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New epilepsy therapies in development

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Abstract:

Epilepsy is a common brain disorder, characterized by spontaneous recurrent seizures, with associated neuropsychiatric and cognitive comorbidities and increased mortality. Although people at risk can often be identified, interventions to prevent the development of the disorder are not available. Moreover, in at least 30% of patients, epilepsy cannot be controlled by current antiseizure medications (ASMs). As a result of significant progress in epilepsy genetics and the development of novel disease models, drug screening technologies, and innovative therapeutic modalities over the past 10 years, over 200 novel epilepsy therapies are currently in the preclinical or clinical pipeline, including many treatments that act by new mechanisms. Assisted by diagnostic and predictive biomarkers, the treatment of epilepsy is undergoing paradigm shifts from symptomatic-only ASMs to disease prevention, and from broad trial-and-error treatments for seizures in general to mechanism-based treatments for specific epilepsy syndromes. In this review, we assess recent progress in ASM development and outline future directions for the development of new therapies for the treatment and prevention of epilepsy.

[H1] Introduction

Epilepsy is one of the most common and disabling brain disorders, affecting approximately 1% of the population of all ages [1], equating to ~3.3 million people in the US and 70 million worldwide, with related psychiatric and neurocognitive impairments, and other comorbidities, psychosocial decline, and risk of premature death.[1, 2] In 2017, epilepsy was re-defined to include a single unprovoked seizure with a ≥60% risk of further unprovoked seizures, emphasizing the propensity to develop epilepsy following an epileptogenic injury, with an epileptogenic lesion or in an epilepsy-prone genetic condition.[3] About 20% of epilepsies are caused by acute CNS injuries such as traumatic brain injury (TBI), stroke (cerebrovascular accident [CVA]), and infection.[4] In these conditions, epilepsy onset is delayed by weeks to years after the insult, presenting a window of opportunity to intervene, but preventive or disease-modifying treatments do not exist. Once epilepsy is established, a third of patients do not respond to symptomatic treatment with antiseizure medications (ASMs) despite the availability of over 30 licensed ASMs.[5] The molecular mechanisms underlying drug-resistant epilepsy (DRE) are incompletely understood.[6]

The last 10 years have seen breakthroughs in understanding epilepsy genetics and in the development of novel tools for target-driven approaches. There is a large preclinical and clinical treatment pipeline, and the principles of precision medicine are being applied to epilepsy.[7] We are entering a new era in the treatment of epilepsy, moving from symptomatic seizure suppression to treatment of syndromes and disease prevention. The development of several potently effective antiepileptogenic treatments in animal models and the identification of biomarkers to enrich the targeted patient population have made clinical trials of epilepsy prevention after acute CNS insults both feasible and timely.[4] Furthermore, there is an explosion of mechanism-based projects aiming to treat rare and genetic epilepsies.

In this review, we discuss the ASM pipeline, focusing on novel mechanisms, and review opportunities, challenges, and potential solutions to develop more effective, disease-modifying and preventive treatments for epilepsy.

[H1] Epilepsy: complex disease and treatment

Our understanding of the pathophysiology of seizure types and epilepsy subtypes has evolved since its inception in the 1960s, and with this, classifications of seizures and epilepsies have been continuously updated based on scientific progress.[8] The latest epilepsy classification has three levels: the first classification level defines the seizure type and seizure onset, which can be focal or generalized seizure (i.e., it classifies the symptoms of the disease by type) [3]; the second level classifies the disease (i.e. the epilepsy type, which reflects the first classification based on predominant seizure type) and can be focal epilepsy, generalized epilepsy, combined generalized and focal epilepsy, or unknown; the third level refers to the epilepsy syndrome diagnosis, a characteristic cluster of seizure types, clinical and EEG features, often supported by specific etiological findings, which can be structural, genetic, infectious, metabolic, immune, or of unknown etiology.[9]

Developmental and epileptic encephalopathies (DEEs) represent a large heterogeneous group of rare but devastating and largely intractable neurodevelopmental disorders characterized by seizures and abnormal neurocognitive development.[10, 11] There are over 250 DEEs and the number continues to grow; widely known DEEs include Dravet syndrome (DS), which, in 80% of cases, is caused by a loss-of-function mutation in one copy (haploinsufficiency) of the *SCN1A* gene that encodes the alpha subunit of voltage-gated sodium channel $Na_v1.1$.[12]; tuberous sclerosis complex (TSC), which, in 70% of cases, is an autosomal dominant disorder caused by mutations in *TSC1* or *TSC2* resulting in non-cancerous (benign) tumors in the brain and several body organs[13]; and (iii) Lennox-Gastaut syndrome (LGS), which has diverse causes, many of which are not genetic.[10, 11, 14-16]

Recently, the scientific community changed the designation of "antiepileptic drugs" (AEDs) to "antiseizure medications" (ASMs) because the drugs' effect is mainly symptomatic and they do not impact comorbidities or, in general, the underlying mechanisms of the disease.[17] ASMs can be classified depending on their mechanism of action (MOA) into four broad classes [18] (Fig. 1)*:*

(1) modulators of *voltage-gated ion channels*, including sodium, calcium, and potassium channels;
(2) enhancers of *GABA-mediated inhibition* acting on $GABA_A$ receptors, the GABA transporter (GAT1), the GABA-synthesizing enzyme glutamic acid decarboxylase (GAD), or the GABA-metabolizing enzyme GABA aminotransaminase (GABA-T);
(3) inhibitors of synaptic excitation mediated by *ionotropic glutamate receptors,* including α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptors;
(4) modulators of *synaptic neurotransmitter release*, targeting the presynaptic release machinery, including synaptic vesicle protein 2A (SV2A) and the α2δ subunit of voltage-gated calcium channels.

Ideally, correct diagnosis and classification of the epilepsies should guide treatment decisions, and response to treatment should be part of the syndrome classification. However, there are at present very few diagnosis- or ictogenic mechanism-specific treatments. For the vast majority of epilepsy syndromes, correct diagnosis leads at best to the avoidance of specific ASMs which are known to exacerbate seizures in a particular syndrome, such as carbamazepine and ASMs with a similar mode of action for idiopathic generalized epilepsies [19] or DS [20].

Most of the ASMs were developed to suppress or reduce focal- or generalized-onset seizures across different syndromes and etiologies (Table 1) with little difference in efficacy between ASMs licensed for monotherapy treatment. About 45-50% of newly-diagnosed patients respond to the first ASM treatment, whichever ASM is tried, whilst chances for significant improvement are less than 5% after failure of three ASMs, with about 35% of patients not achieving long-lasting seizure-freedom.[21, 22] Worldwide, this equates to approximately 25 million people with epilepsy who are not satisfactorily treated with any combination of the over 30 available ASMs.

ASMs are selected for an individual patient primarily on the basis of the patient's potential for certain side effects caused by a particular ASM or the presence of co-morbidities which may be improved as an off-target effect of an ASM, or the potential specific off-target effects, drug-drug interactions and pharmacokinetics of the ASM, and, in a few rare syndromes like DS, the underlying pathophysiology.

Multiple pathophysiological mechanisms are involved in the development of different epilepsies, be it genetic, acquired, or unknown, and the heterogeneity of various epilepsy syndromes and subtypes complicates the preclinical and clinical development of new ASMs. Double-blind, placebo-controlled randomized controlled trials (RCTs) of adjunctive therapies in patients with drug-resistant focal epilepsies continue to be the primary tool to obtain regulatory approval for novel ASMs.[23] The existence of ~30 ASMs on the market creates major hurdles for demonstrating the superior efficacy of any novel compound, discouraging large pharmaceutical companies from investing in ASM development. By contrast, there is increasing interest, particularly among small and medium-sized companies, in developing novel molecules for orphan indications (i.e., rare genetic epilepsies) where unmet needs are particularly large.

In the last decade, eight new ASMs have been approved for use in the treatment of epilepsy (Supplementary Table 1). Despite these advances and the lessons learned from these past approvals (Box 1), several challenges and unmet needs remain.

First, DRE continues to be one of the most important challenges in epilepsy management, and novel strategies are needed to find more effective ASMs for DRE therapy. Second, the heterogeneity of epilepsy types and the highly variable inter-individual response to the therapies makes disease management challenging. Thus, individualized prediction of antiseizure response and adverse events of ASMs would markedly improve therapy and quality of life for patients. Third, better understanding mechanisms of seizure generation (ictogenesis) in humans, specifically individual patient-based ictogenesis, is an important prerequisite to target precision medicine, and several such approaches of targeted treatment are currently being developed (Table 2). Fourth, status epilepticus (SE), a continuous seizure of >5 minutes duration or recurrent seizure activity without recovery between seizures, is refractory to ASM treatment in about 30% of patients. Refractory SE is a life-threatening medical emergency. More effective SE therapies are urgently needed. Fifth, epilepsy is one of a few diseases where people at risk can often be identified and are in medical care at the time of onset of the disease process, before the disease appears, allowing a window of opportunity to use preventive

treatment to alter, stop or prevent the process and the disease. At present no treatment exists to prevent or modify the development of the disease. Thus, prevention of acquired and genetic epilepsies is an important goal.

[H1] Advances in ASM discovery and development

For many decades, the fundamental commonality across pharmacotherapies for epilepsies has been the modulation of neuronal excitability.[23-26] However, because patterned neuronal excitation is essential to normal brain function, disrupting this activity leads to adverse effects. Indeed, almost all approved ASMs have the potential to cause adverse CNS effects such as drowsiness, dizziness, fatigue, and others.[23] To be effective, therapies have to be taken chronically and for this, they have to be well tolerated. Medication non-adherence is a major cause of "breakthrough seizures".[27] Approaches that allow selective targeting of critical populations of cells and particular pathways in the brain have the potential to both reduce side effects and improve efficacy.[28]

In line with these goals, large ASM screening programs such as the Epilepsy Therapy Screening Program (ETSP, formerly termed the Anticonvulsant Screening Project [ASP]) of the National Institute of Neurological Disorders and Stroke (NINDS) of the US National Institutes of Health (NIH) have included batteries of both acute and chronic animal models of drug-resistant focal seizures and models of drug tolerability in drug screening (Box 2).[29-31] The ETSP provides opportunities for researchers in the US and abroad to submit compounds for testing in the rodent models.[30] The screening program has tested over 32,000 compounds submitted from more than 600 participants from both industry (~40%; usually small biotech companies but also pharmaceutical companies) and academia (~60%). Most compounds tested by the program are small molecules, but there has been an increase in large molecular therapeutics (or biologics, i.e., complex molecules or mixtures of molecules such as antibodies, proteins, peptides, ASO/RNA, cell therapies, gene therapies and biological products) over the last 10 years, with 13% of all submitted therapeutics being biologics in the last two years (Brian Klein [NINDS], personal communication).

The ETSP has been instrumental in discovering or characterizing numerous novel ASMs, several of which have been approved for epilepsy therapy in the last decade.[29, 30] A genetic mouse model of DS is currently in development within the program to address the unmet clinical needs of this population.[31, 32] In addition, animal models targeting the discovery of therapies for disease prevention and modification have recently been implemented (Box 2).

[H2] New in vivo and in vitro models

Through technological advances in genome editing, the past ~5 years have produced a flurry of new mouse models of genetic epilepsies, primarily for DEEs, which are now increasingly being used in drug discovery.[33, 34]

While mouse and rat models of seizures and epilepsy discussed in Box 2 have been the cornerstone of ASM discovery for decades, novel *in vivo* models, such as genetically engineered mice and zebrafish, and several *in vitro* models, such as induced pluripotent stem cells (iPSCs) or brain organoids, have entered ASM drug discovery recently.[35-38] Ultra-rapid, single-base-pair editing technologies can be applied to create and validate human gene defects in mouse models and patient-derived iPSC- and organoid-based models, creating *in vivo* and *in vitro* biological test systems to search for targeted therapies.[15] As illustrated in Fig. 2, iPSCs can also be generated from patients with a genetic epilepsy and grown as cerebral organoids to study the disease and test new therapeutic targets.[39, 40] This technique has recently been used for generating a human model for TSC by growing cerebral organoids from patients with mutations in the *TSC2* gene.[41] The organoid model recapitulated the emergence of both brain tumors and dysplastic cortical regions and allowed investigators to identify a specific interneuron progenitor population that gives rise to both tumor and cortical tuber lesions. Epidermal growth factor receptor inhibition in the brain organoids reduced tumor burden, identifying potential new treatment options for TSC and related disorders.[41] Cerebral organoids placed on multi-electrode arrays can be used to study neuronal network hyperexcitability in monogenetic epilepsies as a tool for drug discovery pipelines.[42] The use of iPSCs (“iPSC villages”)

from several unrelated donors in a single dish is interesting because it may reduce some of the limitations of these techniques such as the variability between iPSC lines.[43]. These techniques could also help uncover pathways or targets in the non-genetic epilepsies where iPSC studies have been lacking.

Heterologous expression systems (e.g., human embryonic kidney [HEK293] cells, Chinese hamster ovary cells, *Xenopus laevis* oocytes) allow rapid introduction and screening of many different mutations in a cost-effective manner (Fig. 2).[36] However, without validation in more advanced models, data obtained by heterologous expression systems may yield misleading information. A prominent example of this is quinidine, which was reported to reverse *KCNT1* potassium channel gain-of-function mutations in a *Xenopus laevis* oocyte assay.[44] *KCNT1* mutations have been implicated in different intractable types of DEE with reports of open-label use of quinidine in DEEs due to *KCNT1* mutations.[45-48] The effect on seizures in most cases was reported to be dramatic, sometimes with seizure cessation. However, a cross-over RCT of quinidine in DEE patients with *KCNT1* mutations failed to show efficacy; dose-limiting cardiac side effects were observed.[49] This suggests that the effect of a disease-associated genetic variant on protein function in neurons may not be fully recapitulated in a heterologous expression system. As illustrated in Fig. 2, iPSC and organoid models of epilepsy may bridge the gap between functional studies in heterologous expression systems, animal models, and human clinical presentation of epileptic disorders. It is important to note that in translating *in vitro* to *in vivo* it is often not possible to achieve sufficient target engagement *in vivo* due to pharmacokinetic issues and/or dose-limiting toxicity.[50]

Among genetically engineered mouse models of DEEs, mouse models of DS, the most common DEE, are increasingly being used for drug screening.[38, 51] There are now numerous genetic mouse models for DS which aim to replicate the *SCN1A* loss-of-function.[34, 51] One of these models is used for drug screening in the ETSP (Box 2).

Genetically engineered zebrafish larvae offer an alternative *in vivo* model for assessing the antiseizure efficacy of ASMs and experimental compounds for DS.[51] *SCN1A*-deficient zebrafish

have spontaneous seizures that can be reduced by the antihistamine and 5-HT agonist clemizole. This was discovered by phenotypic screening of drug libraries in the zebrafish model.[52] In a subsequent study, a second blind screening of an active drug library identified a compound with a similar MOA to clemizole that had been brought to market only a few years earlier for treatment of obesity: the anorectic drug lorcaserin.[53] Consistent with the zebrafish findings, lorcaserin reduced seizure frequency in a small number of patients with intractable DS, providing a proof-of-concept of the strategy of repurposing existing drugs after a zebrafish screen. However, seizure reduction was not seen in all patients and was not maintained in all patients in whom it occurred.[53] Both clemizole and lorcaserin are currently in phase II/III trials in patients with DS (Table 2). This was the first 'aquarium-to-bedside' example of evaluating drugs to treat epilepsy and suggested that a zebrafish-based platform holds great potential for achieving effective personalized medicine [37, 51, 54]. However, zebrafish models of epilepsy have several limitations.[36, 38] Zebrafish do not recapitulate the genetic background, lifespan, and complex neural structures of human patients. iPSCs might be able to address some of these weaknesses and provide another complementary model.[36] The combination of a battery of *in vitro* and *in vivo* models as illustrated in Fig. 2 brings new capabilities to model the complex pathophysiology of genetic epilepsies.

[H2] New strategies and tools

As in other areas of drug discovery, both rational (target-based) strategies and phenotypic screening are used in the search for new, more effective epilepsy therapies (discussed in more detail below). The field of epilepsy has also started to apply principles of precision medicine to treatment, i.e., therapy targeted to patients' disease-specific pathophysiology.[55-57] The discovery of specific genetic mutations in monogenic epilepsies, driven by the remarkable advances in sequencing technologies, has provided numerous targets for precision medicine.[7] This allows researchers to either develop new targeted treatments or to repurpose drugs used in unrelated conditions, such as the anti-histaminergic drug clemizole discussed above. For the identification of such drugs, novel approaches to drug

screening in *in vitro* and *in vivo* models are used as illustrated in Fig. 2. Examples of successful repurposing are the anorectic amphetamine derivative fenfluramine for DS and LGS, and the immunosuppressant everolimus as an antiseizure therapy in patients with TSC and other mTORopathies.[58]

One may argue that precision medicine efforts for epilepsy by developing or repurposing small molecule drugs are unnecessary as we move towards targeted gene or oligonucleotide-based therapies.[36, 57] After decades of evolution, the past 10 years have seen a renaissance in the field of gene therapies, leading to the first approved therapies, including oligonucleotide-based and *in vivo* gene therapies.[59-61]

It is estimated that the aetiology of up to 70% of epilepsies have a genetic component.[62] As described above, in addition to long-known genetic epilepsies such as DS or TSC, there is an increasing number of newly discovered inherited DEEs with functionally characterized gene defects, which could pave the way toward novel gene therapies. Various approaches are currently being evaluated for monogenic epilepsies, both in experimental models and clinically (Table 2).

For monogenic epilepsy syndromes, gene therapies target neurons in the CNS. This requires either intrathecal delivery to bypass the blood-brain barrier (BBB) or systemic administration of the therapy that crosses the BBB to reach the whole brain.[57] Most gene therapies are packaged in adeno-associated viruses (AAV) that have tropism for the brain, typically AAV9 because of its BBB-permeable capsid. However, one of the major drawbacks of current AAV therapies is the low efficiency of transduction of target cell populations in the brain following systemic (i.v.) administration. This may be overcome in part by intrathecal or intracerebral administration of AAVs, which, however, has notable drawbacks.[63] Recently, this has been partially addressed with the development of better cross-species capsids with up to 100 times better transduction in the brain following i.v. injection in mice and nonhuman primates.[64] In addition to multiple delivery methods, there are also multiple approaches to gene therapy that can be subdivided into gene supplementation, gene editing, and gene expression modification, including antisense oligonucleotides (ASOs), splice

modulating oligonucleotides (SMOs), RNA interference, transfer RNA technology, regulatory RNA technology, and CRISPR-mediated transcriptional activation.[12, 14, 57, 62] A detailed description of these methods is beyond the scope of this review but the first ASO, STK-001 developed by Stoke Therapeutics, is currently undergoing a phase II trial in patients with DS; preliminary efficacy results have been modest [65] (Table 2).

In addition to monogenic epilepsies, targeted molecular therapies, including optogenetic and chemogenetic approaches, are currently being evaluated in preclinical models of acquired focal epilepsy.[28, 66, 67] Optogenetics can be used in a closed-loop paradigm in which the light source is activated only when seizures are detected. Proof-of-concept of optogenetic use in the therapy of neurological diseases in humans has recently been demonstrated.[68] The therapeutic use of optogenetics typically involves two components: a gene therapy medicinal product that induces long-term expression of light-reactive proteins within a specific subset of cells, and an active implantable device to stimulate the light-sensitized cells. As shown in animal models of epilepsy, either optogenetic inhibition of excitatory principal cells, or activation of a subpopulation of GABAergic stops seizures rapidly upon light application.[67]

In addition, novel optogenetic approaches, compatible with high-throughput capability, are now increasingly being applied in the discovery of novel ASMs. Chemogenetic methods, which combine the selective expression of designer receptors with designer drugs thus providing a method for selective and “remote” control of neuronal activity, have rapidly grown in use in the neurosciences, including epilepsy. One such approach is in the preclinical pipeline for focal epilepsy (Table 2). Epilepsy may well be the first indication to reach a clinical trial for this strategy.[69]

Novel genetic approaches have also been used to target acquired focal epilepsies, including a systems genetic approach for ASM discovery that predicted the tyrosine kinase receptor Csf1R as a potential therapeutic target [70], a sophisticated, localizable new genetic strategy enabling on-demand inhibition of neural activity [71], and a new Connectivity Map (CMapP) based target validation and discovery approach.[72] Overall, various novel concepts and tools for target-driven approaches to

epilepsy therapy have evolved over the past 10 years through the combined efforts of academia and industry. This has led to an enormous increase in the number of new treatments in the preclinical or clinical pipeline (discussed below). Some of the novel targeted molecular therapies in development may not only suppress seizures (like current ASMs) but allow disease modification or even cure.[35]

[H1] Novel epilepsy therapies in development

We identified 203 novel epilepsy treatment discovery or development projects (Supplementary Table 2) but the actual number may be higher because not all industry projects are in the public domain. Some 900 novel potential epilepsy therapies were evaluated by the ETSP in the past 10 years (Brian Klein, personal communication. This high number reflects an increased interest of the pharmaceutical industry (particularly small to medium-sized companies) in this area and has several reasons, including the enormous progress in understanding the molecular causes of epilepsy (most novel treatments are target-based) and the business model of orphan drug designation for rare genetic epilepsies, which gained attractiveness in recent years.[73, 74] Fig. 3 illustrates the most promising or advanced strategies and targets in development based on the data shown in Supplementary Table 2, and also highlights that epilepsy is more than a disease of neurons but includes alterations in other cell types that form potential targets for novel drug development.

Table 2 illustrates our selection of the most interesting epilepsy treatment development projects in the preclinical or clinical pipeline, for which details on MOA and antiseizure effects are available. Orphan designations for rare epilepsies have increased dramatically in the past 10 years.[73] About half of the novel epilepsy treatments are for the treatment of DEEs. The growing number of new DEE therapies is also illustrated by the fact that at least 25 new gene or molecular therapies are under development (Table 2), while the majority of the treatments listed in Table 2 are small molecules. Among the small molecule projects, 23 target different types of ion channels, and 19 the GABAergic system, making the GABA system one of the commonest target in new ASM development. Several of the GABAergic compounds act as positive allosteric modulators (PAMs) at the $GABA_A$ receptor,

demonstrating the renaissance of an old concept (for review see also[75, 76]).

The richness and diversity of novel therapeutic approaches in epilepsy is impressive, but some clear trends and particularly promising molecules can be identified. This is particularly noticeable in orphan and genetic epilepsy indications. For example, as discussed below, next-generation approaches exploiting the importance of serotoninergic mechanisms, initially exemplified by the approval of fenfluramine, are advancing (e.g., LP352). Similarly, precision therapy approaches for genetic epilepsies such as DS using ASO therapeutics (e.g STK-001) or GRIN disorders (e.g. radiprodil) appear to hold significant promise. Finally, selective targeting of particular miRNAs involved in drug-resistant epilepsies could be the beginning of a novel trend, as signaled by quite remarkable preclinical results obtained with NMT-001.

[H2] The renaissance of GABAergic compounds

For over 100 years, PAMs of $GABA_A$ receptors or "GABAkines" have been widely used in epilepsy, anxiety, sleep disorders, general anesthesia, and other indications.[77] As illustrated in Fig. 4, one of the first ASMs, phenobarbital, a nonselective $GABA_A$ receptor PAM, and other barbiturates act via barbiturate binding sites at the $GABA_A$ receptor to potentiate GABA's inhibitory effect (Fig. 5).[78] The benzodiazepines (BDZs, e.g., diazepam, clonazepam), which were introduced some 50 years after phenobarbital, act as PAMs via another allosteric binding site to enhance the effect of the neurotransmitter.[79, 80]

Soon after the discovery of GABA as the main inhibitory neurotransmitter in the brain, it was thought to be critically involved in the pathogenesis of epilepsy.[81, 82] Consequently, the first rationally developed ASMs, such as vigabatrin and progabide, were designed to enhance GABAergic transmission.[83] As shown in Fig. 5, this can be achieved via numerous targets, including GABA synthesis, uptake and degradation, and $GABA_A$ receptor modulation at different binding sites of the receptor.[84] About one-third of all ASMs act, at least in part, via one of these targets.[23] However, increased GABAergic inhibition is associated with the dampening of neuronal activity, and

GABAergic ASMs are associated with sedative adverse effects. These effects also limited the use of BDZs as anxiolytics.[85] Thus, soon after the discovery of the "BDZ receptor" in the 1970s [86, 87] several pharmaceutical companies (Roche, Schering/Bayer, Lundbeck and others) started programs to design ligands for the BDZ binding site that lack the sedative, hypnotic, muscle relaxant and dependence-inducing effects of traditional BDZs. The main strategies were to develop partial agonists and subtype-selective agonists that only act on certain subtypes of the $GABA_A$ receptor.[77, 85, 88] Importantly, the BDZ site recognizes not only BDZs but also ß-carbolines (e.g., abecarnil), imidazopyridine derivatives (e.g., zolpidem), imidazolone derivatives (e.g., imepitoin), and various other chemical structures.[88, 89]

$GABA_A$ receptors are ligand-gated chloride channels composed of five subunits that can belong to different subunit classes.[79] As illustrated in Fig. 5, most of these receptors are composed of two α, two β, and one γ subunit. The existence of 19 different subunits gives rise to a multiplicity of $GABA_A$ receptor subtypes with distinct subunit composition; regional, cellular, and subcellular distribution; and pharmacology.[79] BDZ binding sites are located at the α/γ interface of the heteropentameric receptor (Fig. 5). $GABA_A$ receptor subunit diversity creates unique opportunities for selective pharmacological modulation of the BDZ site.[90-92] α1-subunit containing $GABA_A$ receptors were found to mediate sedation and hypnosis, anterograde amnesia, and part of the antiseizure activity of BDZs, whereas α2- and α3-$GABA_A$ receptors mediate anxiolysis and antiseizure effects without sedation in preclinical models. This led to the development of α1-preferring PAMs such as zolpidem as sedative/hypnotics [93, 94] and, more recently, the α2/α3-subunit selective PAMs for seizures, including KRM-II-81[95], AZD7325[96, 97], and the α2/α3/α5-subunit selective PAMs darigabat and ENX-101[98-100] (Table 2) (Fig. 4). Darigabat exerts lower intrinsic efficacy than classical BDZs, acting as a partial agonist at the BDZ site of the $GABA_A$ receptor. This combination of α-subunit selectivity and partial agonism is not new. It was used in the 1980s and 1990s in the search for non-sedating ("anxioselective") anxiolytics, like the β-carboline derivative abecarnil, which exhibited a large dose separation between anxiolytic-like and antiseizure actions vs. adverse effects, i.e. ataxia and

sedation, without tolerance development and dependence in animal models.[77, 85, 101-103] Unexpectedly, it induced potent sedative and inconsistent anxiolytic activity in clinical trials.[85] Similar clinical failures were also reported for other partial agonists, subtype-selective molecules, and hybrid solutions such as bretazenil, alpidem, and ocinaplon.[85] The one exception was the imidazolone derivative imepitoin, which acts as a low-affinity partial agonist at the BDZ site of the $GABA_A$ receptor and was approved by the EMA in 2013 as a nonsedative ASM for the treatment of canine epilepsy.[104]

In addition to the synaptic localization illustrated in Fig. 5, $GABA_A$ receptors have considerable extrasynaptic localization where they are activated by low concentrations of ambient GABA to mediate 'tonic' inhibitory currents, distinguishable from 'phasic' synaptic transmission.[79] Extrasynaptic δ-subunit-containing $GABA_A$ receptors are insensitive to traditional BDZs but several endogenous and synthetic neuroactive steroids, including allopregnanolone (brexanolone) and ganaxolone act as agonists at both synaptic and extrasynaptic $GABA_A$ receptors.[105] Ganaxolone was recently approved for the treatment of seizures associated with CDLK5 deficiency (Table 1) and brexanolone for the treatment of postpartum depression.[77] Several other neuroactive steroids are in clinical development for intractable epilepsy (Table 2), SE, depression, and other indications.

In addition to the apparent disconnect between the preclinical profile and the human adverse event profile reported for several $GABA_A$ receptor PAMs[77, 85] it is important to note that epilepsy itself alters the subunit composition of $GABA_A$ receptors[106, 107]. This likely affects the pharmacology of GABAkines. It remains to be seen how it affects the clinical utility of the GABAkines in development.

In addition to $GABA_A$ receptor PAMs, novel GABAergic compounds that enhance GABA-mediated inhibition via presynaptic targets are in preclinical development, including two highly selective inhibitors of the GABA degrading enzyme GABA aminotransferase (GABA-T) and one inhibitor of GABA uptake by the GABA transporter 1 (Table 2). The next-generation GABA-T inhibitors are thought to lack the retinal toxicity that limits the clinical use of the approved GABA-T

inhibitor vigabatrin. Inhibiting GABA-T has been shown to effectively dampen excessive neural activity without affecting basal neuronal firing, whereas $GABA_A$ receptor agonists continuously interact with the receptor, which - depending on $GABA_A$ receptor subtype selectivity (see above) – may lead to adverse effects related to GABAergic hyperactivation.[108]

In addition to the compounds listed in GABA system-targeted sections in Table 2, several compounds listed in other sections also exert GABAergic effects. They include bumetanide analogs, KCC2 activators, the ASO STK-001, the transgene ETX-101, and the neuroactive peptide NRP2945. Another, alternative and innovative approach to restore GABAergic function in mesial temporal lobe epilepsy (mTLE) (not listed in Table 2) is stereotactic cerebral implantation of GABAergic interneurons (NRTX-1001; Neurona Therapeutics) derived from human PSCs.[109] This approach, which was shown to be effective in the kainate mouse model of mTLE, has recently entered clinical development in patients with drug-resistant unilateral mTLE.

[H2] Other target-driven strategies

[H3] Metabotropic or ionotropic glutamate receptor modulators. Fourteen of the pipeline drugs shown in Table 2 and Supplementary Table 2 target metabotropic or ionotropic glutamate receptors, including two compounds (JNJ-55511118 and CERC-611) that act as negative modulators of AMPA receptors containing the transmembrane AMPA receptor regulatory protein TARP γ8, an auxiliary receptor subunit that is enriched in the hippocampus.[110, 111] Negative modulation of AMPA receptors containing TARP γ8 offers the possibility of selectively reducing excitatory transmission within brain circuits associated with epilepsy, avoiding direct inhibitory effects on brain regions involved in motor coordination and wakefulness.[112] Furthermore, AMPA receptor γ8–negative modulators do not completely inhibit AMPA receptor signaling, which is an added advantage. To define the target engagement of AMPA receptor γ8–negative modulators, AMPA receptor γ8 positron emission tomography (PET) ligands have been developed and used in monkey PET studies.[113] Clinical validation of the PET ligands is ongoing.

[H3] Glutamate transporter modulators. The glial glutamate transporter EAAT2 (rodent homolog is GLT-1) plays a major role in glutamate clearance, a critical function for maintaining low extracellular glutamate concentrations and preventing excitotoxicity.[114] Several studies have reported decreased EAAT2 function in animal models of TLE, posttraumatic epilepsy (PTE), and human TLE. Recently, the first selective small molecule PAM of EAAT2 has been described and is currently being developed as a new ASM (Table 2).[115] Previous attempts to target glutamate signaling in epilepsy revealed important safety concerns in clinical studies, thus particularly careful assessment of these aspects is needed. Nevertheless, at least during preclinical studies, the EAAT2 PAM compound was well tolerated and showed a remarkably broad therapeutic window [115].

[H3] Serotonergic drugs. Initiated by the findings in the zebrafish DS model[52] and the clinical efficacy of fenfluramine (discussed above), several 5-HT receptor agonists and one 5-HT reuptake inhibitor are being developed for DS (Table 2). These include three drugs (clemizole, lorcaserin, trazodone) that are repurposed from other indications, as well as new selective $5HT_{2C}$ modulators. Promising results of this approach include a recent phase IIa study of adjunctive treatment with bexicaserin (LP352) in patients with DEEs of diverse causes; median seizure frequency reduction was 53.3% with bexicaserin vs 20.8% with placebo, and included 72.1% seizure frequency reduction in patients with DS.[116]

[H3] Modulators of voltage-gated ion channels. Six of the pipeline compounds shown in Table 2 activate neuronal Kv7 (KCNQ) potassium channels, including one drug (retigabine or ezogabine) that was previously approved for the treatment of focal epilepsy in adults and is now being developed as a precision medicine for the treatment of patients with *KCNQ2* mutations-caused DEE. Such mutations lead to decreased activity of the potassium channel which also underlie the autosomal dominant benign familial neonatal epilepsy (BFNE).[57] Similarly, two of the novel sodium channel modulators shown in Table 2 are being developed for DEEs, including *SCN8A* mutation-related DEEs such as early infantile epileptic encephalopathy-13 (Ohtahara Syndrome). Five other pipeline compounds target voltage-dependent T-type or P/Q-type calcium channels (Table 2). Currently there is

not enough clinical data to fully assess how safe and efficacious these subtype-selective ion channel modulators are going to be. While selective Kv7 potassium channel activators (e.g. XEN1101) appeared promising in Phase 2 studies, other compounds targeting specific sodium or calcium channels so far failed to achieve positive clinical proof of concept (e.g. NBI-921352, NBI-827104).

It has been suggested that cenobamate's high efficacy may be due, at least in part, to its effect on the persistent sodium current (I_{NAP}) of voltage-dependent sodium channels.[117] I_{NAP} is a small fraction (1-2%) of the total sodium current, fails to inactivate significantly, even with prolonged depolarization, and can amplify a neuron's response to synaptic input and enhance its repetitive firing capability.[118] The importance of I_{NAP} in sodium channelopathies and possibly also in acquired focal epilepsies has led to the development of novel compounds such as PRAX-330 and PRAX-562 that act as preferential inhibitors of persistent sodium channels (Table 2).

[H3] Cation-chloride cotransporters. While many of the approved ASMs act by targeting voltage-dependent sodium or calcium channels (Fig. 1), a new category of compounds target cation-chloride cotransporters, i.e., the K-Cl cotransporter KCC2 or the Na-K-2Cl cotransporter NKCC1 (Table 2), which have been implicated in the generation of seizures and epileptogenesis.[119-121] KCC2 is exclusively expressed at the plasma membrane of CNS neurons, including pyramidal neurons in the hippocampus, where it pumps Cl^- out of the cell to maintain Cl^- homeostasis, promoting fast hyperpolarizing postsynaptic GABAergic inhibition.[120] In contrast to KCC2, NKCC1 is expressed by many cells in and outside of the CNS and facilitates the Na^+-driven uptake of Cl^- into cells. Deficits in neuronal KCC2 expression or function in neurodevelopmental disorders and after brain injury are often associated with decreased efficacy of GABAergic inhibition, which can provoke seizures. NKCC1's role in this process is a matter of debate.[120, 122, 123] Its evaluation is challenged by a lack of selective and brain-permeable NKCC1 inhibitors.[123]

The potent loop diuretic bumetanide inhibits both NKCC1 and the renal cotransporter NKCC2 and penetrates only poorly into the brain. Bumetanide has been investigated as an adjunct to phenobarbital for neonatal seizures. Clinical data available thus far are inconsistent and bumetanide

increases the risk of irreversible ototoxicity in neonates.[124, 125] Using an integrated *in silico* and *in vitro* screening approach for developing brain-permeant NKCC1-selective inhibitors, researchers reported some novel NKCC1 inhibitors[126, 127], one of which (IAMA-6) is currently in development (Table 2). However, the NKCC1-selectivity and brain permeability of these compounds have been questioned.[123] Based on our own studies and the protein structure and drug binding sites of NKCC1 and NKCC2, we think that it may be impossible to design NKCC1-selective drugs.[123] NKCC1 is expressed as two splice variants, NKCC1a and NKCC1b, which differ by alternative splicing of the exon-21.[128] Using advanced RNA methods and NKCC1 splice variant selective antibodies, Kurki et al.[122] recently showed that CNS neurons predominantly express NKCC1b, substantiating previous reports in mice[128] and humans[129] Thus, theoretically, a drug that is selective for the NKCC1b splice variant would mainly target neuronal NKCC1; however, previous attempts to discover NKCC1b-selective compounds failed.[130] One strategy to overcome the poor BBB permeability of bumetanide is to develop lipophilic prodrugs of this drug.[131] One such prodrug, bumetanide dibenzylamide (NPT-2042), is currently in clinical development (Table 2).

Pharmacological targeting of KCC2 in neurological disorders such as epilepsy is more promising [121]. Until recently no selective KCC2 activators were available.[132] Gagnon et al.[133] designed an HTS assay that led to the identification of the KCC2 activator CLP257 and the carbamate prodrug CLP290, which has an improved pharmacokinetic profile. Sullivan et al.[134] reported that CLP290 increased KCC2, rescued the antiseizure effect of phenobarbital on neonatal seizures, and prevented the development of epileptogenesis in a model of ischemia-induced KCC2 hypofunction in neonatal mice. It has been questioned whether CLP290 directly modifies KCC2 surface expression and activity [132, 135, 136], but its effectiveness in multiple neuropathological paradigms is encouraging.[137] Recently, Astra Zeneca, using a multi-tiered drug screening cascade to screen 1.3 million compounds for potentiation of KCC2, identified a series of fused aminopyrimidine compounds .[138] Medicinal chemistry optimization resulted in OV350, a compound that directly binds to the KCC2 co-transporter with high affinity and potentiates KCC2 activity without modifying its plasma

membrane accumulation or key regulatory phosphorylation sites. OV350 was shown to terminate treatment- resistant SE, restore the efficacy of BDZs, and reduce neuronal cell injury and death following SE in a kainate mouse model. Ovid Therapeutics has partnered with AstraZeneca to develop OV350 (Table 2).

[H3] Multiple targets. Based on the role of neuroinflammation and oxidative stress in certain types of SE and epilepsy, several anti-inflammatory and anti-oxidant compounds are in preclinical or early clinical development.[139] Table 2 and Supplementary Table 2 list numerous compounds with other diverse mechanisms, many of which are novel mechanisms for epilepsy therapy. They include inhibition of glycolysis by 2-deoxy-D-glucose[140], inhibition of cholesterol 24-hydroxylase by soticlestat [141, 142], and a compound (ataluren) that targets genetic disorders by interacting with translation and preventing premature termination caused by early stop codons.[143] However, data from a small phase II trial do not support the clinical efficacy of ataluren in nonsense variant-mediated DS and CDKL5 deficiency.[144]

The last two categories of small molecules in development shown in Table 2 and Supplementary Table 2 consist of 11 compounds that act by multiple mechanisms. We have previously suggested that single-target treatments that focus exclusively on a single protein or individual biochemical pathway may be less effective than multi-target treatments that act on different proteins or pathways involved in an epileptic network.[24] Whether any of the multimodal compounds shown in Table 2 will be more effective than approved ASMs remains to be determined. For example, padsevonil, a drug that combines two mechanistic targets (presynaptic interaction with SV2 isoforms and postsynaptic enhancement of GABAergic inhibition (see below), recently failed in phase 3 trials [145]. However, the success of cenobamate, which also acts by at least two MOAs, argues in favor of multimodal compounds for epilepsy therapy.

[H2] Gene and oligonucleotide-based therapies

As shown in Table 2 and reviewed in detail recently ([62] [57] [146-148]), a variety of genetic

approaches are in preclinical development but only a few have entered clinical efficacy testing. One example is STK-001. As noted, most of DS is caused by de novo loss-of-function mutations in the *SCN1A* gene, leading to decreased expression of the voltage-gated sodium channel isoform $Na_V1.1$, which results in impaired activity of inhibitory GABAergic interneurons and seizures.[14] Targeted augmentation of nuclear gene output (TANGO) of *SCN1A* by the ASO STK-001 increased *Scn1a* mRNA levels, increased $Na_V1.1$ protein expression, restored the function of GABAergic interneurons, reduced seizures, and improved survival in the *Scn1a*$^{+/-}$ mouse model of DS.[149] An ongoing open-label phase I/IIa MONARCH trial aims to assess the safety, tolerability, and pharmacological properties of intrathecally administered STK-001 in children and adolescents with DS. Interim analyses showed that 71% of the patients experienced a median reduction of 17-37% in convulsive seizure frequency.[65] Data to date indicate that doses of STK-001 up to 30 mg administered every 4 months are well-tolerated with no significant safety concerns. The overall efficacy of this novel genetic approach will not be known until the final analysis of this study.

[H2] Failure of some rational strategies

Failures in drug development are often not reported but in general, the attrition rate of CNS drugs in development is higher compared with non-CNS drugs, mainly because of a lack of efficacy in large RCTs.[150, 151] Padsevonil, the first rationally designed multimodal ASM, is a good example.[152] Based on the success of levetiracetam, which acts by modulating SV2A[153], UCB Pharma initiated a rational medicinal chemistry design program to develop a single molecular entity that could target both different SV subtypes (SV2A, SV2B, SV2C) and the BDZ site of $GABA_A$ receptors.[154] The resulting drug displayed robust antiseizure efficacy across several validated seizure and epilepsy models, including models that are resistant to levetiracetam and various other ASMs.[155] Target engagement in humans was demonstrated by PET, which allowed the projection of a quantitatively based dosing rationale for clinical trials.[156] A randomized, double-blind, placebo-controlled adjunctive treatment phase IIa proof-of-concept trial of padsevonil in patients with very frequent drug-

resistant focal seizures showed antiseizure efficacy.[156] However, in subsequent larger randomized placebo-controlled phase IIb and phase III add-on trials, padsevonil had only a modest effect in focal DRE and did not separate from placebo in the primary endpoints.[145] Thus, the positive animal model data on the antiseizure efficacy did not predict the negative outcome of the clinical studies. The predictivity of models of seizures and epilepsy is generally considered to be excellent.[157] It is less certain whether the same is true for drug-resistant seizure models.[38]

Another example is the neurosteroid brexanolone (allopregnanolone). Based on preclinical evidence and case studies in patients with super-refractory SE (SRSE; a life-threatening form of SE that continues or recurs despite ≥24 hours of anesthetic treatment), it was evaluated in an open-label multicenter phase I/II study in 25 SRSE patients.[158] The study indicated high efficacy in terminating SRSE, suggesting a potential new treatment approach in SRSE. The rationale to use brexanolone in SRSE was biologically plausible. SE is thought to become resistant to BDZs by endocytosis-mediated internalization of synaptic $GABA_A$ receptors whereas extrasynaptic $GABA_A$ receptors, which can be targeted by neurosteroids but not by BDZs, do not endocytose.[159, 160] However, a subsequent larger double-blind placebo-controlled phase III trial failed to demonstrate a significant difference between brexanolone and placebo in patients with SRSE.[161] This might have been due to insufficient target engagement since relatively low doses of brexanolone were used.

A third notable failure is the NKCC1/2 inhibitor bumetanide discussed above. Given the complexity of the cellular expression of NKCC1 within and outside of the CNS, it is not possible to selectively target neuronal NKCC1 with drugs such as bumetanide. Furthermore, with the low systemic doses of bumetanide that are approved for humans, the drug does not reach NKCC1-inhibitory brain levels.[123]

[H1] Paradigm-shift in epilepsy treatment

The recent change in epilepsy definition emphasizing the enduring predisposition to generate seizures and neurobiological consequences of this condition shifts the goal from the treatment of seizures to

modulating the development and progression of epilepsy.[3] As far back as Cushing's operations of brain-injured World War I soldiers, there has been an interest in preventing epilepsy after acute CNS injuries such as TBI.[162] The delay between TBI and other CNS injuries such as stroke or infection and the subsequent development of epilepsy offers a window of opportunity to intervene with treatment to prevent or halt epileptogenesis and thus prevent epilepsy (Fig. 6). Despite this realization and intense interest, relatively few controlled studies have been done to try to prevent epilepsy after TBI[163], none after infection, and only two after CVA, in the current studies of eslicarbazepine and perampanel.[164, 165] Only five medications have been evaluated for the prevention of PTE in phase III RCTs in humans.[163, 166] Four were ASMs (phenytoin, phenobarbital, carbamazepine, valproate), on the assumption that a medication that stops seizures may also prevent them. The fifth one was magnesium sulfate, also used for seizure control in pre-eclampsia. All failed. The ASMs were evaluated at a time when there was little knowledge of epileptogenesis, and in preclinical studies either lacked antiepileptogenic effects or required doses too high for human use. Failure of these studies generated reluctance to conduct further preventive studies in PTE. In essence, though, there has never been a preventive RCT after TBI of a drug with proven preclinical antiepileptogenic activity at a clinically applicable dose.[166]

More recently, a third-generation ASM, levetiracetam, which has been reported to exert antiepileptogenic and disease-modifying effects in several animal models of epileptogenesis [167] (Table 3), showed an antiepileptogenic potential in an open-label phase IIa study when administered within 8 hours of TBI [168], although this was a feasibility study not powered to show efficacy. In another study of patients with DRE who were treated surgically, patients who received levetiracetam peri-/postoperatively had greater seizure freedom at 5 years post-surgery than patients treated with any other ASMs, even though they had more severe epilepsy preoperatively. The study suggested an antiepileptogenic effect of levetiracetam preventing epilepsy recurrence after surgery.[169]

Several clinical studies suggest possible antiepileptogenic/disease-modifying effects of statins. [170] These drugs have been reported to be antiepileptogenic in more published clinical and preclinical

studies and in a wider range of brain insults than any other compound.[171] These effects are cholesterol-independent and are likely explained by the immunomodulatory, anti-inflammatory, and anti-excitotoxic properties of statins [172](Supplementary Table 3). Statin treatment after CVA reduced the risk of poststroke early onset seizures, and reduced the risk of poststroke epilepsy (PSE) in CVA patients with early poststroke seizures.[173] Other studies in patients with new ischaemic CVA [174] suggests the antiepileptogenic and PSE-reducing effect of statins are dose dependent.

In a study of patients with intracranial hemorrhage, post-stroke (but not pre-stroke) use of statins reduced PSE, again dose-dependently.[175] In a study of older adults with cardiovascular disease treated with revascularization, there was a dose-dependent reduced risk of epilepsy-related hospitalization for current and past statin users, with no benefit with non–statin cholesterol-lowering drugs, beta-blockers, and angiotensin-converting enzyme inhibitors.[176] A retrospective cross-sectional study of risk factors for new-onset geriatric epilepsy of US veterans showed that statin prescription was associated with a lower likelihood of epilepsy[177] These uncontrolled interventional, observational, and epidemiological studies suggest the intriguing possibility of a statin antiepileptogenic effect. However, RCTs are needed to properly evaluate this suggestion.

[H2] Clinical proof-of-concept for epilepsy prevention

In a knock-out mouse model of TSC, treatment with vigabatrin, an inhibitor of GABA aminotransferase, before the onset of spontaneous seizures prevented seizures and reduced mortality. [13] Vigabatrin also decreases mTOR activity, suggesting a mechanistic basis for a potential antiepileptogenic effect in TSC.[178] Using a biomarker (EEG) to identify the initial change in excitability and epileptogenesis [179], a recent multicenter RCT (EPISTOP) demonstrated that vigabatrin treatment at the time of the first detection of EEG interictal epileptiform discharges, but before the onset of clinical seizures, reduced by ~half the incidence of epilepsy and associated neurocognitive and behavioral co-morbidities in infants diagnosed with TSC soon after birth.[180] In a pilot study [179], these effects were long lasting, including after drug withdrawal.[181] [182]

This proof-of-concept study heralds a potential paradigm shift in the treatment of epilepsy from symptomatic treatment to disease prevention, including its neurobiological, cognitive, psychological, and social consequences. However, the EPISTOP study findings were not confirmed in a more recent US multi-center Phase IIb randomized, double-blind, placebo-controlled trial, PREVeNT (Preventing Epilepsy using Vigabatrin in Infants with TSC), which used a similar treatment approach but a double blinded placebo controlled design.[183] Reasons for the difference between the two studies are unclear, but may include a slightly younger age at enrollment and treatment initiation in the EPISTOP trial and differences in vigabatrin dosing.

[H2] Preventable acquired epilepsies

Similar paradigms can now be extended to people with genetic or acquired conditions, such as TBI or CVA who are at high risk of developing epilepsy. For both PTE and PSE, seizures are divided into insult-associated "early" (within 7 days after injury for TBI, 14 days for CVA) and spontaneous "late". Only about 25% of patients with early seizures develop subsequent late seizures. Early seizures are therefore not considered epilepsy but may be due to acute post-injury factors such as hypoxia, sepsis, increased intracranial pressure, and metabolic disarray. About 90% of spontaneous "late", or unprovoked seizures after TBI and 50-66% of late post-stroke seizures are followed by further seizures[4]; thus, a single late unprovoked seizure is defined as PTE or PSE, in agreement with the recently modified definition of epilepsy by the International League Against Epilepsy (ILAE).[3]

Suppression of early seizures with ASMs does not prevent PTE or PSE.[184, 185] Incidence of PTE varies by TBI severity, affecting 2.1%, 4.2%, and 16.7% of patients with mild, moderate, and severe TBI 30 years after TBI, where severe is defined as TBI with intracranial hemorrhage or >24 hours post-traumatic loss of consciousness or amnesia.[186] The risk of PSE after CVA ranges from 2-14%[4, 187], and of epilepsy after acute infections from 10-60 %.[188-191] Certain injury elements common to all three conditions increase the risk of epilepsy. They include the presence of intracranial blood, breakdown of BBB, localization (temporal or frontal), early seizures, and severity of TBI or

stroke.[4]. These increase the risk for both PTE and PSE two years after injury to up to 60%.[192]

Approximately 1/3 of PTE present within 3 months of injury, 50% within 6 months, 65% within 1 year, and 80% within 2 years.[168, 193-195] PSE latency after CVA is generally longer, with the probability of developing PSE after CVA being ~ 1.5% by 3 months, 3-4% by 1 year, 5% by 2 years, 7-9% by 5 years, and 9-12% by 10 years.[196] The epileptogenic process after meningoencephalitis is also slower with only 58% of epilepsies starting within 5 years.[188] Thus, preventive treatment trials may be more feasible in TBI or stroke than following infection, because of the shorter time to epilepsy presentation.

Pharmacological strategies for epilepsy prevention in animal models

During the last 10 years, ≥ 20 treatments have been shown to prevent or modify the development of acquired epilepsy in animal models, including eleven tested in PTE models.[197] [166, 167, 198](Supplementary Table 3). Fifteen of these 20 treatments are FDA-approved repurposable drugs. However, the design of many of the preclinical studies makes them difficult to translate to clinical studies. Studies with all but three of these 15 FDA-approved, repurposable drugs were done without blood levels to target human dosing or with clinically unrealistic therapeutic windows such as treatment starting before or at the time of injury. There are now at least five in vivo PTE models, i.e. the fluid percussion injury (FPI), controlled cortical impact (CCI) models, a weight drop model, BBB disruption model, and ballistic injury model, and three in vitro PTE models, i.e., the undercut model, CCI combined with slice excitability, and cerebral iron injection model.[197, 199-201] The models vary in methodological details both within and between models, such as force and site of injury, age of animals, rodent strains, and anesthetic agents and depth used, which results in PTE outcomes and treatment results often not being reproducible across different models and between laboratories with the same model. Most in vivo models do not have sufficient seizure incidence or density and short enough PTE latency to allow pre-clinical preventive trials [197, 199], although recently a mouse CCI model was described with ~45% at 3 and 58% PTE rate at 5 months after injury.[202]

Two multi-lab projects, the NIH-funded "EpiBioS4Rx" project (https://epibios.loni.usc.edu) and the Department of Defense/CURE funded project "Team Approach to Prevent Post-Traumatic Epilepsy", TAPTE [203] are developing standardized FPI and CCI models with validation across different labs and have produced models ready for therapeutic testing.[202, 204] In addition, the porcine CCI PTE model has been developed using CCI to allow PTE research in gyrated brain species that are closer to humans.[205]

Positively tested treatments open for translation include the repurposed drugs levetiracetam, gabapentin, pregabalin, ceftriaxone, drug combination treatment, focal cooling, and ASOs directed against micro RNA-134 (see supplementary Table 3). Numerous other treatments have been tried with less success.[187, 198, 199] Box 3 discusses the challenges of clinical antiepileptogenesis trials and the potential role of biomarkers.

Apart from the prevention of epilepsy by antiepileptogenic treatments administered after the brain insult, some preclinical studies indicate that the progression of epilepsy (or the "secondary epileptogenesis" as illustrated in Fig. 6) can be modified or halted even after the onset of epilepsy [206, 207]. Detlev Boison's lab [206] used bioengineered silk implants to deliver a defined dose of adenosine over 10 days to the brains of epileptic rats, which reversed the DNA hypermethylation seen in the epileptic brain, inhibited sprouting of mossy fibers in the hippocampus, and prevented the progression of epilepsy for at least 3 months. Iori et al.[207] used an epigenetic approach by injecting a synthetic mimic of microRNA-146a that impairs interleukin (IL)-1 receptor/Toll-like receptor 4 signal transduction, or blocked receptor activation with anti-inflammatory drugs. Both interventions when *transiently* applied to mice *after* epilepsy onset, prevented disease progression and reduced chronic seizure recurrence, while the ASM carbamazepine was ineffective.

PTE prevention by cortical cooling in the rat FPI model was associated with normalization of the injury-induced reduction in the α/δ power.[208] In longitudinal EEG recordings after TBI in animal models, these changes occur gradually.[205, 209] Such electrophysiological biomarkers of the evolution of epileptogenesis would allow targeted timing of preventive treatment initiation and

duration.

Conclusions and future directions

We have observed a paradigm shift in the discovery of novel ASMs in the last decade, which is a consequence of significant progress in epilepsy genetics, the availability of novel disease models, drug screening technologies, and innovative therapeutic modalities. The result is a rich pipeline of potential future treatments for epilepsy, including potential disease-modifying treatments, an explosion of gene-modifying treatments for the rare DEEs, and mechanistically-guided precision treatments. The development of these new treatments could also be harnessed to address another major area of need in the treatment of epilepsy, namely the development of individualized predictors of treatment response, both for efficacy and side effects, to replace the current, deficient and outdated trial-and-error treatment approach.

It is now widely accepted in many therapeutic areas ranging from oncology to neurology that stratified patient populations increase the probability of success in clinical development.[210] We believe that this trend is going to be increasingly applied to the development of future epilepsy therapies, and the focus on well-defined orphan or genetic syndromes is an important step in this direction. Moreover, utilization of various types of stratifying biomarkers, i.e. genetic, molecular or imaging, is also dramatically increasing the probability of success in clinical development and may lead to better response to treatment.[210]

We are at the beginning of a new era of disease prevention and modification. A major need to facilitate this development is the identification of biomarkers for early PTE detection and determination of the course of human epileptogenesis after acute CNS injury. Such tools could allow proof of concept studies, shorter phase III studies, and study design for targeted treatment initiation and duration. Tools such as the recently developed long-term 24/7 continuous EEG monitoring [211], longitudinal serial EEG recordings, and longitudinal quantitative MRIs may allow the development of these biomarkers in the near future. Preventive clinical trials would benefit from EEG seizure detection

because clinical seizures are often unreported or unreliably reported in patients with epilepsy and because PTE may start before the patient's first clinical seizure (as occurs in animal models).[212] Finally, the new frontier in the development of future epilepsy therapies will likely be based on the application of advanced analytics and AI that offer multimodal data integration capability to potentially predict treatment outcomes for individual patients.[213, 214]

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Pavel Klein has served as a consultant, advisory board member or speaker for Abbott, Angelini, Aquestive, Arvelle Therapeutics, Aucta Pharmaceuticals, Dr. Reddy's, Eisai, Jazz Pharmaceuticals, Neurelis, Neurona, Paladin, SK Life Science, Sunovion, UCB Pharma, UNEEG, UniQure, is a member of the Medical Advisory Board of Stratus and of the Scientific Advisory Board of OB Pharma, is on DSMB of Neurona Therapeutics for the NRTX-1001 trial, is co-founder and the CEO of PrevEp, and has received research support from CURE/Department of Defense and from the NIH/SBIR.

Matthias Koepp has served as a consultant, advisory board member or speaker for Angelini, Arvelle Therapeutics, Bial, Eisai, GE, Novartis, Jazz Pharmaceuticals, UCB Pharma, is co-founder of PrevEp, has received research support from Epilepsy Research UK, Epilepsy Society, MRC and Wellcome Trust.

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Table 1. Types of seizures or epilepsy syndromes

Seizure type or epilepsy syndrome	**Description**	**Pathophysiology**
Focal-onset seizures	Seizures starting in one area (focus) of the brain	Various subtypes and numerous causes (acquired and, less often, genetic); origin often from temporal lobe, particularly hippocampus; often loss of inhibitory GABAergic interneurons, functional changes of voltage-gated ion channels and many other molecular and structural changes
Generalized-onset tonic–clonic seizures	Seizures affecting both cerebral hemispheres from seizure onset and resulting in whole body tonic spasm followed by generalized convulsion	Numerous causes (acquired and genetic); involvement of the thalamus and brainstem
Generalized absence seizures	Seizures affecting both cerebral hemispheres from seizure onset resulting in brief arrest of consciousness and purposeful behavior, without other behavioral or generalized motor symptoms	Occur in multiple idiopathic and genetic generalized epilepsies; caused by multifactorial inheritance, including pathogenic variation in *GABRG2*, *GABRG3* and *CACNA1A2* genes
Generalized myoclonic seizures	Seizures affecting both cerebral hemispheres from seizure onset and resulting in bilateral jerks, without loss of consciousness	Numerous causes (acquired and genetic)
Infantile spasms (West syndrome)	Seizures with brief sudden body flexion	Not well understood (range of possible structural, metabolic, and genetic etiologies, including pathogenic variation in *SCN2A*, *KCNQ2*, *STXBP1* and *CDKL5*)
Dravet syndrome	Frequent, prolonged seizures of multiple types, usually starting in first year of life, and neurodevelopmental regression	Mutations in *SCN1A* in 70-80% of individuals; may result in inhibition of GABAergic interneurons, leading to excessive neuronal excitation
Lennox-Gastaut syndrome	Rare and severe form of epilepsy; typically begins at 3- 5 yeaers, with multiple seizure types, intellectual disability, and specific EEG abnormalities	Caused by various conditions, including brain malformations, tuberous sclerosis, perinatal asphyxia, severe head injury, CNS infection and inherited genetic, degenerative or metabolic conditions, including pathogenic variation in *SCN2A* and *CDKL5*.
Tuberous sclerosis	Multi-organ disease affecting brain, skin, heart,	Caused by mutations in, *TSC1* or *TSC2* in 70% of

complex	lung and kidneys; often starting in first year of life with infantile spasms, then focal and generalized seizures, with neurodevelopmental delay or regression	cases, resulting in overactivation of mTOR signaling pathway
Developmental epileptic encephalopathies (DEEs)	Group of severe neurological disorders characterized by early-onset seizures, developmental delay or regression, and cognitive impairment, usually starting in infancy or early childhood	Heterogeneous group of monogenetic neurodevelopmental disorders caused by a variety of genetic variants most commonly of *SCN1A*, *KCNQ2*, *PCDH19*, *CDKL5*, *SCN2A*, and *SCN8A*

Modified from Löscher and Klein [23]and guidelines discussed in this paper. Note that several additional childhood epilepsy syndromes are not included.

Table 2. Selected novel epilepsy therapies in development

Drug	Companies	Mechanism of action	Indication	Status
PAMs at $GABA_A$ receptors (GABAkines)				
Darigabat (formerly PF-06372865 and CVL-865)	Cerevel Therapeutics	α1-sparing, $\alpha_2/\alpha_3/\alpha_5$-selective	Adult focal epilepsy	Phase II
ENX-101	Engrail Therapeutics	$\alpha_2/\alpha_3/\alpha_5$-selective, α1-blocking	Focal onset seizures	Phase I
SAN-2219	Saniona	$\alpha_2/\alpha_3/\alpha_5$-selective	Epilepsy	Preclinical
KRM-II-81	RespireRx Pharmaceuticals	α_2/α_3-selective	Epilepsy	Preclinical
BAER-101	Avenue Therapeutics	α_2/α_3-selective	Focal epilepsy	Phase IIa
SAN-711	Saniona	α_3-selective	Generalized seizures	Phase I
Alogabat (RG-7816)	Roche	α_5-selective	Angelman syndrome	Phase II
Ganaxolone (allopregnanolone analogue)	Marinus Pharmaceuticals	Neurosteroid analog PAM on synaptic and extrasynaptic $GABA_A$ receptors	Refractory SE and TSC	Phase II/III
Zuranolone (SAGE-217)	SAGE Therapeutics	Synthetic neurosteroid analogue PAM on synaptic and extrasynaptic $GABA_A$ receptors	Seizures	Phase I
SAGE-324 (BIIB-124)	SAGE Therapeutics	Synthetic neurosteroid analog PAM on synaptic and extrasynaptic $GABA_A$ receptors	Epileptiform disorders	Phase I/II
SAGE-689	SAGE Therapeutics	Second-generation neuroactive steroid PAM on synaptic and extrasynaptic $GABA_A$ receptors	Resistant status epilepticus	Phase I
Gaboxadol (OV101; THIP)	Ovid/Healx	Orthosteric agonist of $GABA_A$ receptors with high affinity at extrasynaptic δ-subunit-containing receptors that mediate tonic inhibition	Angelman syndrome and FXS	Phase I/II
ETX-155	Eliem Therapeutics	Neuroactive steroid PAM on synaptic and extrasynaptic $GABA_A$ receptors	Focal onset seizures	Phase Ib
CPT-021	Mercaptor Discoveries	$GABA_A$ receptor PAM	Epilepsy	Preclinical
GRX-917 (deuterated version of etifoxine)	GABA Therapeutics	$GABA_A$ receptor PAM and activator of TSPO (increases synthesis of endogenous neurosteroids)	Epilepsy	Phase 1
Inverse agonists (or NAMs) at $GABA_A$				

receptors				
Basmisanil (RG-1662)	Roche	α_5-selective	Angelman syndrome, Dup15q syndrome	Phase II
Presynaptic effects on GABAergic transmission				
OV329	Ovid Therapeutics	Inhibitor of GABA-degrading enzyme GABA-T	Infantile spasms	Phase I
CPT-004	Mercaptor Discoveries	Inhibitor of GABA-degrading enzyme GABA-T	Epilepsy	Preclinical
E2730	Eisai	Selective non-competitive GAT1 inhibitor	Epilepsy	Phase I
PAMs, NAMs or antagonists at glutamate receptors				
ADX71149 (JNJ-40411813)	Addex Therapeutics/ Janssen	PAM of mGlu2	Adult focal onset epilepsy	Phase IIa
Acamprosate	Confluence Pharmaceuticals	Antagonist of mGlu5; also modulates NMDA receptors	FXS	Phase III
Basimglurant	Noema Pharma	Antagonist of mGlu5	Seizures in TSC	Phase II
Tezampanel (LY293558)	Proniras	Antagonist of AMPA and kainate subtypes of ionotropic glutamate receptor	Epilepsy	Preclinical
JBPOS-0101	Bio-Pharm Solutions	Antagonist of mGlu1, mGlu4 and mGlu7	DEEs, refractory SE	Phase II
JNJ-55511118	Janssen	NAM of AMPA receptors containing TARP-γ8	Epilepsy	Phase I
CERC-611 (LY3130481)	Eli Lilly/Cerecor/Avalo Therapeutics	NAM of AMPA receptors containing TARP-γ8	Focal seizures	Preclinical
Radiprodil	GRIN Therapeutics/UCB Pharma	NAM of NR2B-NMDA receptors	Gain of function variants of *GRIN2B*	Phase II
AV-101	Vistagen	Prodrug of 7-chloro-kynurenic acid, a selective antagonist of glycine co-agonist site of NMDA receptor	Epilepsy	Phase I
PAM of the glutamate transporter EAAT2 (GLT-1)				
iQ-007	iQure	PAM of astrocytic glutamate transporter EAAT2	DRE	Preclinical

Serotonergic (5-HT) mechanisms				
EPX-100 (clemizole HCl)	Epygenix	Probably modulation of 5-HT receptors	Dravet syndrome	Phase II
EPX-300 (trazodone HCl)	Epygenix	SSRI	Dravet syndrome	Phase I
Lorcaserin (E2023)	Eisai	5-HT_{2C} receptor agonist	Dravet syndrome	Phase III
Bexicaserin (LP352)	Longboard Pharmaceuticals	5-HT_{2C} receptor agonist	DEEs	Phase Ib/IIa
BMB-101	Bright Minds Biosciences	5-HT_{2C} receptor agonist	Dravet syndrome	Phase I
NLX-101	Neurolixis	5-HT_{1A} receptor agonist	Rett syndrome and FXS	Phase I
Potassium channel modulators				
XEN1101	Xenon Pharmaceuticals	PAM of neuronal Kv7.2-7.5 (KCNQ2-5) channels	Adult focal epilepsy	Phase III
Pynegabine (HN37)	Chinese Academy of Sciences/Hainan Haiyao Company	PAM of neuronal Kv7.2-7.5 (KCNQ2-5) channels	Epilepsy	Phase I
BHV-7000 (KB-3061; BNP-25203)	Knopp Biosciences/Biohaven Pharmaceuticals	Kv7.2/7.3 modulator	Seizures associated with *KCNQ2* DEE	Phase I
CB-003	Zhimeng Biopharma	Kv7.2/7.3 modulator	Epilepsy	Phase I
ZM-003	Protheragen	Kv7.2/7.3 modulator	Epilepsy	Preclinical
ETX-123	Eliem Therapeutics	Kv7.2/7.3 modulator	Epilepsy	Preclinical
AUT-00206	Autifony Therapeutics	Kv3.1/3.2 positive modulator	Fragile X syndrome	Phase II
AUT-00201	Autifony Therapeutics	Kv3.1/3.2 positive modulator	Orphan epilepsy syndromes	Phase I
PRAX-020	Praxis Precision Medicines/UCB Pharma	Inhibitor of KCNT1 (T type) channels	KCNT1-related DEE	Preclinical
Sodium channel modulators				
NBI-921352 (XEN901)	Xenon Pharmaceuticals/Neurocrine Biosciences	Selective inhibitor of $Na_v1.6$ sodium channels	SCN8A DEE and adult focal epilepsy	Phase II
TD567	OB Pharmaceuticals	Na_V modulator	Posttraumatic epilepsy	Preclinical
PRAX-562	Praxis Precision Medicines	Preferential inhibitor of persistent sodium channels	SCN2A and SCN8A DEEs	Phase II

PRAX-628	Praxis Precision Medicines	Next-generation Na_V blocker	Focal epilepsy	Phase I
SKL-24741	SK Life Science	Possible Na_V inhibitor; exact mechanism not known	Epilepsy	Phase I
Calcium channel modulators				
ACT-709478* (NBI-827104)	Idorsia/Neurocrine Biosciences	Blocks T-type calcium channels ($Ca_v3.1$, $Ca_v3.2$, and $Ca_v3.3$), inhibiting thalamocortical circuit	Electrical status epilepticus of sleep	Phase II
CX-8998	Cavion/Jazz Pharmaceuticals	Blocks T-type calcium channel (Ca_v3), inhibiting thalamocortical circuit	Idiopathic generalized epilepsy with absence seizures	Phase II
FV-137	Trillium Therapeutics	Inhibits P/Q type Ca^{2+} channels $Ca_v2.1/\beta4/\alpha2\delta1$ and $Ca_v2.2/\beta3/\alpha2\delta1$ and $Na_v1.6$ and $Na_v1.7$	Focal and generalized seizures	Preclinical
NIP-301	Nissan Chemical	Blocks T-type (Ca_v3) calcium channels	Epilepsy	Preclinical
Modulators of cation-chloride-cotransporters				
OV350	Ovid/AstraZeneca	Activator of KCC2	DRE	Preclinical
AXN-006	Axonis	Activator of KCC2	DRE	Preclinical
NPT-2042 (bumetanide dibenzylamide)	NeuroPro Therapeutics	Lipophilic prodrug of bumetanide	Adjunct for medically intractable epilepsy	Phase I
IAMA-6	IAMA Therapeutics/Evotec	Inhibitor of NKCC1	TSC and other types of refractory epilepsy	Preclinical
Anti-inflammatory/anti-oxidative mechanisms				
GAO-3-02 (synaptamide derivative)	GAOMA Therapeutics	Anti-inflammatory	Epilepsy	Preclinical
Rozanolixizumab	UCB Pharma	FcRn inhibitor	Autoimmune epilepsy syndromes	Phase II
Anakinra	Various academic sites	Antagonist at recombinant human IL-1 receptors	Febrile-infection-related epilepsy syndrome	Case series
Other mechanisms				
2-Deoxy-D-glucose	NeuroGenomeX/University of Wisconsin	Inhibits glycolysis in response to neural activity	SE and acute repetitive seizures	Preclinical; phase II planned for SE
Blarcamesine (ANAVEX2-73)	Anavex Life Sciences	Sigma 1 receptor agonist; decreases protein misfolding and reduces	Rett syndrome, infantile spasms, FXS, Angelman syndrome	Phase I-III

		oxidative stress		
Pridopidine	Prilenia Therapeutics	Selective sigma 1 receptor agonist	FXS and Rett syndrome	Phase III
Vatiquinone	PTC Therapeutics	15-lipoxygenase inhibitor	DEE (mitochondrial epilepsy)	Phase III
Soticlestat (OV935/TAK-935)	Ovid Pharmaceuticals & Takeda Pharmaceuticals	Cholesterol 24-hydroxylase inhibitor	Dravet and Lennox Gastaut syndromes	Phase III
Zatolmilast (BPN14770)	Tetra Therapeutics/Shionogi	Selective PDE4D allosteric inhibitor	FXS	Phase III
SPN-817 (synthetic huperzine A)	Supernus Pharmaceuticals/Biscayne Neurotherapeutics	Suppresses AChE activity in cortex, increasing cholinergic and GABAergic signaling	Focal impaired awareness seizures	Phase II
PQR530, PQR620, PQR626	Piqur Therapeutics	Inhibition of mTORC1/2 or PI3K/mTORC1/2	TSC	Preclinical
Palomid (P529)	Paloma Pharmaceuticals	Inhibition of mTORC1/2	TSC	Preclinical
NRP2945	CuroNZ	Peptidomimetic analog of CAPS-2 protein; modulates anti-inflammatory pathways and upregulates $GABA_A$ receptor expression	Genetic generalized absence epilepsy and Lennox-Gastaut syndrome	Phase II/IIa
ACT-03	Accure Therapeutics	Peptidomimetic inhibitor of MMP2 and MMP9	Focal epilepsy	Preclinical
PKL-021	Pikralida	MMP9 inhibitor	Epilepsy	Preclinical
Ataluren	PTC Therapeutics	Promotes read-through of premature stop codons to increase protein expression	Dravet syndrome and CDKL5 deficiency disorder	Phase II
Cannabidivarin	Greenwich Biosciences/Jazz Pharmaceuticals	Non-psychoactive naturally occurring cannabinoid; specific antiseizure mechanism not known	Focal epilepsy	Phase III
SUPERA-CBD	MyMD Pharmaceuticals	Synthetic cannabidiol; specific mechanism of antiseizure effect not known	Epilepsy	Preclinical
NNI-351	NeuroNascent	Promotes proliferation of neuronal progenitors in hippocampus by modulation of *DYRK1A* pathway that increases mRNA translation	FXS	Preclinical
PTI-5803	PannTherapi	Pannexin-1 inhibitor	Epilepsy	Preclinical
Tricaprilin	Cerecin	Medium chain triglyceride designed to induce ketosis	Epilepsy	Phase I
DPM-1003	DepYmed	Inhibits PTPN1	Rett syndrome	Preclinical

ACT01	DRI Biosciences	Inhibits the dopamine transporter (SLC6A3)	FXS	Preclinical
MC-1	Medicure	Provides PLP, a naturally occurring metabolite of pyridoxine (vitamin B6)	PLP-dependent epilepsy	Phase III
Calpain-2 inhibitors	NeurAegis	Calpain-2 inhibitors	SE	Preclinical
PAX-101 (i.v. suramin)	PaxMedica	Antagonist at purinergic P2 receptors	FXS	Phase II
ReS-3T	reMYND	Targets PDE6δ to reduce neuron hyperactivity	Dravet syndrome	Preclinical
TRV-045	Trevena	Selective modulator of S1P1R	Epilepsy	Phase I
EPGN-1370	Epigen Biosciences	70 kDa ribosomal protein S6 kinase inhibitor	FXS	Preclinical
EPGN-2036	Epigen Biosciences	70 kDa ribosomal protein S6 kinase inhibitor	FXS	Preclinical
HRP-12975	Herophilus	Small molecule reactivator of silenced *MECP2*	Rett syndrome	Preclinical
AMP-X-0079	AurimMed Pharma	Not known (no effect on >140 common targets)	Epilepsy	Preclinical
BL-001	Bloom Science	Live biotherapeutic; aims to reduce neuronal hyperexcitability	Dravet syndrome	Phase II
VAL-1221	Parasail/Valerion	Fusion protein that delivers recombinant human acid alpha glucosidase to cytosol and lysosomes	Lafora disease	Phase I
RAP-219	Rapport Therapeutics	Targets hippocampus-specific receptor-associated proteins	DRE	Phase I
Compounds with multiple mechanisms				
Carisbamate	SK Life Science	Blocks Na_Vs, T-type Ca^{2+} channels, and AMPA- and NMDA-receptor mediated neurotransmission	Lennox-Gastaut syndrome	and III
FV-082	Trillium Therapeutics	Possibly interacts with androgen receptors, MAO-B and $Na_v1.8$	Focal and generalized seizures	Preclinical
Propofol (EP103)	Epalex	Multiple mechanisms	Drug-resistant seizures and SE	Phase I
Ergoloid mesylate	Purposeful	Dihydroergocornine, dihydroergocristine and dihydroergocryptine; causes partial agonism/antagonism of adrenergic, dopaminergic and serotonergic receptors	FXS	Phase II
AAV-based gene therapy approaches				
CG01	CombiGene/Spark Therapeutics	Neuropeptide Y and receptor Y2	Focal epilepsy	Preclinical
ETX-101	Encoded Therapeutics	*SCN1A*-specific transcription factor (eTF^{SCN1A})	Dravet syndrome	Phase I/II

		that upregulates $Na_V1.1$ expression in GABAergic interneurons		
ACTX-101	Alcyone Therapeutics	X reactivation	Rett syndrome	Preclinical
WWOX gene replacement	Mahzi Therapeutics	*WWOX* gene replacement	WOREE and SCAR12	Preclinical
RT-101	Regel Therapeutics	Delivers dCas and epigenetic modulator; *SCN1A* activator	Dravet syndrome	Preclinical
STRX-220	Stride-Bio/Sarepta Therapeutics	Activates *UBE3A*	Angelman syndrome	Preclinical
STRX-230	Stride-Bio/Sarepta Therapeutics	Activates *MECP2*	Rett syndrome	Preclinical
STRX-240	Stride-Bio/Sarepta Therapeutics	Activates *SCN1A*	Dravet syndrome	Preclinical
TSHA-102	Taysha Gene Therapies	Replaces/activates *MECP2*	Rett syndrome	Phase I/II
TSAH-105	Taysha Gene Therapies	Replaces *SLC13A5*	*SLC13A5* deficiency disorder (DEE)	Preclinical
FBX-101	Forge Biologics	Replaces *GALC*	Krabbe disease	Phase I
FBX-201	Forge Biologics	Stimulates FMR1	FXS	Preclinical
NGN-401	Neurogene	Restores MeCP2 protein production	Rett syndrome	Phase I/II
NGN-101	Neurogene	Replaces *CLN5*	CLN5 disease	Phase I/II
AGIL-AS	PTC Therapeutics	Replaces *UBE3A*	Angelman syndrome	Preclinical
CAP-002	Capsida Biotherapeutics	Replaces *STXBP1*	DEE with *STXBP1* mutations	Preclinical
Coda71	Coda Biotherapeutics	Engineered chimeric ligand-gated chloride channels activated by α7 nAChR agonists	Focal epilepsy	Preclinical
Antisense oligonucleotides				
STK-001	Stoke Therapeutics	Increases $Na_v1.2$ protein in GABAergic interneurons	Dravet syndrome	Phase I/IIa
PRAX-222 (RC-222)	Praxis Precision Medicines/RogCon Biosciences	*SCN2A* ASO	*SCN2A* gain-of-function DEE	Phase I
PRAX-080	Praxis Precision Medicines	*PCDH19* ASO	PCDH19-associated DEE	Preclinical
PRAX-090	Praxis Precision	SYNGAP1 activator	SYNGAP1 loss-of-function DEE	Preclinical

	Medicines			
RCUR-212	RogCon Biosciences	Downregulates *SCN2A* expression	*SCN2A* gain-of-function DEE	Preclinical
RCUR-313	RogCon Biosciences	Upregulates *SCN2A* expression	*SCN2A* loss-of-function DEE	Preclinical
ION-582	Ionis Pharmaceuticals	UEB3A modulator	Angelman syndrome	Phase II
NMT.001	NEUmiRNA	miRNA-134 ASO	Focal epilepsy	Preclinical
MECP2 ASO	Vico Therapeutics	Targets *MEC2-R255X* by RNA editing to activate MECP2	Rett syndrome	Preclinical
GTX-102	Ultragenyx Pharmaceutical	Inhibits expression of paternal *UBE3A* antisense	Angelman syndrome	Phase II
Rugonersen	Roche	Locked-nucleic acid (LNA)-modified ASO that reduces UBE3A silencing	Angelman syndrome	Phase I
Other RNA-based therapies				
LSP-GR1	LifeSplice Pharma	Splice-modulating oligonucleotide that decreases AMPA receptor GluA1-flip subunit expression	Epilepsy	Preclinical
LSP-SCN8	LifeSplice Pharma	Splice-modulating ASO that reduces expression of *SCN8A*	Dravet syndrome	Preclinical
AMT-260	uniQure/Corlieve Therapeutics	miRNA that suppresses aberrantly expressed kainate receptors in the hippocampus	Temporal lobe epilepsy	Preclinical
Enhancer and suppressor tRNAs	Tevard Biosciences	Enhancer tRNAs increase expression of healthy *SCN1A* allele; suppressor tRNAs allow production of full-length protein from faulty *SCN1A* allele	Dravet syndrome	Preclinical
CMP-SCN	CAMP4	Regulatory RNA technology; upregulates endogenous *SCN1A* expression by targeting natural antisense transcripts	Dravet syndrome	Preclinical
CUR-1916	OPKO Health	siRNA technology; targets an antisense non-coding RNA to boost protein production from functional *SCN1A*	Dravet syndrome	Preclinical

A complete list of all 203 novel epilepsy treatment discovery or development projects that we identified by text mining in the public domain is shown in Supplementary Table 2. See Löscher and Klein [23] Bialer et al.[215], Chilcott et al.[12], Goodspeed et al.[62] Pong et al.[216]. Zimmern et al.[57] and the Epilepsy Pipeline Tracker [217] for details and literature. Abbreviations: AAV, adeno-associated virus; AChE, acetylcholinesterase; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ASO, antisense oligonucleotide; Ca_V, voltage-gated calcium channel; CDKL5, cyclin-dependent kinase-like 5; CLN5,

ceroid-lipofuscinosis neuronal protein 5; DEE, developmental and epileptic encephalopathy; DRE, drug-resistant epilepsy; DYRK1A, dual specificity tyrosine phosphorylation regulated kinase 1A; EEAT2, excitatory amino acid transporter 2; FcRn, neonatal Fc receptor; FMR1, fragile X messenger ribonucleoprotein 1; FXS, fragile X syndrome; GABA, γ-aminobutyric acid; GABA-T, GABA aminotransferase; GALC, galactosylceramidase; GAT-1, GABA transporter 1; 5-HT, 5-hydroxytryptamine; IL-1, interleukin-1; KCC2, potassium–chloride cotransporter 2; MAO-B, monoamine oxidase type B; MECP2, methyl CpG binding protein 2; mGlu, metabotropic glutamate receptor; MMP, matrix metalloproteinase; mTOR, mechanistic target of rapamycin complex 1/2; nAChR, nicotinic acetylcholine receptor; NAM, negative allosteric modulator; Na_V, voltage-gated sodium channel; NKCC1, sodium–potassium–chloride cotransporter 1; NMDA, N-methyl-D-aspartate; PAM, positive allosteric modulator; PCDH19, protocadherin 19; PDE, phosphodiesterase; PLP, pyridoxal 5'-phosphate monohydrate; PTPN1, protein tyrosine phosphatase non-receptor type 1; SCAR12, autosomal recessive spinocerebellar ataxia type 12; *SCN*, voltage-gated sodium channel gene; SE, status epilepticus; S1P1R, sphingosine-1-phosphate subtype 1 receptor; STXBP1, syntaxin binding protein 1; SYNGAP1, synaptic ras GTPase-activating protein 1; TARP-γ8, transmembrane AMPA receptor regulatory protein γ8; TSC, tuberous sclerosis complex; TSPO, translocator protein (18 kDa); UBE3A, ubiquitin protein ligase E3A; WOREE, WWOX-related epileptic encephalopathy; WWOX, WW domain containing oxireductase

Figures

Figure 1

Molecular targets of clinically approved antiseizure medications (ASMs). Current ASMs act by diverse molecular mechanisms. ASMs can be categorized into drugs that act selectively via a single target (e.g. several of the sodium channel modulators) or act more broadly via several targets (marked by asterisks; e.g., valproate, topiramate, felbamate, and cenobamate). ASMs in current clinical use typically act via several targets. The actions of most ASMs on molecular targets can be categorized into four broad groups: (1) modulation of voltage-gated ion channels (e.g., benchmark ASMs such as carbamazepine); (2) enhancement of GABA-mediated inhibition (e.g., valproate and cenobamate); (3) inhibition of synaptic excitation mediated by ionotropic glutamate receptors (e.g., perampanel); and (4) direct modulation of synaptic release through effects on components of the release machinery (e.g., levetiracetam and gabapentin). The result of the interactions at these diverse targets is to modify the intrinsic excitability properties of neurons or to alter fast inhibitory or excitatory neurotransmission. By these actions, ASMs reduce the probability of seizure occurrence by modifying the bursting properties of neurons (reducing the capacity of neurons to fire action potentials at a high rate) and reducing synchronization in localized neuronal ensembles. In addition, ASMs inhibit the spread of abnormal firing to adjacent and distant brain sites. ASMs that were approved in the last 10 years and are described in more detail in the text are highlighted in red (including perampanel, which was approved by the EMA and FDA in 2012 but only released for non-investigational use in the USA in 2014). Note that an inhibitory and excitatory synapse are merged for display purposes, whereas in reality the same nerve terminal does not release both GABA and glutamate. Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; 5-HT, 5-hydroxytryptamine; GABA, γ-aminobutyric acid; GABA-T, GABA aminotransferase; GAT-1, GABA transporter 1; KCNQ, K_v7 potassium channel family; mTOR, mechanistic target of rapamycin; NMDA, N-methyl-D-aspartate

Commented [KK1]: Au: in figure 1 legend, is intended meaning preserved in sentence beginning "ASMs in current clinical use…"

Figure 2

Paths to the development of targeted therapeutic strategies in genetic epilepsy. Genetic screening is identifying large numbers of causative mutations. As shown in the upper row, for ion channels or receptors, functional analysis of the mutation usually involves heterologous expression systems and genetically engineered mice or zebrafish that provide good preclinical models on which disease mechanisms can be determined and on which treatments can be tested. This can lead to the development of various targeted therapies that can be based on small molecules or large, complex molecules (e.g., genes or oligonucleotides). The lower row shows an alternative or supplemental strategy involving patient-specific induced pluripotent stem cells (iPSCs;derived by ectopic co-expression of transcription factors in cells isolated from a skin biopsy) and cerebral organoids. This technology allows both studying the disease phenotype and screening for disease-specific therapies as illustrated in the middle. For details, see Parent and Anderson [40], Oyrer et al.[220] and Rowe and Dailey [221].

Figure 3

Evolving views on epilepsy pathophysiology drive therapy development. A: Epilepsy has been traditionally viewed as purely neuronal disease, and the main targets for all currently approved antiseizure medications (ASMs) are almost exclusively neuronal (see also Fig. 1). However, in recent years it has become evident that non-neuronal cells play an important role in modulating seizure activity. Astrocytes shape the function of neuronal circuits[222] and resident microglia and immune cells infiltrating the brain from the systemic circulation release inflammatory mediators with neuromodulatory effects.[139] Thus, restoring balanced activity of neuronal and non-neuronal cell populations in discrete brain circuits is essential for seizure control. B: The current development status of epilepsy therapies is summarized based on the analysis of 203 publicly disclosed programs (see Supplementary Table 2 for additional details). Neurotransmitter systems, mainly ɣ-aminobutyric acid

(GABA) and glutamate receptors, as well as voltage-gated ion channels are still the most common targets for future epilepsy therapies under development but other targets, such as serotonin, cannabinoid and purinergic receptors, are increasingly being pursued. Several drug discovery and development programs target the mammalian target of rapamycin (mTOR) and the ubiquitin-protein ligase E3A (UBE3A) for treatment of rare genetic epilepsies. Normalization of neuronal excitability is still the most dominating biological process targeted by candidate epilepsy therapeutics. However, processes related to neuroplasticity and circuit remodeling as well as neuroinflammation are increasingly targeted. Interestingly, energy metabolism and epigenetics are beginning to appear as biological pathways targeted by drugs under development. The spectrum of therapeutic modalities considered for future epilepsy therapies is also beginning to increasingly diversify, although small molecules still represent approximately three quarters of the total. Antisense oligonucleotides (ASO) and RNA therapeutics are an exciting new modality being increasingly pursued for epilepsy therapeutics. A sizable number of gene therapy projects are also under development. Implementation of these new therapeutic modalities reflects a growing understanding and interest in rare and ultra-rare genetic syndromes, which are currently the most pressing unmet medical need in epilepsy. As expected, early-stage programs (from discovery to Phase I) represent most of the pipeline projects. However, more than one quarter of the most promising projects are already in Phase II/III development, which could signal the advent of novel epilepsy therapies that may become available to patients within the next few years.

Figure 4

The long and winding road of development of $GABA_A$ receptor PAMs. The first $GABA_A$ receptor positive allosteric modulators (PAMs), the barbiturates, and benzodiazepines (BDZs) were developed mainly as sedatives/hypnotics without knowing their mechanism of action. Following the discovery of GABA in the brain in 1950 and its establishment as a major inhibitory neurotransmitter in the subsequent two decades, the role of GABA in the mechanism of action of the sedative/hypnotic,

anticonvulsant, and anesthetic activities of the barbiturates was investigated.[78] The barbiturate binding site at the $GABA_A$ chloride ionophore receptor complex (see Fig. 5) was first described in 1980.[223] The BDZ binding site at the $GABA_A$ receptor, by which BDZs act to allosterically increase the inhibitory effect of GABA on neuronal membranes, was described in the late 1970s.[86, 87, 126] Understanding the molecular pharmacology of the $GABA_A$ receptor and its subunits allowed the rational development of $GABA_A$ receptor PAMs, including the sedative/hypnotic α1-preferring 'Z-drugs' (zolpidem and zaleplon) and non-sedative anxiolytic ("anxioselective") partial PAMs such as bretazenil, abecarnil, alpidem, and ocinaplon, which, however, were discontinued because of toxicity, unexpected sedative effects in patients, or low efficacy.[85] One of these compounds, abecarnil, was shown to exert antiseizure effects.[103, 224] A new strategy was the characterization and development of endogenous (e.g., allopregnanolone [brexanolone]) and synthetic neurosteroids, one of which (ganaxolone) was recently approved as an antiseizure medication.[225] Synthetic neuroactive steroids and the dual-mechanism drug cenobamate are the only PAMs that act at both synaptic and extrasynaptic $GABA_A$ receptors, impacting both phasic and tonic GABA currents. The most recent strategies are "new age" PAMs (or GABAkines) that act as α2/3- (KRM-II-81; AZD7325), α2/3/5- (darigabat), or α3- (SAN711) selective PAMS.[77, 95]

Figure 5

The $GABA_A$ receptor as a target of antiseizure medications. In a GABAergic neuron (shown on the left), GAD catalyses the decarboxylation of glutamate to GABA which is packaged into vesicles at nerve terminals. Following GABA release, uptake into GABAergic nerve terminals and astrocytes is mediated by GAT-1; degradation of GABA to succinic semialdehyde (SSA) is catalysed by GABA-T, which is present in both neurons and astrocytes. All these processes are targeted by ASMs and investigational candidates (discussed in the main text) as illustrated (but note that the GAD activators valproate, gabapentin, and pregabalin also exert GABA-independent mechanisms.[18]) The pentameric subunit structure of a typical $GABA_A$ receptor chloride ionophore complex is shown, with

cross-sectional views of the extracellular domain (ECD) and the transmembrane domain (TMD) of the receptor. The $GABA_A$ receptor protein contains multiple functional domains including the GABA binding site, benzodiazepine (BDZ) and barbiturate binding sites, chloride channel, and sites for other modulatory drugs (not all shown).[226]. Only one of the two GABA binding sites in the ECD of the pentamer is illustrated. In the schematic representation of the TMD, two barbiturate recognitions sites are shown. The TMD contains additional binding sites, e.g., for etomidate, propofol, and neuroactive steroids. By binding to the BZD site in the ECD, BZDs and other drugs act as positive allosteric modulators (PAMs or GABAkines) of the $GABA_A$ receptor, leading to increased chloride channel opening frequency, increased chloride influx and, consequently, to hyperpolarization of the membrane and inhibition of the postsynaptic neuron.[80] Barbiturates such as phenobarbital bind to a distinct site in the TMD and potentiate GABA by increasing open channel probability. [26] The new PAMs or GABAkines indicated in the figure are selective for certain α-subunit (α2/3/5 or α2/3)-containing synaptic $GABA_A$ receptors (see text), but their specific binding sites are not illustrated here. In addition to synaptic $GABA_A$ receptors, extrasynaptic receptors, which differ in subunit composition from synaptic receptors, are targets for neurosteroids.

Figure 6

Paradigm-shift in the treatment of epilepsy from symptomatic-only to syndrome modulation and disease prevention. The figure illustrates a concept of the multi-step development and progression of epilepsy and possible therapeutic interventions. Following an initiating event such as head trauma, the majority of patients will not develop epilepsy but, depending on the severity of the trauma and several other factors, a subset of patients will develop epilepsy by a process termed epileptogenesis. The term epileptogenesis includes processes that take place before the first spontaneous seizure occurs to render the brain susceptible to spontaneous recurrent seizures (primary epileptogenesis) and processes that intensify seizures and make them more refractory to therapy (progression; secondary epileptogenesis). Primary epileptogenesis occurs in the latent period between brain injury and onset of epilepsy and is

characterized by numerous functional and structural brain alterations. In current epilepsy therapy regimens, antiseizure medications are given after epilepsy has started and aim to symptomatically suppress the seizures. The goal of antiepileptogenic or disease-modifying therapies in development is to administer treatment shortly after the initial brain insult to stop or modify epileptogenesis. In addition, disease-modifying therapies may be administered after the onset of epilepsy to prevent epilepsy progression.

Box 1. Lessons learned from recently approved ASMs

In the last 10 years, eight new antiseizure medications (ASMs) have been approved for the treatment of epilepsy (Supplementary Table 1). Two of these new ASMs are breakthroughs in the treatment of drug-resistant epilepsy (DRE): cenobamate for focal epilepsies [23, 117, 227, 228] and fenfluramine for Dravet syndrome (DS).[229-231] With both drugs, the achieved seizure freedom in previously drug-resistant patients is considerably higher than with any other ASM approved since 1990. Five of these ASMs were developed by rational target-based strategies (perampanel, brivaracetam, everolimus, cerliponase alfa, ganaxolone), one by phenotypic screening (cenobamate), and two based on anecdotal clinical observations (cannabidiol, fenfluramine).

The serendipitous discovery of fenfluramine as a highly effective treatment for DS with its proposed MOA including an increased release of serotonin (5-hydroxytryptamine, 5-HT), agonist activity at the 5-HT_{2A}, 5-HT_{2B}, 5-HT_{1D}, and 5-HT_{2C} receptors, and modulation of the σ1 receptors [232] led to a novel rational 5-HT-based strategy for the treatment of DS. Several 5-HT modulating drugs are in development targeting more selectively the $5HT_{2C}$ receptor to avoid adverse effects mediated by the 5 HT_{2B} receptor (see Table 2).

The efficacy of cerliponase alfa demonstrates that the development of precision medicines for genetic epilepsies is possible. Injected intrathecally, cerliponase alfa provides effective targeted precision therapy with a notable impact on both seizures and the underlying disease through replacing the missing enzyme tripeptidyl peptidase 1 in the extremely rare genetic disorder of neuronal ceroid lipofuscinosis type 2 or Batten's disease.[233, 234]

In contrast, precision medicine with everolimus, a selective inhibitor of the mechanistic target of rapamycin (mTOR), the disease-specific molecular pathway in TSC [235], shows only poor brain penetration and efficacy in epilepsy not substantially different from other ASMs in TSC [236]. Everolimus is more effective the earlier it is given suggesting that multiple other mechanisms are involved in chronic TSC-related epilepsy than just mTOR overactivity. Still, this and other recent findings have stimulated various other gene and molecular therapies (Table 2).

The rationally designed AMPA glutamate receptor antagonist perampanel brought relatively little improvement in efficacy compared to existing ASMs. Similarly, the novel SV2A modulator brivaracetam, a purpose-designed second-generation racetam molecule which has a 15-30 fold higher binding affinity for the target SV2A than the parent drug levetiracetam, is also not more effective than levetiracetam, but, paradoxically, is better tolerated. The lack of superior efficacy of perampanel and brivaracetam in the treatment of DRE may indicate that highly selective ASMs, which act by a single mechanism, may be inferior to drugs such as cenobamate, which act by at least two different MOAs (Supplementary Table 1). This appears to be especially relevant in heterogenous populations of patients with DRE, who share similar seizure semiology that could be driven by very different pathophysiological mechanisms.

Finally, the high efficacy of cenobamate and fenfluramine demonstrates that despite the ever-growing knowledge about molecular targets for epilepsy therapy (see Table 2), phenotypic screening and serendipity still play an important role in drug discovery. Notwithstanding the breakthroughs in DRE treatment with cenobamate and fenfluramine, significant unmet medical needs and treatment challenges remain in several areas of epilepsy treatment.

Box 2. The Epilepsy Therapy Screening Program (ETSP)

In 1975, the US National Institute of Neurological Disorders and Stroke (NINDS) initiated the Anticonvulsant Screening Project (ASP; renamed ETSP in 2015) to stimulate the discovery and development of new chemical entities for the symptomatic treatment of human epilepsy.[29] The ETSP covers three key areas of research ('performance areas'). The pharmacoresistance performance area (see the figure) includes a large battery of both acute and chronic models of drug-resistant seizures and epilepsy for drug screening. Performance area 2 includes animal models of genetic epilepsies (such as Dravet syndrome) and special epilepsy populations (such as viral encephalitis-induced epilepsies). Performance area 3 includes chronic epilepsy models (such as the kainate model) to identify investigational compounds that prevent the development of epilepsy or are disease-modifying. [31]

The mission of the ETSP is to facilitate the discovery of new therapeutic agents that address unmet medical needs in epilepsy, i.e., drug resistance and disease prevention or modification. The program provides opportunities for researchers from academia and industry in the US and abroad to submit compounds for testing, thus assembling compelling efficacy packages that serve to facilitate the advancement of new compounds toward the clinic for the symptomatic control of seizures.[30] As shown in the figure on the current testing scheme for pharmacoresistant epilepsy, the workflow of compound testing starts with an identification phase, including assays such as the MES test and the 6-Hz model that allow for higher throughput. Furthermore, etiologically relevant chronic models of epilepsy such as corneal kindling have been included in the early identification stages of compound evaluation. Because the 6-Hz and corneal kindling models in mice and rats are pharmacoresistant to numerous ASMs, investigational compounds found to be effective in these models without significant tolerability issues may be advanced into the differentiation phase of the testing scheme. In this phase, several chronic TLE models with seizures that are resistant to several ASMs are used. These models are more etiologically relevant than the models used in the identification phase but - due to the economic, labor, and time constraints associated with these models – not suited for high throughput

screening. As the baseline comparison for the experimental compounds, all FDA-approved ASMs were evaluated in the screening models of the ETSP.

An important milestone for the ETSP has been the release of a publicly accessible database termed PANAChE (Public Access to Neuroactive and Anticonvulsant Chemical Evaluations)[237], which provides detailed information on tests, procedures, and workflows used by the ETSP. Furthermore, it provides a searchable repository for non-confidential efficacy data on compounds tested by the program.[30]

Box 3. Epilepsy prevention trials – challenges and biomarkers

Approximately 20% of all epilepsy is caused by acute CNS insults such as traumatic brain injury, stroke and infection. There is a latency between the insult and the onset of epilepsy which offers a window of opportunity to use treatment to prevent epilepsy, but several challenges remain.

Treatment initiation, duration, and selectivity. We currently lack knowledge of the timing of onset, evolution and completion of human epileptogenesis needed to guide targeted treatment initiation, duration, and selectivity. PTE preventive trials done since the 1980s used the earliest clinically feasible intervention, ranging from 8-24 hours after injury [166, 168, 194, 238, 239], based on the untested assumption that the sooner after injury treatment is started, the better the chance of catching the onset of epileptogenesis. Treatments lasted from 1-18 months, applying intuitive considerations of "longer is better" and feasibility considerations of lesser likelihood of treatment discontinuation with shorter treatments. Two successful preclinical preventive treatments (inhibition of injury-activated ADK with 5-iodotubercidin (5-ITU) and post-traumatic enhancement of the glutamate transporter GLT-1 with ceftriaxone) were both targeted to time the start and duration of treatment based on temporally defined epileptogenic mechanisms of astrocytic ADK activation and GLT-1 depression after injury.[240, 241] Mechanistically guided timing of treatment initiation and duration is not at present available in humans.

Distinguishing between adaptive and maladaptive response to injury. We do not know whether epileptogenesis results from an aberration of adaptive processes, such as astrocytosis, neuroinflammation, axonal sprouting, neuronal regeneration, and synaptogenesis, to maladaptive ones, in either quantity or quality or from adaptation-independent processes. Antiepileptogenic treatment should target the maladaptive response while sparing the beneficial recovery response. An example of the need for selective targeting is the modulation of the brain-derived neurotrophic factor (BDNF)BDNF/TrkB pathway as the beneficial neuroprotective and the harmful epileptogenic process

(potentiation of excitatory synapses) are mediated by two distinct TrkB downstream effects. This insight opened the path to selective targeting of the epileptogenic effect, phosphorylation of tyrosine 816 of TrkB, and to the development of selective treatment, the peptide pY816, which blocks the epileptogenic effect in rodents without impairing the neuroprotective effect.[242]

Logistical challenges of PTE prevention studies include a large study sample size, required because not all TBI patients develop PTE, and long follow-up duration, needed because of the latency to PTE. Past studies have evaluated patients with an overall 20% risk of PTE at 2 years after TBI based on clinical risk factors. With an approximate 30% subject attrition rate due to death (~10%) and loss of follow-up (~20%), this requires approximately 520 patients for a two-arm study to show 50% PTE reduction with 0.05 significance and 80% power.[166, 238] Preventive studies' outcome is PTE, defined as first late post-traumatic seizure. Because 80% of PTE starts within 2 years, this has been the follow-up duration in PTE prevention studies.

Biomarkers

Biomarkers may help to overcome these challenges. Biomarkers serve several purposes.[209, 243, 244] For epileptogenesis, they should: define the onset, timeline, and duration of epileptogenesis to guide the timing and duration of treatment, improve PTE risk prediction to reduce the sample size, and detect PTE before first clinical seizure, to shorten follow up and study duration and allow proof of concept studies and potentially also serve as a surrogate outcome.

Potential biomarkers for disease prediction include clinical, genetic, epigenetic, protein, electrophysiological, and neuroimaging markers.

Clinical and lesional neuroimaging: Subdural hemorrhage (SDH) requiring surgery, SDH and parenchymal hemorrhages, multifocal parenchymal hemorrhages including bitemporal and bifrontal hemorrhages, depressed skull fracture and penetrating injury combined carry a ~30% PTE risk.[195]

Genetic: Variation within genes encoding regulation of astrocytic control of adenosine homeostasis

(ADK and NT5E), of glutamate transport (SLC1A1), and of the pro-inflammatory cytokine IL1-β have identified TBI patients with 40-50% PTE risk.[245-248]

Electrophysiological: Early clinical seizures (≤ 7 days after TBI) carry an approximately 25% risk of PTE.[193, 249] Detection of subclinical, electroencephalographic (EEG) seizures may increase that risk, as suggested in two uncontrolled studies.[195, 250] Interictal epileptiform abnormalities on cEEG within 5 days of TBI also predict PTE risk (64% positive in PTE vs 36% in non-PTE patients at 1 year.[251] Another potential biomarker of PTE prediction are EEG high-frequency oscillations with fast ripples (HFOs, frequency 250-500 Hz).[252, 253] [254] The ongoing EpiBioS4RX project is evaluating early subclinical seizures and HFOs as a potential biomarker of PTE prediction.

Potential biomarkers for early PTE detection include subclinical EEG seizures, other electrophysiological changes such as interictal epileptiform discharges, HFOs, spectral, connectivity and sleep pattern changes, MRI changes of persisting inflammation [255] and blood biomarkers, e.g. inflammatory proteins such as HMGB1[256], and miRNA[257], although these have not been validated in clinical prospective studies. Biomarkers of early detection of PTE are crucial for the development of preventive treatment of PTE, because they will enable proof of concept studies which are currently not feasible because of the long time to clinical PTE onset. Lack of a feasible POC study approach has been the major impediment to development of preventive treatment in the last 20 years. With two exceptions (levetiracetam and topiramate in the 2000s) none of the ~20 treatments with demonstrated preclinical antiepileptogenic efficacy have progressed to the clinic.

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Epilepsy: A chronic brain disorder that is characterized by partial or generalized spontaneous (unprovoked) recurrent epileptic seizures and, often, comorbidities such as anxiety and depression.

Ictogenic mechanism: the processes or factors that trigger or contribute to the generation of seizures in epilepsy; derived from the Greek word “iktos”, meaning “seizure”.

Antiseizure medications (ASM): Also termed anticonvulsant or antiepileptic drugs, compounds that inhibit or control seizures that are associated with epilepsy or other conditions.

Breakthrough seizures: epileptic seizures that occur despite the ongoing use of ASMs or other seizure management strategies.

Intrathecal delivery: drug delivery directly into the CSF in the space surrounding the spinal cord, bypassing the bloodstream.

Epileptogenesis: the gradual process by which normal brain tissue becomes epileptic, encompassing the events in the latent period between an initial brain injury and the onset of recurrent seizures.

Anti-epileptogenic drugs: Compounds that, when administered immediately following a brain insult, prevent or reduce the long-term consequences of the insult after drug washout, including the development of epilepsy, neurodegeneration and cognitive or behavioural alterations.

Disease-modifying drugs: Compounds that alter the development or progression of epilepsy by affecting the underlying pathophysiology and natural history of the disease.

Temporal lobe epilepsy: A common, difficult-to-treat epilepsy characterized by focal seizures originating from medial (hippocampus or amygdala) or lateral temporal lobe regions.

Blood–brain barrier: A dynamic interface that separates the brain from the circulatory system and protects the brain from potentially harmful chemicals, while regulating the transport of essential molecules and maintaining a stable environment.