontinued Patients

		Phenserine	
	Placebo	**10-mg BID**	**15-mg BID**
Reason for Discontinuation			
Adverse event	6.1	13.6	5.9
Noncompliance	3.7	1.1	0.0
Withdrew consent	4.9	8.0	10.6
Protocol violation	2.4	3.4	4.7
Other: All other reasons	0.0	1.1	2.4
All Reasons	17.1	27.2	23.6

Table 5

Percentage of Patients with Adverse Events in Each Treatment Group for Events Occurring in at Least 3% of Patients

		Phenserine	
Adverse Event	**Placebo**	**10-mg BID**	**15-mg BID**
Nausea	0	9	19
Vomiting	1	5	11
Dizziness	0	8	8
Headache	1	3	8
Nasopharangitis	7	3	2
Hypertension	3	3	4