Review XX

Dear Authors,

Dear Editorial Members Committee,

Thanks for the opportunity to rate the submitted paper. During reading, I evaluate the theme as actually and very important. Because I am deeply interested in the topic, some questions come to my mind. Your replies, supported by literature (by experimental research), could be incorporated into the manuscript for the article readability growth after the agreement of all authors. Except for the points below, I have no problem with the paper publishing in the chosen journal. Good luck.

Query #1

Why did you choose the ACTAT1 isoform (SOAT1 gene)? Is a *model enzyme* for your research? There are more manners to treat AD or cancer... In what way is the submitted proposal **much better than other available paths** (effectiveness, speed, low cost, simplicity of preparation, statistical success, etc.)? Pick one up.

Query #2

Why should the **ACTAT1-SOAT1 gen over-expressed** in brain cells and **contribute** to the neurodegenerative pathway?

Which factor initialize SOAT1 gen - and thus ACTAT1 (hyper-) **activity**? Is it a heritable problem or could it be the consequence of some external factor? (chemical, physical or biological factors, toxins, neurotoxins, or injury?) I think the **statistical occurrence** of Alzheimer's disease in some localities should guide researchers to find this **risk factor** to **eliminate** it from the **environment** for human health **protection** and the protection of **younger generations**.

Query #3

Please explain the pathway of **how apolipoprotein E** (ApoE) - extracellular cholesterol transporter - can **start to belong to the most significant risk factor of later AD development**. What could happen to this biomacromolecule? Is it almost a **genetic** reason, a consequence of natural **ageing** or another **biochemical mechanism's failure**? Would it be possible to add information about **the most probable pathological origin connected to AD (+ApoE relation)** development?

Query #4

Why did you choose **K-604** and **F12511**? For **which** medical **problems was this substance used/tested** already? In **which pathology** was this drug **the most successful**? Many thanks.

Query #5

Please, repeat briefly, from which natural substance was **K-604** and **F12511** derived? Or, which natural substance do these two potential drugs **simulate**? The **natural source** for **K-604** and **F12511** drugs lab synthesis **was**…

Query #6

I am not an expert in clinical **practice**. For these reasons, the experimental part with cell line acronyms caused difficulty for my reading, which slowed down. I had problems with **cell line identification by symbols and acronyms**. What kind of cell line this symbol represents (some animal, human or bacterial cell line? Healthy/vital or pathological/i.e. somehow destroyed lines? From which organ?)

** My note based on a submitted manuscript material, for example:*

Figure S4 – Table shows MAM proteins identified in proteomic analysis - N9 cells.

Is it possible to identify another gene (another information for concrete protein biosynthesis) in another cell line? Would it depend on **cell age** or external **gen segment intercalation during some concrete cell life**?

Before the patient drug therapy treatment setting (usually aggressive, especially in cancer treatment), the proteomic analysis for attacked/ affected tissue has to be realized. Such tissue has to be detected using diagnostic methods, e.g. except for external symptoms and suspicions, a combination of various diagnostic tools should be applied (biochemical tests or imaging diagnostic technologies).

Query #7

The **rich number** of used **methods**, based on **standard or modified processes**, **seems** to be described in detail. **Extracted results interpretations were explained clearly**, without unnecessary particulars.

Query #8

I agree similarities between the harmfulness and benefits of the way you solved are in **mutual contradiction**. Therefore it is necessary to search for details in **future research**. Despite mentioned apparent contradictions, additional (bio-) physical explanation of *"membrane phenomena"* and *"membranes contact and communication"* research could contribute to the development of **biophysical treatment of incurable patients**.

Cholesterol as an important **energy source can accumulate locally where there are high energy needs to "process something".*

Query #9 - *Notes: Cite this idea above, if applicable, please.*

The cholesterol level is regulated STRICTLY because of its importance in membrane function management (from the paper text).

*As I understand from the text (?), if the ATP-binding cassette (regulation through the **liver**) is inactive, thus, one possibility is to **knock out** the (hyper-) **active ACAT**. This enzyme catalyzes the esterification of cholesterol. The **accumulated product** of this reaction was detected in the **brain of AD patients and it is associated with cancer risk**.*

*I recommend authors propose some **scheme** of this **pathological** (probably cascade or **linear**) **phenomenon** with all **mentioned bio-macromolecules**. Also, **graphical abstract of simple suggested pathway mechanisms between ER-mitochondria** can attract readers and help students of pathological focus.*

*As I understand, some **uncertainty** about **pathological mechanisms** containing **partial explanations of the ER-mitochondria relationship** has to be clarified by **independent** 1.) ER, and 2.) mitochondria **function recognition**. See **mitochondria evolutional origin** (mitochondria absorption by another object or two "objects fusion"). (Theoretically, thanks to the membrane's presence, mitochondria can fully operate in a suitable environment). **Knockout of one or a few biochemical manifestations** of the cell **does not mean the end of a complex organism**. As I mentioned (query #2), I believe the **treatment with drugs** and then future **avoidance of detected irritant agents occurring** in the environment might **return health** to the complex organism.*

*As you mentioned in the discussion, a **deep analysis of healthy and unhealthy enhancement of MAM** can help maintain its full function (**balance** maintaining by **regulation** of a.) **oxidation** – oxygen, oxidants, versus b.) **reduction** – vitamins and reduction supplements...)*

Conclusion

*The paper is well written. I am not a native English speaker, so I will not assess the English grammar. Despite that, I had no problem understanding this problematics concerning cholesterol regulation, connectivity, communication and signal transport between ER and mitochondria. In addition, mentioned genetic knockout lab testing could be an effective way to show the phenotype rescue of species with the last chance for survival. For these reasons, such a scientific approach is valuable for life and life quality protection against future potential external attacks/factors that can initialize neurodegenerative pathways in risk-irritated sensitive organisms. Strengthening connectivity by cholesterol modulation can be one of many possibilities of biochemical mechanisms grip for life quality control from the outside help. Paper is beneficial from my point of view. I am **grateful** for this work. Please, continue.*

Kind regards

Reviewer XX