Dear Sir / Madam,

We are writing to express grave concerns regarding Cassava Sciences as a sponsor of clinical studies using Simufilam to treat Alzheimer's disease (AD). These concerns arise from an assessment of virtually every aspect of their program that has been made available for public scrutiny. We find serious deficiencies in the scientific integrity of the sponsor, Cassava Sciences, who exhibits concerning signs of misleading behavior. We show, using publicly available evidence, that Cassava sciences has not fulfilled the responsibilities that your agency requires of sponsors in the conduct of clinical studies and the monitoring of patient's safety (21 CFR 312).

We are familiar with the recent Citizen's Petitions (CP), FDA-2021-P-0930 as well as FDA-2021-P-0967, and support the allegations made. However, in this document we present additional concerns not addressed by the petitions and its supplements. More importantly, we reveal a pattern of deliberate, coordinated misconduct involving both Cassava Sciences and their academic collaborator at CUNY, Dr. Hoau-Yan Wang. As documented below, our analysis identifies numerous critical issues which include:

i) fabrication of pre-clinical and clinical evidence across the entire Simufilam program
ii) inadequate and unreliable safety studies for Simufilam
iii) serious misconduct in the analysis and reporting of clinical trial data
iv) improper and opaque study conduct by the sponsor and its collaborators

Considering the questionable pre-clinical research on Simufilam (first discussed in the CPs to FDA) we take a critical look at the data reported from clinical trials so far and, more importantly, the conduct of Cassava Sciences as a sponsor. What follows is a description of the methods by which, we allege, Cassava Sciences has either obfuscated or fabricated data during these clinical trials; from the Phase 2a (Ph2a) to the ongoing Open Label (OL) study. Where direct access to the raw data was not available to the sponsor - mainly data from the cognitive assessment of patients - elaborate post-hoc exclusion criteria and suspiciously large alterations in patient population characteristics were devised to alter outcomes. On the other hand, we demonstrate that the CSF biomarker data generated by Cassava scientific advisory board (SAB) member Dr. Wang through an opaque process, yielded improbable values. This leads to the strong suspicion that the data have been entirely fabricated.

Given these issues, there is a material concern regarding the sponsor's credibility and very real risk of exposing thousands of patients to a compound with unknown risk, for which there is no evidence of clinical benefit to justify this risk.

A Precarious Preclinical and Pharmacological Foundation

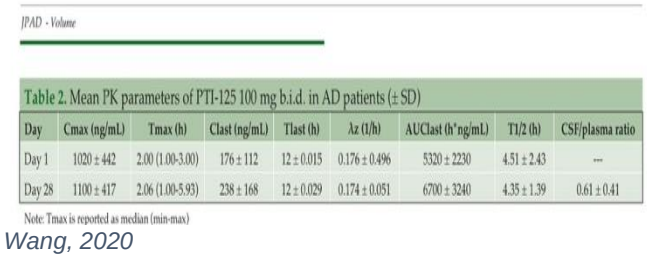
Our investigation was triggered by the striking inaccuracies, image manipulation and incomprehensible rationale of Cassava Sciences' pre-clinical research referenced in the CPs. Putting aside that literally no other lab has replicated Cassava's putative findings regarding Simufilam or a connection between Filamin-A function in AD, we call into question the logic and biophysical plausibility of the proposed mechanism and the conduct of the laboratory studies supporting this drug candidate.

Briefly, the discovery of Simufilam was predicated on the reported binding of Naloxone to the Filamin-A (FLNA) protein (Wang, Frankfurt, and Burns, 2008). The discovery and structure of Simufilam (formerly known as PTI-125) has never been properly described in a peer-reviewed paper. However, the development program is summarized in a review describing a related molecule (Burns & Wang, 2010) and described in a series of patents. Notably, the patents claim that Simufilam was found in an *in vitro* screen (in competition with Naloxone) for binding against an isolated pentapeptide, VAKGL.

Cassava - at the time known as Pain Therapeutics, and developing Remoxy, an in-licensed reformulation of oxycodone that was ultimately rejected multiple times by FDA - was initially interested in Naloxone analogs and Filamin-A binding molecules for analgesia. Miraculously, several years later, Dr. Wang claimed, based on his signature immunoprecipitation and western blot experiments, that FLNA interacts with several proteins potentially involved in AD pathogenesis. According to his research, FLNA is mysteriously 'altered' in AD in a manner that affects interactions and signaling, and that Simufilam 'restores' Filamin-A to its native structure and function. Thus, the just-so story of Simufilam for AD began.

Filamin-A is an actin-binding protein with diverse mechanical and molecular scaffolding functions, and interacts with dozens of other structural and signaling proteins. While abundant in certain tissues such as smooth muscle, FNLA is not highly expressed in the adult brain. Naloxone, a drug that has been intensively studied for over 50 years, has never been observed by any other researchers to bind Filamin-A, nor is it distributed in vivo to tissues with high FLNA expression, including smooth muscle (Pert and Snyder, 1973). These simple observations evoke profound and troubling questions about whether Simufilam actually binds its supposed target, and whether the molecule was discovered in the manner claimed by Cassava Sciences.

Moreover, Cassava Sciences claims that Simufilam binds FLNA with sub-picomolar affinity. Based on the pharmacokinetic data reported for the Phase 2a study, Cassava is currently administering doses that achieve >3 micromolar concentration in the plasma, with high penetration to the CSF. This represents a **3-million-fold overdosing** and illustrates that the clinical trials are in no way related to the purported mechanism of action of Simufilam.



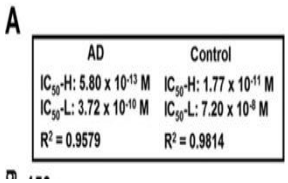
JPAD - Volume

Table 2. Mean PK parameters of PTI-125 100 mg b.i.d. in AD patients (± SD)

Day	Cmax (ng/mL)	Tmax (h)	Clast (ng/mL)	Tlast (h)	λz (1/h)	AUClast (h*ng/mL)	T1/2 (h)	CSF/plasma ratio
Day 1	1020 ± 442	2.00 (1.00-3.00)	176 ± 112	12 ± 0.015	0.176 ± 0.496	5320 ± 2230	4.51 ± 2.43	---
Day 28	1100 ± 417	2.06 (1.00-5.93)	238 ± 168	12 ± 0.029	0.174 ± 0.051	6700 ± 3240	4.35 ± 1.39	0.61 ± 0.41

Note: Tmax is reported as median (min-max)

Wang, 2020



Simufilam Affinity for FLNA
Wang, 2017

The reported high affinity of Simufilam for its target would also imply that at the doses achieved, the drug binds all Filamin-A throughout the body to saturation. Even more worryingly, a recent paper co-authored by the Cassava CSO, Dr. Lindsay Burns (Zhang, 2020) claims an effect of Simufilam on a mouse genetic model of focal cortical malformations. This paper confusingly asserts that Simufilam acts as an **inhibitor** of FLNA function, exerting an effect equivalent to genetic knockdown. If this were

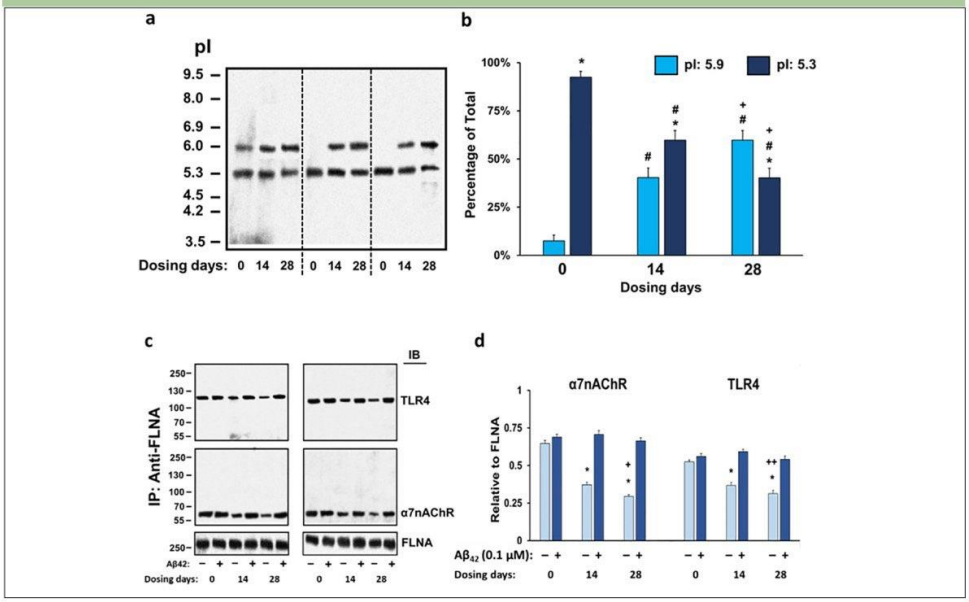
true, one may expect to see widespread effects on scaffolding and signaling function in tissues in which Filamin-A is abundant, which have not been reported by either pre-clinical or clinical research with Simufilam so far.

Even if one ignores this overdosing and potential for on-target toxicity, the proposed mechanism of Simufilam in AD is supported by virtually no accepted scientific findings. The Sponsor attempts to provide supporting evidence in the “More Information” section of the clinical trial entry (http://clincialtrials.gov for NCT04079803) with links to four publications. One is a review and has no data, however the other three are critical to the rationale for using Simufilam to treat Alzheimer’s disease. Each of these publications has been flagged on http://pubpeer.com for possible image manipulation by, among others, international expert in scientific fraud detection Dr. Elisabeth Bik. The central author common to these papers is none other than Dr. Wang. In addition to being the key scientific contributor to Cassava Sciences and a named inventor on the Simufilam patents, Dr. Wang is currently under investigation by the City University of New York (CUNY) for scientific misconduct for work directly related to these publications. In fact, concerns have now been raised on PubPeer on twenty-four of Dr. Wang’s publications spanning his entire independent career over the last two decades.

Target Engagement in Lymphocytes.

The biological implausibility of the Simufilam story extends to Cassava’s clinical claims. In a paper describing the results of the Ph2a trial, the authors claim that FLNA is present in an altered state in the lymphocytes of AD patients – but not healthy individuals – and that this misfolded version of the protein is restored to sits native structure after a 28-day course of Simufilam.

Figure 4. PTI-125 restores FLNA’s native shape and reduces FLNA linkages to α7nAChR and TLR4



a, b Restoration of FLNA’s native shape. Isoelectric focusing gel (a) and its quantitation (b) show that 93% of FLNA isolated from lymphocytes prior to treatment is in the altered conformation (pI 5.3), with just 7% in the native shape (pI 5.9). PTI-125 treatment for 28 days shifts this distribution to 40% in the altered and 60% in the native conformation. *p < 0.0001 comparing 5.9 to 5.3, #p < 0.0001 vs. dosing day 0, +p < 0.0001 vs. dosing day 14. c,d, Reductions in FLNA linkages to TLR4 and α7nAChR found in lymphocytes. Reductions are illustrated by TLR4 or α7nAChR levels detected using immunoblotting with specific antibodies in solubilized anti-FLNA antibody immunoprecipitates of lymphocytes. Additionally, exogenous Aβ42 added in vitro to lymphocytes reversed these reductions in FLNA associations, returning levels to pre-treatment baseline. Blots (c) were assessed by densitometric quantitation (d). *p < 0.001 vs. dosing day 0, +p < 0.01 and ++p < 0.05 vs. dosing day 14. N=13 with one missing value. Error bars are SEM.

IIt is worth noting that this hypothesis suggests that despite Alzheimer’s patients’ lymphocytes containing almost entirely the misfolded variant of Filamin A, they exhibit only Alzheimer’s disease

and no significant immune-related maladies. If true, this would represent an entirely new dogma not only for neuroscience but for immunology as well.

We confidently assert that the proposed mechanism of action of Simufilam is irrational and not supported by accepted evidence. Prospective investigators and patients in the currently recruiting studies must be clearly alerted to the highly controversial nature of the trial immediately, and the preclinical rationale in the Investigator's Brochure provided to the IRB and to investigators must be updated, pending the findings of multiple investigations into Dr. Wang's reported misconduct currently underway. The current state of evidence supporting a mechanism of action of Simufilam raises many questions that should be answered before trials continue:

Key Questions

1. Is there any evidence supporting Simufilam mechanism of action that does not rely on Dr. Wang's discredited data?

2. Given the expression of Filamin-A outside of the brain, has Cassava Sciences ever evaluated the tissue distribution of Simufilam in a preclinical model?

3. What is the biophysical mechanism for high affinity or selective binding of Simufilam to the isolated VAKGL pentapeptide, or to the native Filamin-A protein, given that the VAKGL sequence is in a region lacking any potential binding pocket?

4. Does Simufilam interact with the dozens of other human proteins that also contain the VAKGL sequence?

5. Why is Simufilam being dosed BID at concentrations that are supra-supramaximal for the reported mechanism?

6. Given the million-fold excess dosing, how is it possible that any dose response could ever be observed between the 50mg and 100mg doses?

7. Why, despite the central role of Filamin A, zero reports exist of Alzheimer's patients presenting with severe comorbidities affecting the immune or other systems?

Inadequate And Unreliable Evaluation of Safety

Cassava Sciences frequently asserts that Simufilam is well-tolerated and safe. However, an evaluation of available data reveals little rational basis for initial dose selection and no consideration of potential on-target toxicity. Moreover, the clinical studies that form the basis for the presumption of Simufilam safety were conducted by investigators whose deficiencies in trial conduct have already been well documented by FDA investigations.

Remarkably, for a drug intended for chronic use, the Phase I safety study tested only a **single** administration of the drug, with subjects monitored for only one week. The doses studied were chosen based on an estimate of a safe dose from a NOAEL in preclinical toxicology studies (PTI-125-01 Protocol) but apparently without regard to the purported mechanism of action or pharmacology.

Of even greater concern, safety data from the Ph2a and Ph2b studies cannot not be relied upon due to concerns raised about the conduct of a key investigator only very recently and while Cassava Sciences' studies were ongoing at the same clinic. It is alarming to observe that one of only two investigators common to both studies, Dr. Evelyn Lopez-Brignoni, received a Warning Letter from the CDER Office of Scientific Investigations in March 2021, describing conduct that "raises concerns about the validity and integrity of the data collected at [the] site". While this inspection and enforcement action appear to have been associated with a different, but contemporaneous trial, it implies that the conduct at this site was woefully deficient. Specifically, the Warning Letter states:

- "Subjects may have taken placebo only instead of the required study drug, or less than the full intended dose of the study drug"
- "The investigator failed to ensure that subjects adhered to the dosing regimen"
- "The investigator failed to conduct the clinical studies in accordance with the investigational plan"

If similar deficiencies in dosing and trial conduct occurred in the Cassava trials at this site under the supervision of Lopez-Brignoni, neither efficacy nor safety data reported by the Sponsor for the Ph2a or Ph2b Simufilam trials can be relied upon. While Simufilam might be safe, as suggested by the absence of (reported) serious adverse events to date, the data available are unreliable due to improper study design and questionable conduct, and thus insufficient to properly assess risk in large-scale and longer Phase 3 trials.

Key Questions

1. Were the Phase 2 studies conducted according to the investigational plans at all sites, including the IMIC Inc. site under the supervision of Dr. Lopez-Brignoni?

2. Why did Cassava Sciences choose to work with the specific sites and clinical investigators used to conduct the Phase 2a and 2b trials?

3. What oversight did the Sponsor provide to ensure that the investigators conducted the clinical studies in accordance with the investigational plan?

4. What studies have been conducted to evaluate short- or long-term effects of Simufilam on Filamin-A function in the many tissues in which it is highly expressed, including immune cells and in cardiac and visceral smooth muscle?

A Series of Improbable Biomarker Data

Biomarker values reported across the entire Simufilam clinical program are biologically and statistically implausible. While the CPs allege errors or manipulation in the Ph2b Study (Supplement 2, Paragraph 4), we demonstrate a continuous, consistent pattern of data fabrication that involves key clinical biomarkers; including inflammatory cytokines that could provide insights to the safety of Simufilam administration. Beyond the improbable values, we discovered some questionable research practices with the most notable instance being, of course, the re-analysis of the Ph2b samples.

The Re-do

This unusual "re-do" of the bio-marker analysis has already been documented in two CPs filed to FDA, however we note several additional concerns around this decision. Cassava Sciences stated (Top-line Results from a Phase 2b Study of PTI-125 in Alzheimer's Disease Does Not Meet Primary Endpoint | Cassava Sciences, Inc.) that on initial analysis an undisclosed external lab produced data with an unacceptably high variation in CSF tau and p-tau readings. Yet, that very same lab successfully analyzed CSF samples at baseline which were accepted by the company at that time and used to recruit patients with an appropriate CSF tau/Aβ42 ratio (≥ 0.28). Furthermore, the company was happy to report that CSF IL-1beta analysis from the same lab showed a favorable trend: "*PTI-125 significantly reduced a secondary endpoint, CSF levels of IL1-beta (p<0.035), a core biomarker of neuro-inflammation, from baseline to Day 28*". This belated dissatisfaction raises strong suspicions that Cassava Sciences only deemed the lab's analysis to be inadequate after receiving undesired results. As a consequence of this "re-do" decision, the majority of the bio-marker data found in the Clinicaltrials.gov records originate from a second analysis of the samples performed under Dr. Wang's supervision. Surprisingly, the IL-1beta levels from patients reported after the first "failed" analysis have subsequently never been reported again, despite being listed as efficacy endpoints in the study protocol as well as in the statistical analysis plan.

Non-sensical Albumin Levels

In analyzing albumin, a key biomarker of blood-brain barrier (BBB) integrity, Dr. Wang employed semi-quantitative Western blotting to measure its concentration in CSF and plasma samples. Albumin is the most abundant protein in plasma and CSF and routinely quantified in clinical and research settings either by BCG staining, ELISA or immuno-nephelometry assay. We emphasize this aberration from the norm as it speaks to the motives behind the company's insistence on Dr. Wang's analysis.

▼ Analysis Population Description

Three subjects who had no detectable simufilam in plasma at return visits were removed from analyses (two in the 100 mg arm and one in the 50 mg arm). As noted in the Participant Flow, one subject in the 50 mg arm did not complete the study. One additional subject in the 50 mg arm was missing a Day 28 CSF sample.

Arm/Group Title	Placebo Cohort	Simufilam (PTI-125), 100 mg Tablets Cohort	Simufilam (PTI-125), 50 mg Tablets Cohort
▼ Arm/Group Description:	Placebo oral tablets administered twice daily (BID)	Simufilam, 100 mg oral tablets administered twice daily (BID)	Simufilam, 50 mg oral tablets administered twice daily (BID)
Overall Number of Participants Analyzed	22	19	18
Mean (Standard Deviation) Unit of Measure: pg/mL; optical density for albumin & IgG			
CSF IL-6	-1.1 (2.0)	-3.7 (1.8)	-3.3 (1.9)
CSF sTREM2	-77.3 (510)	-426 (274)	-424 (386)
CSF HMGB1	19.4 (172.3)	-143 (51.3)	-152 (50.1)
CSF albumin	-240 (1620)	-2292 (1760)	-1245 (1735)
CSF IgG	-574.8 (2518)	-2350 (2517)	-2444 (2097)

In the company's Ph2b publication pre-print (Effects of simufilam on cerebrospinal fluid biomarkers in Alzheimer's disease: A randomized clinical trial | Research Square) the reduction in CSF albumin is reported as 15% and 29% for the 50mg and 100mg arms respectively. Using the referenced reduction, we back calculated the baseline albumin values for each group; these are 8300 and 7903 pg/ml for the 50mg and 100mg arms respectively. Comparing these to the expected levels for adults

over 55 years old, which are in the range of 8 to 55 (012229: Albumin, Cerebrospinal Fluid | Labcorp) we find these are lower by 10,000-fold. Furthermore, the reported baseline values are surprisingly similar, given that the SD for each group is over 1700 pg/ml.

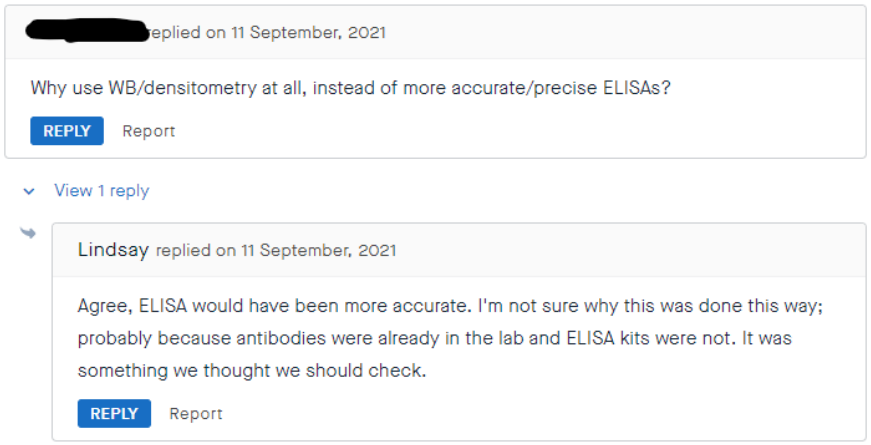
Beyond the CSF readings, more issues arise with the CSF/plasma albumin ratios reported by the company. These are ~0.25 in their pre-print, which - after adjusting for a multiplier of x1000 to match the official QAlb formula – becomes 250. This contrasts with the expected range of 5 – 9 (Chalbot 2011, Skillback 2017, Velpen 2019) by over 27 times and would imply a near total breakdown of the BBB in AD patients(!). This raises serious questions as to the specificity and accuracy of the unorthodox quantification approach used, and to whether these numbers were even the result of any sample analysis. Strikingly, the company relied on these values to claim a major scientific breakthrough.

Clinical Dataset to be Presented November 7th at CTAD 2020 Conference

AUSTIN, Texas, Nov. 04, 2020 (GLOBE NEWSWIRE) -- Cassava Sciences, Inc. (Nasdaq: SAVA) today announced additional clinical data of a Phase 2b study with sumifilam, its lead drug candidate, in patients with Alzheimer's disease. In a clinical study funded by the National Institutes of Health (NIH), sumifilam decreased levels of a protein called HMGB1 and improved measurements of the integrity of the blood-brain barrier (BBB). The ability of a drug candidate to decrease HMGB1 and improve BBB integrity in patients with Alzheimer's disease has not been previously reported in the science literature. Sumifilam is a proprietary, small molecule (oral) drug that restores the normal shape and function of altered filamin A protein in the brain.

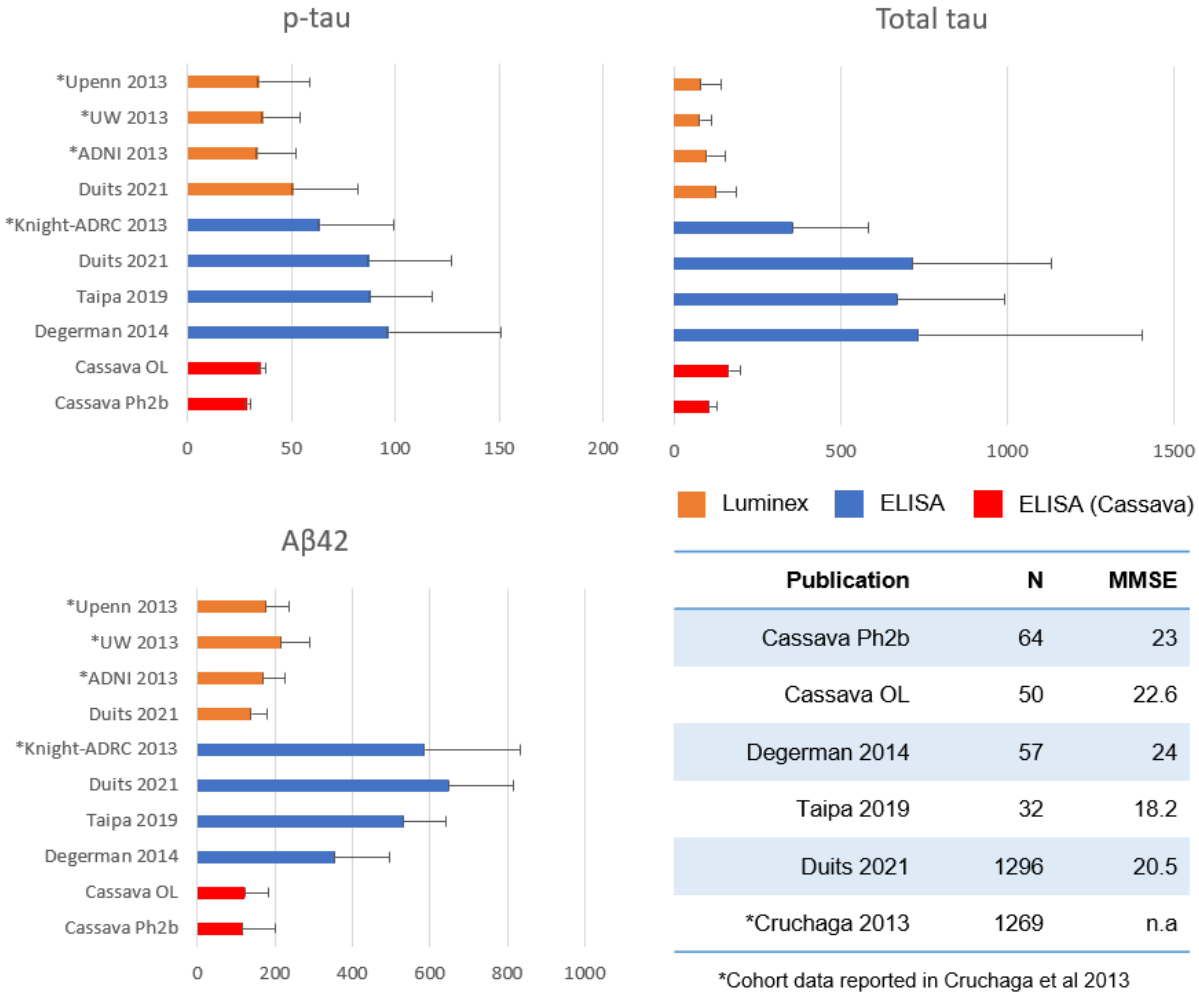
"The ability to improve multiple biomarkers of disease with one drug is a unique achievement," said Remi Barbier, President & CEO of Cassava Sciences. "We believe these exciting clinical results create a time of rapid strategic momentum for the Company, to include development plans to evaluate sumifilam in a Phase 3 clinical program in patients with Alzheimer's disease."

We believe the intent of this unusual albumin analysis was to support this "unprecedented discovery" with a publication utilizing Dr Wang's questionable methods in Western blot image "preparation". Yet, even the company's CSO Dr. Burns indicates in one of her responses to ResearchSquare comments that this assay was likely employed due to practical considerations and the results warranted further examination. We wholeheartedly agree that any responsible and credible sponsor should and would use validated methods accepted by the scientific community; especially prior to announcing biomedical breakthroughs such as the one reported by Cassava Sciences.



Inexplicable Tau and Amyloid-beta Values

Another distinctly worrying pattern emerges when surveying the data reported by Cassava for their Ph2b and Open Label (OL) study of biomarkers analyzed by ELISA in Dr. Wang's lab (Aβ42, total-tau, phosphor-tau). Note that in this report we refer to the 9-month OL data presented at AAIC 2021 and available from Final Results of a Phase 2b Study of Simufilam in Alzheimer's Disease (cassavasciences.com) unless stated otherwise. We compared these values to other published studies in AD populations with similar MMSE scores (all references available upon request). Surprisingly, while the values reported by Cassava are many-fold lower than those in comparable research utilizing ELISA assays; they align much better with values reported by Luminex analysis of CSF samples. The differing outcomes of the two analytical assays in CSF samples from Alzheimer's patients are discussed and contrasted in numerous publications.



Publication	N	MMSE
Cassava Ph2b	64	23
Cassava OL	50	22.6
Degerman 2014	57	24
Taipa 2019	32	18.2
Duits 2021	1296	20.5
*Cruchaga 2013	1269	n.a

*Cohort data reported in Cruchaga et al 2013

Because the two assays differ fundamentally in underlying technology and equipment, it is inconceivable that Dr. Wang's team has been using an assay other than ELISA. This is clearly stated in study protocols, posters, publications and public remarks from Dr. Lindsay Burns, the company's CSO.

Lindsay Burns

Cassava Sciences, Inc.

Posted: 30 Sep 2021

Thanks for your comment. Aβ42 was measured using Invitrogen's ELISA kit. Different antibodies can produce very different results, even though all translate data to pg/mL. I can say that the R2 values for the standard curves for all ELISAs were 0.88 or higher. I cannot access this full paper—which ELISA plates were used? Simufilam is not LSD. That was plugged into a presentation once when we were not yet disclosing structure.

We are left to conclude that the values reported may have been fabricated to simulate those in relevant literature albeit from a reference using Luminex rather than the claimed ELISA. This conclusion is further backed by findings discussed below and would be consistent with the allegations of Dr. Wang's systematic manipulation in images of Western Blots.

Questionable Biomarker Readings

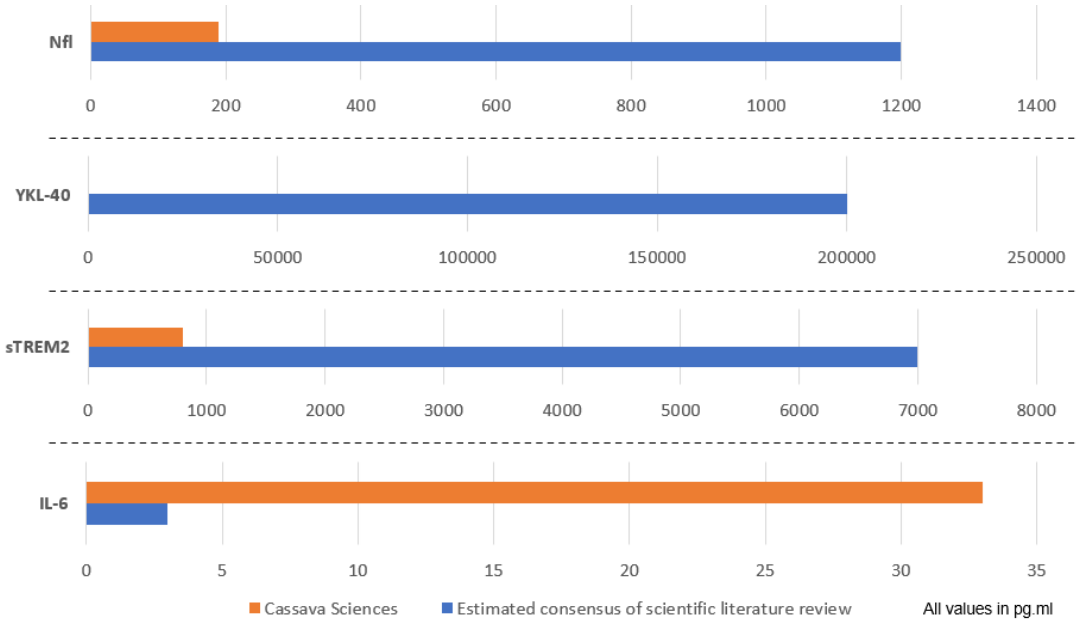
After reviewing the literature for the remaining biomarkers we uncovered more values consistent with the pattern of clumsy data fabrication described so far. The key ones are listed below:

- Nfl levels at the Ph2b study baseline have a reported range of 161 – 219 pg/ml. These however do not correspond with the Ph2a values; ranging from 400 to 700 pg/ml. Further, they appear extremely low compared to the literature [fnagi-11-00254-t001.jpg (1144×1314) (frontiersin.org)]

- YKL-40 levels reported are in the 200 – 250 picogram/ml range while other researchers report 100s of nanograms (Wang et al. 2016, Lleo et al 2019) which is 1000-times higher. The values would have made sense if reported in ng/ml instead, yet Cassava have consistently presented these in pg/ml concentrations.

- IL-6 and sTrem2 levels reported are extremely high and don't align with reported literature as shown previously in CP Supplement 1

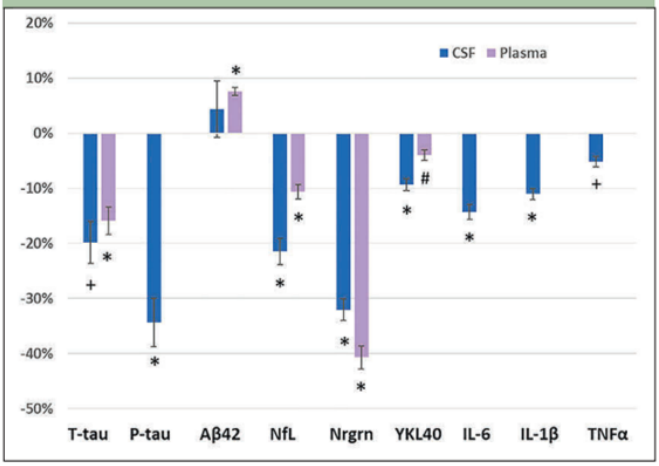
The above adds to an extended series of implausible and entirely unrealistic values reported for nearly every CSF biomarker analyzed by Cassava Sciences.



In the Phase 2a study (PTI-125 Reduces Biomarkers of Alzheimer's Disease in Patients - PubMed (nih.gov)), we are presented with the remarkable finding that a 28 day treatment with Simufilam "improves" levels of the AD biomarker, neurogranin. A large reduction of 40% is reported in the plasma of patients after treatment. The large reduction is consistent with that reported in the CSF. The problem is that plasma neurogranin is NOT a biomarker of AD and does not differ between patients and healthy individuals (De Vos 2015, Kvartsberg 2015).

Finally, it is readily apparent that the SD values of mean change reported for the Ph2a study (below, right) are extremely narrow and unrealistic. Particularly a 1% SD in the mean change for the inflammatory cytokines Il-6, Il-1β and TNF-α over a 28-day interval is not consistent with human biology.

Figure 1. PTI-125 treatment reduces CSF and plasma biomarkers



▼ Analysis Population Description

[Not Specified]

Arm/Group Title	PTI-125
▼ Arm/Group Description:	PTI-125 100 mg oral tabl PTI-125, 100 mg tablets:
Overall Number of Participants Analyzed	13
Mean (Standard Deviation) Unit of Measure: % change from baseline	
Total tau	-19.8 (0.04)
Abeta42	4.3 (0.05)
p-tau181	-34.4 (0.05)
Neurogranin	-32 (0.02)
Neurofilament light chain	-22 (0.02)
YKL-40	-9 (0.01)
IL-6	-14 (0.01)
IL-1 beta	-11 (0.01)
TNF alpha	-5 (0.01)

Inconsistent Baseline Reports

A hallmark of fraudulent data is inconsistency. Comparing the baseline values reported for patients recruited in the Ph2b with those in OL study we find an inconsistent shift in the mean values of biomarkers most egregiously in the values for Neurogranin, sTrem2 and hmgb1.

	Ph2b						OL		
	Pbo	Pbo	50mg	50mg	100mg	100mg	100mg	100mg	
Biomarker (pg/ml)	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Ph2b-to-OL diff in means*
CSF Aβ42	125	152	108	54.8	117	51.4	122.8	62.4	**+5%**
CSF total tau	104	32	101	17.6	106	27.9	163.5	33.7	**+58%**
CSF P-tau181	28.5	0.73	29	1	29.7	1.5	35.7	2.1	**+23%**
CSF neurogranin	1200	365	1352	614	1551	751	2147.6	575.7	**+57%**
CSF NfL	161	42.8	181	64.4	219	95.3	291.6	55.1	**+56%**
CSF YKL-40	206	29.5	194	26	203	22.7	250.4	35.8	**+25%**
CSF IL-6	32.5	1.2	33.6	1.7	33.6	1.8	-	-	
CSF sTREM2	878	435	882	476	861	421	1165.8	421.2	**+33%**
CSF HMGB1	424	48	454	70.6	446	67.3	722.6	98.6	**+64%**

These differences are difficult to explain based on patient variability alone. Note that the OL study is an extension of the Ph2b – with the very same limited number of clinical sites recruiting until mid-2021. Therefore, it is safe to assume that a significant proportion of the 50 reported patients are made up of those previously enrolled in the Ph2b study. This dramatic change in baseline values is puzzling and cannot be attributed to a different patient population or even a plausible effect from prior Simufilam dosing, as the values are higher for the OL study patients.

We also used estimates of the mean baseline values from the publication of the Ph2a study and again compared to the Ph2b baseline data. The analysis uncovered yet more inexplicable differences, particularly in the levels of p-tau, Nfl, YKL-40 and IL-6. It is additionally worth noting that the range of IL-6 cytokine values reported in both Ph2a and Ph2b studies are inconsistent with values reported in the literature (Wennstrom 2015, Wu 2015, Albrecht 2021). Such discrepancies between studies using the same lab and assays again raise suspicion that the reported values are not genuine.

	Ph2b						Approximate Ph2a values		
	Pbo	Pbo	50mg	50mg	100mg	100mg	100mg	100mg	
Biomarker (pg/ml)	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Ph2b-to-Ph2a diff in means*
CSF Aβ42	125	152	108	54.8	117	51.4	176	-	**+51%**
CSF total tau	104	32	101	17.6	106	27.9	168	-	**+62%**
CSF P-tau181	28.5	0.73	29	1	29.7	1.5	8	-	**-74%**
CSF neurogranin	1200	365	1352	614	1551	751	971	-	**-29%**
CSF NfL	161	42.8	181	64.4	219	95.3	541	-	**+189%**
CSF YKL-40	206	29.5	194	26	203	22.7	74	-	**-63%**
CSF IL-6	32.5	1.2	33.6	1.7	33.6	1.8	19	-	**-44%**

Key Questions

Based on the above findings, the sponsor must answer:

1. Why was a method with known limitations in quantitation used to assay a high concentration protein (albumin) in CSF and plasma – both from precious clinical samples?

2. Why, despite both the range of values and ratio reported being entirely incompatible with scientific literature and clinical references, was Cassava Sciences eager to announce a never-before finding of such major significance without taking steps to validate the results using other assays?

3. Why are the Aβ42 and Tau values published similar to those reported by researchers using the Luminex immunoassay when analysis was conducted by ELISA?

4. How can the clinical safety and efficacy of simufilam be assumed based on biomarker data that is – in all cases we investigated – entirely out of line with literature in AD?

5. What is the explanation for the wide variation in baseline values for these biomarkers between studies?

6. Why did the sponsor discard the original biomarker measurements, and elect to re-do the measurements using non-validated methods in an academic laboratory?

7. Why has the company relied entirely on a close collaborator and SAB member with direct interest in the success of the clinical program to perform assays that are routinely performed by qualified, accredited commercial laboratories? What safeguards are in place to protect the integrity of the study from this direct conflict of interest?

8. Why have the results of the IL-1β analysis from the Ph2b study never been presented?

Selective Analysis of Cognitive Outcomes

Post-hoc Analysis of Cognitive Testing

The evidence for any putative benefit of Simufilam is based on reports of non-statistically significant trends in a single Randomized Clinical Trial. As is well known, standard analysis of such a protocol is established pre-hoc in the Statistical Analysis Plan, which routinely requires analysis of the full analysis set. The Study Protocol and the Statistical Analysis Plan state that "*All patients who receive study medication will be included in analyses for safety, biomarkers and cognition*" and "*All subjects for which data is available for Day 1 and Day 28 will be included in the cognition analysis*" (protocol section 10.2 and statistics plan section 3.0).

The data handling and reporting of cognitive data (secondary outcomes 7 and 8) is in violation of the Study Protocol and Statistical Analysis Plan. Outcome 7 "Paired Associates Learning Test" employs a complicated Analysis Population Description method for eliminating subjects based on scores that are too high, scores that are too low, pill counts and detectable levels of drug in plasma; none of which are found in the Protocol. This resulted in the post-hoc elimination of 40% of subjects (27 of 64).

While individual case records are not available, it can be reasonably assumed that subjects who were too ill to comply (scores too high) were in fact non-responsive due to floor effect and those "too healthy" (scores too low) could also show no improvement due to a ceiling effect. It is not unreasonable to assume that through arbitrary and post-hoc selection of which scores defined these boundaries (11 and 54) the sponsor was able to arrive at the desired trend. Similar post-hoc manipulation is seen in Outcome 8. Here, examining drug plasma levels, pill counts and "didn't understand" criteria (all also not in the Study Plan) resulted in the exclusion of 11% of the study subjects (7 of 64). That all the subjects excluded from outcome 8 analysis were from the drug treatment groups rather than randomly distributed further suggests this exclusion scheme was devised post-hoc with a desired treatment effect outcome in mind. These obvious violations of the data treatment plan are clearly designed to skew the data in a favorable direction and obscure the lack of benefit of Simufilam on cognition.

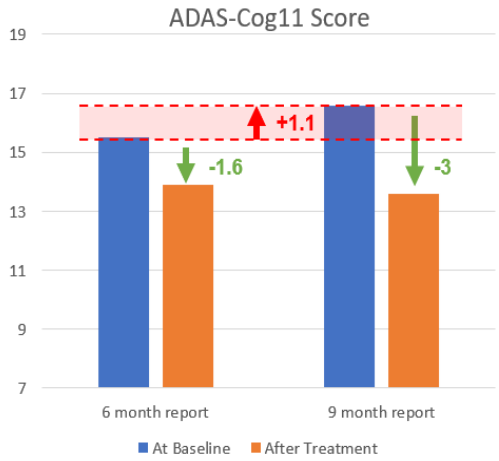
Endpoints	Cognitive		Plasma	
Study outcomes	**# 7 PAL Test**	**# 8 SWM Test**	**#11 pTau**	**#12 SavaDx**
Score too high or low	⃠	⊗	-	-
Pill count	⃠	⃠	⊗	⊗
Plasma levels	⃠	⃠	⊗	⃠
CVs too high	-	-	⃠	⊗
If(x/y>150% AND x-y>2.5)	-	-	⃠	⊗
Total Patients excluded	**41%**	**10%**	**17%**	**8%**
Placebo	36%	0%	9%	0%
100mg Tx	38%	14%	19%	10%
50mg Tx	50%	15%	25%	15%

⃠ Protocol violation ⊗ Criteria not applied

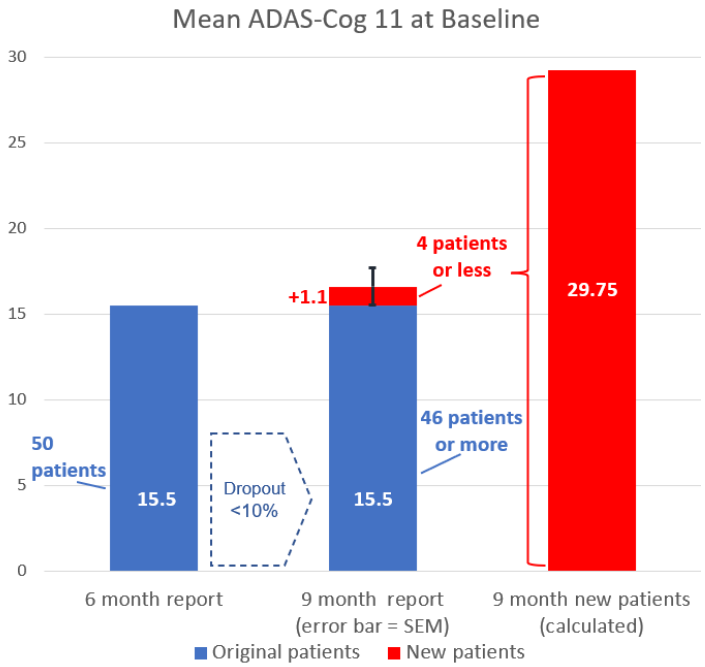
Similar post-hoc data manipulation has been applied in this trial to plasma-based biomarkers pTau and SavaDx (outcomes 11 and 12) and those are discussed in detail in our "*Plasma-based biomarkers and SavaDx*" section.

Inconsistencies Across Studies

This pattern of misleading the public, prospective patients and investigators through questionable reporting and data manipulation has continued past the initial Phase 2 and into the current Open Label extension. In Cassava Science's Phase 2B Open Label Study ADAS-Cog 11 scores for the first 50 patients have been reported after six, nine, and 12 months. On February 2nd, 2021, a press release was issued in which the company announced that at 6 months, ADAS-Cog 11 scores had improved 10%, dropping on average 1.6 points from a reported mean baseline score of 15.5. This means the actual observed mean score was 13.9. On July 29th the company presented the 9-month interim results at the Alzheimer's Association International Conference 2021. There, the reported ADAS-Cog 11 mean baseline was 16.6, with an observed mean improvement of 18% or 3 points, meaning that the actual observed mean score was 13.6



Not only does this mean that the observed mean ADAS-Cog 11 scores after 6 and 9 months are virtually the same – 13.9 vs. 13.6 – but also a considerable change in baseline score occurred. In this same presentation it was stated that the dropout rate was <10%. Final Results of a Phase 2b Study of Simufilam in Alzheimer's Disease (cassavasciences.com) . Given that less than 10% of patients dropped out there could have been only four patients who were replaced. If this is true, those new patients must have had a baseline score that was on average 13.75 points higher than that of those patients who dropped out [calculated: (50x16.6-50x15.5)/4=13.75].



ADAS-Cog values for new patients at 9 months are calculated based on the assumption that the mean score for drop-outs didn't differ from that of other patients

When compared to the reported baseline standard deviation of 7.7 points and the observed improvement of 3 points, a difference of 13.75 points between dropped-out and newly included patients is suspiciously large. Whereas in Ph2b Cassava was able to obscure the effect of Simufilam on cognition through imaginative use of outlier exclusion criteria, in the Open Label Study they appear to have swapped subjects from 6 to 9 months in order to include those with extremely high ADAS-Cog scores. This observation becomes even more crucial as the ADAS-Cog test shows different sensitivity for patients with mild vs. moderate AD. In light of this obfuscation of baseline changes and uncertainty regarding the process of determining drop-outs, the presented results become inconclusive, as they can easily be explained by regression to the mean (i.e. patients who by chance exhibit "extreme" scores the first time tend to have more "normal" scores the second time). After this criticism had been publicly stated online, the 12 month ADAS-Cog 11 scores were reported in a press release on September 22nd, 2021. In this press release a mean improvement of 3.2 points was reported but, unlike previous releases, there was no mention of baseline or percent change.

The skewing of clinical data has further implications. Because the sponsor has claimed there is "benefit," they extend and exacerbate this claim by suggesting there are biomarkers indicative of improvement. This is misleading since no clinical improvement was demonstrated according to protocol. Thus, clinical investigators along with patients being recruited into the two Phase 3 studies are being misled into believing there are biomarkers indicating benefit based on a study where no benefit was shown.

Key Questions

The Sponsor's conduct in the analysis of cognitive outcome measures in this trial raises serious questions:

1. Why was the Statistical Analysis Plan not followed?

2. Were the outlier criteria designed to give an inaccurate understanding of Simufilam's effects?

3. How can the shift in the cognitive baseline values be explained when the two groups being reported at 6 and 9 months were >90% the same subjects?

4. What are the exact criteria for excluding patients in the OL study? Since the drop-in patients must have had much higher baseline values than those that dropped out, how can it be assured that we are not dealing with two different sub-populations for which ADAS-Cog has a different sensitivity?

5. What are the individual progression trajectories of patients as measured with ADAS-Cog at baseline, six months, nine months and 12 months? What were the mean ADAS-Cog values at 6 and 9 months for those patients for whom 12-month ADAS-Cog values were presented?

6. What are the missing values for baseline, standard deviation and percentage change for the 12-month ADAS-Cog data and why were they omitted?

Plasma-based biomarkers and SavaDx

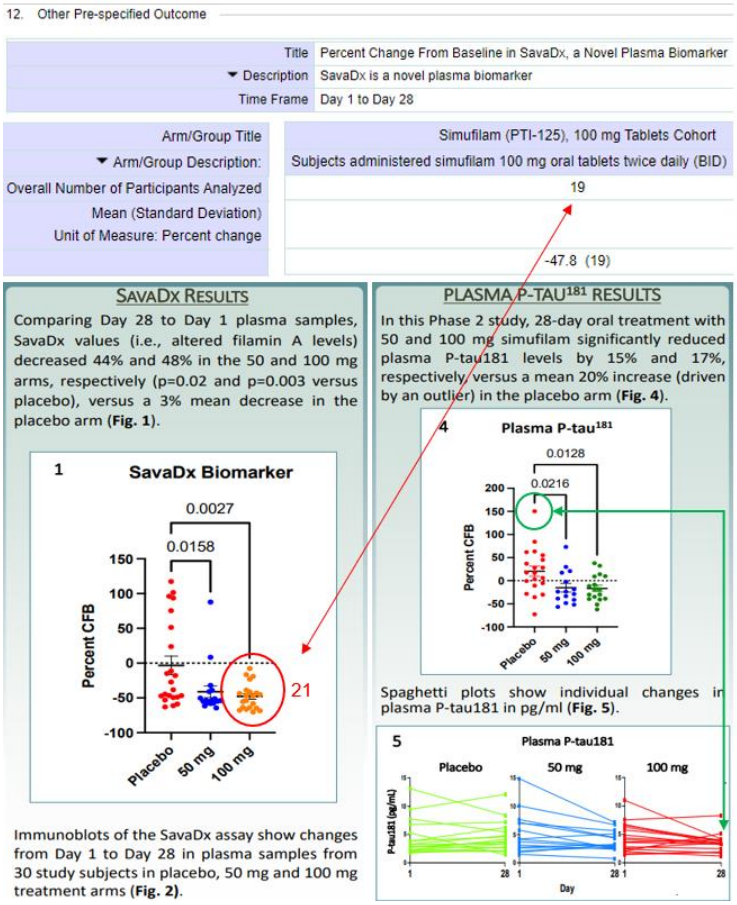
Post-hoc Data Selection

Turning our attention to the plasma-based pTau and SavaDx biomarkers (outcomes 11 and 12 of Ph2b), we see the same degree of post-hoc data manipulation in violation of the Study Protocol as in the other outcome measures discussed earlier. Plasma pTau is the only biomarker that has been analyzed by an external lab, not by Dr. Wang. Therefore, they are data-points which Cassava Sciences cannot interfere with directly. Manipulation would likely occur indirectly through post-hoc sample data manipulation. This appears to be the case as outlined below.

The Analysis Population Description for these analyses is complex and arbitrary in terms of data exclusion and has no clinical or statistical rationale. While assurances of including all the data were made in the Study Protocol and Statistical Analysis Plans, outcome 11 “Plasma P-tau181” outlines an elaborate decision matrix for excluding data including: coefficients of variation between duplicate measures of samples compiled after screening for variability, missing blood samples, and a novel (to these writers) IF [X/Y>150% AND (X-Y)>2.5]. This last “creative” criterion was added after it was pointed out that a subject’s data had been moved from the 100mg treatment arm to the placebo group, which allowed for statistical significance to be claimed where none existed prior to the move and was provided as an “explanation” (visualized by the green arrow below). It is noteworthy that this decision matrix was compiled and entered in the Clinical Trial record only after the data had been presented publicly extensively questioned by many, including Dr. Elisabeth Bik and in the CP.

Furthermore, data presumably gathered from the same blood samples and used in outcome 12 “SavaDx” were not subjected to a similar set of criteria (no mention of variation of replicates or compound IF clauses). However, when Cassava publicly presented the data from outcome 12, the values that were excluded on the Clinical Trial record were included in the poster and YET the values of the means suspiciously did not change. This is most easily observed (screenshot below) by counting the number of points on the poster in the 100mg treatment group of figure 1 (4) and comparing to the data entry of outcome 12. In the record there are 19 analyzed and on the poster there are 21 points PowerPoint Presentation (cassavasciences.com). Furthermore, the criteria used for eliminating 2 subjects in the record but not on the poster was that there was “no detectable plasma Simufilam” which, if reasonable, implies low SavaDx readings were obtained from subjects without drug in plasma, thus undermining a conclusion that the drug has an effect. That these two exclusion schemes differ from the schemes (also post-hoc and in violation of the Statistical Analysis Plan) used in the other biomarker data treatments further supports the conclusion that the aim here was not a better understanding of the effects of Simufilam, but rather the obfuscation.

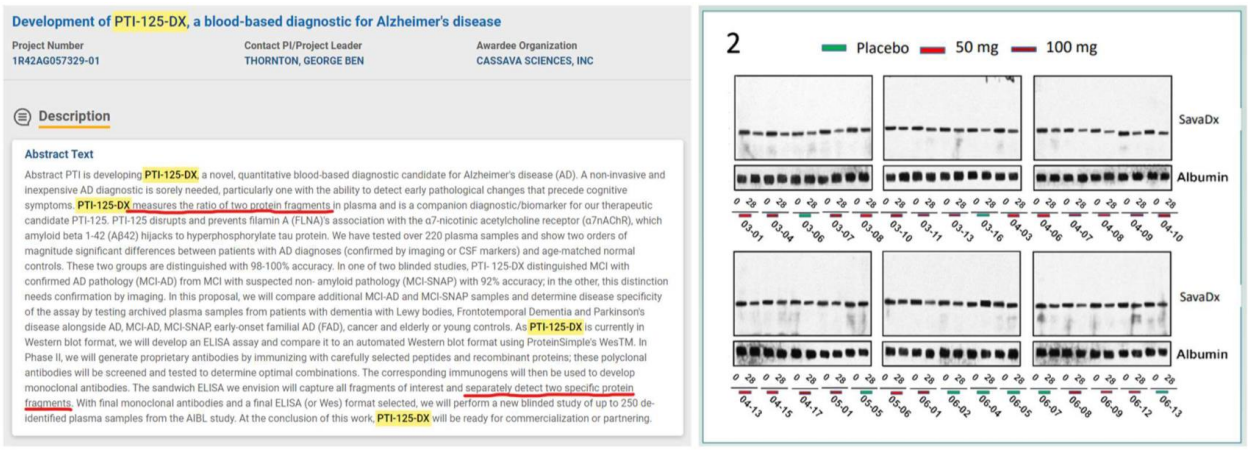
To understand why Cassava Sciences would need to interfere with the outcomes of this analysis, we discuss some revealing findings along the SavaDx development path in the following section.



A Mysterious Diagnostic Breakthrough

In parallel with their Simufilam clinical program Cassava Sciences have been developing a blood-based diagnostic that is – according to the company - able to accurately differentiate Alzheimer's patients from cognitive normal individuals with 98 – 100% accuracy and from MCI-AD patients with 92%(!) RePORT ⟩ RePORTER (nih.gov). Notably, the relevant studies, while being referred to in the company's 2021 AAIC poster and in NIH grant applications since 2017, have never been published in any format. The company has received nearly $2M in NIH funding towards the development of SavaDx.

This novel diagnostic was developed to "separately detect two specific protein fragments" and "measure the ratio" of these fragments. In contrast, looking at the AAIC 2021 poster, only a single band appears for the SavaDx label (screenshots below). To state the obvious, it is not possible to measure a ratio when only one value is available. It appears likely that this measurement, which forms the basis of SavaDx, is not being reported accurately.



Development of PTI-125-DX, a blood-based diagnostic for Alzheimer's disease

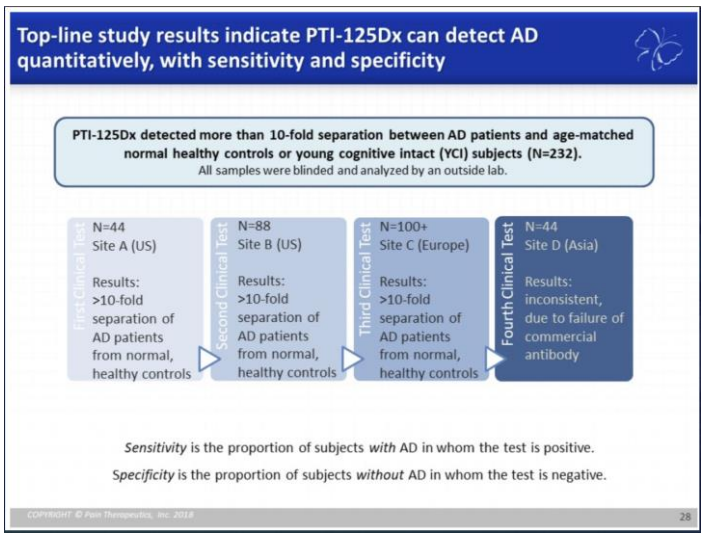
Project Number	Contact PI/Project Leader	Awardee Organization
1R42AG057329-01	THORNTON, GEORGE BEN	CASSAVA SCIENCES, INC

Description

Abstract Text

Abstract PTI is developing PTI-125-DX, a novel, quantitative blood-based diagnostic candidate for Alzheimer's disease (AD). A non-invasive and inexpensive AD diagnostic is sorely needed, particularly one with the ability to detect early pathological changes that precede cognitive symptoms. PTI-125-DX measures the ratio of two protein fragments in plasma and is a companion diagnostic/biomarker for our therapeutic candidate PTI-125. PTI-125 disrupts and prevents filamin A (FLNA)'s association with the α7-nicotinic acetylcholine receptor (α7nAChR), which amyloid beta 1-42 (Aβ42) hijacks to hyperphosphorylate tau protein. We have tested over 220 plasma samples and show two orders of magnitude significant differences between patients with AD diagnoses (confirmed by imaging or CSF markers) and age-matched normal controls. These two groups are distinguished with 98-100% accuracy. In one of two blinded studies, PTI- 125-DX distinguished MCI with confirmed AD pathology (MCI-AD) from MCI with suspected non- amyloid pathology (MCI-SNAP) with 92% accuracy; in the other, this distinction needs confirmation by imaging. In this proposal, we will compare additional MCI-AD and MCI-SNAP samples and determine disease specificity of the assay by testing archived plasma samples from patients with dementia with Lewy bodies, Frontotemporal Dementia and Parkinson's disease alongside AD, MCI-AD, MCI-SNAP, early-onset familial AD (FAD), cancer and elderly or young controls. As PTI-125-DX is currently in Western blot format, we will develop an ELISA assay and compare it to an automated Western blot format using ProteinSimple's WesTM. In Phase II, we will generate proprietary antibodies by immunizing with carefully selected peptides and recombinant proteins; these polyclonal antibodies will be screened and tested to determine optimal combinations. The corresponding immunogens will then be used to develop monoclonal antibodies. The sandwich ELISA we envision will capture all fragments of interest and separately detect two specific protein fragments. With final monoclonal antibodies and a final ELISA (or Wes) format selected, we will perform a new blinded study of up to 250 de-identified plasma samples from the AIBL study. At the conclusion of this work, PTI-125-DX will be ready for commercialization or partnering.

While the initial set of studies involving SavaDx has never been published, we were not surprised to discover from a company presentation in 2018 that SavaDx yielded "inconsistent results" in a 4th and final study. In an act of foreshadowing, the sponsor blamed outside commercial labs for unacceptably high variability (screenshot below). This is not mentioned in the company's 2018 or 2020 grant applications.



Despite our best efforts to understand the mechanism of this revolutionary diagnostic, the limited published data are vague and uninformative. Our understanding is that it may be based on the discovery of Drs. Wang and Burns (Wang et al., PTI-125 Reduces Biomarkers pf Alzheimer's Disease in Patients, *J Prev Alz Dis.* 2020) that the lymphocytes of AD patients contain ~93% misfolded filamin A (FLNA) and that this is restored to a 40% misfolded vs 60% native conformation within 28 days of Simufilam treatment. Based on a cryptic response from Dr. Burns (screenshot below) in the comment section of the Ph2b pre-print manuscript, it appears that rather than targeting the misfolded filamin in lymphocytes, SavaDx detects something else to reveal misfolded filamin in the brain. We find this limited explanation unsatisfactory in that it would require novel actions of filamin which do not exist in the literature to date as well as contrast the company's own research to date.

[redacted] commented on 18 September, 2021

Hello, Can you comment on how FLNA has altered conformation in blood? Is this in lymphocytes and that is how it is monitored by SavaDx? Or is the altered FLNA due to increase in binding sites? It seems this is potentially applicable for many diseases.

REPLY Report

View 1 reply

Lindsay replied on 19 September, 2021

What SavaDx detects in plasma is an indicator of altered FLNA in brain. We haven't disclosed much more than this.

REPLY Report

A Missing Blinded 3-Month Study

Cassava Sciences have received generous public funding for their clinical trials involving Simufilam and SavaDx to date, amounting altogether to $17,809,563. According to official NIH records, among other grants, in 2018 Cassava Sciences received NIH funding for extending their Phase 2 study from a duration of 1 month to 3 months - grant number 3R44AG060878-01S1, amounting to $374,500. Subsequently, in 2020, they received an NIH grant for an “Open-label extension of a 3-month blinded clinical trial of PTI-125” - grant number 1R44AG065152-01, amounting to $2,499,896, and finally, also in 2020, another grant for extending the study's amount of patients to 60 - grant number 3R44AG060878-02S1, amounting to $374,500.

While the open label extension and the increase to 60 participants are well documented, the 3-month extension neither shows up on clinicaltrials.gov nor have its results been reported anywhere else. Both of the blinded Phase 2 studies had a duration of only 28 days, thus the question remains as to what happened with the planned blinded 3-month extension. Communication with the Sponsor suggests this planned study was dropped in favor of a protracted two-year cognitive maintenance study (email between JB and CFO Eric Schoen). This decision by the Sponsor does not seem one designed to expeditiously resolve whether the cognitive trends reported in Ph2b were genuine and persisted. Knowing whether this changes after a longer period of time – disease progression would be expected to be more uniformly detrimental and possible positive effects of medication more pronounced after 3 months – would have been crucial for realistically assessing efficacy of Simufilam going forward. We find it suspicious that a planned study which would have quickly addressed the authenticity of the cognitive trends was changed to a two-year study that only started enrolling in May of this year.

Key Questions

Our investigation of Cassava Sciences’ tactics during the clinical development of Simufilam and their companion diagnostic (SavaDx) has mainly left us in the dark and – we allege – that is by design. For the sake of transparency and the company’s own credibility we believe the following points require clarification:

1. How can the discrepancies between the clinicaltrials.gov record and the AAIC SavaDx poster be reconciled when different samples present with the same mean?

2. How can the SavaDx diagnostic target two distinct protein fragments yet be detected in a single Western Blot band? What is the purpose of albumin as a control if SavaDx measures the ratio of these two fragments?

3. By what novel scientific mechanism is SavaDx able to detect proteins in plasma that inform the conformation of FLNA in the brain? And why does that mechanism rely on the detection of the nearly entirely misfolded filamin A supposedly found in lymphocytes?

4. Why in the face of stellar, unprecedented Phase 2b data did the company decide to steer away from the planned 3 month duration blinded study?

5. Why was there no measurement after 3 months? Was this originally part of the blinded 3-month extension grant that seems to have been silently dropped and/or not reported??

6. Why has Cassava not been able to publish these unbelievable results despite a nearly yearlong effort?

Summary

In sum, we have presented a series of evidence that directly challenge the integrity of research findings reported by Cassava Sciences during its entire clinical program. These involve:

i) Questions on the validity of the data presented and published for both
   - critical data of key biomarkers of inflammation and BBB integrity that inform the safety evaluation of Simufilam, and
   - central biomarkers of AD diagnosis and staging that map disease progression in patients

ii) Evidence of methodical post-hoc data manipulation directly contrasting predefined SAP with a clear intention to:
   - Overstate the efficacy of Simufilam based on cognitive outcomes in patients
   - Present favorable findings of plasma-based biomarkers

iii) Systematic attempts either to obscure or over-state research findings and behavior entirely incompatible with the conduct of scientific research and clinical trials, such as:
   - lack of quality control or validation either within their own studies or to scientific consensus
   - lack of transparency in the conduct and outcomes of clinical sample analysis and elusive presentation of data
   - inexplicable choices and alarming inaccuracies in the choice of analytical methodologies with no plan to address the direct conflict of interest of the collaborators involved

These behaviors, beyond directly violating the SAP, reflect a clear attempt to obscure evaluation of the effect of Simufilam. Contrary to the Sponsor's public assertions, Simufilam treatment is not free of risk and in fact possible side-effects include convulsions and changes to liver cell size and function (Prot_SAP_ICF_000.pdf (clinicaltrials.gov)). Given the obfuscation of clinical benefit outlined in this document, no proper assessment of risk-benefit can be made from these studies and there is no justification for large-scale exposure to this drug.

The evidence that we have presented shows that Cassava Sciences is not meeting its obligations as an IND sponsor under 21 CFR 312. Specifically:

- The pattern of errors and misconduct in measuring and reporting biomarker and cognitive outcomes, as well as the reliance on clinical investigators whose conduct has been flagged by FDA inspections and Warning Letters, calls into question whether the investigators leading the Simufilam program are qualified to conduct the trial
- In light of the misleading and erroneous clinical and preclinical results communicated to date, the Investigator Brochures for the Phase 3 trials are necessarily misleading and erroneous and require amendment
- Given the incongruous and apparently manipulated clinical and preclinical data, the Simufilam IND does not contain sufficient information to properly assess the risks to subjects.

Ultimately, only the conduct of a full, thorough investigation of the data, investigators, sponsor, and collaborators can provide reassurance. We, therefore, request that an immediate clinical hold be placed on the entire clinical program of Cassava Sciences. Furthermore, we strongly believe that the conduct of the company and its program application be reviewed by the FDA's Application Integrity Policy Committee (AIP-C) and proper action taken.

Cassava Sciences, through persistent obfuscation and exaggeration of the effects of Simufilam, have exposed study participants to incalculable risk with unknown consequences for their health and misled investigators and patients into choices that affect their wellbeing. This presents a clear and ongoing harm to the public and considering that an investigation process may be lengthy, and patients are currently being administered with Simufilam, immediate action is warranted.

Sincerely,

Enea Milioris, PhD

Adrian Heilbut, PhD

Jesse Brodkin, PhD

Patrick Markey, PhD