

## New avenues for anti-epileptic drug discovery and development

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**Abstract** | Despite the introduction of over 15 third-generation anti-epileptic drugs, current medications fail to control seizures in 20–30% of patients. However, our understanding of the mechanisms mediating the development of epilepsy and the causes of drug resistance has grown substantially over the past decade, providing opportunities for the discovery and development of more efficacious anti-epileptic and anti-epileptogenic drugs. In this Review we discuss how previous preclinical models and clinical trial designs may have hampered the discovery of better treatments. We propose that future anti-epileptic drug development may be improved through a new joint endeavour between academia and the industry, through the identification and application of tools for new target-driven approaches, and through comparative preclinical proof-of-concept studies and innovative clinical trials designs.

### 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.

Epilepsy is a life-shortening brain disorder affecting approximately 1% of the worldwide population<sup>1</sup>. Although repeated epileptic seizures are the clinical hallmark of epilepsy, the disease process (epileptogenesis) begins before the first seizure and may also lead to the progression of epilepsy after the onset of seizures. Epilepsy is diverse, with over 15 different seizure types and over 30 epilepsy syndromes<sup>2</sup>, and is associated with substantial comorbidity, including depression, anxiety and increased mortality<sup>3</sup>.

During the past three decades, the introduction of over 15 third-generation anti-epileptic drugs (AEDs) has provided physicians and patients with more options for the treatment of many types of seizures<sup>4</sup>. However, although approximately 70–80% of patients with new-onset epilepsy eventually enter remission with current AEDs, these medications fail to control seizures in 20–30% of patients<sup>5,6</sup>. Furthermore, no AED has been shown to prevent the development of epilepsy in patients prior to the first seizure; these drugs seem to purely act to symptomatically suppress seizures once they occur<sup>7,8</sup>. For some AEDs, an anti-epileptogenic effect has actually been suggested in certain preclinical epilepsy models<sup>9,10</sup>, but this has not been proven in humans. Indeed, with the exception of traumatic brain injury<sup>7</sup>, none of the therapies found to be effective in preclinical studies has been adequately tested using an appropriately designed clinical trial in humans.

Unfortunately, there are few aetiologically relevant animal models used in epilepsy research today that have

been validated at the clinical level — a fact that obviously hampers clinical trial design using the appropriate patient population. In addition, there is no compelling evidence that third-generation AEDs are generally much better tolerated<sup>11–13</sup>. However, individual modern AEDs such as gabapentin (Neurontin; Pfizer) or levetiracetam (Keppra; UCB Pharma) cause fewer or no dermatological hypersensitivity reactions. Also, non-enzyme-inducing modern AEDs such as gabapentin or levetiracetam do not induce the drug interactions seen with older AEDs that have been reported to substantially lower the efficacy of other medications, including other AEDs given in combination<sup>14</sup>.

AEDs are also unable to prevent or reverse the development of drug-resistant epilepsy, to treat comorbidities or to reduce the burden of disease in a holistic sense<sup>4</sup>. A particularly disquieting aspect of current epilepsy treatments is that we have not made substantial progress in seizure control over the past 40–50 years since the introduction of carbamazepine and valproate to the market<sup>4,15</sup>.

The consequences of the standstill in the development of more efficacious drugs for the treatment of epilepsy are several-fold. Patients and physicians are increasingly disappointed and have thus become less interested in using recently developed, pricier AEDs. Payers are hesitant to pay premium prices for drugs that do not differentiate from established low-cost generic medications, and the pharmaceutical industry is losing interest in developing novel compounds for epilepsy (BOX 1).

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**Box 1 | Business challenges and opportunities for anti-epileptic drug development**

In the 1990s, epilepsy presented an opportunity to enter a therapeutic space in which there was a good chance for return on investment. Drivers for this included a significant unmet need with few treatment options (especially for patients with refractory epilepsy), good potential for reimbursement at competitive pricing with few competitors in the field, as well as manageable technical and regulatory hurdles.

The adjunctive or add-on treatment paradigm in the clinical management of refractory epilepsy was well suited for bringing forward new agents to the market. The placebo-controlled adjunctive model for evaluating the efficacy of a test compound in refractory patients established efficacy and tolerability at an early stage and could be performed using cost-efficient short-term clinical studies. Furthermore, following the introduction of felbamate (Felbatol; MedPointe) to the market, a new regulatory path existed for the clinical development and labelling of anti-epileptic drugs (AEDs).

Together, these commercial, scientific, technical and regulatory factors drove confidence and reduced the risk associated with developing and obtaining a value-returning marketable product for epilepsy. This template provided an incentive for several companies to confidently invest in bringing new AEDs to the market.

**Loss of industry interest in AEDs**

Prior incentives for investment in AED development are now negatively balanced by the drug development challenges facing industry overall<sup>144–146</sup>. Payer reimbursement requires that future AEDs bring additional value or differentiation (principally an improvement in efficacy) to an already crowded, highly generic AED field. No AED to date has convincingly been demonstrated to be superior in efficacy to any other AED in adjunctive therapy for partial seizures, and differentiation by safety profile for new AEDs is not a principal component for optimizing pricing and reimbursement.

New regulatory hurdles have also evolved over the past 15–20 years. A generally lower risk tolerance for new drugs and recent class labelling regarding safety signals (that is, suicide) have affected opportunities in non-epilepsy indications and had an impact on the overall value proposition for AEDs. New AEDs can require commitments for long-term safety data in a variety of age populations, and paediatric investigational plans necessitate the development and testing of new formulations in very young patients (babies who are ≥1 month old). Commercialization models indicate that the adjunctive indication alone for a marginally differentiated product is not adequate. Product promotion for additional uses requires those specific indications to be established in the label. A monotherapy indication can move an AED earlier into the epilepsy treatment paradigm. However, the approval of a monotherapy has so far required the prior approval of an adjunctive therapy and this causes a considerable time delay.

**Future business opportunities for AED development**

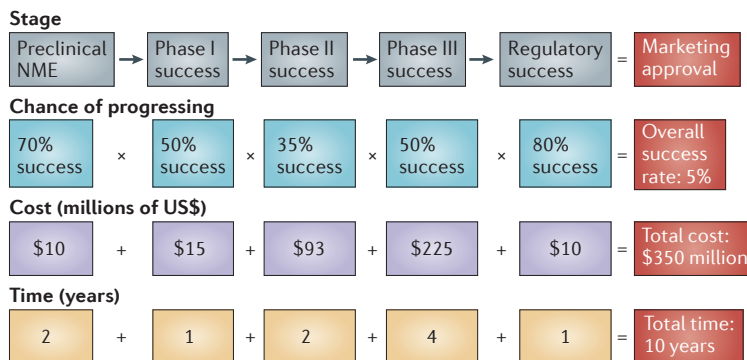
Interesting business cases seem to exist for the very disabling epilepsy syndromes — which are associated with an increased risk of premature death — such as infantile spasms and Lennox–Gastaut syndrome. These may present viable business opportunities for orphan indications, for which tax incentives are provided, investments are smaller and there is a potentially less demanding path for approval.

Another more immediate business opportunity may involve the repurposing of drugs from other therapeutic areas that possess either relevant disease-modifying properties for epilepsy or a novel mechanism of action that provides substantial synergistic efficacy against drug-resistant epilepsy when combined with an existing AED therapy. This would markedly reduce the level of investment necessary for discovery and development, and also potentially lower the technical hurdles and regulatory data requirements, thereby improving the premises for a very positive business case.

A substantial level of investment, beyond that required for traditional AED development, will be necessary for the future development of new AEDs that have evidence of superior efficacy against a relevant standard of care for the treatment of drug-resistant epilepsy, or that have the ability to markedly alter the course or the prognosis of epilepsy. However, as these types of new epilepsy therapies address a major unmet medical need, they also offer a promising business case to drive incentive for future AED development.

The figure illustrates a hypothetical investment example for the development of an AED: a new molecular entity (NME) transitions from discovery into clinical development to be ultimately approved for marketing authorization. From discovery, the lead molecule passes through late-stage preclinical toxicology testing and chemistry scale-up into clinical testing at a cost of US\$10 million and a success rate of 70%. The NME passes through each stage with an overall success rate of about 5% at a total cost of \$350 million. A key inflection point is at the Phase II stage prior to the most significant spending investment in Phase III. A reduction of risk at this stage can greatly influence the overall success rate and total expenditure for the development of an AED. Note that a cost-effective proof-of-differentiation step early in Phase II can further reduce the investment risk, cost and time. Sales and marketing costs add to the investment and can be of a similar

magnitude to the development costs. Following marketing approval, there are costs for sales and marketing, launch, sales force, Phase IV medical affairs studies and post-marketing regulatory commitments. Investments in the initial monotherapy indication and an alternative non-epilepsy indication could add up to approximately \$50–250 million.



**Epileptogenesis**

The gradual process (also termed latent period) by which epilepsy develops in the normal brain following brain insults or gene mutations.

**Anti-epileptic drugs (AEDs)**

Also termed anticonvulsant or anti-seizure drugs. Compounds that, when administered systemically in animal models or to patients, inhibit or control seizures that are associated with epilepsy or other conditions.

**MES seizure test**

(Maximal electroshock seizure test). A model in which a short (0.2-second) transcorneal or transauricular application of a 50 or 60 Hz electrical stimulus in rodents induces generalized tonic-clonic seizures that are mediated by brainstem structures.

**Pentylenetetrazole**

(PTZ). A chemical convulsant that, when administered systemically to rodents, induces characteristic myoclonic and clonic convulsions that are mediated by forebrain structures.

**Amygdala kindling**

Repeated administration of an initially subconvulsive electrical stimuli via a depth electrode in the amygdala, which induces seizures that progressively increase in severity and duration; once established, the increased susceptibility to the induction of kindled seizures is a permanent phenomenon.

**GAERS rat**

(Genetic absence epileptic rat from Strasbourg). A genetic rat model that displays characteristic 6–7 Hz spike-wave electrographic seizures and a pharmacological profile that is consistent with generalized absence epilepsy.

**6-Hz psychomotor seizure model**

A seizure model in which a prolonged (4-second) transcorneal application of a 6-Hz electrical stimulus in mice induces limbic seizures that are characterized by a stun, vibrissae chomping, forelimb clonus and a Straub tail; these seizures are resistant to phenytoin and some other anti-epileptic drugs.

**Non-inferiority trial design**

A clinical trial design that determines whether a test compound is inferior to another compound; the lower limit (95% confidence interval) of a test compound's treatment efficacy or effectiveness is to be compared to a preset lower boundary of efficacy or effectiveness relative to the adequate comparator's point estimate of efficacy or effectiveness.

In this Review we briefly examine the experimental and clinical strategies for AED discovery and development over the past few decades and discuss why these approaches may have failed to address unmet medical needs. We also outline the challenges for the pharmaceutical industry that have had an impact on its attitude towards the discovery and development of AEDs. Given the serious unmet clinical needs in epilepsy treatment, we present new ideas on how to revitalize the pharmacological and clinical development of better AEDs that could provide the foundation for a new, joint endeavour between academia and the industry.

**Previous AED discovery and development**

Until recently, the discovery and development of a new AED almost exclusively relied on preclinical testing in animal seizure models to establish anti-seizure efficacy prior to conducting clinical trials in humans<sup>16</sup>. This approach has been successful and crucially contributed to the development of numerous clinically effective AEDs<sup>4,17</sup>. Indeed, animal models with a similarly high predictive value do not exist for other central nervous system (CNS) disorders, such as bipolar disorders or migraine<sup>18</sup>.

Since Merritt and Putnam<sup>19</sup> first described the use of an electroshock seizure model to assess drugs for anti-seizure properties in 1937 (FIG. 1 (TIMELINE)), simple models of acute seizures — such as the MES seizure test and the subcutaneous pentylenetetrazole (PTZ) seizure test in mice and rats — have been widely used in AED discovery. These models were considered to be ideal for AED discovery, which necessitates the screening of large numbers of compounds; acute seizure models should therefore be easy to perform, time- and cost-efficient, and predictive of clinical activity. The rodent MES test created by Toman, Swinyard and Goodman<sup>20</sup> in 1946 is still the most commonly used first screen in the search for new AEDs and is quite effective in identifying drugs that block generalized tonic-clonic seizures in patients<sup>17</sup>. The MES test has also repeatedly been proposed to identify drugs that are active against partial seizures in patients, but this test failed to detect several AEDs (for example, levetiracetam and vigabatrin (Sabril; Lundbeck)) that are effective against partial seizures in patients; therefore, other models such as amygdala kindling are better for identifying anticonvulsant effects against partial seizures<sup>21</sup>.

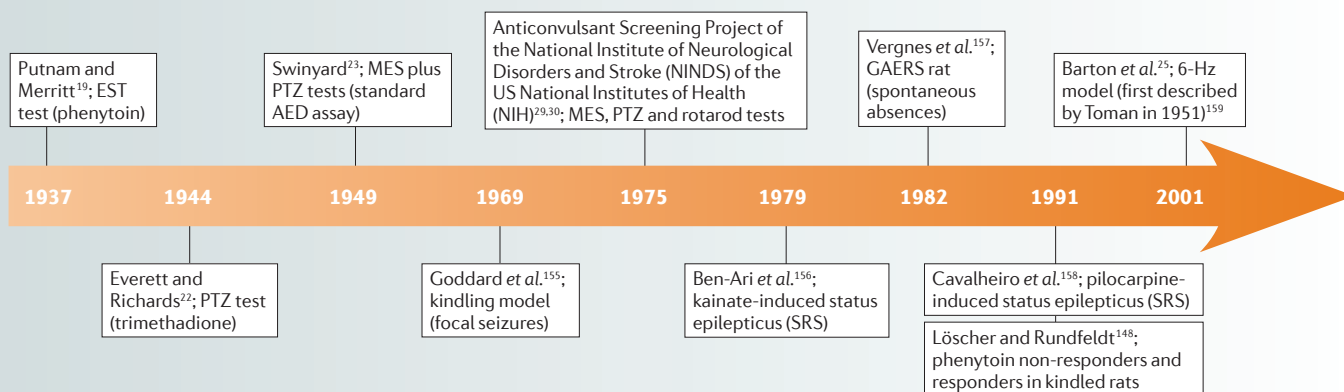
Following the report of Everett and Richards<sup>22</sup> in 1944 that the PTZ test can identify the anti-absence efficacy of AEDs, two simple animal models — the MES and PTZ tests — were thought to be sufficient for differentiating among AEDs with different clinical effects. This subsequently formed the basis for the proposal made by Swinyard and colleagues<sup>23,24</sup> that the MES and subcutaneous PTZ tests in mice and rats be used as standard procedures for predicting the clinical anticonvulsant activity of investigational drugs (FIG. 1 (TIMELINE)). However, because of false-positive and false-negative findings in these models, more complex chronic epilepsy models that were developed in the 1980s and 1990s (FIG. 1 (TIMELINE)) have subsequently been included in

later-stage screening to further characterize anti-epileptic efficacy — the most notable of these models being the kindling model and genetic models of epilepsy, such as the absence-epilepsy-prone GAERS rat. More recently, the 6-Hz psychomotor seizure model in mice has been introduced for differentiating an investigational AED from existing AEDs. This model is resistant to some of the old AEDs and enables the screening of a large number of compounds<sup>17,25</sup>, which is not possible with more elaborate models such as the kindling model.

**Preclinical strategies.** Three strategies have been used in AED discovery: first, the random, phenotypic screening of newly synthesized compounds of diverse structural categories with as yet unknown mechanisms; second, the structural variation of known AEDs; and third, hypothesis-driven, target-based drug design<sup>4,17,18</sup>. All three strategies have generated clinically useful AEDs but only very few AEDs have been identified by rational, target-based strategies. These have been based on previously presumed mechanisms of seizure generation: that is, impaired GABA ( $\gamma$ -aminobutyric acid)-ergic inhibition and increased glutamatergic excitation, resulting in AEDs that either potentiate GABA transmission (such as vigabatrin and tiagabine) or inhibit glutamate receptors (such as perampanel (Fycompa; Eisai))<sup>17</sup>. However, the old reductionistic view that seizures or epilepsy are due to an imbalance between GABAergic inhibition and glutamatergic excitation ignores the complexity of the alterations within these neurotransmitter systems in the brain of a patient suffering from epileptic seizures<sup>26</sup>.

**Clinical strategies.** Marketing approval of new AEDs for the treatment of epilepsy has been routinely obtained by adjunctive therapy placebo-controlled Phase III trials in adult patients with refractory seizures<sup>27</sup>. In the 1960s and 1970s, when few AEDs were available<sup>4</sup>, the enrolment of patients into these trials was straightforward and the use of placebo treatments was deemed acceptable given the lack of alternative treatment options<sup>28</sup>. This clinical strategy was very successful and has resulted in over 15 new AEDs entering the market since the 1980s (TABLE 1). Many AEDs that are marketed for adjunctive treatment are subsequently tested in monotherapy trials in patients with either refractory or previously untreated epilepsy. Because regulatory guidelines for monotherapy approval differ between Europe and the United States, sponsors need to pursue two separate and costly development programmes. The monotherapy development paradigm currently used in Europe for new-onset epilepsy is the non-inferiority trial design, which establishes a preset limit for the allowed difference in outcome between the test drug and a standard AED<sup>27</sup>. In the United States, the preferred development path is conversion to monotherapy in refractory patients using historical controls. These designs have demonstrated that several AEDs are efficacious as monotherapies, including levetiracetam and zonisamide (Zonegran; Eisai) in Europe and lamotrigine (Lamictal-XR; GlaxoSmithKline) in the United States<sup>28</sup>.

Timeline | Milestones in the development of animal models for AED discovery and development\*



AED, anti-epileptic drug; EST, electroshock threshold; GAERS, genetic absence epilepsy rat from Strasbourg; MES, maximal electroshock; PTZ, pentylenetetrazole; SRS, spontaneous recurrent seizures. \*All animal models shown (except for the SRS models described by Ben-Ari *et al.*<sup>156</sup>, Vergnes *et al.*<sup>157</sup> and Cavalheiro *et al.*<sup>158</sup>) are those in which seizures are electrically or chemically induced. All models, except for the EST method in cats described by Putnam and Merritt<sup>19</sup>, are still used in the development of new epilepsy therapies<sup>21</sup>. Various models are important for different purposes in epilepsy research<sup>21</sup> and can be assigned to four major categories: first, acute seizure models in which single seizures are electrically or chemically induced in healthy, neurologically intact rodents, such as the MES, subcutaneous PTZ or 6-Hz tests; second, chronic seizure (or epilepsy) models in which single or multiple seizures are electrically or chemically induced in rodents with chronic brain alterations, such as amygdala kindling; third, genetic animal models with inborn chronic epilepsy, such as the GAERS rat (which is better suited than the PTZ test to identify drugs that are active against absence seizures); and fourth, chronic epilepsy models in which epilepsy with SRS is induced by brain insults, such as status epilepticus (for example, induced by pilocarpine or kainate) or traumatic brain injury<sup>21</sup>. The MES and subcutaneous PTZ tests, which were developed more than 60 years ago, have been widely used in the search for new AEDs but they obviously do not predict efficacy against difficult-to-treat (or pharmacoresistant) seizures<sup>4</sup>. Löscher and Rundfeldt<sup>148</sup> were the first to describe a chronic model of pharmacoresistant seizures in which AED-resistant rats were selected from large groups of amygdala-kindled rats by repeated testing with phenytoin. Later, Löscher *et al.* also described the selection of AED-resistant subgroups of rats for post-status epilepticus models of temporal lobe epilepsy with SRS<sup>21,160</sup>.

**Limitations of previous strategies**

Despite the development of various new AEDs since the early 1990s, the available evidence indicates that there has been a failure to deliver drugs with improved efficacy<sup>4</sup>. What are the reasons for this apparent failure to discover drugs that can effectively control drug-refractory seizures and comorbidities as well as prevent or modify the disease?

**Problems with preclinical models.** Simple seizure models such as the MES and PTZ tests in rodents have been instrumental in the identification of most AED candidates. The advantages of such acute seizure models are their technical simplicity and the ability to screen large numbers of compounds. A disadvantage is that the seizures do not mirror epilepsy (that is, spontaneous seizure occurrence) and occur in ‘normal’, non-epileptic brains. Furthermore, older AEDs provide complete seizure suppression in these tests, hampering the identification of new AED candidates with greater efficacy, including those that might be effective in patients who are resistant to the older drugs.

More recently, large AED screening programmes such as the Anticonvulsant Screening Project (ASP) of the National Institute of Neurological Disorders and Stroke (NINDS) of the US National Institutes of Health (NIH), which was initiated in 1975 to stimulate the discovery and development of new chemical entities for the symptomatic treatment of human epilepsy<sup>29,30</sup>, have included models for pharmacoresistant partial seizures in drug screening. One particular model is the 6-Hz mouse test, which was also introduced to avoid missing

the identification of compounds like levetiracetam; levetiracetam is ineffective in the MES and PTZ models but is among the most effective AEDs in the clinic<sup>16,25,31</sup>. However, although several novel AEDs — including brivaracetam, retigabine (Potiga; Valeant Pharmaceuticals/GlaxoSmithKline) and carisbamate — are highly effective in the 6-Hz mouse model, they are not more effective in patients with pharmacoresistant partial seizures<sup>21</sup>.

Thus, it seems that the simple acute seizure screening models used in the ASP and other programmes fail to differentiate between compounds with promising potential for efficacy against drug-resistant seizures and compounds that work through mechanisms that are not detected by these models. Importantly, chronic seizure models, such as the lamotrigine-resistant kindled rat<sup>32</sup>, in which seizures are induced in animals with chronic brain alterations, were therefore recently included in the ASP. However, none of the emerging models of therapy-resistant epilepsy (FIG. 1 (TIMELINE)) has actually been validated at predicting clinical success in the therapy-resistant patient population. Thus, it remains to be established whether the use of chronic models such as kindling or models with spontaneous recurrent seizures will lead to the identification of more effective anti-epileptic treatments, but we consider this approach to be much more viable than the exclusive use of simple acute seizure models, particularly when testing hypothesis-driven, target-based strategies of drug development<sup>21</sup>.

**Problems with broad-spectrum approaches.** An important aim of previous research and development (R&D) efforts was to discover novel AEDs that exert a broad

Table 1 | Characteristics of clinically approved AEDs\*

AED	Companies	Year of approval	Presumed main mechanisms of action	Approved indications	Main utility	Main limitations
<i>First-generation drugs</i>						
Potassium bromide	Dow	1857 <sup>†</sup>	GABA potentiation?	GTCS, myoclonic seizures	Broad use for focal and generalized seizures	Currently for adjunctive use only, not in wide use anymore; acts as a sedative
Phenobarbital	Bayer	1912 <sup>†</sup>	GABA potentiation	PGCS, sedation, anxiety disorders, sleep disorders	Broad use for focal and generalized seizures	Enzyme inducer; skin hypersensitivity; no absence seizures
Phenytoin	Parke-Davis/Pfizer	1938	Sodium channel blocker	PGCS	First-line AED, i.v. use	Enzyme inducer; skin hypersensitivity; NLPK; not useful for absence or myoclonic seizures
Trimethadione	Abbott	1946	T-type calcium channel blocker	Absence seizures	Rare use for absence seizures	Not in wide use anymore; teratogenic
Primidone	Imperial Chemical Industries	1954	GABA potentiation	PGCS	Broad use for focal and generalized seizures	Enzyme inducer; skin hypersensitivity; no absence seizures; acts as a sedative
Ethosuximide	Parke-Davis/Pfizer	1958	T-type calcium channel blocker	Absence seizures	First-line AED, no skin hypersensitivity	Somnolence, loss of appetite, nausea, vomiting, singultus, depression, psychotic episodes, insomnia, rare aplastic anaemia
<i>Second-generation drugs</i>						
Diazepam	Roche	1963	GABA potentiation	Convulsive disorders, status epilepticus, anxiety, alcohol withdrawal	Broad use for focal and generalized seizures, i.v. use, no clinical hepatotoxicity, no skin hypersensitivity	Currently for adjunctive use only; emergency use only; acts as a sedative; leads to tolerance (loss of efficacy)
Carbamazepine	Novartis	1964	Sodium channel blockade	PGCS, trigeminal pain, bipolar disorder	First-line AED	Enzyme inducer; skin hypersensitivity; not useful for absence or myoclonic seizures
Valproate	Sanofi/Abbott	1967	Multiple (for example, GABA potentiation, glutamate (NMDA) inhibition, sodium channel and T-type calcium channel blockade)	PGCS, absence seizures, migraine prophylaxis, bipolar disorder	Broad use for focal and generalized seizures, first-line AED, i.v. use, no skin hypersensitivity	Enzyme inhibitor; substantial teratogenicity; weight gain
Clonazepam	Roche	1968	GABA potentiation	Lennox–Gastaut syndrome, myoclonic seizures, panic disorders	Broad use for focal and generalized seizures, no clinical hepatotoxicity	Currently for adjunctive use only; acts as a sedative; leads to tolerance (loss of efficacy)
Clobazam	Hoechst Roussel/Lundbeck/Sanofi	1975	GABA potentiation	Lennox–Gastaut syndrome, anxiety disorders	Broad use for focal and generalized seizures, no clinical hepatotoxicity	Currently for adjunctive use only; acts as a sedative; leads to tolerance (loss of efficacy)

Table 1 (cont.) | **Characteristics of clinically approved AEDs\***

AED	Companies	Year of approval	Presumed main mechanisms of action	Approved indications	Main utility	Main limitations
<i>Third-generation drugs</i>						
Progabide	Synthelabo	1985	GABA potentiation	PGCS, Lennox–Gastaut syndrome, myoclonic seizures, muscle hypertonia	Rarely used for focal seizures	Clinical hepatotoxicity, not in wide use anymore
Vigabatrin	Sanofi/Lundbeck	1989	GABA potentiation	Infantile spasms, complex partial seizures (currently for adjunctive use only)	No clinical hepatotoxicity	Not useful for absence or myoclonic seizures; vision loss; weight gain
Lamotrigine	GlaxoSmithKline	1990	Sodium channel blocker	PGCS, Lennox–Gastaut syndrome, bipolar disorder	Broad use for focal and generalized seizures, first-line AED	Enzyme inducer; skin hypersensitivity
Oxcarbazepine	Novartis	1990	Sodium channel blocker	Partial seizures	First-line AED	Enzyme inducer; skin hypersensitivity; not useful for absence or myoclonic seizures
Felbamate	Carter-Wallace/MedPointe Pharmaceuticals	1993	Multiple (GABA potentiation, glutamate (NMDA) inhibition, sodium and calcium channel blockade)	PGCS, Lennox–Gastaut syndrome	Broad use for focal and generalized seizures	Currently for adjunctive use only; aplastic anaemia; clinical hepatotoxicity; skin hypersensitivity; clinical hepatotoxicity; not in wide use anymore
Gabapentin	Parke-Davis/Pfizer	1993	Calcium channel blocker ( $\alpha 2\delta$ subunit)	PGCS, postherpetic and diabetic neuralgia, restless legs syndrome	No clinical hepatotoxicity	Currently for adjunctive use only; weight gain; not useful for absence or myoclonic seizures
Topiramate	Janssen/Johnson & Johnson	1995	Multiple (GABA potentiation, glutamate (AMPA) inhibition, sodium and calcium channel blockade)	PGCS, Lennox–Gastaut syndrome, migraine prophylaxis	Broad use for focal and generalized seizures, first-line AED, no clinical hepatotoxicity	Somnolence, dizziness, cognitive impairment, speech problems, kidney stones, weight loss
Tiagabine	Novo Nordisk	1996	GABA potentiation	Partial seizures	No clinical hepatotoxicity	Currently for adjunctive use only; not useful for absence or myoclonic seizures
Levetiracetam	UCB Pharma	2000	SV2A modulation	PGCS, partial seizures, GTCS, JME	First-line AED, i.v. use, no clinical hepatotoxicity	Not useful for absence or myoclonic seizures
Zonisamide	Elan/Eisai	2000	Sodium channel blocker	Partial seizures	Broad use for focal and generalized seizures, no clinical hepatotoxicity	Currently for adjunctive use only; acts as a sedative
Stiripentol	Biocodex	2002	GABA potentiation, sodium channel blocker	Dravet syndrome	No clinical hepatotoxicity	Currently for adjunctive use only
Pregabalin	Pfizer	2004	Calcium channel blocker ( $\alpha 2\delta$ subunit)	Partial seizures, neuropathic pain, generalized anxiety disorder, fibromyalgia	No clinical hepatotoxicity	Currently for adjunctive use only; not useful for absence or myoclonic seizures; weight gain

Table 1 (cont.) | Characteristics of clinically approved AEDs\*

AED	Companies	Year of approval	Presumed main mechanisms of action	Approved indications	Main utility	Main limitations
Rufinamide	Eisai	2004	Sodium channel blockade	Lennox–Gastaut syndrome	No clinical hepatotoxicity	Currently for adjunctive use only
Lacosamide	UCB Pharma	2008	Enhanced slow inactivation of voltage-gated Na <sup>+</sup> channels	Partial seizures	No clinical hepatotoxicity	Currently for adjunctive use only
Eslicarbazepine acetate	Bial/Eisai	2009	Sodium channel blocker	Partial seizures	Adjunctive drug for partial seizures	Enzyme inducer; currently for adjunctive use only
Retigabine (ezogabine)	GlaxoSmithKline	2011	Potassium channel activator	Partial seizures	Adjunctive drug for partial seizures, only when other suitable AEDs have failed	Currently for adjunctive use only; blue colouration of lips and nails; retinal dysfunction; not useful for absence or myoclonic seizures; not in wide use anymore
Perampanel	Eisai	2012	Glutamate (AMPA) receptor antagonist	Partial seizures	Adjunctive drug for partial seizures	Currently for adjunctive use only; not useful for absence or myoclonic seizures

AED, anti-epileptic drug; AMPA,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GTCS, generalized tonic–clonic seizures; i.v., intravenous; JME, juvenile myoclonic epilepsy; NMDA, N-methyl-D-aspartate; NLPK, nonlinear pharmacokinetics; PGCS, partial and generalized convulsive seizures; SV2A, synaptic vesicle glycoprotein 2A. \*For details, see REFS 4, 17, 52, 161. The year of approval indicates the year in which the drug was first approved or marketed in the United States or Europe. †Anti-epileptic effect discovered by clinical observation and subsequently used for the treatment of epilepsy.

spectrum of activity against different seizure types: that is, a ‘one for all’ blockbuster concept. Indeed, some of the most useful drugs in clinical practice are those with broad-spectrum activity. However, none of these broad-spectrum drugs, such as valproate or topiramate, is more efficacious for specific seizure types than narrow-spectrum drugs, and for new-onset complex partial seizures carbamazepine was found to be more efficacious than valproate<sup>4,33,34</sup>. In view of the different mechanisms and possible aetiologies<sup>35</sup> underlying diverse types of seizures or epilepsy syndromes, there is a growing concern that the broad-spectrum concept may not be best suited to identify drugs with higher efficacy in difficult-to-treat patient populations. This view is supported by the fact that several new AEDs have shown highly selective efficacy, such as stiripentol (Diacomit; Biocodex) for Dravet syndrome, vigabatrin (Sabril; Sanofi) for West syndrome or rapamycin for seizures in tuberous sclerosis complex<sup>36</sup> (which are often resistant to broad-spectrum AEDs).

**Problems with clinical trial designs.** Issues with clinical trial designs may have contributed to the lack of progress in the discovery of more effective anti-seizure drugs. Frequent use of clinically irrelevant controls, such as placebo or a substandard dosage of AEDs, has prevented previous trial designs from identifying agents with improved efficacy for drug-resistant epilepsy<sup>4</sup>. Achieving this goal would require a comparative trial design using an optimal dosage of an accepted standard-of-care AED

for the given patient population. Additional concerns associated with placebo controls include the unpredictable and unexpectedly high placebo response rates, which have been held responsible, at least in part, for the failure of new AEDs to show efficacy in placebo-controlled add-on trials<sup>37,38</sup>. In addition, placebo use seems to be associated with an increased rate of unexplained sudden death<sup>39</sup>. Clinical baseline features such as a history of epilepsy surgery or prior lifetime exposure with up to seven or more AEDs have also been shown to be associated with a low placebo response<sup>28,40</sup>, which may maximize the treatment effect of the experimental AED versus placebo. If variations of placebo mechanisms are left uncontrolled, it will be more difficult to document any specific effects of a drug<sup>41</sup>.

Current trial designs have also failed to acknowledge the heterogeneity of disease severity among trial participants with drug-resistant epilepsy. It is well known that clinical features such as lifetime exposure to an increasing number of AEDs are associated with a decreased likelihood of remission in patients with new-onset epilepsy<sup>6,42,43</sup>. Yet, current trial designs do not stratify patients based on disease severity — for example, by the number of prior AEDs the patients have been prescribed. Although the flawed clinical trial designs used at present have led to the identification of many novel AEDs, most were of similar — and some of lesser — efficacy to older AEDs. Another drawback of development programmes is reflected by the frequent lack of clinical trials evaluating

the efficacy and safety of experimental AEDs in individual epilepsy syndromes — particularly those seen first in childhood<sup>44,45</sup> — as well as a lack of well-designed, properly conducted epilepsy trials for patients with generalized seizures and for children in general<sup>46</sup>.

**Anti-epileptogenesis.** Another major unmet medical need is the lack of treatments for preventing epilepsy in patients who are at risk of developing seizures — for example, after epileptogenic brain insults such as traumatic brain injury, stroke and prolonged acute symptomatic seizures such as complex febrile seizures or status epilepticus<sup>47</sup>. Typically, following brain insults there is a seizure-free interval (known as a latent period) lasting a few months to several years before the onset of spontaneous recurrent epileptic seizures. Such a latent period is also typical for genetic epilepsies. The processes occurring during this latent period, which is of variable length in different patients and ultimately leads to chronic epilepsy, are called epileptogenesis<sup>47</sup>. The latent period after brain insults offers a window of opportunity in which an appropriate treatment may prevent or modify the epileptogenic process induced by a brain insult<sup>48</sup>.

Based on this concept, several clinical trials have been carried out to evaluate whether prolonged prophylactic administration of an AED prevents the development of epilepsy after traumatic brain injury, but no beneficial prophylactic effects have been discovered<sup>7,47</sup>. This is not surprising because AEDs have been developed for the symptomatic suppression of seizures and not for the prevention of epilepsy or for disease modification, which — together with the imperfect clinical trial design — probably explains why previous discovery strategies failed to identify anti-epileptogenic drugs. Indeed, the molecular mechanisms underlying epileptogenesis and ictogenesis probably differ, but some mechanisms — such as inflammatory processes — might be relevant for both<sup>47</sup>.

Numerous animal models of epilepsy exist that can be used in the search for anti-epileptogenic or disease-modifying drugs. Previously, amygdala kindling was widely used for this purpose, but most researchers currently prefer post-status epilepticus models of temporal lobe epilepsy, such as the pilocarpine or kainate models<sup>47</sup>. In these models, compounds are evaluated for their anti-epileptogenic potential by administering them during the latent period following status epilepticus before the onset of spontaneous seizures<sup>47</sup>. A major challenge of this approach is that models cannot be validated by a clinically established anti-epileptogenic drug because such compounds do not yet exist. More recently, researchers have started to use traumatic brain injury and genetic models of epilepsy for the evaluation of potential anti-epileptogenic compounds<sup>9,10,49,50</sup>.

However, the concept of a seizure-free, pre-epileptic latent period between brain injury and clinical epilepsy has recently been criticized<sup>51</sup>. Based on observations in post-status epilepticus models of epileptogenesis, Sloviter and Bumanglag<sup>51</sup> suggested that the latent period is a state of 'epileptic maturation' rather than a prolonged period of 'epileptogenesis', and therefore the anti-epileptogenic therapeutic window may be narrow

and treatment may only be effective during the first few days after injury. Thus, the timing of any intervention with these processes will be critical, and this is something that was not fully addressed in earlier clinical trials. However, in the clinic, epileptic maturation — including the progression of epilepsy — may take a few months to several years and so a window of opportunity to modify the disease may be open for a considerable period of time<sup>47</sup>.

### Future AED discovery and development

In view of the various limitations and challenges described above, it is mandatory to revisit conventional AED discovery and development. There is an urgent need for the development of new strategies that can address both the remaining unmet medical needs in epilepsy and also simultaneously provide a favourable business case that can be successfully executed by the pharmaceutical industry. The focus should be on new treatments that address key unmet medical needs: that is, pharmacoresistant epilepsy, comorbidities and epilepsy prevention. Furthermore, treatments that modify the natural history of epilepsy, rendering the disease less progressive and easier to treat, would be highly welcome given that new-onset epilepsy is progressive in as many as one in three patients<sup>5</sup>.

We believe that the marked improvement in our understanding of the complex molecular and cellular alterations leading to epilepsy and recurrent seizures now permits the definition of novel targets for new AEDs<sup>48</sup>, which could not only suppress seizure expression but also affect the underlying pathophysiology of epilepsy, thereby altering the course and prognosis of the disease (anti-epileptogenesis). New druggable targets should be extensively validated by pharmacological and genetic approaches before the onset of substantial drug discovery efforts. To facilitate this goal, major attention should be devoted to biomarker identification and validation (see below), which would allow rapid translation to early clinical proof-of-concept trials. In addition to the traditional models of acute seizures that are used to identify anti-seizure properties, chronic models of epilepsy now exist (FIG. 1 (TIMELINE)) that have already proved to be instrumental in evaluating several of the novel targets described below. In the future, conclusive preclinical trials should derive from properly conducted comparisons, using these models, between new AED candidates and the standard of care (if any).

### New target-driven approaches

The AEDs that are currently approved for the treatment of epilepsy act by diverse mechanisms, mainly involving the modulation of voltage-activated ion channels, potentiation of GABA and inhibition of glutamate receptors<sup>52,53</sup>. Surprisingly, the anti-epileptic efficacy of these drugs in initial add-on trials does not seem to differ substantially, which indicates that seemingly similar anti-seizure activity can be obtained by diverse targets. It has even been debated whether the mechanism of action matters for epilepsy therapy<sup>54–56</sup>. However, as discussed above, many AEDs have a shared mechanism or

#### Anti-epileptogenic drugs

Compounds that, when administered systemically in animal models or to patients immediately following a brain insult, prevent or reduce the long-term consequences of the insult after washout, including the development of epilepsy, neurodegeneration and cognitive or behavioural alterations.

#### Ictogenesis

The complex mechanisms that initiate and maintain a seizure, involving the transition from the interictal (or pre-ictal) to ictal state with abnormal, excessive, hypersynchronous discharges from an aggregate of central nervous system neurons.

#### Disease-modifying drugs

Compounds that alter the development or progression of epilepsy by affecting the underlying pathophysiology and natural history of the disease, thus altering the severity of epilepsy or the development of pharmacoresistance, neurodegeneration and cognitive or behavioural alterations.

#### Temporal lobe epilepsy

A common, difficult-to-treat type of epilepsy that is characterized by simple partial or complex partial seizures originating from medial or lateral temporal lobe regions such as the hippocampus or amygdala.



mechanisms of action, and most AEDs have been identified by screening in seizure models without targeting the specific mechanisms involved in ictogenesis or epileptogenesis. The mechanism of action was only determined after the discovery of anti-seizure effects, and mechanism-driven drug discovery was largely ignored.

We believe that recent progress in our understanding of the mechanisms involved in ictogenesis and epileptogenesis now permits a shift towards target-based drug discovery approaches that are underpinned by validation studies in animal models of refractory epilepsy or epileptogenesis. Systems biology approaches are a promising source for targets, as they take advantage of newer high-throughput technologies to profile large numbers and types of molecules using functional genomics, transcriptomics, epigenomics, proteomics and metabolomics, and they enable the identification of causal pathways from the myriad of competing hypotheses and thus assist in defining candidate targets<sup>57</sup>. Molecular profiling of brain tissues from animal models of epilepsy and from patients with epilepsy also holds promise for identifying new ictogenic and epileptogenic drug targets, and it might be possible to discover a final common pathway of genes that are consistently induced at human epileptic foci<sup>57</sup>. This is supported by the recent identification of various promising pathways and potential drug targets. Some particularly interesting examples, illustrated in FIG. 2, are discussed below.

**mTOR pathway.** The mammalian target of rapamycin (mTOR) signalling pathway regulates cell growth, differentiation, proliferation and metabolism in the brain<sup>58,59</sup>. Loss-of-function mutations in upstream regulators of mTOR have been highly associated with dysplasias and neurodevelopmental disorders<sup>59,60</sup>. These include tuberous sclerosis, where mutations in the genes encoding the tumour suppressors tuberous sclerosis 1 protein (TSC1; also known as hamartin) or TSC2 often result in intractable epilepsy with a poor prognosis<sup>59,60</sup>. Increasing evidence also implicates mTOR dysregulation in the pathogenesis of acquired forms of epilepsy, such as temporal lobe epilepsy<sup>59,60</sup>. In 2008, Zeng *et al.*<sup>61</sup> reported that rapamycin prevents epilepsy in a mouse model of tuberous sclerosis complex, which stimulated a significant interest in rapamycin as a potential anti-epileptogenic compound.

Currently, the clinical use of rapamycin for preventing epilepsy in patients with tuberous sclerosis is being evaluated by a consortium (directed by Martina Bebin at the UAB Tuberous Sclerosis Clinic in Birmingham, Alabama, USA) that is funded by the NINDS (which is part of the NIH) in the United States. Furthermore, the rapamycin analogue everolimus is being assessed for its ability to reduce the frequency of seizures in patients with tuberous sclerosis, and has shown promising preliminary results<sup>60</sup> ([ClinicalTrials.gov](http://ClinicalTrials.gov) identifier: NCT01070316). Thus, therapeutic intervention in the mTOR pathway may lead to both anti-epileptic and anti-epileptogenic drug candidates if druggable targets with improved tolerability can be identified within this pathway.

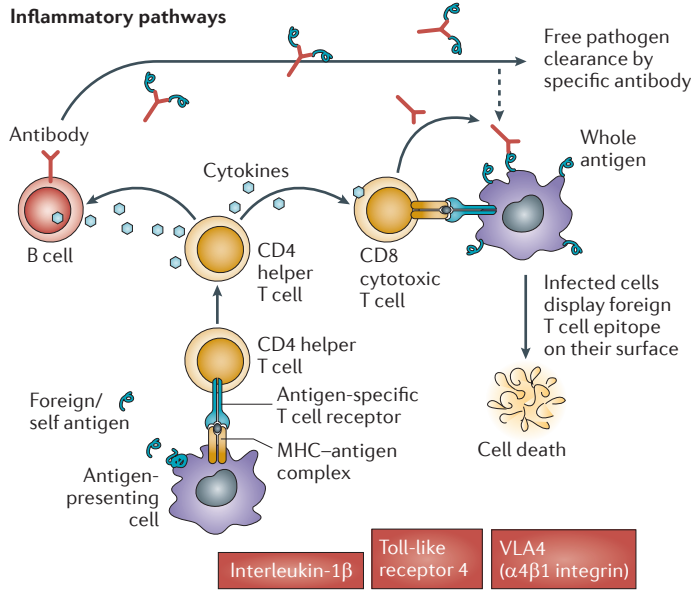
**Cation chloride co-transporters.** Epileptogenic brain insults often lead to a replay of development programmes, resulting in a recapitulation of immature-like transmitter and ion channel functions in brain nuclei that are involved in epileptogenesis. One prominent example is the landmark study of Miles and co-workers<sup>62</sup>, who reported that hippocampal slices from patients with temporal lobe epilepsy generated interictal paroxysmal activity that was attributable to depolarizing GABA<sub>A</sub> receptor-mediated transmission in a subpopulation of principal neurons. This shift from inhibitory to excitatory GABA-mediated transmission was later shown to be related to changes in the intracellular chloride concentration caused by altered expression of the cation chloride co-transporters electro-neutral potassium chloride co-transporter 2 (KCC2; also known as SLC12A5) and bumetanide-sensitive sodium-(potassium)-chloride co-transporter 1 (NKCC1; also known as SLC12A2)<sup>63</sup>. In neonates, increased expression of the chloride inward transporter NKCC1 and decreased expression of the chloride outward transporter KCC2 is also associated with excitatory GABA<sub>A</sub> receptor-mediated transmission, which is thought to explain why neonatal seizures are resistant to GABA-potentiating AEDs such as phenobarbital or diazepam<sup>64</sup>.

In a rat model, Dzhala *et al.*<sup>64</sup> reported that neonatal seizures can be blocked by the NKCC1 inhibitor bumetanide, which formed the basis for two large ongoing proof-of-concept clinical trials with bumetanide in children with neonatal seizures in the United States and Europe (US Food and Drug Administration investigational new drug number: 101690; [ClinicalTrials.gov](http://ClinicalTrials.gov) identifier: NCT00830531; European Union Seventh Framework Programme [NEMO](http://www.nemo-project.eu) ('treatment of neonatal seizures with medication off-patent: evaluation of efficacy and safety of bumetanide')). Furthermore, using a rat model in which epilepsy develops in adult animals after inducing complex febrile seizures at postnatal day 11, Koyama *et al.*<sup>65</sup> recently reported that treatment with bumetanide after the induction of febrile seizures prevented the development of epilepsy, which indicates that bumetanide has an anti-epileptogenic effect. The Löscher group<sup>66</sup> demonstrated that bumetanide, in combination with phenobarbital, also exerts disease-modifying activity in an adult rat model of epileptogenesis, and the first anecdotal reports indicate that bumetanide may be useful in adult patients with pharmacoresistant partial epilepsy<sup>67</sup>.

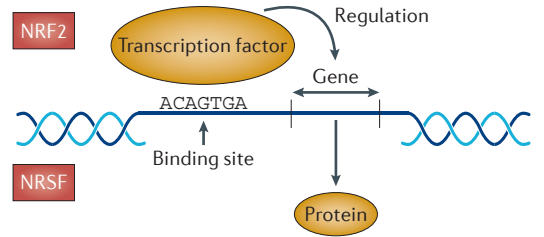
However, the highly potent diuretic effect of bumetanide limits its chronic use and can lead to hypokalaemic alkalosis, which may promote seizures<sup>63</sup>. Furthermore, bumetanide is highly ionized at physiological pH, so it only poorly penetrates into the brain<sup>66</sup>. These problems can be resolved by designing bumetanide derivatives that specifically target the brain. Novel bumetanide derivatives with decreased diuretic properties but increased anti-epileptic and disease-modifying efficacy are being developed<sup>63</sup>.

**Inflammatory pathways.** A rapidly growing body of evidence indicates that inflammatory mediators released by brain cells and peripheral immune cells are involved in both the origin of individual seizures and in

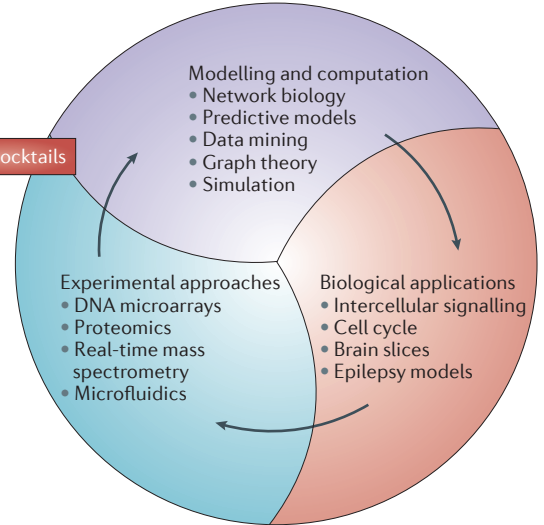
**Inflammatory pathways**



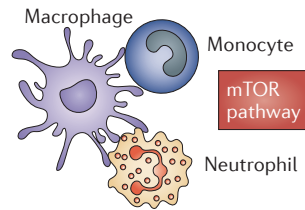
**Transcription factors**



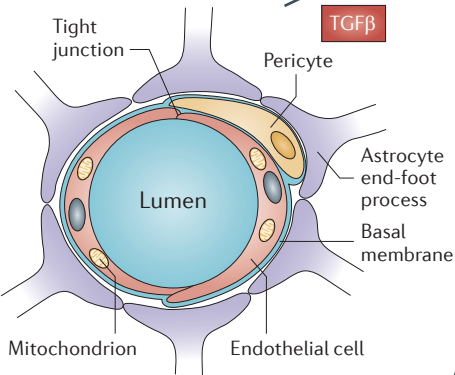
**Systems biology (network) approaches**



**Immune functions**



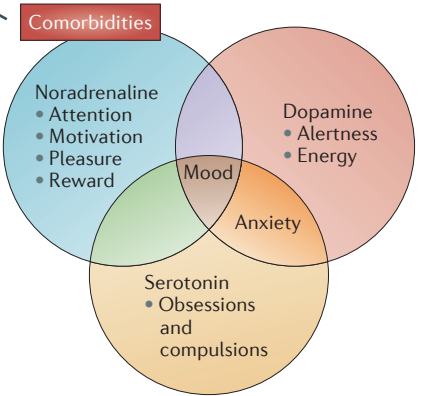
**Blood-brain barrier**



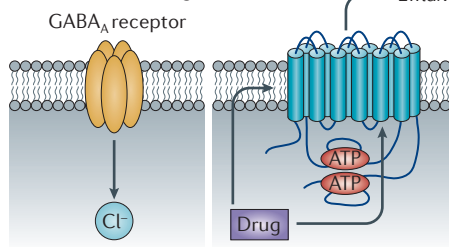
AED targets, transporters and others

NKCC1

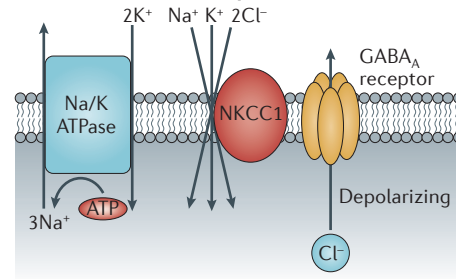
**Monoaminergic system**



**Mechanisms of drug resistance**



**Cation chloride co-transporters**



◀ **Figure 2 | Novel anti-epileptic or anti-epileptogenic drug targets.** Examples of novel targets that are particularly interesting for the development of anti-epileptic drugs (AEDs) or anti-epileptogenic drugs are shown. All of the targets and approaches illustrated in the figure are described and discussed in the main article. In short, accumulating evidence suggests that inflammatory pathways are involved in both epileptogenesis and ictogenesis, which makes anti-inflammatory drug targets promising for new epilepsy therapies. The same is true for treatments that target the immune system, with the mammalian target of rapamycin (mTOR) pathway as one example. Blood–brain barrier dysfunction may participate in epileptogenesis, which indicates that treatments targeting the mechanisms of this dysfunction may offer a novel strategy for preventing or modifying the development of epilepsy. Mechanisms of drug resistance (BOX 2) involve alterations in the structure and/or functionality of AED targets, such as GABA<sub>A</sub> (γ-aminobutyric acid type A) receptors or voltage-dependent sodium channels, but they also involve increases in the expression and functionality of drug efflux transporters such as P-glycoprotein at the blood–brain barrier, which lead to insufficient AED concentrations in the brain. Pharmacological modulation of these or other mechanisms of drug resistance (BOX 2) may counteract AED resistance in epilepsy. Cation chloride co-transporters, such as the bumetanide-sensitive sodium-(potassium)-chloride co-transporter 1 (NKCC1), can undergo dramatic changes in expression within epileptic brain tissue, causing a shift from hyperpolarizing to depolarizing GABA currents in adult neurons, which may crucially contribute to the chronic hyperexcitability of epileptic neurons. NKCC1 inhibitors such as bumetanide represent interesting proof-of-concept tools for determining whether NKCC1 is a suitable target for seizure control or disease modification. The monoaminergic system has a dominant role in psychiatric diseases, including mood disorders, anxiety and psychoses, but is also involved in regulation of the seizure threshold. Thus, noradrenergic, dopaminergic or serotonergic neurotransmission offer promising targets for new epilepsy therapies that not only block seizures but also reduce comorbidities of epilepsy. Systems biology (or network) approaches allow the development of drugs or drug combinations that target the complex alterations underlying epileptic networks in the brain by acting on different proteins or pathways involved in this network. Preclinical findings show that the network pharmacology approach is often more effective and associated with fewer adverse effects than treatments acting on a single protein or on individual biochemical pathways. Similar to drugs or drug combinations that act on several targets within a network, such a network effect can be also obtained by targeting single transcription factors that modulate several pathways that are altered in the epileptic brain. MHC, major histocompatibility complex; NRF2, nuclear factor erythroid 2-related factor 2; NRSF, neuron-restrictive silencer factor; TGFβ, transforming growth factor-β; VLA4, very late antigen 4.

the epileptogenic process<sup>68–70</sup>. Clinical evidence, particularly in children, indicating that steroids and other anti-inflammatory treatments displayed anticonvulsant activity in some drug-resistant epilepsy syndromes provided the first evidence for a potential role of inflammation in human epilepsy<sup>69</sup>. Additional evidence came from febrile seizures, which always coincide with (and are often caused by) a rise in the levels of pro-inflammatory cytokines<sup>71</sup>.

Chronic brain inflammation, which comprises the activation of microglia, astrocytes, endothelial cells of the blood–brain barrier (BBB) and peripheral immune cells, as well as the concomitant production of inflammatory mediators, was first observed in patients with Rasmussen encephalitis<sup>72</sup>. Since then, evidence has emerged that alterations in immune and inflammatory pathways might be a consequence as well as a cause of different types of epilepsy, including temporal lobe epilepsy<sup>69</sup>. A key player in this respect is the cytokine interleukin-1β (IL-1β), which is produced by glia (microglia and astrocytes), endothelial cells of the BBB

and leukocytes, and contributes to alterations in the BBB, neuronal injury and the hyperexcitability of neurons during epileptogenesis<sup>69,71</sup>. The induction of IL-1β-converting enzyme (ICE; also known as caspase 1) and the activation of the IL-1β–IL-1R1 (IL-1 receptor 1) axis both occur in human epilepsy and contribute to experimentally induced acute seizures<sup>73</sup>.

Inhibition of IL-1 biosynthesis by the selective ICE inhibitor VX-765 reduced acute seizures and drug-resistant chronic epileptic activity in mice<sup>74</sup>. A proof-of-concept trial in patients with refractory partial-onset seizures suggested a possible clinical efficacy of VX-765, which triggered a double-blind, placebo-controlled, multicentre, international study in AED-resistant patients with partial-onset seizures. However, Vertex recently made a business-related decision to stop further enrolment in this study<sup>75</sup>. Furthermore, the human recombinant IL-1β receptor antagonist anakinra (Kineret; Amgen), which is approved for the treatment of rheumatoid arthritis, rapidly terminated seizures, prevented their recurrence and resolved seizure-associated BBB breakdown in animal models<sup>76–78</sup>. Combinations of VX-765 and anakinra are currently being evaluated for their anti-epileptic and anti-epileptogenic effects in animal models.

Another potentially interesting target is Toll-like receptor 4 (TLR4), which is a key receptor of innate immunity<sup>79</sup>. TLRs, which are transmembrane proteins that are expressed by immunocompetent cells such as antigen-presenting cells, share common cytoplasmic domains with the IL-1R family and use partially overlapping signalling molecules with IL-1R1 (REF. 73). The activation of IL-1R–TLR signalling in neurons and glia is thought to be pivotal for initiating the inflammatory brain response following seizures or epileptogenic brain insults<sup>73</sup>. Antagonists of TLR4 retard seizure precipitation and decrease acute and chronic seizure recurrence in rodents<sup>79</sup>. Furthermore, Fabene *et al.*<sup>80,81</sup> have suggested that leukocyte–endothelial adhesion mechanisms have a role in epilepsy, and leukocyte integrins such as very late antigen 4 (VLA4; also known as α4β1 integrin) may also constitute novel drug targets. The adhesion molecule antagonist natalizumab (Tysabri; Elan/Biogen Idec) would be an interesting probe compound in this respect; natalizumab binds to VLA4, which is expressed on the surface of immune cells, and inhibits VLA4-dependent transmigration of circulating immune cells across the vascular endothelium into the brain<sup>82</sup>.

**Blood–brain barrier breakdown.** Dysfunction of the BBB is a hallmark of epileptogenic brain injuries, regardless of their aetiology<sup>83–85</sup>. Damage to the BBB microvasculature during brain insults leads to extravasation of serum albumin into the cerebral cortex microenvironment, which activates a transforming growth factor-β receptor (TGFβR)-mediated signalling cascade in astrocytes and causes local inflammation<sup>70,86</sup>. Astrocytic dysfunction results in impaired homeostasis of the extracellular brain environment, which leads to enhanced neuronal excitability. Blockade of TGFβ signalling in the albumin model of epileptogenesis reversed

**Blood–brain barrier (BBB).** 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. It is formed by highly specialized endothelial cells that line brain capillaries and are connected by extensive tight junctions that restrict paracellular penetration of compounds.

inflammation and transcriptional patterns associated with activated glia and prevented the development of epileptiform activity<sup>87</sup>, which indicates that TGF $\beta$  represents an interesting novel target that interferes with epileptogenesis. Recent data suggest that losartan, which is a clinically used angiotensin II type 1 receptor blocker that also antagonizes TGF $\beta$  signalling, is an interesting probe compound in this respect (A. Friedman, personal communication).

### Genetic and epigenetic targets

Given the complex pathophysiology of epilepsy, targeting the epigenetic mechanisms involved in transcriptional regulation seems to be an attractive option for therapeutic intervention<sup>48,88,89</sup>. In fact, it has recently been demonstrated that targeting a single molecular entity that modulates multiple molecular pathways by transcriptional repressors, such as neuron-restrictive silencer factor (NRSF; also known as REST), or via epigenetic mechanisms, offers new strategies for epilepsy therapies<sup>90–92</sup>. Targeting transcription factors may also have similar therapeutic potential. For instance, nuclear factor erythroid 2-related factor 2 (NRF2; also known as NFE2L2), which is an important transcription factor that is involved in orchestrating the cellular response to oxidative stress, was found to be activated in hippocampal tissue from patients and mice with temporal lobe epilepsy<sup>93</sup> and was the most highly connected gene in the hippocampus of animals with kainate-induced seizures in a systems-level functional genomic analysis of chronic epilepsy<sup>94</sup>. NRF2 was previously found to be differentially expressed in the lesioned versus non-lesioned hippocampi in animals with seizures, and *Nrf2*-knockout mice were more sensitive to kainate-induced seizures, which indicates a role for NRF2 in the neural cell defence response of the adult brain<sup>89,95</sup>.

Further support for the therapeutic potential of NRF2 in epilepsy was recently obtained in a study showing that overexpression of NRF2 via an adeno-associated virus vector, after the onset of recurrent and spontaneous seizures following pilocarpine-induced status epilepticus in mice, resulted in a significant reduction in seizures, microglial activation and loss of hippocampal neurons<sup>93</sup>. Dimethyl fumarate (BG-12), which is a compound that upregulates NRF2, exerts neuroprotective effects and has proven to be effective in multiple sclerosis, would be an interesting probe compound for further exploring the role of NRF2 in epilepsy<sup>96</sup>.

Parallel analysis of differentially expressed genes provides an unbiased approach for identifying the genes and pathways that are associated with complex disease aetiologies, and it allows the identification of key regulatory networks that are likely to be modulated by transcription factors<sup>97</sup>. In models of acquired epilepsy, alterations in gene expression appear to be time-specific and underlie various processes that are linked to epileptogenesis, such as cell death and survival, neuronal plasticity or immune responses<sup>98</sup>. Thus, genetic and epigenetic alterations in epilepsy are interesting sources for the identification of new targets for both seizure suppression and anti-epileptogenesis.

### Network pharmacology

Most epilepsies do not develop from alterations in a single target; rather, they arise from complex alterations resulting in an epileptic network in the brain<sup>99,100</sup>. The only existing cure for epilepsy in suitable patients is resective surgery, in which the regional epileptic network or part of this network is removed<sup>101</sup>. Thus, single-target treatments that focus exclusively on a single protein or individual biochemical pathway may be less effective than multiple-target treatments that act on different proteins or pathways involved in the network. The latter approach — multi-target treatment — has been recently termed ‘network pharmacology’ or ‘pleotherapy’ and relates to principles of systems biology<sup>102,103</sup>. The principle of network pharmacology is to develop combinations of existing drugs or novel drugs that modulate several mechanisms and regulate activity via different targets within a biological network, to treat diseases that do not sufficiently respond to single-target treatments or for which no treatment yet exists.

Systems biology-based approaches of network pharmacology have recently been proposed for the development of anti-epileptic and anti-epileptogenic treatments<sup>47,57,104</sup>, and some drug combinations have demonstrated substantial synergy and are strikingly more effective in models of seizure and epilepsy than each compound alone<sup>66,105–107</sup>. This approach does present both opportunities and complications for intellectual property rights and commercialization. For existing drugs, the benefits include diminished development time and costs by repurposing. Indeed, interest in the pharmaceutical industry does appear to exist and large pharmaceutical companies, biotech companies and academic laboratories are forming consortia for network pharmacology<sup>103</sup>. In addition to combining drugs in network pharmacology, an alternative option is one target that modulates several pathways — as illustrated by the mTOR pathway or NRF2 and other transcription factors discussed above (FIG. 2).

Network pharmacology could also tackle another challenge of drug development — namely, the fact that many drugs work on only a subset of patients. By performing network analysis of individual patients, inter-individual variations of disease mechanisms can be identified. This will enable clinical trials to be carried out in groups of patients who share the same underlying molecular condition<sup>103</sup>. This concept is extremely interesting for personalizing epilepsy treatment, not only because of inter-individual differences in the pathophysiology of epilepsy but also because of inter-individual differences in the mechanisms of resistance to AED treatment<sup>108</sup>. For instance, by using multimodal brain imaging, patients with a specific mechanism of resistance can be identified and treated with a drug targeting this mechanism<sup>109–111</sup>. Indeed, biomarkers for a specific mechanism of pharmacoresistance would be required before the clinical trial investigator could effectively recruit a sufficiently large enough population of patients to a trial that would prove the concept.

### Targeting specific types of seizures or epilepsy

One way to provide added clinical value of new AEDs is to develop highly effective compounds for specific types of seizures or rare epilepsy syndromes. This has

**Comparator drug**

A current standard-of-care drug given at a recommended daily dose for the same setting as the intended use of the test drug with an absolute minimum point estimate for an efficacy or effectiveness of 50%; this prevents the use of well tolerated but inefficacious drugs as comparators.

**P-glycoprotein**

A well-characterized efflux transporter that transports a variety of substrates across extra- and intracellular membranes, including endothelial cells of the blood–brain barrier, and protects cells from intoxication by potentially harmful lipophilic compounds, thereby restricting the distribution of many therapeutically used drugs.

been convincingly shown in the past after the introduction of vigabatrin for West syndrome<sup>44</sup> and stiripentol for Dravet syndrome<sup>45</sup>, albeit long after the drugs were tested for common types of seizures. The development of drugs that target syndrome-specific mechanisms and are tested in syndrome-specific models — for example, Dravet syndrome or infantile spasms — provides a basis for syndrome-specific clinical trials and for targeting specific types of seizures. Although it may be challenging to show effects on specific types of seizures in epilepsy syndromes, AEDs that are specifically effective in the treatment of very disabling types of seizures such as drop attacks or tonic seizures would address a significant unmet medical need and provide an attractive business case. For example, there is an increased risk of death in children with infantile spasms and Lennox–Gastaut syndrome<sup>112</sup>, which may in part be due to the poor efficacy of current drug treatment. The development of drugs that are specific for these syndromes may have a great impact on mortality, morbidity and injury rates and therefore present a compelling business case.

An additional methodological and statistical issue has been to provide substantial evidence for the effect of the treatment on generalized tonic–clonic seizures versus other types of seizures in focal epilepsy<sup>113</sup>. Furthermore,

specific types of seizures may not be observed during the baseline phase but may be revealed during the treatment phase. The concern of seizure aggravation arising as a result of an AED's novel mechanism of action can be assessed by analysing the comparator drug or placebo control<sup>38</sup>.

**Targeting mechanisms of pharmacoresistance**

Pharmacoresistance constitutes a major challenge in the management of epilepsy, and its mechanisms still remain to be fully elucidated<sup>108,114–116</sup>. Current theories on the causes of drug resistance in epilepsy include the transporter hypothesis, the target hypothesis, the network hypothesis, the gene variant hypothesis and the intrinsic severity hypothesis (BOX 2; FIG. 3). However, none of these hypotheses is currently a stand-alone theory that is able to convincingly explain how drug resistance arises in human epilepsy<sup>116</sup>.

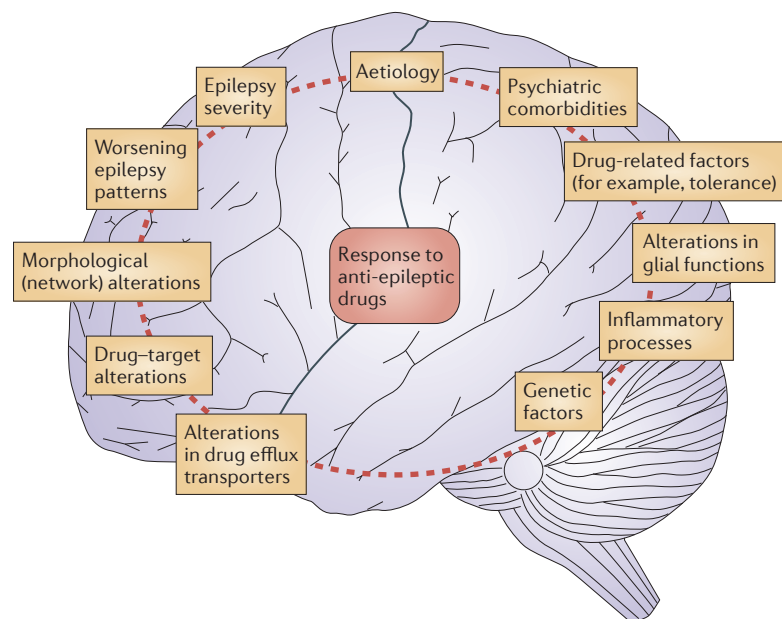
Experimental and clinical evidence has accumulated for the transporter hypothesis, which suggests that increased expression of efflux transporters at the BBB in focal tissue limits the penetration of AEDs to the focus<sup>108,117</sup>. A proof-of-concept clinical trial with an inhibitor of the efflux transporter P-glycoprotein (also known as MDR1 or ABCB1) reversed resistance to AED treatment in a rat

**Box 2 | Mechanisms of resistance to anti-epileptic drugs**

Drug-resistant epilepsy is defined as follows: a failure of adequate trials of two tolerated, appropriately chosen and commonly used anti-epileptic drug (AED) schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom<sup>147</sup>. Using the same definition, several animal models of pharmacoresistant epilepsy, such as the phenytoin-resistant kindled rat<sup>148</sup> or the phenobarbital-resistant epileptic rat<sup>149</sup>, have been developed and used to determine potential mechanisms of drug resistance<sup>21</sup>. Such models are ideally suited for studying mechanisms of AED resistance because AED responders and non-responders can be selected from the same model and directly compared. Several findings in these models are in line with clinical findings in patients with AED-resistant seizures, including a high frequency of spontaneous seizures prior to the onset of AED treatment, psychopathology and hippocampal damage as poor prognostic factors for treatment, alterations in AED targets and transporters in resistant individuals and a role of genetic factors (FIG. 3).

Based on these findings in animal models and patients, five hypotheses have been proposed that may — at least in part — explain resistance. The first is the transporter hypothesis, which suggests that inadequate penetration of AEDs across the blood–brain barrier (caused by increased expression of efflux transporters such as P-glycoprotein) leads to insufficient drug levels in epileptogenic brain tissue. The second is the target hypothesis, which suggests that acquired alterations to the structure and/or functionality of target ion channels and neurotransmitter receptors lead to insufficient pharmacodynamic activity of AEDs in the brain. The third is the network hypothesis, which proposes that structural brain alterations and/or network changes (for example, hippocampal sclerosis) are involved in resistance to AEDs. The fourth is the gene variant hypothesis, which suggests that there is an inherent resistance that is governed by genetic variants of proteins that are involved in the pharmacokinetics and pharmacodynamics of AED activity. The fifth is the intrinsic severity hypothesis, which suggests that an increased disease severity leads to drug intractability<sup>21,108,114,115,121,150</sup>.

Clinical proof of concept has been achieved for the network hypothesis in that surgical resection of the altered network counteracts AED resistance and may even cure epilepsy<sup>101</sup>. Preclinical proof of concept has also been obtained for the transporter hypothesis in that inhibiting the efflux transporter P-glycoprotein counteracted resistance to AEDs in a rat model of pharmacoresistant temporal lobe epilepsy<sup>118</sup>. Evidence for the other hypotheses, including the popular target hypothesis, is currently more limited. A reduced sensitivity of major targets for many of the clinically established AEDs<sup>32</sup>, such as the voltage-gated sodium channel and the GABA<sub>A</sub> (γ-aminobutyric acid type A) receptor, has been suggested to have a role in AED-resistant chronic human and experimental epilepsy<sup>114,115</sup>. Thus, AEDs acting through other targets that are not downregulated in epilepsy may offer substantial advantages and promise for future AED discovery. A complicating factor for strategies that are aimed at developing more effective therapies is the possibility that AED resistance is not caused by a single mechanism but is instead due to several mechanisms, which may even occur in the same patient<sup>114</sup>. Overcoming AED resistance represents a challenge and will necessitate the combined efforts of basic and clinical epilepsy researchers.



**Figure 3 | Possible determinants of AED resistance in human and experimental epilepsies.** In recent years, various potential mechanisms of anti-epileptic drug (AED) resistance or factors predicting poor outcome have been implicated in patients with epilepsy and in animal models of drug-resistant seizures, which indicates that intrinsic or acquired resistance to AEDs is a multifactorial phenomenon. Based on these findings, various hypotheses have been proposed to explain AED resistance, including the target, transporter and network hypotheses. These hypotheses are not mutually exclusive but may be relevant for the same patient, thus complicating any strategy to counteract or reverse pharmacoresistance. For further detail see the main article, BOX 2 and REF. 116.

model of pharmacoresistant temporal lobe epilepsy<sup>118</sup>. A recent clinical study involving positron emission tomography (PET) imaging of AED-resistant patients showed that ~40% of the patients had increased P-glycoprotein functionality in the epileptic focus, which demonstrates that these patients are likely to benefit from P-glycoprotein inhibition<sup>111</sup>. However, as several AEDs are apparently not substrates of P-glycoprotein<sup>119,120</sup>, other mechanisms seem to contribute to the overall problem of AED resistance (BOX 2).

The target and gene variant hypotheses (BOX 2) suggest that genetic or acquired alterations in protein expression (for example, in voltage-gated ion channels or neurotransmitter receptors) govern drug resistance; the validity of these hypotheses is supported by several studies that have reported specific mutations and expression profiles of genes and proteins in experimental models and tissue samples obtained by surgical resection from patients who have shown AED resistance<sup>121</sup>. These studies could be applied in the context of the network hypothesis, which suggests that structural brain alterations or network changes are involved in drug-refractory epilepsy. Together, these hypotheses hold interesting potential for combining relevant molecular targets in improved synergistic treatments, which is relevant for the complex pathophysiology of drug-refractory epilepsy. Indeed, a vast number of reports in the literature have shown the striking benefits of combination therapy with existing

AEDs in preclinical models<sup>122,123</sup>, but clinical validation of these combinations has not yet been demonstrated. This is probably because most of these studies have been conducted in simple seizure models and focused on improving the potency of seizure protection, as determined by isobolographic analysis<sup>124</sup>. As insufficient seizure control is the principal unmet need in drug-refractory epilepsy, this is likely to represent the key reason why these preclinical studies have not yet translated into an improved outcome of rational combination therapy in patients, perhaps with the exception of combined treatment with lamotrigine and valproate<sup>125</sup>.

Unfortunately, appropriately designed clinical trials involving a combination therapy (for example, one that aims to determine the best or most optimal AED combination in the patient population of interest) have never been attempted in patients with drug-resistant epilepsy. This is a case where translation of data from patients back to the animal models could be very informative for preclinical research. Future drug discovery efforts should identify genes and proteins that are inherent to the refractory condition and then rationally assess synergistic interactions that improve efficacy in animal models of drug-refractory epilepsy with the aim of identifying major treatment benefits. The cellular and molecular alterations involved in the progression of epilepsy (or in ongoing epileptogenesis) may also contribute to pharmacoresistance in chronic epilepsy<sup>114</sup>.

#### Developing AEDs with fewer adverse effects

The adverse effects of AEDs are common, they can have a considerable impact on the quality of life and they contribute to treatment failure in up to 40% of patients<sup>126</sup>. These adverse effects include issues with CNS tolerability, hypersensitivity reactions and weight gain. Modern AEDs manifest these adverse events to varying degrees but all AEDs exhibit issues with CNS tolerability<sup>127</sup>. This is probably because all current AEDs have been developed to counteract the hyperexcitability of neurons by targeting mechanisms that also interfere with normal neurotransmission; this is why they all — to a large extent — have similar issues associated with CNS tolerability as doses are increased<sup>4,127</sup>. Furthermore, the classical preclinical screening models such as the MES and PTZ tests have consistently selected drugs with significant CNS side effects, apparently as a result of these models identifying compounds with specific molecular targets<sup>128</sup>. The only exception seems to be levetiracetam, which was devoid of anticonvulsant activity in the conventional screening models and has been shown to be well tolerated in preclinical testing and clinical studies<sup>31</sup>. Targeting mechanisms that specifically address the pathology for drug resistance or the progression and maintenance of the disease, as proposed in this Review, has the potential to improve the CNS tolerability of future therapies.

Another important aspect that may also help to develop better-tolerated AEDs is that epilepsy is associated with multiple changes in the function and subunit composition of ion channels and receptor molecules. This may not only result in the loss of efficacy of drugs acting on such targets but also change their adverse effect

**Box 3 | New clinical trial designs for refractory epilepsy**

Active-control trial designs (in which the new drug is directly compared to a standard anti-epileptic drug (AED)) in an add-on setting have several important advantages; they avoid the disadvantages associated with placebo-controlled trials (discussed in the main article) and allow a better assessment of sustained efficacy over 6–12 months compared to the 3-month trial period that is typical for clinical trials with exposure to placebo. Most importantly, they provide information on whether the new drug is superior, inferior or similar to an established AED.

Although active-control trials are informative, they may be associated with challenges regarding patient recruitment, dose selection<sup>38</sup> and a no-difference outcome<sup>151</sup>. Concerns have been voiced that active-control trials may set the bar too high unless you have a very efficacious drug. This, however, is exactly the kind of drug that is needed to address the needs of patients with seizures that are uncontrolled by current medications. Another concern is that one could discover a drug with a high efficacy resulting in seizure freedom in 20% or more patients in a placebo-controlled trial, which would therefore not require an active control. Although very welcome, such a result cannot provide much needed direct evidence for the added benefit of the drug over existing AEDs. Finally, concerns have been raised about the longer duration of active-control trials. They would involve freezing dosages of concomitant treatments, which may be difficult for 6–12 months, except in those patients in whom seizure freedom is achieved or unless one introduces escape criteria.

These issues are markedly counteracted by the benefit of conducting early Phase IIA studies involving superiority trials, which can determine whether a signal for the superior efficacy of a new AED candidate exists before advancing to costly Phase III trials (FIG. 4).

Given the disadvantages of placebo, efforts are underway to de-emphasize its use in clinical trials of epilepsy<sup>28,41</sup>. Novel trial designs, such as the time to nth add-on seizure design versus placebo in refractory epilepsy, minimize placebo exposure and give rapid answers about the efficacy of the treatment without keeping non-responders in the clinical trial<sup>28</sup>. The active-control trial design can be supplemented by adding a placebo arm if this is deemed necessary by regulatory authorities (see the ‘Guidance for Industry’ document on the US Food and Drug Administration website) for confirmatory Phase III studies. If backed by a positive signal for superior efficacy from early Phase IIA studies, this additional investment is not likely to discourage drug sponsors.

profile<sup>128–131</sup>. An early example illustrating this problem is that of competitive antagonists of the NMDA (*N*-methyl-D-aspartate) subtype of glutamate receptors, which were well tolerated in healthy volunteers but induced serious CNS adverse effects in patients with epilepsy<sup>130</sup>. These NMDA receptor antagonists had an enhanced potential to induce severe adverse effects in epilepsy, which was correctly predicted in kindled rats (a chronic model of epileptogenesis) but not in non-epileptic rodents<sup>129,130</sup>. Thus, kindled or epileptic animal models should be included in preclinical testing for adverse effects<sup>128,131</sup>. For a comprehensive assessment of the drug-induced impact on CNS function, models beyond the classical rotarod test should be used.

A difficult issue relates to the risk of serious adverse events that may only be discovered at a late stage in the adoption of new AEDs, such as idiosyncratic events or toxic effects that are difficult to identify and predict from preclinical development programmes. Felbamate (Felbatol; MedPointe), vigabatrin and, most recently, retigabine are relevant examples. With respect to such adverse effects, the emerging evidence for the role of polymorphisms will certainly have a positive impact and could result in the development of personalized medicines.

**Developing AEDs targeting major comorbidities**

Independently of seizure control, patients with epilepsy often suffer from substantial cognitive impairment and psychiatric comorbidity associated with significantly increased mortality<sup>3</sup>. Although some of the marketed AEDs such as lamotrigine are useful in the prophylaxis of bipolar disorders<sup>132</sup>, none of the current AEDs has been shown to effectively reduce the incidence of

epilepsy-associated depression or anxiety, and some (but not all) AEDs can be associated with treatment-emergent psychiatric problems that can lead to suicidal ideation and behaviour; the actual suicidal risk has yet to be established but it seems to be very low<sup>133</sup>. Thus, a promising avenue for future AED discovery and development is to focus on mechanisms that suppress seizures and reduce comorbidities<sup>134</sup>. A case in point is huperzine A, a dual inhibitor of acetylcholinesterase and glutamate (NMDA) receptors, which is in clinical development and being explored for its potential to improve cognitive performance beyond seizure suppression<sup>135</sup>.

Another discovery approach to consider is to target a single mechanism that is involved in both seizure generation and comorbidities. This is illustrated by naluzotan, a selective 5-hydroxytryptamine 1A (5-HT<sub>1A</sub>) receptor agonist, which is also in clinical development with a potential to induce both seizure suppression and antidepressive effects (see the [Proximagen pipeline](#) for further information). Indeed, the potential of naluzotan is consistent with several recent experimental studies showing that the monoaminergic system modulates mechanisms of seizure generation as well as depression and anxiety<sup>136–138</sup>.

A further strategy to overcome competition in the AED market could be to first obtain approval for a new AED for diseases that currently appear to have more attractive market potential, such as bipolar disorder or neuropathic pain, and to subsequently obtain approval for epilepsy. AEDs that are approved for neuropathic pain and bipolar disorder can effectively reduce the incidence of comorbidities associated with epilepsy. However, major downsides of this strategy are that the development of new AEDs would depend on advances

**Active-control trial**

A clinical trial design comparing the outcome of an experimental compound to a drug whose efficacy has been established.

**Superiority trials**

Studies that are designed to detect a difference in the primary outcome (that is, efficacy and/or effectiveness) between the study treatment and the comparator using an intent-to-treat analysis; the limit of a >20% absolute (rather than relative) difference is arbitrary, context-specific, and subject to change with time.

**Box 4 | New clinical trial designs for epilepsy prevention and disease modification**

Although the task of defining clinical trials for anti-epileptogenesis is difficult without knowing what the intervention would be like, any clinical trial to evaluate treatments that could prevent epileptogenesis prior to the first seizure or to control epileptogenesis in ongoing epilepsy (that is, disease modification) has to meet two essential requirements. First, the clinical trial design has to include a randomized treatment phase versus a control, usually placebo or preferably a standard anti-epileptic drug (AED), to assess anti-seizure effects, if any. Second, and very importantly, a study of anti-epileptogenic effects should be carried out after drug washout<sup>5</sup>. Trials that do not study patients after drug washout cannot differentiate between anti-seizure effects (that is, 'on-drug' seizure reduction) and prevention or modification effects (that is, 'off-drug' seizure reduction)<sup>6</sup>. New clinical trial designs have been used for children with tuberous sclerosis (see main article). It has been suggested that clinical trials in patients who have had a stroke should take into consideration the potential existence of a therapeutic window<sup>47,152,153</sup>. End points include measures of seizure frequency or remission as in conventional anti-seizure trials. However, epilepsy prevention trials are more complex, lengthy and costly than standard anti-seizure treatment trials for many reasons. Issues revolve around the selection of suitable participants, consent for participation, duration of treatment, length of follow-up, and the selection of an appropriate end point<sup>152</sup>. Key parameters of feasible clinical trial designs will need to be adapted to the specific intervention, preferably based on translational data. Most previous anti-epileptogenesis trials with standard anti-seizure drugs that were aimed at preventing epilepsy following traumatic brain injury or stroke have been unsuccessful<sup>7,153</sup>. The failure of these past trials might be related to problems in the patient populations with traumatic brain injury and stroke as well as respective problems associated with clinical trials in such populations<sup>153,154</sup>. Treatment effects for the prevention of epilepsy can be optimized by narrowing down subgroups of populations with the highest risk of developing epilepsy from the following groups: genetically predisposed individuals, as well as patients with traumatic brain injury, stroke, central nervous system (CNS) infections or *de novo* status epilepticus. In addition, data on risks as a function of time after insult in the different patient populations at risk may be helpful in determining whether therapeutic windows exist to optimize the design of prevention trials. As a successful anti-epileptogenic trial design is still largely a terra incognita, alternative approaches to test for the effects of an anti-epileptogenic drug may include disease modification by starting treatment after the first seizure or in patients with drug-resistant epilepsy. Disease modification can also be assessed prospectively in a double blind-design in patients with epilepsy who are seizure-free after surgery and thus plan to discontinue AED treatment.

in therapies for psychiatry and pain, and the resulting delays would reduce the opportunity for return on investment in epilepsy indications. In addition, this may stifle epilepsy research for discovery purposes and is likely to restrain new AEDs to conventional mechanisms such as interfering with an imbalance in hyperexcitability. In addition, the unfortunate stigma associated with epilepsy could possibly lower the acceptance of the drug at least for some patients with neuropathic pain or bipolar disorder.

**Biomarkers to optimize AED development**

Optimal translation of preclinical findings to clinical studies necessitates robust and objective biomarkers that can assess target engagement, the impact of target interaction on downstream biological processes and disease activity, as well as predict the response to therapy<sup>139</sup>. Currently, large research programmes in Europe and the United States have started to search for biomarkers that would: diagnose epileptogenesis (that is, identify individuals who are at a high risk of developing epilepsy after brain insults); predict the severity of epilepsy; and predict therapy responses<sup>88,139,140</sup>. The search for anti-epileptogenic or disease-modifying treatments would be markedly facilitated by the availability of biomarkers that can predict the development and progression of the disease<sup>139</sup>. Given the complexity of epilepsy, it is unlikely that a single biomarker will be sufficient for predicting epileptogenesis; rather, a combinatorial approach may be necessary to identify appropriate biomarkers at different stages of the evolution of the disease.

Potential biomarkers that need to be validated experimentally and clinically in this respect include blood biomarkers of brain injury, inflammation and BBB damage, microRNA and epigenetic factors, biomarkers resulting from multimodal brain imaging, including magnetic resonance imaging (MRI) and PET, as well as remote sensing technologies such as actigraphy and ambulatory three-point electroencephalography (EEG) or electrocardiography (ECG)-dependent algorithms to supplement subjective seizure counts. Whether utilizing objective seizure count measures will reduce the placebo responder rate or its variation in clinical trials remains to be seen. Potentially useful EEG alterations include pre-ictal and interictal spikes as well as high-frequency oscillations (known as ripples)<sup>141</sup>. Bioinformatics and network-based systems biology approaches, as already used in neurotrauma and Alzheimer's disease research<sup>142,143</sup>, will need to be applied to identify the most predictive combination of biomarkers for the various types of epilepsy.

**New clinical trial design**

Future clinical trial design for epilepsy drugs should determine drug efficacy (preferably by objective seizure counts) during early stages of clinical development, demonstrate superiority to the standard of care at the optimum dosage and be capable of assessing the ability of new drug candidates to prevent epilepsy prior to the first or second seizure in those individuals who are at risk of developing epilepsy. After the onset of seizures, clinical trial designs are available to test whether new



Stage	Key activities
Target identification	Identification of novel targets and/or repurposing of compounds with novel mechanisms from other therapeutic areas
Target validation	Genetic validation by transgenic animals and/or pharmacological validation with relevant probe compounds
Hit identification, hit-to-lead, lead optimization	Drug discovery searching for hits and translation of these into leads with drug-like properties
Candidate selection	Selection of candidates with optimal drug-like properties, including confirmation of target validation by comparative preclinical proof-of-concept studies
Preclinical development	Conventional GLP-driven programme to permit onset of Phase I studies, including preclinical studies with relevant PET ligand and validation of biomarkers
Phase I and initial proof-of-concept 'light' studies	Conventional Phase I programme to determine safety, tolerability and DMPK properties, and initial proof-of-concept 'light' studies with PET ligands and biomarkers to assess target engagement and its biological consequences
Phase II proof-of-concept studies	Proof-of-concept study versus comparator and placebo assessing potential for differentiation
Phase III confirmatory studies	Confirmatory studies versus comparator and placebo (optional) to prove superior efficacy for drug approval and marketing authorization

Figure 4 | **Roadmap for the discovery and development of medications for drug-resistant epilepsy and for epilepsy prevention or disease modification.** Following the identification of novel targets or compounds with the potential to be re-purposed, extensive pharmacological and/or genetic validation is required before making the decision to initiate further drug discovery efforts. These efforts aim to identify a preclinical candidate (or candidates) that can subsequently be validated in comparative, preclinical proof-of-concept studies. Translation to Phase I studies involves the use of positron emission tomography (PET) ligands and other biomarkers to assess target engagement and to conduct early, decisive proof-of-concept 'light' studies, which reveal whether a biological consequence of target engagement can be detected by imaging, electroencephalography (EEG) or other biomarkers. This is followed by a comparative, add-on Phase II study in patients, in which the magnitude of the efficacy signal determines the potential of pursuing confirmatory add-on Phase III studies at a later stage, which would involve making a direct comparison between the drug and the standard of care, if any. DMPK, drug metabolism and pharmacokinetics; GLP, good laboratory practice.

drug candidates can positively modify the course of the underlying epilepsy (that is, disease modification). Future development strategies should translate preclinical findings using robust and objective biomarkers in Phase I trials as well as in early and decisive (but 'light'; that is, less costly) clinical proof-of-concept studies. Comparative Phase II trials with the standard of care (if any) should be conducted to permit early de-risking and determination of the differentiation potential before investment in confirmatory Phase III studies. Future clinical trial designs using placebo treatments need to control the variation of the placebo response seen with the traditional clinical trial design<sup>40</sup>. New clinical trial designs for these different aims are described in BOX 3, BOX 4 and FIG. 4.

### Conclusions and future directions

New strategies for the discovery and development of AEDs that also offer a compelling case for industry investment must be pursued in order to provide new and improved treatment options for patients with epilepsy. We propose that recent progress in the understanding of the molecular and cellular events leading to epilepsy now permit a focus on novel target-driven approaches for the discovery of more efficacious and better-tolerated AEDs

and anti-epileptogenic drugs. This may include the repurposing of compounds with novel mechanisms from other therapeutic areas (FIG. 4). Future development strategies should involve validation using comparative, preclinical proof-of-concept studies, as well as translation using robust and objective biomarkers into early, decisive proof-of-concept 'light' clinical studies and comparative Phase II trials, which will allow early de-risking and the determination of the differentiation potential from the standard of care before investment in confirmatory Phase III studies.

A major incentive for the industry to adopt this strategy and to execute it successfully will be the availability of valid and druggable targets, interpretable and target-population-relevant preclinical proof-of-concept studies, disease and target-related biomarkers, diagnostic methodology for the identification of the specific patient populations, and innovative clinical trial designs. Fortunately, various initiatives from major public and private funding bodies in the United States and Europe have recently stimulated a focus on further identification of these tools, and this has led to new concerted efforts between academia and industry. This holds great potential for the revitalization of AED discovery and development, bringing us closer to the ultimate goal of curing epilepsy.

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### Competing interests statement

The authors declare **competing financial interests**: see Web version for details.

### FURTHER INFORMATION

ClinicalTrials.gov website: <http://clinicaltrials.gov>  
 NEMO Europe: <http://www.nemo-europe.com>  
 Proximagen pipeline: <http://ikonix.co.uk/science/pipeline>  
 US Food and Drug Administration 'Guidance for Industry' document (E 10 Choice of Control Group and Related Issues in Clinical Trials): <http://www.fda.gov/downloads/Guidances/UCM073139.pdf>

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