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Fire in the Ashes: Can Failed Alzheimer's Drugs Succeed with Second Chances?

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Abstract

Background—Since Cognex over 200 Alzheimer's disease (AD) drug candidates have failed. Investigations have identified vulnerabilities of these AD drug developments to methodological errors. (–)-Phenserine has been discussed as possibly failing due to flawed methods and practices in development

Methods—We analyzed documentation of (–)-phenserine's development for vulnerabilities to errors and designed interventions for a redevelopment that could provide fair or unbiased assessments of (–)-phenserine target engagement, target relevance for human diseases, and adequate presumptive evidence of efficacy as a therapeutic for one or more diagnoses to justify registration required clinical trials.

Results—Similar to studies of 40 other AD developments, with (–)-phenserine we found little evidence of preemptive interventions against potentially invalidating errors, grounds to judge progress in development through stages as not scientifically justifiable, and variance excess and placebo group improvements as capable of accounting for outcomes from various studies in the development. We propose to compare a redevelopment resourced to counter these deficiencies to the original development as historical control to evaluate further our hypothesis that errors in development accounted for the (–)-phenserine failure specifically and other AD drug failures potentially.

Conclusions—We find support for our earlier proposal that (–)-phenserine did not fail but the methods of development failed to provide conditions where efficacy could be tested. We propose that redevelopment under conditions aimed to correct methodological deficiencies common in AD drug developments will successfully test efficacy for (–)-phenserine and hopefully lead to a disease modifying addition to the AD therapeutic armamentarium.

Keywords

AD drug development; (–)-phenserine; validity; scientific practices

1. Introduction

The 200 failed Alzheimer's disease (AD) drug candidates daunt academic and industry efforts to develop therapeutic advances against AD [1]. Basic knowledge of AD pathology with possible relevance to therapy has grown dramatically in the last 30 years; yet, applications of this knowledge to arrest AD disease progression hang like fruits out of reach for investigators and pharmaceutical firms hungry to be the first with a cure. We have been asking whether there are lessons to be learned from the repeated defeats in AD drug development [2–8]; lessons that might arm clinical neuroscientists to translate discoveries more effectively and advance Alzheimer's therapy beyond current transient and marginal symptomatic benefits for patients. In the contexts of relentless AD drug candidate failures and emerging awareness that current AD drug development practices are vulnerable to biases that can invalidate the research [6–13], we now ask how we as investigators can resource studies to insure a valid test of efficacy for (–)-phenserine, an AD drug candidate.

2. Background

2.1 (–)-phenserine

(–)-Phenserine was discovered by investigators at the National Institute of Aging to provide dose-dependent acetylcholinesterase inhibitory activity (AChEI) [14]. Greig et al. [15] subsequently uncovered that (–)-phenserine also reduces amyloid precursor protein (APP), sAPP- α and sAPP- β , and A β ₄₂ levels by post-transcriptionally regulating APP mRNA translation at the 5'-untranslated region (5'-UTR) [16]. After licensing (–)-phenserine for commercial development, Axonyx demonstrated reductions of APP synthesis and A β ₄₂ concentrations up to 50% in cell culture, mice and 20% in humans [17,18]. A Phase I study of 32 healthy elderly volunteers given single doses of 5, 10, and 20 mg (–)-phenserine produced nausea and vomiting at the 20 mg dose but no serious adverse events [19]. In an ensuing series of studies including two Phase II and three Phase III timed trials Axonyx safely administered phenserine to over 500 humans for up to 18 months; but, failed to demonstrate efficacy for either AChEI or APPSI activities of (–)-phenserine. This led to (–)-phenserine being abandoned as a 'failed' drug when in fact, as we later demonstrated, the development most probably was sufficiently biased that it did not provide a fair test for either efficacy or the lack of efficacy and left APPSI activities, most relevant to an advance in AD therapy, untested [20].

2.2 Untangling the web of risks to experimental validity

After developing methods to analyze AD and other drug developments for possible invalidation due to excessive imprecision in measurements [21], we demonstrated that neglect to control variance could have accounted for the failure of early phenserine clinical trials [20]. We then surveyed the drug development literature to identify whether other methodological errors would be capable of putting at risk the validity of NP clinical drug study outcomes and their interpretations [2]. Somewhat to our surprise we found with ease a surfeit of candidate biases present in and capable of invalidating AD and other neuropsychiatric (NP) clinical studies: problems with dosing [7,22,23], biases [24,25], ineptitude and deception [25,26], protocol violations [24], measurement errors [11,21,27],

inadequate experience and skills to participate in CTs [12,28], and other factors associated with failures of NP trials to reproduce positive results with further testing [29]. With items from this list we then determined, in 40 randomly selected AD drug developments, which risks were potentially present and what actions the investigators undertook to avoid risks and their effects on outcomes [4]. Finding very low levels of documentation that risks were being assessed and addressed by investigators, we then studied some recent AD drug developments and found that biases introduced by methods could have determined the outcomes of these studies [5–8]. We concluded that lax applications of scientific standards in clinical, compared to basic, neuroscience investigations may dispose otherwise efficacious AD drugs to fail [8]. In a preliminary study we found little evidence to change our view that lax standards of scientific practice might be playing an important role in opening clinical neuroscience studies to error risks and unjustified failures [29]. We then analyzed documentation of Axonyx's potentially miscarried development of phenserine for AD [20] and found statistically significant methodological effects on the outcomes for one clinical trial we had data to analyze and similarities of vulnerabilities to errors and patterns of actual errors across all Axonyx studies. Based on the presence of errors in the phenserine studies at levels found in our reviews of 40 randomly chosen AD drug developments and statistically significant predictions of outcomes by variance in measurements and placebo group responses at study sites we concluded that, due to bias, phenserine had never been tested for efficacy in spite of years of investigation at a cost of many tens of millions of dollars. Given the similar patterns of risks we identified in the phenserine and other AD developments we have been able to study, we propose that an evenhanded redevelopment of phenserine will require markedly higher levels of controls against invalidating biases and that these changes in design and study management may potentially find wider applications in AD drug developments.

2.3 The restructuring of AD drug developments

Clinical trials have not lacked proposals to make them more effective [10,30]. Recently Lansbury [31] integrated the old-fashioned testing of drug candidates in cells, in animals and then people with parallel more modern validations of drug candidates using target molecules and drug library screenings. Paul et al. [32] emphasized early human, Phase I, proof of concept (POC) testing to best focus the design of human development stages. Klein [33] laments the exclusion of serendipity from drug developments. Pangalos [23] and Markou [24] drew attention to the need to insure the integrity of preclinical preparations before proceeding into human investigations. Experts have endorsed shifting the research focus onto pre-AD as more therapeutically promising [34]. Industry has called for research collaborations while they slash budgets and personnel to staff research and development collaborations. [35]. We have expressed concerns with attitudes that appear to guide investigators: too uncritical beliefs that evidence for efficacy provides proof of efficacy and that scientific practices can be selectively employed as good practice standards in NP and AD drug developments [8]. Our concern is that these attitudes inappropriately elevate regulatory hurdles to status as adequate ultimate goals for what should be fully scientifically informed clinical neuroscience drug development studies. While there have been offered these and other proposals to modify AD drug development, the effectiveness of adding the resources needed to avoid bias intrusions into drug developments has not been tested.

Critics of AD and other NP drug developments appear to be in agreement that fundamental problems confound clinical neuroscience: neurodegenerative diseases are heterogeneous; difficult to diagnose; often present as not pure clinically defined disorders but rather as clinical outcomes from more than one independent pathology; slowly unfold in time for affected persons making reliable assessments of changes in clinical status challenging; the absence of definitive biomarkers undermines early diagnosis and the development of surrogate endpoint outcomes; extensive hidden damage to the nervous system occurs prior to clinical symptoms appearing (it is estimated in macular degeneration that 25% and in Parkinson's and Alzheimer's that 60–80 % of neuronal loss may occur prior to measurable functional impairments); early diagnosis is difficult and sometimes out of reach; and so forth. On the one hand, amazing advances in investigative technological capabilities for imaging and bioassays are already in place and promised for the future. On the other hand, the hopes fueled by initial NP successes in drug developments for depression, schizophrenia, anxiety, Parkinson's disease, Alzheimer's disease, and other disorders have gone largely unfulfilled [8,36].

A historical divergence between basic and clinical, investigative technological and clinical therapeutic, applications of neuroscience has unfolded during the last half-century. Our research suggests that clinical neuroscience has not dealt effectively with a dysfunctional tension between the need for drug developments to function as tablets upon which the drugs themselves will write their story and the requirement that industry accelerate its drugs towards efficacy testing to constrain costs, to husband patent life, to maximize return on investment, to compete for market position, and simply to bring new therapies to patients as rapidly as the slow pace of drug development allows. We propose that this tension arose, at least in part, as an unanticipated consequence from the international consensus among governments that private industry, not governments, would take responsibility for the clinical developments of pharmacological therapies. This consensus shifted government research funding for academia away from clinical and towards basic neurosciences. Basic neurosciences have flourished while clinical neurosciences relevant to drug developments have not. We find these tensions and the negative consequences for the clinical neuroscience of drug development dysfunctional.

Commercial organizational structuring of drug development brings high levels of investment and powerful technological resources to bear on therapy problems but, we are concerned that at the same time economics, patent life, regulatory and other realities, dysfunctionally distract both industry and academic clinical neuroscientists from independent research into clinical pharmacology and therapeutics methods and designs. Our research into (–)-phenserine and other AD drug developments reveals errors that are compromises of pharmacological and therapeutic sciences [8]. Pharmacology and therapeutics, by their mandates to characterize molecules first as drugs—compounds able to exert defined and predictable effects in organisms—and then as therapeutics, by their complementary obligations to delineate drug benefits and toxicities and to find balances for benefits and safety for organisms, are disciplines with integrities separate from and basic to successful drug developments. To pursue medical benefits pharmacology and therapeutics need funding for research and development, but not the commercial goals and priorities that today accompany this funding.

We propose that the progressive NIH reductions in levels of basic research funding coupled with the shift to fund specialized academic consortia [38,39] that collaborate with industry have encouraged present day AD drug development research methods to become overly aligned and inappropriately influenced by with commercial priorities. Unlike NIH employed scientists who are precluded from benefitting personally from associations with commercially based researchers, the modern clinical neuroscience academic, lacking the clinical laboratory resources and funding needed for AD drug development, develops a network of commercial ties to and funding received for services from industry. We suggest that published acknowledgements of potential conflicts of interest cannot discharge subtle and not so subtle influences on the clinical neuroscience practices and priorities of recipients of industrial payments for services. Industry is obliged to act only in ways that promise to provide the greatest profits possible to investors. This may distract from adherence to vital neuroscience practices and put clinical neuroscience academics, devoted to science and science-derived human benefits, into irresolvable conflicts with industry. One example is the decades long industrial defense of not publishing or spinning in publications human research results as business properties [39,40]; practices recognized for decades as scientific misconduct [41] and clearly compromises of the international conventions that enable uses of humans in research [2]. AD may gain funding from the present industry investments in drug developments; but, not realize pharmacology's aim, the best drug development that money can buy.

The problems for the clinical neurosciences are further complicated by the relatively, if not dramatically, novel conditions that current ND disease realities impose. Most currently available drug therapies target diseases or symptoms that are consequence of a foreign or native intruder such as with infections or cancers, or due to a deficiency of a naturally occurring hormone or transmitter. When faced with mechanisms to which drug investigators have many times before responded effectively, time-tested strategies can frame the developments of primary and secondary preventive interventions. In NDs arresting the death of cells presents a never before successfully implemented drug intervention challenge to neuroscience. With modern medical resources surgeons very effectively replace limbs and lenses with prostheses and transplant organs; but, clinical neuroscience has not yet transplanted or re-grown for patients functional replacements for dead neurons or arrested the death of neurons that account for the various NDs. AD drug development is asked to write the operating manual while flying the airplane and managing the nuclear plant.

Analogous to the responses demanded of operators when nuclear plants and aircraft encounter novel mishaps [7], the plethora of failures in AD drug developments may force on clinical neuroscientists a Hobson's choice to live with events as they unfold because clinical neuroscience does not have the resources to diagnose developments' current miseries and respond more effectively. In other words, we find the need for possibly implementing fundamental changes to existing clinical neuroscience drug development practices if we are to assure, in its second chance redevelopment, that (-)-phenersine receives unbiased assessments of its properties as a drug and of the possible efficacies of those properties as therapeutics in human diseases.

2.4 Why did phenserine appear to fail?

(–)-Phenserine, an ACHEI and APPSI, developed by Axonyx for Alzheimer's disease showed indicators of desired activities in preclinical [15–17] and clinical [18,42,43] developments but failed in Axonyx's hands to display the statistically significant group differences from placebo that would have driven it onward to registration. Did phenserine fail or, as our early analysis showing possible invalidation of two phenserine clinical trials due to excessive variance in outcome ratings indicated [20], did the development methods fail to provide phenserine fair and unbiased conditions in which the drug could be tested for efficacy? We found in the available (–)-phenserine development documentation supports to the contention that the methods failed the drug. Investigators' attention to risks from biases did not differ from levels we found in reviews of 40 randomly selected AD drug developments [4]. Axonyx documentation and publications offered no evidenced dismissals of methods, conditions, or assumptions as possible sources for study outcomes. Axonyx proceeded on to the next phase of development before adequately grounding the progression with research from earlier phases and, for one clinical trial, placebo group variance, improvements, and declines during the double blind predicted the outcomes thus invalidating that trial and adding to the evidence of invalidity for other trials comprising the (–)-phenserine clinical development.

3. Offering second chances to AD drug candidates: redeveloping phenserine

In the (–)-phenserine documentation and in other AD and NP studies others and we have found a range of potentially invalidating practices we regard as necessary to avoid in any phenserine redevelopment. We intend that our redevelopment serve as the active arm of a clinical trial with Axonyx's development of (–)-phenserine as the historical control. If the Axonyx development failed due to the lack of resources needed to control against bias intrusions, then, by providing the resources needed to avoid these biases we should be able to demonstrate (–)-phenserine efficacy or lack of efficacy. In addition, if our assessments are correct that existing preclinical and clinical data indicate an ability of phenserine to lower rates of APP synthesis, then, we should be able to demonstrate reduced APP metabolites, including $A\beta_{42}$ and $A\beta_{40}$ and either no effects from this APPSI function of (–)-phenserine on the disease progressions of the disorders we will investigate or disease modifying effects of (–)-phenserine in one or more of these diseases. Any of these outcomes will confirm our hypotheses; that biases accounted for the failed Axonyx outcomes, that similar biases may account for other recent NP and AD drug development failures, and that with access to resources needed to exclude biases AD drug developments could become more effective.

3.1. The Redevelopment Strategy

We seek to implement our views that drug developers must have available the resources needed to avoid surprises able to derail sequentially evolving activities [2] and to design developments to provide guidance adequate for practitioners to optimize therapies with their patients [8]. To provide a fair and unbiased redevelopment we give priority to using the following resources: 1) More rigorous compliance with core scientific practices, ideally

compliance at a level equal to that observed in basic neurosciences, and 2) A defined sequencing of activities to first, characterize the molecule and its (–)-enantiomer pharmacologically, pharmacokinetically, identify potential target(s), and optimize engagements of therapeutic candidate targets and second, to confirm target engagements in humans and then test for efficacies available from optimized drug-target(s) interactions. In our view this strategy requires that progress through the development be i) ruthlessly stepwise yet flexibly able to invoke bench, animal, or human investigations in whatever sequences needed to be able to predict and then confirm new findings before proceeding to next steps [2], ii) neuroscience-theory-relevant hypothesis driven [3,5], iii) disciplined by a new logic that defines a drug as a compound with properties to be accepted only after evidence documents that the outcomes on which the properties are based occur independent of factors that are part of the development or use of the compound [8], and iiiii) committed to provision of the resources needed to provide these structural supports to the development. Our interpretation of this strategy requires two responsibilities to be met as development proceeds through traditional phases: the pharmacological characterization of (–)-phenserine as a drug or compound available to produce specified effects in multiple species with defined risks of toxicity and the subsequent therapeutic characterization of various drug-target engagements by demonstrating the presence or absence of efficacy associated with specified disease conditions.

Further problems complicate the redevelopment strategy. No outcome measures have been validated to distinguish clinical effects that derive from slowing of the rate of progression of AD pathology from symptomatic effects such as occur with ACHEIs. No criteria for accepting an interpretation of an outcome as a disease modification are available. Accompanying these problems are human limitations such as excessively high floor limits for the levels of precision available with rating instruments of clinical states in AD [21] and unproven abilities to insure accuracy, compliance with protocols (for example, to exclude pathology inflation as site investigators strive to meet entry criteria), adequacy of training at and monitoring of sites to insure compliance with the protocol, and so forth [11–13]. Also absent are validated robust surrogate biomarker outcomes [44]. These limitations undermine the effectiveness of the long duration disease modification studies required by the slow progressions in AD. In response, we set out the aim of sustained symptomatic and functional impairment suppression (SISS) to distinguish phenserine from donepezil and, by inference, from other current symptomatic AD therapies. The reported tendencies of ACHEI treated comparison groups to decline more slowly in clinical trials than predicted [45] presents a strong challenge that will add to any confidence that can be placed in a phenserine SISS superiority compared to donepezil. An advantage from this SISS outcome is the potential for development and translation of adequately precise markers of benefits into clinical practice to guide dosing and thus optimize therapeutic effectiveness with individual patients [8]. To support that option, we will time clinical assessments and use tools available to practitioners, such as the MMSE [46], to provide patient clinical status assessment and dosing guidelines for practitioners [8].

3.2. Pharmacokinetics

The existing Axonyx (–)-phenserine documentation supports a revised view that (–)-phenserine operates, not as a drug, but as a prodrug for its metabolite (–)-N1,N8-bisnorpheneserine (BNP). BNP is shown by Axonyx data to be present, at all times after dosing when (–)-phenserine is detected, at 8 or more times (–)-phenserine tissue concentrations and active at APPSI targets at 10 to 14 % of the concentrations required for pheneserine's non-chirally dependent APPSI. The lack of evidence for prolonged tissue effects beyond 1.5 times the 2 to 2.5 hour $t_{1/2 \text{ elim}}$ of (–)-phenserine, BNP, and other metabolites requires revisions to Axonyx's b.i.d. dosing. Consequently we have in development a 12 hour, 20% release of drug every 2–2.5 hours, sustained release formulation already tested *in vitro* and to be tested in dogs and then humans. The final formulation tablets will provide a 50 to 250 nanoM steady state BNP concentration option adequate to bracket the BNP APPSI IC_{50} .

3.3. Pharmacology

The existing pharmacology for (–)-phenserine almost entirely centers on applications in AD. Replicated Axonyx studies in tissue culture and small animals demonstrated dose responsive APPSI by BNP. Inflammatory cytokines, such as IL-1 and TGF β , as well as iron, all elevated in AD brain, have been demonstrated to upregulate APP expression via this 5'-UTR of APP mRNA [15–17]. Preliminary evidence indicates that (–)-phenserine returns oxidative stress induced elevated IL-1 levels to normal. Since (–)-phenserine has not been detected at concentrations able to act at the 5'-UTR in humans, it is necessary to assume that BNP provides the clinical APPSI activities [47]. One study in humans observed a 2 hour peak 20% reduction in plasma AB associated with a simultaneous 3 ng./ml. pheneserine peak plasma concentration or, estimating from independent human metabolic data, an accompanying 25 ng/ml. BNP concentration [18]. One additional potential benefit from pheneserine may derive from lower plasma APP metabolites which, by sink effects, could facilitate removal of AB₄₂ and AB₄₀ from nervous and vascular tissues.

A number of potential therapy targets for pheneserine interactions with APP mRNA and reduced APP product consequences are unexplored. We are characterizing pheneserine in relation to conditions known to present with A β amyloidosis: AD, Macular Degeneration (MD), Cerebral Amyloid Angiopathy (CAA), and mild Traumatic Brain Injury (mTBI) induction of AD and Chronic Traumatic Encephalopathy Dementia (CTE-D). A β , its oligomers, and aggregated plaques are currently regarded as contributing to and possibly causal, in part, of AD and CAA pathology; but, as with other amyloidosis diseases, both the small molecule precursors and plaques may also contribute to or account for pathologies in other organ systems.

- a. AD. We judge the preclinical data we have available to us and the dosing formulation being developed as adequate to ground Phase IIa and subsequent IIb clinical studies. We are currently scheduling a Phase IIa study of 40 mild cognitively impaired (MCI) subjects under double-blind, donepezil controlled conditions to i) demonstrate sAPP alpha and sAPP beta reductions in CSF from pheneserine subjects, ii) demonstrate that

sAPP alpha and sAPP beta reductions in CSF and plasma and AB₄₂ and AB₄₀ reductions in plasma, up to 30–50 % of baseline and possibly to normal or less levels, are BNP tissue concentration dependent, iii) demonstrate that the conditions of the clinical trial will control for ACHEI symptomatic effects on cognitive and functional outcomes such that long term reductions in cognitive loss and functional impairments with phenserine can be tested using donepezil as a control, and finally iv) to provide extended baseline evaluations of subjects whom we intend to follow under these protocol conditions for up to 18 month to determine whether the group comparisons support SISS for phenserine with reduced ADCS-Cog [48] and ADCS-ADLs [49] losses.

- b.** MD. With aging, drusen form in human retinas between the retinal pigmented epithelial layer (RPE) and the underlying coriocalpilaris [50]. These drusen appear to be benign until amyloid accumulates [51], although no pathogenic effects have been definitively linked to the appearance of amyloid. Candidate source(s) for amyloid are RPE cells, endothelial cells, plasma amyloid, or retinal neurons. We are studying RPE cells in tissue culture for responses to Aβ₄₂ and its oligomers including the induction of APP synthesis and the vasogenic factor, vascular endothelial growth factor-A (VEGF). VEGF, produced normally by RPE, is increased when MD becomes ‘wet’ producing capillary growth and retinal disruption [52]. Antibodies to VEGF in humans can reverse this capillary growth, its disruption of the retinal structure, and the accompanying loss of visual acuity [53]. Our hypotheses are that (–)-phenserine may be able to reduce or reverse amyloid accumulations in drusen. We hope with (–)-phenserine to reduce both toxic effects on retinal neurons, the integrity of RPE tight junctions, and levels of VEGF secreted by RPE thus slowing the progression of pathology to and in wet MD. We will administer (–)-phenserine and donepezil to volunteers about to undergo lens replacements for glaucoma to obtain aqueous humor samples for assay of Aβ₄₂, Aβ₄₀ and BNP concentrations to set dosing conditions for a Phase IIa POC trial.
- c.** CAA. CAA, a condition present in the majority of AD subjects and sharing properties of amyloidosis with atherosclerosis [54]; is also found independent of identified ND diseases, and has been associated with vascular dementia pathology both in NDs, including AD, and independent of ND diagnoses. We consider study of CAA as potentially informative about the effects of reducing tissue amyloid load by APPSI for the different NDs. We are studying, in a CAA transgenic mouse, the effects of (–)-phenserine on concentrations of AB₄₂, AB₄₀ and other metabolites of APP and on vessel and brain amyloid accumulations in hopes of discerning to what degrees this pathology is small molecule, oligomer, fibrillary, or amyloid plaque dependent. If indicated we will test (–)-phenserine in humans for potential to reduce progression into CAA

vascular pathology and the clinical deterioration of subjects with CAA, including AD subjects.

- d. mTBI. Since mTBI has been associated with progression into AD and CTE-D we are studying, in wild and transgenic type mice, the biochemistry of mTBI and of (–)-phenserine intervention. Particular foci for this work are: the amyloid abnormalities induced by mTBI, the tau related pathology, and the known ACHEI and the newly identified (–)-phenserine neuroprotection of neurons against the organophosphate soman. Based on this preclinical work we will study potential phenserine applications in war, terrorism, and other sources for mTBI or organophosphate intoxications.

3.4. Interventions against bias in clinical studies

Our strategy and aim to demonstrate methodological benefits compared to the initial conditions of the Axonyx development require improved compliance with common features of scientific practice. We have chosen to emphasize revisions in the logic used in the development, improved compliance with features commonly held as characteristic of science as used in basic neurosciences, and special attention to the preemption of errors.

a). The logic of reasoning in the drug development—Our strategy requires a logical reversal of the direct inference from outcome to efficacy reasoning that Axonyx used to rapidly progress through the regulatory mandated phases of drug development. Instead, we use ‘checklist’ reviews in each phase to identify and correct possible vulnerabilities to invalidation that have gone unaddressed [7] and to ascribe implications of the outcomes to the drug variable only after eliminating, with evidence, alternative origins in the methods, assumptions, conditions, or other features of the study [55 p. 188]. In effect we propose a Spaghetti Western movie like scenario, the last man standing, hopefully efficacy, owns the designation implied by the study outcome.

b) Development as neuroscience—To support a more rigorous overview of our redevelopment and to contribute to scientific knowledge, we are disciplining our clinical designs to include neuroscience theory relevant mechanistic hypothesis testing. Mechanistic hypothesis testing, as opposed to solitary efficacy testing, brings to outcomes, both positive and negative, consequences for the body of scientific knowledge as well as efficacy of the drug being tested [8]. When a test of efficacy fails only the developer suffers losses. When a test of neuroscience knowledge fails the community’s attention is recruited and methodologies are more widely scrutinized, a change we regard as important to progress in NP drug development design and practice. One implication of this assumption is that, to understand a negative outcome’s significance for neuroscience, the design must be able to distinguish failures to engage the mechanism, in which case both the drug and theoretic hypotheses go untested, from a failure of the engaged mechanism to elicit the desired clinical effects, with negative implications for both the drug and the underlying mechanistic neuroscience knowledge.

c) Bias controls—We are giving priority to resourcing our redevelopment to overcome the major deficiencies we find the (–)-phenserine development sharing with other failed NP and AD developments: unconfirmed dosing; excessive variance; evidence of inaccuracy; uncertain compliance with protocol conditions; lack of subject stabilization prior to study assessments; inadequate power; and so forth. As we have already discussed we will provide a steady state in brain of the active drug, BNP, with a slow release formulation of (–)-phenserine. In our Phase IIa study we will document the dose-response relationships in plasma and CSF between BNP and APP metabolite concentrations. With these data we will design a Phase IIb study adequate to provide both presumptive evidence for the target engagement needed to provide SISS and for SISS efficacy. To control variance we will use as data points from each rating instrument the mean of three assessments of each patient [21]. As we have documented, this reduction of variance by the n or 1.7 improves power and replaces a biased statistic with the unbiased mean, a useful addition for later clinical applications [8]. For accuracy we will use as research sites academic centers currently trained by the ADCS [37] in clinical ratings and internally monitor individuals and sites for biasing outcomes and thus compromising validity. We will either provide protocol conditions essential to success, such as circadian timing of APP metabolite sampling, or remove the need for such conditions, such as we have done for timing outcome samples to follow dosing with the provisions for steady state drug effects. To stabilize subjects to study conditions we will initiate trials with a placebo one-month series of assessments. The mean values provide an unbiased baseline and the pill counting an opportunity to test for compliance and remove non-compliant subjects. Finally, we will support vigilance against bias by using checklists to draw to our attention to possible risks for each phase of development.

4. Discussion

We have shown that (–)-phenserine and perhaps other AD drug developments were biased and therefore invalid as tests of the drug as a therapeutic. What may be newsworthy is the extent and diversity of vulnerabilities that have been identified as potentially problematic for AD drug developments and the apparent inadequacy of currently resourced clinical neuroscience to provide the otherwise relatively simple-to-implement innovations we plan for the (–)-phenserine redevelopment.

We anticipate the successful redevelopment of (–)-phenserine as a drug useful at least as pharmacological probe of APP in biological systems including humans and potentially as a therapeutic able to elicit clinical benefits through reductions of APP metabolites in AD, MD, CAA, and mTBI sequelae such as CTE-D. We also predict an improved confidence in our ability to discriminate efficacy from lack of efficacy from biased invalidations of outcomes. We anticipate this confidence to derive from methodological and clinical neuroscience interventions. We regard our (–)-phenserine redevelopment as the research arm in a clinical trial comparison of a risk-averse drug development strategy to a historically available risk-neutral drug development strategy. As such, the outcome of the (–)-phenserine redevelopment will both give or withhold further substance to our concerns with risk effects on AD drug failures and identifying and providing resources for their remediation.

Hopefully these efforts will contribute to the development of a new drug intervention against the disease course of AD.

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Abbreviations used

AChE	acetylcholinesterase
AD	Alzheimer's disease
ADAS-Cog	Alzheimer's Disease Assessment Scale-Cognitive Subscale
ADCS	Alzheimer's Disease Cooperative Study
ADCS-ADL	Alzheimer's Disease Cooperative Study-Activities of Daily Living Scale
APP	amyloid precursor protein
APPSI	amyloid precursor protein synthesis inhibitor
CAA	Cerebral Amyloid Angiopathy
CTE-D	Chronic Traumatic Encephalopathy Dementia
MD	Macular Degeneration
mTBI	mild Traumatic Brain Injury
MMSE	Mini Mental State Examination
BNP	(-)-N1,N8-bisnorphenserine
NIH	National Institutes of Health
NP	neuropsychiatric
POC	proof of concept
RPE	the retinal pigmented epithelial layer
SISS	sustained symptomatic and functional impairment suppression
VEGF	vascular endothelial growth factor-A
5'-UTR	5'-untranslated region

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