



# Epilepsy Benchmarks Area II: Prevent Epilepsy and Its Progression

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## Abstract

Area II of the 2014 Epilepsy Research Benchmarks aims to establish goals for preventing the development and progression of epilepsy. In this review, we will highlight key advances in Area II since the last summary of research progress and opportunities was published in 2016. We also highlight areas of investigation that began to develop before 2016 and in which additional progress has been made more recently.

## Keywords

preventing epilepsy, epilepsy prevention, prevention clinical trials, epileptogenesis, NINDS benchmarks for epilepsy research, epilepsy, benchmarks, progress in epilepsy research

## Introductory Vignette by Shelly Meitzler. Can Epilepsy Be Prevented?

Two of my 3 children have tuberous sclerosis complex (TSC). Ashlin is 18 and Mason is 6. Because of recent research discoveries, their hopes for the future are entirely different.

Ashlin experienced her first seizure at 4 months old and, 4 agonizing weeks later, TSC was confirmed as her diagnosis. I felt defeated as multiple seizures, hospitalizations, life flights, countless failed medications, and endless testing dictated daily life. Status epilepticus when Ashlin was two and a half years old ripped away a piece of my child forever. She came home



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after a 10-day hospital stay with right-sided paralysis, no vocabulary, the inability to feed herself, sit up, crawl, or walk. She sees 10 different specialists, receives in-home therapy 5 days a week, will require assisted care for the duration of her life, and takes 18 doses of 7 different medications to treat the varying manifestations of TSC.

Mason was diagnosed with TSC at 7 months old. He was promptly enrolled in a research study at Boston Children's Hospital, which has been invaluable to Mason's developmental progress. When his infantile spasms began, vigabatrin was started within 6 days, and we've not seen an infantile spasm since. Mason experienced status epilepticus in March 2015 and required so much rescue medication, a code blue was called to resuscitate him. Fortunately, he recovered with no major setbacks.

Over the past 18 years, I've witnessed so much progress and, because of additional options now available, I have so much more hope for Mason's future. While the TSC community is grateful for current treatment options, they do not work for everyone, and the long-term need is to prevent manifestations before onset. We have made huge progress in terms of research and new treatments, but we have more work to do and more answers to find.

Shelly Meitzler of Tuberous Sclerosis Alliance

## Introduction

Area II of the 2014 Epilepsy Research Benchmarks aimed to establish goals for preventing the development and progression of epilepsy. In this review, we will highlight some key advances in Area II since the last summary of research progress and opportunities in this area was published in 2016<sup>1</sup> as well as some areas of investigation that began to develop before 2016 and in which additional progress has been made more recently.

## New Insights into Mechanisms and Modulators of Acquired Epileptogenesis

In the following sections, we summarize 3 themes in research in antiepileptogenic mechanisms: metabolic mechanisms, epigenetic mechanisms, and astrocyte-specific processes that influence epileptogenesis.

### Metabolic Mechanisms

The role of metabolism is an emerging area of epilepsy research. In addition to epilepsy being the direct consequence of pathogenic variants in genes encoding proteins in epilepsy disorders, such as in glucose transporter type 1 deficiency syndromes,<sup>2</sup> it has been shown that maladaptive changes in metabolism contribute to epilepsy development.<sup>3</sup> Conversely, metabolic therapeutic approaches, such as the ketogenic diet (KD), have been shown (1) to influence the epigenome and (2) to prevent epileptogenesis.<sup>4-8</sup> The KD suppresses seizures in some patients, reflecting the antiepileptic effects of specific metabolic changes.<sup>4,6,9</sup> Mechanisms underlying the success

of the KD are the subject of intense research efforts. Dietary compliance is difficult for many individuals on the KD, and lack of complete adherence to this diet can obviate the potential benefits of treatment. Some recent studies have focused on the specific effects of medium-chain triglycerides, both as a component of the KD<sup>9</sup> and independently<sup>10</sup> on both metabolic and antiepileptic effects. Others have suggested that effects of ketone bodies themselves on mitochondrial metabolism may underlie antiseizure effects of the KD, for example, in *Kcna1*-null mice.<sup>11</sup> There has been recent progress toward understanding some of the mechanisms of action of the KD, such as AMPA receptor inhibition.<sup>12</sup> In parallel, there are efforts to include modifications in the diet to make it more tolerable for people with epilepsy.<sup>13</sup>

Traditional antiseizure medication screening has been largely biased toward transmembrane channels and receptors, yet intracellular proteins and enzymes may represent appropriate therapeutic targets. Recently, several studies have emerged demonstrating proof-of-principle for metabolic targets as novel antiseizure medications or antiepileptogenic drugs. One study used a novel screening platform involving *in vivo* bioenergetics screening assays to uncover therapeutic agents that improve mitochondrial health; using an 870-compound screen in *kcnal*-morpholino zebrafish larvae, vorinostat (an histone deacetylase [HDAC] inhibitor) was uncovered as a potent antiseizure agent.<sup>14</sup> This study provides proof-of-principle for metabolism-based experimental therapeutics in epilepsy. Similarly, a study of glucose metabolism in *SCN1Lab* mutant zebrafish (a model of Dravet syndrome, DS) identified a decrease in baseline glycolytic rate and oxygen consumption rate which was rescued by KD, suggesting that mitochondrial hypometabolism contributes to the pathophysiology of DS.<sup>15</sup> Inhibition of lactate dehydrogenase (LDH), a component of the astrocyte-neuron lactate shuttle, hyperpolarized neurons and suppressed seizures *in vivo* indicating that LDH inhibition may represent a promising antiepileptic target.<sup>16</sup> Together, the above and other studies indicate that as our knowledge of specific metabolic manipulations deepens, novel antiepileptic targets for various types of acquired epilepsy are likely to emerge.<sup>17,18</sup> Various dietary or pharmacological therapies may also modify the gut microbiome,<sup>19-21</sup> which may have indirect effects on brain excitability. Indeed, "dysbiosis" may underlie some forms of drug-resistant epilepsy,<sup>22</sup> and a more systemic or "metabolic" viewpoint should be adopted to attempt to develop novel antiepileptogenic strategies that may initially seem "far from the synapse." This area requires more investigation to determine whether there will be evidence to support some of the novel hypotheses related to the microbiome.

### Epigenetic Mechanisms

The role of histone modification in contributing to various neurological diseases including epilepsy is under intense study. Modification of chromatin structure has been implicated in learning, memory, and synaptic plasticity; and recent studies suggest translational relevance to epilepsy. For example, in a



mouse model of tuberous sclerosis complex (TSC), decreased hippocampal histone H3 acetylation levels were observed; HDAC inhibition restored histone H3 acetylation, normalized synaptic plasticity, and suppressed seizures.<sup>23</sup> Interestingly, daily treatment with the HDAC inhibitor sodium butyrate inhibited hippocampal kindling epileptogenesis.<sup>24</sup> Other mouse models of temporal lobe epilepsy (TLE), such as the kainic acid and pilocarpine models, also demonstrate altered histone acetylation, HDAC expression, and DNA methylation.<sup>5,25-27</sup>

Beyond mouse models of epilepsy, another approach is to obtain surgically resected brain tissue from patients with drug-resistant epilepsy and perform genome-wide CpG-DNA methylation profiling to evaluate for specific epigenetic signatures. In one study using this approach, tissue from a patient with focal cortical dysplasia type II was found to demonstrate an epigenetic signature that identified candidate genes and pathways involved in pathogenesis.<sup>28</sup> Similarly, methylation analysis reveals specific profiles of TLE with or without hippocampal sclerosis,<sup>29</sup> and increased expression of DNA methyltransferases has been observed in human TLE.<sup>30</sup> Investigators have also tested the ability of induced epigenetic modification to prevent epileptogenesis. The endogenous anticonvulsant adenosine causes DNA hypomethylation by biochemical interference with the transmethylation pathway, and adenosine and/or adenosine kinase inhibition inhibits epileptogenesis in multiple seizure models.<sup>31,32</sup> Thus, pathological changes in DNA methylation may underlie certain forms of epileptogenesis, and reversal of these epigenetic changes may represent a key antiepileptogenic strategy. The currently used antiepileptic drug valproic acid is also known to be an HDAC inhibitor,<sup>33</sup> and its effects could be compared to some of the novel strategies that emerge in this area. Overall, the above studies suggest a role for chromatin modification in various forms of epilepsy, suggesting novel therapeutic strategies focused on normalizing chromatin structure. Profiling specific pathogenic epigenetic modifications may eventually allow more personalized approaches to treatment for specific epilepsy syndromes.

### Astrocyte-Mediated Mechanisms

Astrocytes play an established role in removal of glutamate at synapses and the sequestration and redistribution of  $K^+$  and  $H_2O$  during neural activity. It is becoming increasingly clear that changes in astrocyte channels, transporters, and metabolism play a direct role in seizure susceptibility and the development of epilepsy.<sup>34</sup> Stimulation of astrocytes leads to prolonged neuronal depolarization and epileptiform discharges.<sup>35</sup> Astrocytes release neuroactive molecules and modulate synaptic transmission through modifications in channels, gap junctions, receptors, and transporters. Further, striking changes in astrocyte form and function occur in epilepsy. Astrocytes adopt reactive morphology, become uncoupled, and lose domain organization in epileptic tissue. These and other changes—such as changes in the expression of the astrocytic enzymes adenosine kinase and glutamine synthetase, astroglial

proliferation, dysregulation of ion channel and glutamate transporter expression, alterations in secretion of neuroactive molecules, increased activation of inflammatory pathways, and aberrant activation of mammalian target of rapamycin (mTOR) signaling—may all contribute to hyperexcitability and epileptogenesis.<sup>36</sup>

Two specific examples of astrocyte involvement in epileptogenesis include:

1. *Tuberous sclerosis complex*: Evidence using astrocyte-specific *Tsc1* conditional knockout mice (mice in which the gene is knocked out only in astrocytes) has provided insight into a potential role of astrocytes in the etiology of TSC. These *Tsc1*<sup>GFAP</sup>cKO mice develop severe spontaneous seizures by 2 months of age and die prematurely.<sup>37</sup> The time of onset of spontaneous seizures in these mice is concordant with increased astroglial proliferation. Further, 2 functions of astrocytes—glutamate and  $K^+$  reuptake—are impaired in these mice, which also display reduced expression of the astrocyte glutamate transporters GLT1 and GLAST.<sup>38</sup> In addition, recent evidence indicates that astrocytes from *Tsc1*<sup>GFAP</sup>cKO mice exhibit reduced activity of inwardly rectifying  $K^+$  channels, and hippocampal slices from these mice demonstrated increased sensitivity to  $K^+$ -induced epileptiform activity.<sup>39</sup> A more recent inducible *Tsc1* knockout mouse in which *Tsc1* gene inactivation in GFAP-expressing cells was induced at 2 weeks of age was sufficient to cause astrogliosis and mild epilepsy (with a less severe phenotype than with prenatal *Tsc1* gene inactivation).<sup>40</sup> Together, these studies demonstrate that in this model, changes in glial properties may be a direct cause of epileptogenesis.
2. *Posttraumatic epilepsy*: Posttraumatic epilepsy (PTE) refers to a recurrent seizure disorder whose cause is traumatic brain injury (TBI). Posttraumatic epilepsy develops in a variable proportion of TBI survivors depending on the severity of the injury and the time after injury.<sup>41,42</sup> Antiseizure medication prophylaxis is ineffective at preventing the occurrence of late seizures.<sup>43-45</sup> Various animal models of PTE have demonstrated characteristic structural and functional changes in the hippocampus, such as death of dentate hilar neurons, increased dentate granule cell neurogenesis, mossy fiber sprouting, synaptic reorganization of dentate granule cells and CA3 pyramidal cells, and altered  $\gamma$ -aminobutyric acid receptor signaling.<sup>46-53</sup> Studies have also implicated altered astrocyte function in PTE models, including impaired  $K^+$  homeostasis in posttraumatic hippocampal glia<sup>54</sup> and impaired astrocyte glutamate transport in a PTE model induced by intracortical ferrous chloride injection.<sup>55</sup> Further studies of the role of glial cells appear warranted with advances in the past decade on PTE animal models.<sup>56</sup> In particular, long-term changes in astrocyte channels and transporters after TBI that may correlate with PTE should be



investigated. Recently, new models of repetitive diffuse mild closed-head TBI in mice have been described that are sufficient to cause PTE,<sup>57,58</sup> interestingly, in one study classic astrogliosis was limited but a population of atypical reactive astrocytes was identified that correlated with the subset of mice that developed PTE.<sup>58</sup> This new study suggests that, in addition to well-described neuronal dysfunction, astrocyte morphological, structural, functional, and molecular changes may contribute to or underlie PTE.

Since TBI is associated with breakdown of the blood–brain barrier (BBB) at the time of the initial event, studies of BBB disruption-induced epileptogenesis are also relevant to mechanisms of PTE. Indeed, transient opening of the BBB is sufficient for focal epileptogenesis.<sup>59</sup> Extravasated albumin can be taken up by astrocytes, which activates the transforming growth factor- $\beta$  (TGF- $\beta$ ) pathway leading to focal epileptogenesis and excitatory synaptogenesis through astrocyte TGF- $\beta$ /ALK5 signaling.<sup>60</sup> This mechanism provides an astrocytic basis for BBB disruption-induced epileptogenesis and suggests antiepileptogenic therapeutic approaches (TGF- $\beta$  inhibition). Indeed, TGF- $\beta$  inhibition through treatment with losartan, an angiotensin-II receptor antagonist and Food and Drug Administration (FDA)-approved antihypertensive medication, was found to exert antiepileptogenic effects in these BBB disruption models.<sup>61–63</sup> It will be of interest in the future to test similar strategies in PTE models for antiepileptogenic efficacy.

## New Targets/Opportunities for Antiepileptogenic Therapies

### Repurposing of FDA-Approved Drugs

Current antiseizure medications have several shortcomings: both in terms of efficacy, failing to control seizures in about one-third of cases, and tolerability, with many associated with adverse cognitive, behavioral, or other side effects. Repurposing of existing FDA-approved drugs to treat epilepsy and/or epilepsy-associated comorbidities may offer some advantages. First, there would be savings in time and cost of drug development. Second, the risk profile of an FDA-approved drug may already be understood and may be very different than current antiseizure medications. Third, existing drugs may be targeted based on specific aspects of cellular or network dysfunction that occur in epilepsy. Several recent studies have proposed such an approach.<sup>64,65</sup> Combinations of therapies can be tested for antiepileptogenic or disease-modifying efficacy in animal models. Another approach is to use extensive literature searches to mine data and create databases of FDA-approved drugs with published efficacy in animal models of epilepsy. Such an effort recently led to a database identifying 173 drugs as potentially appropriate for repurposing.<sup>64</sup> Another approach is to take a disease-based screening approach based on a specific assay. For example, a fluorescence-based sodium flux assay for inhibitory activity in a SCN8A R1872Q mutant cell

line identified 4 FDA-approved candidate drugs for SCN8A-related epilepsy.<sup>66</sup>

Based on the above considerations, we see as research priorities (1) the identification of mechanisms through which existing FDA-approved drugs affect epileptogenesis and (2) the study of existing FDA-approved drugs in a range of cellular and animal models of epileptogenesis. For example, existing immunomodulatory drugs used for multiple sclerosis may have unexplored antiepileptogenic potential and could be repurposed for epilepsy prevention. Fingolimod, which targets sphingosine-phosphate receptors, was found to have antiepileptogenic and anticonvulsant effects in the intrahippocampal kainic acid murine model.<sup>67</sup> Antiepileptogenic and immunomodulatory effects of some statins are another example.<sup>68</sup>

While animal model studies remain crucial, are there any clinical success stories of FDA-approved drug repurposing for epilepsy? After being used off label to treat patients with epilepsy for nearly 3 decades,<sup>69</sup> fenfluramine has recently completed 2 successful phase 3 clinical trials in DS and represents one of the most visible case of drug repurposing in epilepsy.<sup>70,71</sup> Personalized medicine studies in newly described genetic epilepsy syndromes might uncover additional opportunities to use existing drugs. This approach has been tried with the drug quinidine for epilepsy due to gain-of-function in the potassium channel gene KCNT1, although efficacy remains controversial.<sup>72</sup> On a cautionary note, while repurposing is a widely pursued strategy for neurological conditions, there are challenges in final translation to humans due to differences in delivery and efficacy when moving, for example, from mouse to human, and due to the realities of paying for phase III clinical trials for medications that either lack new chemical-entity patents and/or are already on the market in generic forms.<sup>73</sup>

### Gene Therapy

Gene therapy involves the induced expression of a therapeutic gene or manipulation of gene expression in a target tissue to alter cellular and tissue and (ideally) disease phenotype. Various investigations have explored the idea of gene therapy for epilepsy to provide an alternative therapeutic option as many forms of epilepsy are difficult to treat with conventional drugs. Viral vector-mediated gene therapy offers the opportunity to target specific mechanisms and cellular populations. These efforts to date have largely focused on preclinical studies, with delivery of various genes into animal models of epilepsy,<sup>74–77</sup> although it was realized long ago that the viral vector approach may also apply to human epileptic tissue.<sup>78,79</sup> Genes for which positive effects from this approach have been reported in animal models of epilepsy include HSP72,<sup>80</sup> aspartoacylase,<sup>81</sup> GDNF,<sup>82</sup> NPY,<sup>83–87</sup> KCNA1,<sup>88</sup> and adenosine kinase.<sup>89</sup> A distinct approach transduces genes with engineered channels, such as an engineered glutamate-gated chloride channel eGluCl<sup>90</sup> or an engineered potassium channel.<sup>91</sup> Of course, for clinical translation the viruses must accurately target the right



populations of human cells.<sup>92</sup> A roadmap from gene therapy in animal models to clinical trials has been proposed.<sup>93,94</sup>

The strongest rationale for gene therapy approaches is that there is little or nothing in sight for disease prevention or mitigation for certain intractable forms of epilepsy, both for seizures as well as cognitive and other severe comorbidities. For these epilepsy syndromes, we often know the gene as the starting point. There are multiple programs ongoing with the potential to be disease modifying in some forms of epilepsy, which are likely to start clinical trials around 2020, such as an anti-sense oligonucleotide approach for the treatment of DS (Stoke Therapeutics, Bedford, MA), enzyme-replacement therapy using adeno-associated virus (AAV) vectors for CDKL5 deficiency disorder (Ultragenyx, Novato, CA), and delivering NPY and Y2 receptors through an AAV approach (CombiGene, Lund, Sweden). With the development of better approaches for vector-mediated gene transfer to the central nervous system (CNS), we are likely to see new opportunities for the treatment of various epilepsies. Recent success in single-gene replacement therapy for spinal muscular atrophy type 1<sup>95</sup> provides hope in this arena.<sup>74</sup>

## Clinical Trials for Epilepsy Prevention

Ultimately the goal of appropriate target identification for anti-epileptogenic and/or disease-modifying therapy is translation to clinical trials for epilepsy prevention or modification. Several aspects of this process will need to be assessed for each type of epilepsy:

1. *Identification of clinical target populations:* Which populations would be the most appropriate for antiepileptogenic therapy trials? One category would be genetic neurodevelopmental conditions with a latency between clinical recognition of an abnormality and gene diagnosis to epilepsy onset (eg, TSC). Another category would be secondary (acquired) epilepsies with known cause and a delay between the initial event that places an individual at risk for epilepsy and the presentation with epilepsy. Clinical target populations in this category may include patients with PTE, poststroke epilepsy, or postinfectious epilepsy, which is an important cause of epilepsy in the developing world. In these situations, there is a window of opportunity between the inciting stimulus and the development of epilepsy during which one could theoretically intervene to prevent the development of epilepsy. This is in contradistinction to other secondary causes of epilepsy, such as tumor-associated epilepsy, which may present with seizures at the time of diagnosis or not be diagnosed in time for any epilepsy-modifying therapy. In contrast, in the case of PTE, even if the initiating traumatic event is well documented, a challenge may be that the actual development of PTE may occur many years after the initial injury, making enrollment and follow-up for anti-epileptogenic efficacy studies difficult.
2. *Identification of high-value therapeutic targets:* Preclinical research in appropriate animal models offers the capability of identifying and validating new therapeutic targets, both through mechanistic understanding and insights from research using repurposed FDA-approved drugs. Human genetics, meanwhile, continues to advance as a resource to identify and validate targets. Emerging computational tools are also being developed to help combine preclinical and clinical data into a framework to identify drug targets<sup>96</sup> and also to help predict the safety and efficacy of new targets.<sup>97</sup> As new targets are identified and validated, clinical interventional trials can be designed accordingly with either repurposed medications or new drugs.
3. *Stratification with biomarkers:* Stratification of clinical populations with biomarkers that predict epilepsy severity or even the potential for epilepsy to develop (as in assessing the risk of PTE after head trauma) will be important for many reasons, including the notion that trials designed to *prevent* epilepsy after high-risk exposures such as trauma, stroke, or cerebral infection would likely expose people without epilepsy to the effects of antiseizure medications. Careful study of natural history of certain conditions as well as predictive factors that incorporate well-defined and reliable biomarkers of risk of epilepsy or of disease progression will be required before preventive studies can be undertaken. Biomarkers can include susceptibility/risk biomarkers, diagnostic biomarkers, monitoring biomarkers, prognostic/predictive biomarkers, pharmacodynamics/response biomarkers, and safety biomarkers.<sup>98</sup> Each category may have distinct types of biomarkers (eg, serological, cerebrospinal fluid, brain tissue, imaging, electrophysiological, and behavioral/cognitive). The path to validation of each biomarker for each epilepsy syndrome will need to continue with high priority for translational epilepsy research. Thus, intermediate biomarkers that predict longer term outcome measures will take on an important role in determining interim outcomes.
4. *Implementation:* After the above considerations are met, there remain logistics and considerations around implementation. With respect to antiepileptogenesis trials, implementation should include the patient/caregiver perspective—what would be decision-making factors for people to enroll in a clinical trial when they have not yet developed epilepsy? Considerations of this sort may be quite different, for example, in 2 different scenarios: the parents of a newborn with TSC at risk of devastating, intractable seizures and disability versus an adult with TBI who may or may not develop seizures. The design and duration of antiepileptogenesis trials also need to be adapted to each of these scenarios based on the known natural history for each epilepsy type.

Implementation of antiepileptogenesis trials may differ significantly regarding approach in genetic versus other causes.

For genetic syndromes with epilepsy, early diagnosis of course is critical prior to epilepsy onset to enable the enrollment of clinical populations who are at risk, based on electroencephalogram or other biomarkers, but not yet displaying epilepsy. The development of disease-targeted therapeutics for these syndromes will presumably enable preventive treatments and associated clinical trials; examples here would be sodium channel inhibitors for *SCN8A* or *SCN2A* gain-of-function pathogenic variants or mTOR inhibitors for TSC. Some challenges that preventive clinical trials will face in these neurodevelopmental syndromes are the choice of an appropriate time window to delay or prevent epilepsy onset as well as the development and validation of clinical outcome measures for the nonseizure comorbidities. Preclinical genetic models for these syndromes might inform about the temporal window for prevention of epilepsy in these syndromes as well as the potential prevention of comorbidities. Natural history data in patients will also be essential to determine the rate of conversion (ie, development of epilepsy) as well as the duration of the presymptomatic epileptogenic stage to inform trial duration and minimum number of patients for adequate study power.

Distinct causes of secondary epilepsies may warrant support of antiepileptogenesis trials. For example, in some areas of the world, infections of the CNS by neurocysticercosis or other pathogens cause one-third of epilepsies and directly contribute to the higher incidence of epilepsy in resource-poor countries.<sup>99</sup> The 2019 World Health Organization report on Epilepsy, A Public Health Imperative highlighted these and other modifiable risk factors as key opportunities to reduce the global burden of epilepsy.<sup>100</sup>

Antiepileptogenesis trials are currently underway for TSC. A clinical trial for epilepsy prevention using vigabatrin in asymptomatic infants with TSC aiming to lower the risk of developing infantile spasms is currently ongoing (Preventing Epilepsy Using Vigabatrin in Infants with Tuberous Sclerosis Complex, NCT02849457, PREVeNT trial, clinicaltrials.gov). The trial targets a patient population of presymptomatic infants with TSC less than 6 months of age and monitors the developmental impact of epilepsy from birth to 36 months of age. As illustrated in the introductory vignette, the antiepileptogenic or preventive approach is expected to also result in more favorable cognitive, behavioral, and developmental outcomes.

## Conclusion

In summary, successful integrated research programs in antiepileptogenesis will combine<sup>101</sup>: (1) animal model studies; (2) development of new animal models (both for genetic epilepsies and acquired epilepsies); (3) development and validation of biomarkers; (4) stratification of treatment groups and outcome evaluations based on validated biomarkers in both animal and human trials; (5) selection of novel creative high-value targets based on preclinical research (such as metabolic, epigenetic, and astrocytic targets reviewed above); (6) screening and repurposing of FDA-approved drugs; and (7) coordination of

clinical research strategies to understand the best time window for preventive trials and the ideal patient populations.

## Authors' Note

The opinions expressed in this publication are those of the author(s) and do not necessarily reflect the views of the NIH or the AES.

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