CONTACT: Alzheimer's Association Media Line, 312.335.4078, media@alz.org
                    AAIC 2023 Press Office, aaicmedia@alz.org

FROM THE ALZHEIMER'S ASSOCIATION INTERNATIONAL CONFERENCE 2023

CRISPR/GENE EDITING TECHNOLOGY CREATES NEW TREATMENT POSSIBILITIES FOR ALZHEIMER'S DISEASE

Key Takeaways:
- **Two new CRISPR-based strategies offer hope for next-generation Alzheimer's treatments.**
- **One seeks to dampen the impact of the most common Alzheimer's risk gene; the other aims to decrease production of a toxic protein in the brain.**

AMSTERDAM, JULY 16, 2023 — Two new CRISPR-based therapeutic approaches for Alzheimer's were reported today at the Alzheimer's Association International Conference® (AAIC®) 2023, in Amsterdam, Netherlands, and online. One aims to reduce the impact of the strongest known Alzheimer's risk gene, APOE-e4. The other strives to reduce production of a toxic protein in the brain, beta amyloid, which is a hallmark of Alzheimer's disease and the target of recently-approved treatments.

Gene editing using the Clustered Regularly Interspaced Short Palindromic Repeats system, known as CRISPR, is emerging as one of the most powerful tools in the search for new drugs. CRISPR technology is, among other things, making drug target identification faster with the goal of speeding up the drug discovery process, and building platforms for the development of next-generation treatments. This last point is the focus of the new reports at AAIC 2023.

"A pipeline of potential new treatments offers hope for the Alzheimer's and dementia community," said Maria C. Carrillo, Ph.D., Alzheimer's Association chief science officer. "The progress and approvals we've seen, as well as the diversification of potential new therapies over the past few years, provides hope to those impacted by this devastating disease. The anti-amyloid drugs newly approved by the U.S. Food and Drug Administration are an important first step in Alzheimer's treatment, but there is so much more to be done."

"Studies such as these two that focus the most advanced technologies — in this case, CRISPR — on moving Alzheimer's treatment and prevention forward are enthusiastically welcomed, and need to be multiplied many times over," Carrillo said. "We envision a future where multiple treatments address every aspect of this most complex disease. And that, once proven, the treatments can be combined in ways that complement and enhance each other to reduce risk, treat effectively, stop the progression and eventually cure Alzheimer's disease and all other dementia."

CRISPR Treatment Reduces Production of Amyloid Beta in Mice

Brent Aulston, Ph.D., and colleagues in the Subhojit Roy lab at University of California San Diego, have developed a gene-editing strategy that targets the amyloid precursor protein (APP), which Aulston calls "a gene with a central and indisputable role" in Alzheimer's. Depending on how it is cut by various enzymes in the brain, APP can create products that are either protective (sAPPa) or pathologic (beta amyloid). Aulston's approach hopes to reduce the production of beta amyloid while increasing neuroprotective actions.

Testing the process in an Alzheimer's disease mouse model, the researchers found that CRISPR-treatment led to reduction of beta amyloid plaques and associated markers of brain inflammation, an increase in

neuroprotective APP products, and correction of behavioral and nervous system function deficits. In addition, CRISPR-editing did not lead to undesirable side effects in normal mice.

"We believe this demonstrates that, in mice, our potential treatment strategy is both safe and efficacious," Aulston said. "These results justify future studies aimed at getting APP CRISPR editing into human testing."

CRISPR's Potential To Lower Levels of an Alzheimer's Risk Gene

Certain genes can increase the risk of developing dementia, including Alzheimer's disease. One of the most significant genetic risk factors for some populations is called APOE-e4, although inheriting this gene does not guarantee that a person will develop the disease. Having one copy of APOE-e4 increases the risk of developing Alzheimer's two- to threefold. Having two APOE-e4 genes increases risk even more: approximately eight- to twelvefold.

Boris Kantor, Ph.D., associate professor of neurobiology and faculty member at the Center for Advanced Genomic Technologies at Duke University, Ornit Chiba-Falek, Ph.D., professor in neurology and division chief of translational brain sciences, and colleagues described an epigenome therapy platform based on CRISPR/dCas9-editing strategy intended to reduce APOE-e4. The scientists found that their lead candidate can robustly reduce the levels of APOE-e4 in both human induced pluripotent stem cell derived miniature brains from an Alzheimer's patient and humanized mouse models, without changing levels of other APOE variants that are thought to be neutral or protective.

"The findings are incredibly exciting," Kantor said. "They provide proof-of-concept evidence supporting our approach as a high potential new strategy to treat and possibly even prevent Alzheimer's disease, which currently has no cure."

"The goal of our therapeutic strategy is to advance the field of Alzheimer's drug discovery towards precision medicine," Chiba-Falek added. "We believe the results are very promising and support moving forward with IND-enabling studies."

About the Alzheimer's Association International Conference® (AAIC®)
The Alzheimer's Association International Conference (AAIC) is the world's largest gathering of researchers from around the world focused on Alzheimer's and other dementias. As a part of the Alzheimer's Association's research program, AAIC serves as a catalyst for generating new knowledge about dementia and fostering a vital, collegial research community.
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- Brent D. Aulston, Ph.D., et al. In vivo safety and efficacy of a CRISPR-based gene therapy for Alzheimer's disease. (Funders: Alzheimer's Association, Epstein Foundation, U.S. National Institutes of Health)
- Boris Kantor, Ph.D., et al. APOE-targeted epigenome therapy for late onset Alzheimer's disease. (Funder: U.S. National Institute on Aging - R41 AG077992-01)

Proposal ID: 76735
[In-Person Posters]
P2-02: Monday, July 17, 2023
Drug Development: Nonhuman

In vivo safety and efficacy of a CRISPR-based gene therapy for Alzheimer's disease

Background: Our lab has developed a gene-editing strategy to target the extreme C-terminus (C-term) of APP (amyloid precursor protein) – a gene with a central and indisputable role in AD. In physiologic states, the APP protein is cleaved by α-secretases to generate neuroprotective sAPPα; but in AD (both sporadic and familial), the alternative β-cleavage pathway is active, generating pathologic fragments (including Aβ). Specifically, our approach attenuates pathologic β-cleavage products by disrupting a pentapeptide "YENPTY" domain at the APP C-term, and this retains the edited APP (APP-ΔC) at the cell-surface, augmenting neuroprotective and neuroregenerative α-cleavage.

Method: In order to assess the safety and efficacy of our approach in vivo, we used two different CRISPR-editing approaches. First, we generated germline edits in Wt and APP knockin (APP^{NL-G-F}) mice by injecting embryos with APP C-term targeting CRISPRs. We then selected founders to produce stable strains of WT and APP-KI in which the APP C-term was genomically deleted (referred to as WtΔC and KI-ΔC mice respectively). In another set of experiments, we packaged APP C-term CRISPRs into AAV vectors and systemically injected AAVs into APP-KI mice.
Result: WtΔC showed no cognitive deficits or histological abnormalities compared to Wt controls. KI-ΔC showed a dramatic reduction of amyloid beta plaques and associated neuroinflammatory markers and similar results were observed in AAV-treated KI mice. Moreover, both germline and somatic APP C-term editing produced an increase in neuroprotective sAPPα.

Conclusion: In total, these data demonstrate that our approach is safe and efficacious in vivo and validate the feasibility of our APP C-terminus editing approach as a therapeutic for AD.

Presenting author
Brent D Aulston, PhD
brentaulston@gmail.com
University of California San Diego, La Jolla, CA, USA

APOE-targeted epigenome therapy for late onset Alzheimer's disease

Background: Carrying the APOE*e*4 variant significantly increases lifetime risk for LOAD. The accumulating evidence suggest that alteration of the expression of APOE*e*4 and APOE may hold promise as a potential therapeutics target for LOAD. In this study we developed an epigenome therapy platform to reduce *APOE* and *APOEe*4 specifically by targeted modification of the epigenome landscape within *APOE* locus.

Method: Our approach is based on CRISPR/dCas9-editing strategy. The novel system based on the novel bipartite repressor platform, recently developed in the laboratory. We designed targeting of *APOEe*4 in the allele-discriminatory fashion, as such the editing is allele specific and precise. Furthermore, we developed a similar approach to target the regulatory elements within *APOE* promoter. We evaluated our platform *in vitro* using human hiPSC-derived neurons and organoids, as well as *in vivo* by stereotactic injection of the developed system into the hippocampus of the *ApoE*-humanized mice, harboring human *ApoE* loci replaced the mouse ortholog.

Result: Using the system, we demonstrated an efficient and precise editing of APO*e*E4 and *APOE* expressions in the hiPSC-derived neurons and the human isogenic APOEe4 organoids. We showed that the system can robustly reduce the levels of *APOE*-mRNA and the protein in both models. Importantly, the allele-discrimination approach has resulted in no detectable editing of the *e*3 allele in the *isogeneic* hiPSC-derived neurons, and organoids homozygous for the e3 allele. Moving onto *in vivo* studies, the promoter-targeted approach using adeno-associated- dCas9-KRAB-MeCP2 vector injected into the hippocampus of *APOEe4* and *APOEe3* mice, showed 50-70% decrease in the mRNA and the protein levels. Similar effect was observed using lentivirus- CRISPR/Cas system targeted ApoE*e*4 in allele-specific manner. Collectively, our results provided *in vitro* and *in vivo proof-of-concept* for the utility and efficacy of the APOE-targeted epigenome therapy.

Conclusion: Our epigenome therapy strategy for fine-tuning of *APOE* expression based on dCas9 technology is *translational* toward the development of a therapeutics approach to prevent and/or delay LOAD onset. Furthermore, the technology offers the opportunity to refine the platform for the development of gene-specific and even allele- and cell-type- specific therapies, and by that enables the advancement of strategies for precision medicine in LOAD.

Presenting author
Boris Kantor, PhD
boris.kantor@duke.edu
Duke University, Durham, NC, USA