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VIA ONLINE & FEDEX

Division of Dockets Management
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, MD 20852

Re: *Fourth Supplement to Citizen's Petition Associated with Cassava Sciences, Inc. (FDA-2021-P-0930)*

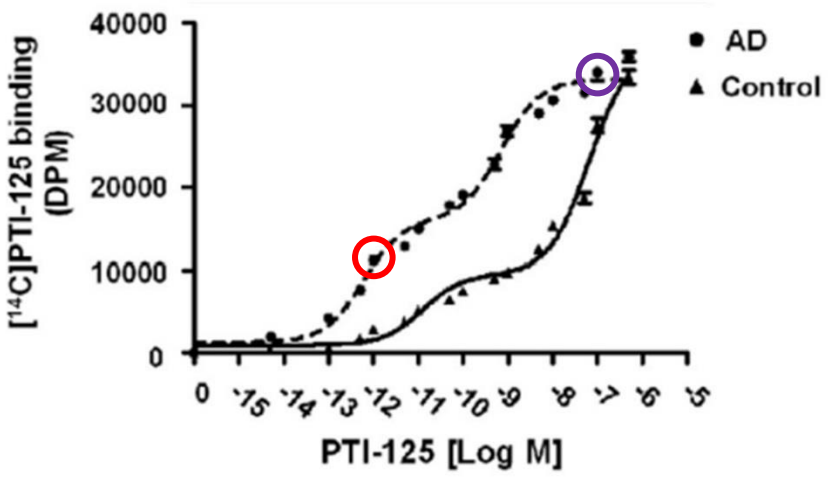
Dear Commissioner Woodcock:

As detailed in my clients' Citizen's Petition and in subsequent filings, including this one, their major concern relates to the mounting evidence that Cassava Sciences has doctored its research and clinical trial results to dupe peer-reviewed journals and to trick the FDA into approving its clinical trials. To confirm the existence and scope of these problems, they have urged the FDA to immediately halt the simufilam (PTI-125) clinical trials, conduct a rigorous audit of all the company's research and clinical trial results, and report the agency's findings to interested law enforcement and regulatory authorities.

Since our last supplemental submission, new analyses by my clients and other independent scientists raise serious concerns about Cassava's foundational claims for the binding of PTI-125 to filamin A and, separately, the methodology and reporting about their diagnostic test for Alzheimer's Disease, SavaDx, which is a key endpoint of two Cassava's Phase 3 trials (NCT04994483 and NCT05026177).

PTI-125 Binding to FLNA in Cassava's Foundational 2017 Paper Seems Impossible

Fundamental to simufilam's activity in Alzheimer's disease is Cassava's claim that PTI-125 (simufilam) potently binds to filamin A. Evidence for this is unique to Drs. Wang, Burns and Cassava and is presented in figure 1B (below) Neurobiology of Aging 2017 **55**:99. No other direct binding studies between PTI-125 and filamin A have been reported. In our Citizen's Petition, we stated that this figure is suspicious/implausible because of (1) the improbably high 580 femtomolar affinity and (2) the gradual increase in binding that spans 6 log changes (10^{-13} to 10^{-7} M) of PTI-125. Binding of drugs to their targets typically occurs sharply within 2 log changes of drug concentration. http://www3.uah.es/farmamol/Public/GraphPad/radiolig.htm



Our recent re-inspection of the Methods section for this crucial experiment shows seemingly irrefutable evidence of data manipulation/fabrication. The section states: "*PTI-125's affinity for FLNA was determined in immunopurified FLNA using [C14]PTI-125* ***(57.7 Ci/mmol)*** *…. Briefly, a binding curve was generated by incubation of* ***0.1 µg*** *immunopurified FLNA from control or AD hippocampus …*"

Importantly, the binding experiments used PTI-125 labeled with carbon-14 [C14], which has a relatively low specific activity (rate of decay). This physical law makes [C14] useful for carbon dating, but completely unsuitable for detecting high affinity binding like that claimed for PTI-125 and filamin A. A few of the **many major problems** are:

Claimed Specific Activity for [C14] PTI-125 is ~1000 Greater Than Physical Laws Allow

Cassava states that their [C14] PTI-125 has a specific activity **57.7 Ci/mmol**. However, pure [C14] has a specific activity of 62.5 mCi/mmol = **0.0625 Ci/mmol**. This is the upper limit for a molecule with one [C14] substitution. Assuming one [C14] as is likely, **Cassava's claimed specific activity for PTI-125 is ~1000 times higher than theoretically possible**. Such an inexplicable error would create insurmountable problems and invalidate the study.
https://www.crbdiscovery.com/technical/radiolabelling/specific-activity/

FLNA Binding [C14] PTI-125 Could Maximally Yield 47 DPM vs >30,000 DPM Claimed in Fig. 1B

Because [C14] has intrinsically low specific activity (0.0625 Ci/mmol), it does not yield sufficient "counts" (DPM, disintegrations per minute) to reliably detect receptor binding. To do such pharmacology experiments, scientists use drugs containing [H3] or [I125] that have specific activity >20 Ci/mmol, ~ 300-fold *higher* than [C14].
https://www.perkinelmer.com/lab-products-and-services/application-support-knowledgebase/radiometric/radiometric-ligand-binding-assays.html

Basic radiochemistry illustrates why [C14]'s low specific activity is a real problem for Cassava Sciences' research:

Assuming the best possible scenario for Cassava, we assign a specific activity of [C14]PTI-125 = 62.5 mCi/mmole.

By definition, 1 Ci = $2.22*10^{12}$ DPM. We can now calculate the maximum number of DPM that could be achieved based on Cassava's methods.

[C14]PTI-125 = 62.5 mCi/mmole × ($2.2 * 10^{12}$ DPM/Ci) = **$1.38 * 10^{14}$ DPM/mole**

Wang et al. (2017) report that they used 0.1 μg filamin A in their binding experiments. Thus, we can calculate the number of moles of filamin A (molecular weight = 290 kDa, or 290,000 g/mole) in their assay:

(0.1 μg filamin A) x (mole/290,000 g) = **$3.4 * 10^{-13}$ moles filamin A in the assay**

In the best-case scenario, *every* molecule of filamin binds one molecule of PTI-125, so one expects **$3.4 * 10^{-13}$ moles PTI-125 bound**.

Now, we calculate the number of counts (DPM) under these favorable (for Cassava) assumptions:

(**$3.4 * 10^{-13}$ moles PTI-125**) × (**$1.38 * 10^{14}$ DPM /mole**) = 47 DPM

This **47 DPM maximum** dramatically clashes with **~30,000 DPM** claimed by Cassava scientists in Fig. 1B (see purple circle).

[C14] PTI-125 Cannot be Used to Detect 1 pM FLNA Binding Affinity

The low specific activity of [C14] precludes its utility to measure ultrahigh affinity binding. Because [C14] has a low rate of decay, there are few "counts" (DPM) in samples with the low drug concentrations that define high affinity binding. In the red circle of Fig. 1B (above), Cassava claims 10,000 DPM binding at 1 pM ($1 * 10^{-12}$ M) PTI-125.

Basic radiochemistry illustrates why this is impossible:

Recall that the specific activity of [C14]PTI-125 (62.5 mCi/mmole), which is 62.5 pCi/pmole.

Recall that 1 Ci = $2.22*10^{12}$ DPM, which is 2.2 DPM/pCi. So,
62.5 pCi/pmole x 2.2 DPM/pCi = 137.5 DPM/pmol

Accordingly, 1 pM [C14]PTI-125 has 137 DPM/liter

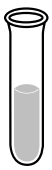
Therefore, for Cassava to detect 10,000 DPM (red circle in Fig 1B), they would require
10,000 DPM ÷ 137 DPM / liter = **73 liters**.

Cassava's binding assays surely were performed in much smaller volumes, and likely used **<5 mL**, if the experiments were done at all.

Volumes for 1 pM solutions of [14C], [H3], and [125I] that contain 10,000 DPM are:
[C14] 0.062 Ci/mmole **73 liter** [H3] 29 Ci/mmole **116 ml** [125I] 2200 Ci/mmol **2 ml**

Wine Barrel

Wine glass



Test tube

This illustrates why high affinity binding requires very high specific activity isotopes like [I125] and why Cassava's [C14]PTI-125 is entirely unsuitable for such experiments.

These issues underscore the implausibility of claiming to measure 580 fM binding affinity with C-14 labeled simufilam. **Indeed, the numerous elementary problems with Cassava's experiments raise troubling questions about whether simufilam binds to filamin A at all.**

It is important to note that no other labs have replicated this alleged potent interaction. Fatal flaws in these critical binding experiments, which form the foundation for their key investigations, raise serious questions about Cassava's hypotheses that filamin A is relevant to Alzheimer's disease and about whether simufilam affects filamin A.

A final note concerns the biodistribution data calculated by Cassava and likely provided to the FDA. Unlike experiments to detect high affinity binding, [C14] labeling *is* typically used to track drug transformations in the body. https://www.britannica.com/science/carbon-14

Indeed, the acknowledgements section of Cassava's 2017 paper states that, "C14-labeling of PTI-125 was produced for biodistribution studies under [Cassava] NIH grant number 4R44AG050301." It will be very important to know what specific activity value [C14] PTI-125 Cassava used in quantifying these biodistribution studies and whether those studies were also compromised by the 1000-fold miscalculation in this paper.

Additional Serious Red Flags Regarding Cassava Science's SavaDx

In addition to simufilam, Cassava is developing SavaDx, which they describe as a simple and accurate blood test for early detection of Alzheimer's disease. A "change from baseline in plasma

biomarker SavaDx" is a key endpoint for Cassava's two Cassava's Phase 3 trials, NCT04994483 and NCT05026177. In their Citizen's Petition to the FDA, they noted that the data behind SavaDx seemed suspicious/implausible. After all, Alzheimer's disease is a complex and poorly understood disease. The Alzheimer's Association website currently states that, "There is no single diagnostic test that can determine if a person has Alzheimer's disease. Physicians (often with the help of specialists such as neurologists, neuropsychologists, geriatricians and geriatric psychiatrists) use a variety of approaches and tools to help make a diagnosis." https://www.alz.org/alzheimers-dementia/diagnosis/medical_tests

Despite the complexity, heterogeneity and ambiguity of Alzheimer's, Cassava Sciences claims that SavaDx can somehow quickly and accurately diagnose the disease. Drs. Burns, Wang and Thornton from Cassava Sciences have received nearly $2,000,000 from the NIH for developing the SavaDx blood test. In their grant application from 2018 (https://grantome.com/grant/NIH/R44-AG057329-02), Cassava claims to have "tested over 220 plasma samples and show two orders of magnitude significant differences between patients with AD [Alzheimer's disease] diagnoses (confirmed by imaging or CSF markers) and age-matched normal controls. These two groups are distinguished with **98-100% accuracy**." Cassava repeats similar assertions on their current corporate website, https://www.cassavasciences.com/SavaDx

As there is not a single gold standard for diagnosing AD, **it seems highly improbable that any test could have 98-100% accuracy**. In their 26 July 2021 poster at the Alzheimer's Association International Conference (AAIC), Cassava claims that SavaDx measures changes in levels of altered filamin A in plasma (the cell free fraction of blood). Because Alzheimer's disease affects a subset of brain neurons, and filamin A is present at relatively low levels in the adult brain, it is scientifically unclear how altered levels of filamin A in plasma could diagnose Alzheimer's disease by reflecting a process in brain. For these and other reasons, Cassava's assertions about SavaDx seem implausible

and have been largely ignored for years by the neuroscience community. In fact, despite being claimed as a revolutionary Alzheimer's Disease diagnostic, a search of PubMed finds that no publications apparently mention "SavaDx."

Nevertheless, Cassava has aggressively touted SavaDx to investors. Specific examples include:

1. Cassava's 26 July 2021 poster at the Alzheimer's Association International Conference (AAIC), "*SavaDx, a Novel Plasma Biomarker to Detect Alzheimer's Disease, Confirms Mechanism of Action of Simufilam*" in which they stated:

   "KEY TAKEAWAY: SavaDx is a plasma assay to detect altered filamin A, a proteopathy in AD. SavaDx detected treatment effects of simufilam in a Phase 2 clinical trial, suggesting potential as a plasma biomarker for Alzheimer's disease."
   https://www.cassavasciences.com/static-files/0854aec6-59b3-4e2b-ac20-c32b7c307b08

2. Cassava's press release from Jul 26, 2021. Title: *Cassava Sciences Announces Positive Data with SavaDx from a Randomized Controlled Phase 2b Study of Simufilam*

   Included in the text of the press release is: "SavaDx Detected Significant Changes in Plasma Levels of Altered Filamin A in Patients with Alzheimer's Disease Before and After Simufilam Treatment… Cassava Sciences, Inc. (Nasdaq: SAVA) today announced positive clinical data with SavaDx, an investigational diagnostic/biomarker to detect Alzheimer's disease with a simple blood test. SavaDx was used to measure plasma levels of altered filamin A before and after simufilam treatment in patients with Alzheimer's disease. In this Phase 2b randomized, controlled trial sponsored by the National Institutes of Health (NIH), simufilam significantly reduced plasma levels of altered filamin A in Alzheimer's patients

treated for 28 days. Plasma levels of p-tau181 also dropped significantly in these same patients.…

About SavaDx

SavaDx is Cassava Sciences' investigational diagnostic to detect Alzheimer's disease. The goal of SavaDx is to make the detection of Alzheimer's as simple as getting a blood test, possibly years before the appearance of any overt clinical symptoms [emphasis added]. SavaDx was substantially funded by a peer-reviewed research grant award from the National Institutes of Health (NIH)."
https://www.cassavasciences.com/news-releases/news-release-details/cassava-sciences-announces-positive-data-savadx-randomized

3. Cassava's press release from July 09, 2020. Title: "*Cassava Sciences to Give Keynote Presentation on SavaDx at a Scientific Conference*", with text stating; "Dr. Burns' talk will focus on SavaDx, the Company's investigational diagnostic to detect Alzheimer's disease with a simple blood test. …

About SavaDx

SavaDx (formerly, PTI-125Dx) is Cassava Sciences' investigational diagnostic to detect Alzheimer's disease. The goal of SavaDx is to make the detection of Alzheimer's as simple as getting a blood test, possibly years before the appearance of any overt clinical symptoms. This clinical-stage program is substantially funded by a research grant award from the National Institutes of Health (NIH)."
https://www.biospace.com/article/releases/cassava-sciences-to-give-keynote-presentation-on-savadx-at-a-scientific-conference/

During the two trading days following their 26 July 2021 poster at the AAIC, "*SavaDx, a Novel Plasma Biomarker to Detect Alzheimer's Disease, Confirms Mechanism of Action of Simufilam*",

https://www.cassavasciences.com/company-presentations the market capitalization of SAVA increased by ~$500,000,000. In my clients' Citizen's Petition to the FDA, they noted that this 2021 SavaDx poster at AAIC had multiple instances of apparent data manipulation. On page 26 of their 10th November, 2021 10-Q, Cassava confessed that the 26 July 2021 poster on SavaDx had "visual errors." That admission was made in response to complaints in the Citizen's Petition, supported by Dr. Elisabeth Bik and other scientists, and involved apparent data manipulation, not visual errors (https://scienceintegritydigest.com/2021/08/30/cassava-sciences-of-posters-and-spaghetti-plots/).

We also re-addressed these issues in our supplemental filing to the Citizen's Petition on 31 August 2021 (https://www.regulations.gov/document/FDA-2021-P-0930-0023). Page 26 of Cassava's November 2021 10Q also acknowledged that Cassava's development of SavaDx has been paused owing to the need for validation studies in patients that they have not done.

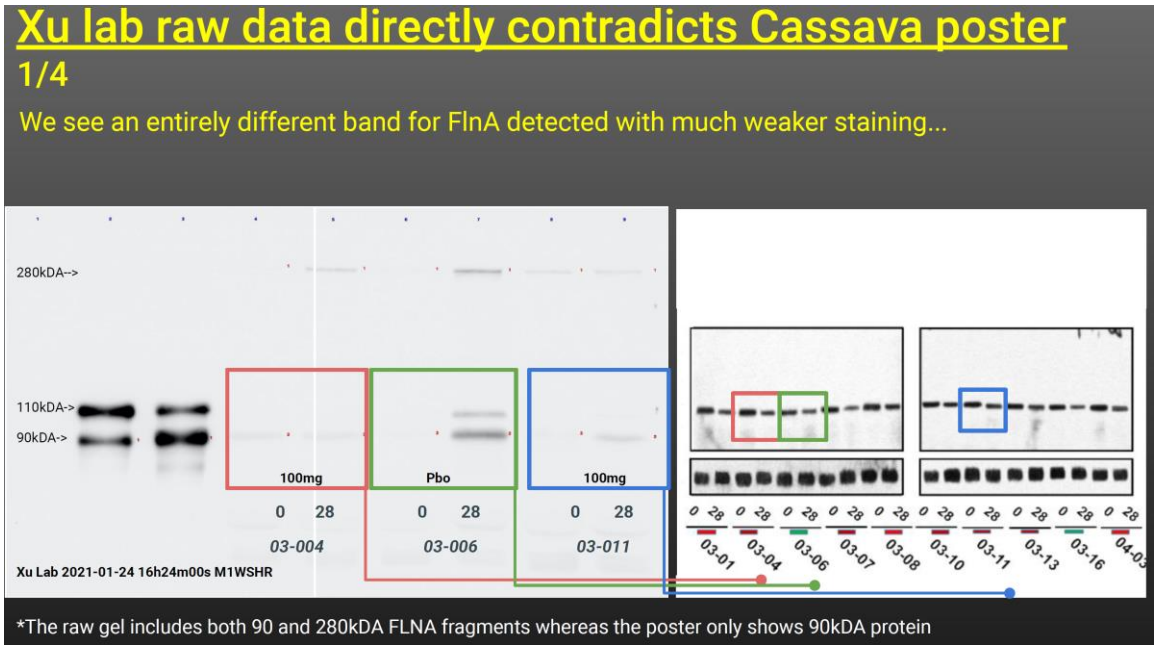
Suddenly pausing SavaDx is another major red flag, as Cassava has described it as fast and inexpensive. As noted above, and more significant to the FDA, SavaDx is specified as a key secondary outcome measure in both of Cassava's on-going phase 3 clinical trials.
https://clinicaltrials.gov/ct2/show/NCT04994483
https://clinicaltrials.gov/ct2/show/NCT05026177

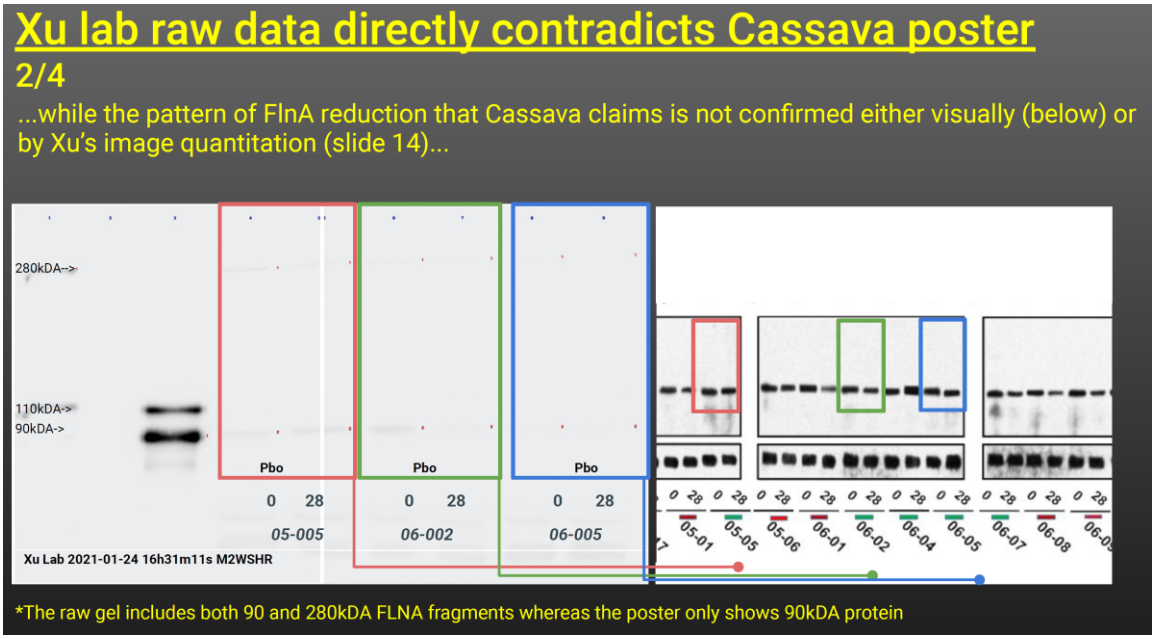
For these and other reasons, we believe that Cassava paused SavaDx and has begun to lower expectations because the problems with SavaDx have been exposed or feared would soon be exposed. Furthermore, potentially powerful, and direct evidence of data manipulation related to SavaDx was documented on 29 November 2021 by a group of scientists independently investigating Cassava. They posted their concerns, of which we were previously unaware, on Twitter (https://twitter.com/jesse_brodkin/status/1465409022199271432). They also made a PDF of the presentation available online at SAVA Dx: Theranos 2.0 (cassavafraud.com).

Through a freedom of information act or law (FOIA or FOIL) request to CUNY, the independent scientists apparently obtained a set of Dr. Wang's emails. Importantly, they found an email to Dr. Wang from Dr. Qiang (John) Xu of Abilene Christian University dated 1/24/2021 10:48:30 PM. This email contains raw data that appear to represent a subset of the experiments presented in Cassava's July 26 poster at AAIC; Dr. Xu is co-author on this poster.

Several apparent red flags arise when comparing the raw data in the FOIAed email with the figures in Cassava's AAIC poster.

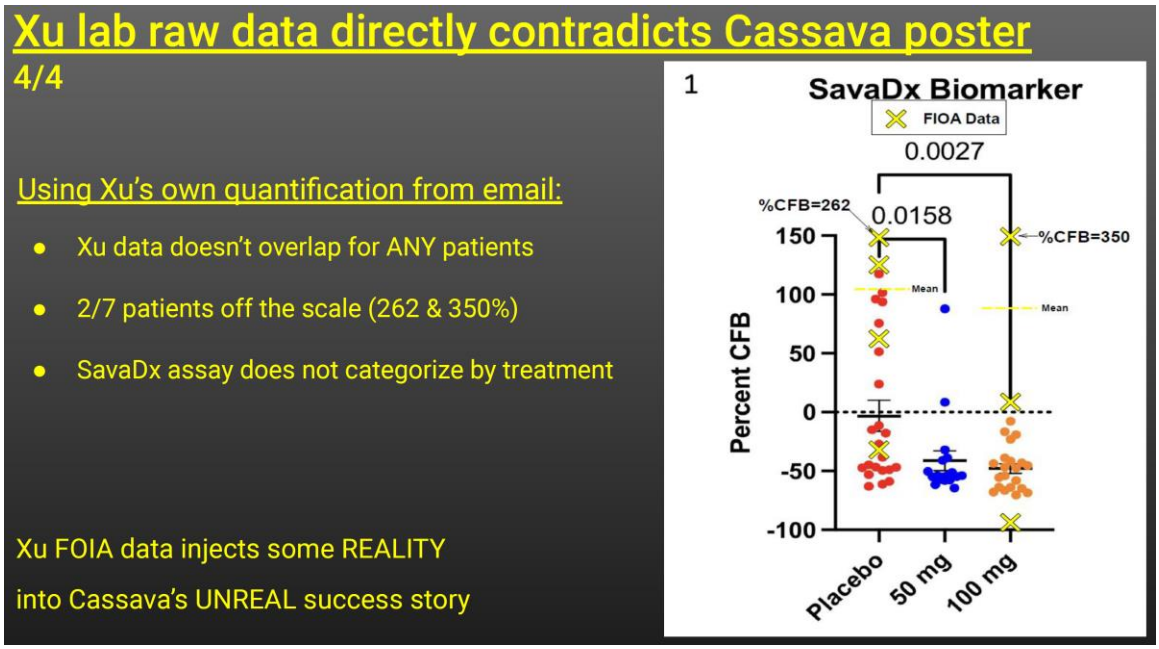
First, the western blot images in the email from Dr. Xu to Dr. Wang look entirely different from those presented in Figure 2 of Cassava's poster. The Western blots in Dr. Xu email are on the left in both panels and the data from Figure 2 of the AAIC poster are on the right in the two figures that follow (bottom of this page and top of next). The color-coded boxes indicate corresponding patient





samples (note the corresponding sample IDs, e.g., 03-04) in Dr. Xu's data and the presentation of those data in Figure 2 of the AAIC poster.

Second, the SavaDx values that the independent scientists calculated from Dr. Xu's data in the email ***look entirely different*** from those presented in Figure 1 of Cassava's poster, as shown on the next page.

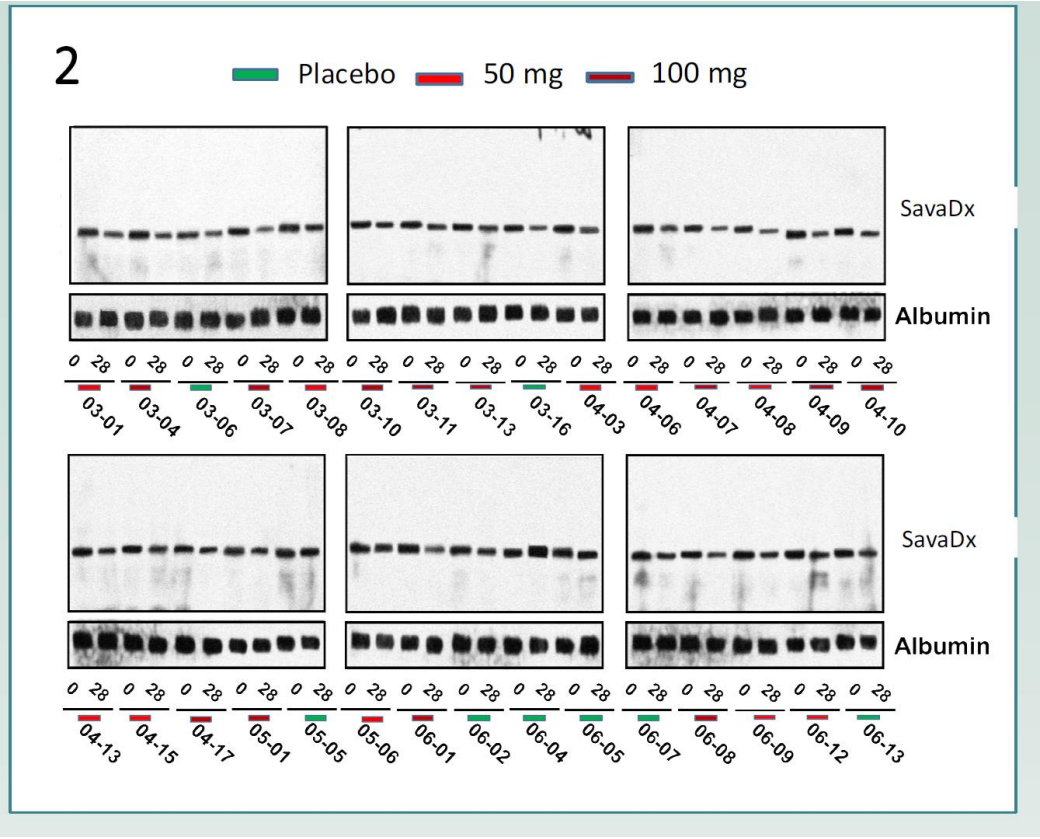


While we do not have access to the original emails obtained by the FOIA request, and we cannot be certain of the independent scientists' calculation of SavaDx, their findings are consistent with the concerns my clients previously raised about the 26th July poster. With the new data from the FOIA emails, there are clear steps to investigate our concerns and those of the independent scientists to determine whether Cassava misled the FDA. Specifically, among many other things, we recommend the FDA staff contact Dr. Xu, Dr. Wang, and Cassava to ask:

1. Who conducted the actual experiments and then who constructed the final Figures 1 and 2 for the July 26 poster at AAIC?

2. Why do aspects of Figures 1 and 2 from this poster appear fundamentally inconsistent with the data Dr. Xu emailed to Dr. Wang on 1/24/2021?

3. Would the company provide the original X-ray films corresponding to the Western blots in Figure 2 from the AAIC poster (below)? Since Cassava's poster was presented just a few months ago, and the data from which they were generated were collected as late as January 2021, the tangible X-ray films themselves should be available for inspection. These hard copy films (or at the very least **high-resolution** scans) should have molecular weight markers and other identifying features to compare with the 2021 poster. To assess authenticity, an image analyst familiar with Western blotting should compare the originals provided with Figure 2 in the poster, as shown below.



In conclusion, while you have 150 days to adjudicate the Citizen's Petition, the need for the FDA to take emergency action couldn't be more urgent. Despite countless red flags associated with the company's foundational research, Cassava Sciences has announced that it has commenced recruiting for a Phase 3 clinical trial of simufilam (NCT0499483), with as many as 750 vulnerable Alzheimer's Disease patients; and plans an additional Phase 3 clinical trial (NCT05026177) with as many as 1,083 patients. Again, the Citizen's Petition simply requests that the agency pause any related clinical trials, until a rigorous audit of all related Cassava Sciences research and clinical trial results can be conducted.

As always, my clients are standing by to answer questions and assist your teams with their important work.

Respectfully submitted,

Jordan A. Thomas