Evaluation of Preclinical & Clinical Simufilam Studies: Strange Observations and Scientific Doubts

The Simufilam Story

- Simufilam (PTI-125) is an investigational drug in Phase 3 trials for AD (NCT04944883, NCT05023177) sponsored by Cassava Sciences (Pam T)
- First proposed as non-opioid analgesic or modulator of opioid signaling / addiction, based on mimicking apocynol retarded paradoxical effects of ultra-low-dose Naloxone [1, 2]
- Wang 2008 [1] reported Naloxone (NLX) bound Filamin-A (FLNA) with pM affinity at specific Smer VAKGL to modulate opioid signaling and that NLX bound the isolated VAKGL peptide, which combined with FLNA to bind NLX
- Simufilam claimed to have been discovered [3] as a competitor to NLX binding to FLNA and FLNA, without opioid signaling / addictiveness, based on mimicking

Reports of Manipulated Images & Data

- In 2021, many scientists independently flagged concerns about images & data in >30 papers authored by Dr. Houa-Yan Wang of City College (CCNY) of University of New York (CUNY) [7] (Petitions & Letters to FDA; PubPeer)

The “Re-Do” & Puzzling Correlations

- 28-day P2B failed when initially reported (Lund analysis)
- Back-up CSF samples sent to Dr. Wang at for “re-do”
- Wang reported highly significant CSF biomarker effects
- Use of Wang’s data was justified based on correlations between changes of different biomarkers in placebo (patient-level; 28d)
- No valid statistical rationale for this justification; in short term, uncorrected random errors are expected in placebo

Implausible Phase 2 Biomarker Data

- Wang analyzed all P2 CSF samples 7 of 9 CSF biomarkers appeared:
  - Inconsistent with scientific literature
  - Inconsistent with human biology
  - Inconsistent with assay
  - (Lumines v ELISA)

Open Label-Cognitive Baseline Shifts

- Reported ADAS-Cog improvement of 1pts at 6mo and 3pts at 9mo
- “Improvement” likely due to 4 (est.) patients dropped-in w/ baseline scores 2 or more as the original cohort
- Absolute scores did not change significantly 6mo >9mo; 1:3.6 vs 1:3.9
- After this was noticed, baseline values were not reported in that 2mo read-out (Δ+1.5pts)

A Foundational Question: Does Simufilam Really Bind Its Purported Molecular Target, VAKGL peptide in Filamin-A?

Claim 1: A New Naloxone Target Naloxone Bind FLN-A

- The only paper [1] to report Naloxone binding FLNA is by Wang and Burns and was retracted by PLoS editors
- There is no reported non-opioid specific binding site for Naloxone [9]; Naloxone does not distribute to tissues with high FLNA expression [10] (50yrs of study)

Claim 2: A Specific New Naloxone Binding Site Naloxone binds VAKGL peptide?

- No reported analogous small molecule ligands for any other pentapeptides
- No binding pocket near VAKGL in structure of the FLNA dimerization domain (SCNA)
- No model ever proposed for how binding to VAKGL causes conformational change, pl shift, or allosteric modulation of FLNA protein interactions or function
- VAKGL occurs in dozens of other human proteins; if Naloxone bound VAKGL (both as a pentapeptide and in native FLNA), unclear why it would not bind other proteins with VAKGL

Claim 3: A Novel Molecule Mimicking Naloxone Simufilam binds VAKGL and Filamin-A?

- Simufilam reportedly discovered in vitro screen against biotinylated VAKGL competing with FITC-tagged NLX [3]
- Simufilam claims to bind AD brain tissue in displacement assay vs * synthesized [5, 6]
- VAKGL claimed to compete vs FLNA for Simufilam & block effects on FLNA in syncytosome prep

Claim 4: High Affinity & Two FLNA Conformations Simufilam binds altered FLNA; IC50?

- Reported Radiolabelled Simufilam Binding Assay

What is ITC? How does it work?

- Molecules interact due to thermodynamic driving forces. Major contributors to non-covalent interactions are hydrogen bonding and van der Waals forces, hydrophobic interactions, entropy, ΔH = ΔTS
- Isothermal Titration Calorimetry directly measures the heat (enthalpy, ΔH) of a molecular interaction [11] as small volumes of a solution containing the ligand are sequentially injected into solution containing target
- As molecules bind (if they bind), heat is released & measured, until all target binding sites are saturated
- Advantages: Easy; automated; does not require labeling molecules; very sensitive; quantitative

Experimental & Results: No Evidence for Naloxone or Simufilam Binding VAKGL By Isothermal Titration Calorimetry

Experimental Design & Methods

- Automated calorimetry: MicroCal Auto ITC 200; 25°C
- No v control exists for a small mol binding any pentapeptide; high-affinity Carbonic Anhydrase II (CAII) inhibitors eg. Acetazolamide (K, 20mM) often used to benchmark detection of binding int
- VAKGL(target) and VAKGL(-ve control) peptides synthesized (GenScript)
- Simufilam HCI (MedchemExpress), Naloxone (Selleckchem), Acetazolamide (Fisher) ligands were dissolved in DMSO
- Peptides,CALI dissolved in dH2O (VAKGL) or PBS
- ITC binding assay in PBS; matched (DMSO) <5%
- Integration & baseline correction using NITPIC [12]

Interpretation & Limitations

- Analysis of published claims together with our experiments suggests that Simufilam does not bind its reported target.
- We believe Simufilam couldn’t have been discovered as patients claim, since NLX doesn’t seem to bind FLNA
- Proving a negative is hard; a single experiment is not determinative. ITC & other expts should be repeated by others
- Simufilam or NLX bound FLNA, it should be easy to determine structure of bound complex by crystallography, cryo-EM, or NMR. No such structures were ever reported.
- Simufilam authors have failed to offer any new experimental data to address concerns for over a year.
- All pre-clinical & clinical claims about Simufilam [3,4,5,6,7,8] are in doubt if they rely biologically or logically upon retracted, invalidated, fabricated, or falsified scientific claims.

Conclusion: In Our Opinion, a Drug That Doesn’t Bind Its Purported Target Has No MoA, Nor Possible Clinical Utility

Ethics & Law of Trials in Humans

- Human experimentation must be “justified on the basis of a favorable risk/benefit assessment” [16]
- Can informed consent be obtained if veracity of science used to justify clinical trials is in question?
- Is offering hope, denying access to alternative trials, & denying lumbar punctures justified if prospect of any clinical benefit is in question?
- Can clinicians & IRB properly evaluate a trial if Investigator Brochure cites dubious papers & scores?
- When should FDA take enforcement action to ensure compliance with 21 CFR 312?