Neurosteroids as Novel Anticonvulsants for Refractory Status Epilepticus and Medical Countermeasures for Nerve Agents: A 15-Year Journey to Bring Ganaxolone from Bench to Clinic

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Abbreviations:
ACh, acetylcholine; AChE, acetylcholinesterase; ASM, antiseizure medication; AP, allopregnanolone; BARDA, Biomedical Advanced Research and Development Authority; BX, Brexanolone; CA1PCs, CA1 pyramidal cells; CAS, Chemical Abstracts Service; CBRN, chemical, biological, radiological, and nuclear; CDKL5, cyclin-dependent kinase-like 5; CNS, central nervous system; DG, dentate gyrus; DGGCs, dentate gyrus granule cells; DH, dentate hilus; DFP: diisopropyl-fluorophosphate; FDA, Food and Drug Administration; GABR-AR, GABA-A receptors; GRAS, generally recognized as safe; FIRES, febrile infection-related epilepsy syndrome; GX, ganaxolone; IV, intravenous; IBA1, ionized calcium binding adaptor molecule-1; MC, medical countermeasure; MTD, maximum tolerated dose; NeuN: neuronal nuclei; NOAEL, no-adverse-effect level; NMDA, N-methyl-D-aspartate; NORSE, new-onset status epilepticus; OP: organophosphate; OPNA, organophosphates and nerve agents; 2-PAM, pralidoxime chloride; PB, pyridostigmine bromide; PD, pharmacodynamic; PK, pharmacokinetic; PKC, protein kinase C; PV: parvalbumin; SE, status epilepticus; RSE, refractory status epilepticus; SRSE, super-refractory status epilepticus; TG, tiagabine; THDOC, allotetrahydrodexocorticosterone; TK, toxicokinetic

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Abstract

This article describes recent advances in the use of neurosteroids as novel anticonvulsants for refractory status epilepticus (RSE) and as medical countermeasures (MCs) for organophosphates and chemical nerve agents (OPNAs). We highlight a decade-long journey to bring the synthetic neurosteroid ganaxolone (GX) from bench to clinic. RSE, including when caused by nerve agents, is associated with devastating morbidity and permanent long-term neurological dysfunction. Although recent approval of benzodiazepines such as intranasal midazolam or diazepam and intranasal midazolam offers improved control of acute seizures, novel anticonvulsants are needed to suppress RSE and improve neurological function outcomes. Currently, few anticonvulsant MCs exist for victims of OPNA exposure and RSE. Standard-of-care MCs for post-exposure treatment include benzodiazepines, which do not effectively prevent or mitigate seizures resulting from nerve agent intoxication, leaving an urgent unmet medical need for new anticonvulsants for RSE. Recently, we pioneered neurosteroids as next-generation anticonvulsants that are superior to benzodiazepines for treatment of OPNA intoxication and RSE. Because GX and related neurosteroids that activate extrasynaptic GABA-A receptors rapidly control seizures and offer robust neuroprotection by reducing neuronal damage and neuroinflammation, they effectively improve neurologic outcomes after acute OPNA exposure and RSE. GX has been selected for advanced, BARDA-supported phase 3 trials of RSE and nerve agent seizures. In addition, in mechanistic studies of neurosteroids at extrasynaptic receptors, we identified novel synthetic analogs with features that are superior to GX for current medical needs. Development of new MCs for RSE is complex, tedious, and uncertain due to scientific and regulatory challenges. Thus, further research will be critical to fill key gaps in evaluating RSE and anticonvulsants in vulnerable (pediatric and geriatric) populations and military persons.

Significance Statement

Following OPNA intoxication, RSE occurs despite benzodiazepine treatment. RSE occurs in 40% of SE patients, with a 35% mortality rate and significant neurological morbidity in survivors. To treat RSE, neurosteroids are better anticonvulsants than benzodiazepines. Our pioneering use of neurosteroids for RSE and nerve agents, led us to develop ganaxolone as a novel anticonvulsant and neuroprotectant with significantly improved neurological outcomes. This article describes the bench-to-bedside journey of bringing neurosteroid therapy to patients, with ganaxolone leading the way.
Introduction

The American Chemical Society’s Chemical Abstracts Service (CAS) registry is a valuable resource for chemical information. The CAS registry contains 110 million chemicals, with about 345,000 categorized as toxic, posing a threat to public health and animal welfare. The U.S. biodefense plan is a multi-agency collaborative effort to enhance medical and public health preparedness in response to current and new threats from chemical, biological, radiological, and nuclear (CBRN) threats and disasters. The federal chemical countermeasures research program is a component of the broader CBRN research plan, specifically focused on advancing research into medical countermeasures (MCs). MCs are promising therapeutic agents (small molecules, biologics, and vaccines) that mitigate acute and/or chronic sequelae after toxic exposure to military chemical warfare agents, biological threat agents, radiation, industrial chemicals, pesticides, nerve agents, and insecticides including, but not limited to, organophosphates, complex environmental exposures, and potent opioids.

Chemical threat agents are toxic compounds that could be used in a terrorist attack or accidentally released from production, storage, shipping, or disasters. Approximately 200 chemicals of concern are known to pose significant threats to public health by producing a range of harmful symptoms in humans and animals. These chemicals are organized into toxidrome groups based on their primary modes of toxicity. The toxidrome classes include: a) anticoagulants (brodifacoum, bromadiolone); b) blood and cellular respiration inhibitors (hydrogen cyanide, hydrogen sulfide); c) cholinergic warfare agents (sarin, soman, VX); d) cholinergic pesticides (parathion, chlorpyrifos, phorate, aldicarb); e) convulsants (picrotoxin, TETS, strychnine); f) hemolytic and metabolic agents (arsenic trioxide, thallium sulfate, arsine); g) pulmonary, agents (chlorine, phosgene oxime, ammonia, sulfur dioxide, chloropicrin, acrolein, phosphine); h) vesicating agents (sulfur mustard, nitrogen mustard, Lewisite, hydrogen fluoride); i) ocular poisons (e.g., mustard, chloropicrin); and j) ultrapotent opioids (fentanyl, carfentanil, sufentanil). The most common chemical threat agents include organophosphates (parathion, chlorpyrifos, carbofuran, pesticides); nerve agents (sarin, soman, VX); vesicants (sulfur mustard, nitrogen mustard, Lewisite); pulmonary toxicants (chlorine, phosgene, phosphine); respiratory poisons (cyanide, hydrogen sulfide); ocular poisons (mustard, chloropicrin); and opioid-based agents (fentanyl). These chemical threats create a great need to define their mechanisms of toxicity and develop efficacious MCs to treat and/or prevent mass morbidity and mortality from occurring after chemical exposure.

This article describes recent advances in the development of anticonvulsant MCs to treat neurotoxicity and SE caused by organophosphates and chemical nerve agents. Neurosteroids, specifically ganaxolone and related neurosteroids that activate extrasynaptic GABA-ARs (GABA-ARs), are highlighted as next-generation anticonvulsants to treat nerve agent seizures and refractory status epilepticus (RSE). Additionally, we discuss current efforts to develop mechanism-based therapies, understand their complexities and uncertainties, and identify challenges to developing new RSE therapies.

Chemical Warfare and Chemical Threats
Organophosphates (OP) and nerve agents (OPNAs) have severe, fast-acting toxic effects on the human body and brain. The OP class of chemical compounds, which contain phosphorus as a central element, are widely used in agricultural, industrial, and household applications as insecticides, herbicides, fungicides, as well as chemical intermediates in the production of plastics, flame retardants, and pharmaceuticals. The most toxic OP chemicals are pesticides used in agriculture (e.g. monocrotophos, parathion, chlorpyrifos, paraoxon, phorate oxon, diazinon, and diisopropylfluorophosphate (DFP), and are also considered credible threat agents (Fig. 1). Nerve agents (aka, nerve gases) are among the most toxic known chemical agents. G-class agents such as tabun (GA), sarin (GB), soman (GD), and cyclosarin (GF) have been used as chemical warfare weapons in combat and as bioterror agents against civilians (Fig. 1). V-class agents such as VX and Russian VX are chemically distinct and more powerful neurotoxic nerve agents. These compounds are hazardous in both liquid and gas form, killing individuals within min of exposure. Other classified nerve agents and Novichoks are highly potent and have unique chemical structures, making them difficult to detect and treat. In the military, nerve agents have been weaponized and deployed in various forms, including aerosol (spray devices), liquid droplets, vapor, and explosive devices such as bombs or artillery shells, which cause widespread dispersal upon detonation. Use of explosive devices combines the destructive force of the explosive with the lethal effects of the nerve agent, increasing the potential for casualties.

Although the history of nerve agents as weapons of mass destruction has been covered elsewhere (Aroniadou-Anderjaska et al., 2020), the roots of chemical warfare can be traced to World Wars I and II, during which chemicals were extensively used, resulting in thousands of deaths. Chemical threat agents were also used during the Iran-Iraq War (1980-88), leading to several thousand fatalities. Over the past few decades, numerous chemical attacks or exposures have occurred among civilian populations. Thus, use of nerve agents is regulated by international treaties, including the Chemical Weapons Convention that prohibits production, stockpiling, and use of chemical weapons. Deployment of nerve agents as weapons is considered a severe violation of international law and a war crime.

Chemical attacks pose a significant global threat, given the ability of several countries to weaponize these lethal agents. Recent incidents involving sarin and VX highlight this alarming trend. In 2013, approximately 1,400 Syrian civilians lost their lives in sarin attacks (Dolgin et al., 2013). Nearly 20 years ago, the Tokyo subway system was attacked with sarin gas, exposing numerous civilians (Okumura et al., 1996) and in 2017 VX was used to assassinate Kim Jong Nam at a Malaysian airport. More recently, a new class of compounds, known as Novichok (or “newcomer” in Russian), has emerged that surpasses the dangers and sophistication of sarin and VX. These compounds are among the most lethal chemical weapons ever created, pose great danger, and are very difficult to identify due to their ill-defined composition. A Novichok exposure incident in Salisbury, England received widespread media coverage when standard detection efforts failed to detect the substance but the patient's response to specific antidotes confirmed its similarity to OPNA toxicity. In addition to threats posed by nerve agents, thousands of OP pesticide poisoning incidents occur annually, resulting from suicides or agricultural accidents (Jokanovic et al., 2010). Approximately 3 million individuals are estimated to be exposed to OPs each year, leading to around 20,000
deaths worldwide (González-Alzaga et al., 2014). Numerous countries prohibit certain OP pesticides to mitigate the acute and long-term toxicities associated with their agricultural and domestic use.

Many OP chemicals and nerve agents produce lethal neurotoxicity via common mechanisms (Fig. 2), primarily causing neurotoxicity by irreversibly inhibiting acetylcholinesterase (AChE) in plasma, red blood cells, tissues, and brain (McDonough and Shih, 1997; Chen et al., 2012; Abou-Donia et al., 2016). Nerve agents and OP pesticides permanently damage AChE, an enzyme with very high catalytic activity, causing excessive accumulation of acetylcholine (ACh) in the synaptic cleft of peripheral and central nervous systems (CNS). This excess ACh leads to rapid cholinergic hyperactivation (cholinergic crisis) in all cholinergic junctions, ganglia, and cholinergic synapses. In a healthy person, ACh is released at the junction of neurons and muscles, acting as an "on" switch that enables the brain to contract muscles and facilitate movement. AChE functions as the "off" switch by breaking down ACh into choline and acetate, causing muscles to cease contraction. Because OPNA exposure blocks the brain’s AChE off switch, excess ACh accumulation leads to widespread nerve excitation and continuous muscle contraction (Fig. 2). The consequences of excess ACh accumulation include muscle spasms, convulsions, continuous seizures, respiratory arrest, and eventual death (Reddy and Colman, 2017; Ciottone et al., 2018). Survivors of OPNA attacks often experience severe secondary neuronal damage, leading to long-term neurologic dysfunction and other debilitating conditions (Savage et al., 1988; Aroniadou-Anderjaska et al., 2016; Reddy et al., 2020; Jett et al., 2020), resulting in significant brain damage. Nerve agents differ in various parameters including LD$_{50}$ (lethal dose for 50% of the population), dose of antidote needed to control lethality, and extent of AChE inhibition in different brain regions and peripheral tissues (Aroniadou-Anderjaska, 2017).

After nerve agent exposure, a distinct set of toxic signs becomes apparent. The specific effects and severity of nerve agent symptoms vary based on dose and route of exposure (Reddy and Colman, 2017; Ciottone et al., 2018), with the most common routes of exposure being inhalation (rapid absorption, with onset in seconds to min), oral ingestion (medium absorption), and dermal contact (slow absorption, with onset in 2 min to 2 hrs). Early signs of OPNA exposure include miosis (pupil constriction) and rhinorrhea (runny nose). However, OPNAs primarily impact skeletal muscles, leading to muscular fasciculation (involuntary muscle twitching). OPNAs also induce bronchoconstriction (airway narrowing) and increased gland secretions, often resulting in chest tightness. Respiratory failure typically occurs later in the progression of toxic signs. In general, symptoms progress similarly in rats, nonhuman primates, and humans however, bronchial secretion, nausea and vomiting, and sweating are not observed in rats. Symptom severity also depends on dose and route of exposure in animals. In most efficacy studies, the OPNA dose is usually the minimum needed to cause consistent seizures in the specific model while the challenge dose is 1 to 2×LD$_{50}$ for MC evaluation.

Severe CNS symptoms associated with OPNA exposure include loss of consciousness, seizure activity, and apnea (temporary cessation of breathing). Status epilepticus (SE) is a hallmark neurological toxicity of acute OPNA exposure characterized by prolonged or recurrent seizures that do not return to baseline between seizures or a continuous seizure lasting over 5 min that develops within min of OPNA exposure. SE can persist up to 30 min, or
longer, and progresses to refractory SE (RSE) (Williamson et al., 2019; Reddy et al., 2021). RSE occurs when seizures persist despite initial treatment with first-line antiseizure medications (ASMs), typically benzodiazepines (e.g. lorazepam or diazepam) or second-line ASMs. New-onset RSE is a life-threatening emergency in which an individual remains in a prolonged, continuous state of convulsions without regaining consciousness. Failure to control RSE promptly and effectively can have tragic consequences, including widespread brain damage or death. The U.S. National Toxicology Program confirmed the long-term effects of acute exposure events (Jett et al., 2020) by assessing physiological effects such as inhibition of AChE, visual and ocular alterations, as well as morphological and histological brain changes. Individuals who were exposed during the sarin nerve agent attacks in Matsumoto and Tokyo reported increased persistent neurobehavioral disorders, trauma, and insomnia five years later (Ohtani et al., 2004). Thus, there is an urgent need to develop effective treatments for acute and long-term neurological consequences of nerve agent exposures.

Medical Countermeasures for OP Intoxication and RSE

Managing a victim intoxicated with a nerve agent or OPNA chemicals requires decontamination, ventilation, and administration of MCs. The three MC approaches used for OPNA intoxication are pretreatment, post-exposure therapy, and complete treatment (Newmark, 2007; Reddy, 2019). A "pretreatment" MC is administered prior to exposure and primarily benefits the military, which can anticipate potential threats when deploying troops to areas susceptible to chemical warfare. In such cases, troops are informed about the risk of nerve agent exposure and provided with pyridostigmine bromide (PB), an FDA-approved oral pretreatment for soman exposure in the U.S. Army. Originally approved to treat myasthenia gravis, PB is taken as a 30mg tablet every 8 hrs in anticipation of a potential chemical attack. In anticipation of an OPNA exposure, PB is an effective pretreatment by reversibly binding to the AChE enzyme. This protective binding prevents irreversible binding of AChE following nerve agent exposure. Approximately 41,650 soldiers received PB as a protective measure during Operation Desert Storm over 1 to 7 days, when facing the threat of a nerve agent attack (Keeler et al., 1991). Although non-incapacitating symptoms were observed, military mission performance was not affected.

A “post-exposure” MC would be administered immediately after a known, or suspected, nerve agent exposure. The post-exposure period is defined as the time immediately after OPNA exposure up to approximately 30 min later. The standard antidote regimen for immediate OPNA exposure therapy involves administering atropine sulfate, a muscarinic receptor antagonist, and 2-pralidoxime (2-PAM), a drug that regenerates AChE activity (Newmark, 2019, Reddy, 2019). It is crucial to administer these antidotes within min of exposure or of symptom onset to improve survival. Currently, two FDA-approved anticonvulsants, diazepam, and midazolam, are used as post-exposure therapies to control OPNA neurotoxicity (Reddy, 2019). These benzodiazepine anticonvulsants help prevent OPNA-induced seizures and acute brain damage when administered within 30 min of OPNA exposure, and their efficacy diminishes significantly if given more than 1 hr after exposure. In the context of chemical warfare and unexpected civilian bioterrorism, this limited timeframe to administer anticonvulsants is unrealistic. Consequently, convulsive seizures and SE often lead to severe and permanent brain damage, resulting in neuronal injury or death. Damage to
the brain is not only due to seizure-related excitotoxicity but can also occur through seizure-independent mechanisms, such as activation of microglia, astrocytes, and cellular inflammation (Banks et al., 2012; de Araujo et al., 2012). Thus, OPNA intoxication has long-lasting effects with a significant risk of enduring neurological and cognitive deficits (McDonough et al., 1999; de Araujo et al., 2012; Flannery et al., 2016).

A “treatment” MC would be administered following a confirmed exposure and/or when a person exhibits symptoms of OPNA exposure. Three available treatment MCs are effective when administered intramuscularly more than 30 min post-exposure, including atropine sulfate, 2-PAM, and midazolam (McDonough et al., 1999; Bajgar et al., 2004; Hulse et al., 2019). This regimen is distributed as a CHEMPACK (a deployable container of nerve agent antidotes with auto-injectors). Atropine is a muscarinic receptor antagonist that very effectively blocks the effects of excess ACh at peripheral sites, with limited effect on the CNS due to its poor entry into the brain. Atropine decreases hypersecretions and relieves bronchoconstriction, allowing for easier breathing. Of note, the nicotinic effects of OPs (e.g., spasms and fasciculations) are not countered by atropine. Indeed, repeated atropine injections are needed until cholinergic crisis is completely dampened. Despite the lifesaving effects of atropine, brain damage persists in survivors. 2-PAM, an AChE reactivator that can break the agent-enzyme bond to release free AChE, is the most commonly used oxime to treat acute OP intoxication by effectively reactivating AChE enzyme inhibited by OPNA compounds. However, 2-PAM has some limitations. Like atropine, 2-PAM has poor brain penetration and can’t reactivate brain AChE. Thus, the effect of 2-PAM is limited to early OPNA effects on the AChE enzyme. However, 2-PAM cannot target ‘aged’ OPNA-AChE enzyme. A recent meta-analysis of 2-PAM’s efficacy in treating OPNA poisoning in humans found that pralidoxime does not significantly improve mortality (Blumenberg et al., 2018). Thus, despite atropine and 2-PAM combination therapy, excess ACh remains uncontrolled in the brain resulting in cholinergic crisis including seizures and RSE (Fig. 2).

While soldiers are equipped with CHEMPACK antidote kits for personal use in the event of a nerve agent attack, civilians generally do not have access to anticonvulsant medications and thus must go to a hospital to receive necessary medication. The process of reaching the hospital and receiving the drugs typically takes at least 40 min (Reddy and Colman, 2017; Apland et al., 2014). This timeframe is crucial, as any anticonvulsant antidote for OPNA chemical seizures must be effective when administered 40 min or more after OPNA exposure. In many emergencies, this timeline is not practical. Consequently, OPNA-induced neurotoxicity can result in long-term brain injury and severe neuropsychiatric dysfunction among chemical attack survivors. Rapid and effective seizure control is critical for neuroprotection and survival (Shih et al., 2003). Several studies conducted after the Matsumoto and Tokyo sarin attacks reported devastating neurological and psychiatric disorders among individuals exposed to sarin, even five years after the incidents (Ohtani et al., 2004; Miyaki et al., 2005; Yamasue et al., 2007). Similarly, thousands of survivors in Syria exposed to sarin may experience lasting effects for the remainder of their lives.

**Benzodiazepine Limitations and Mechanisms of Antiseizure Resistance in OPNA and RSE Models**

Benzodiazepines are the primary class of drugs utilized as the current standard-of-care to treat SE after OPNA intoxication (Reddy and Reddy, 2015). These drugs function as positive allosteric modulators of post-synaptic
GABA-ARs and do not interact with or activate extrasynaptic receptors (Fig. 3) (Reddy et al., 2015). Benzodiazepines, such as diazepam and midazolam, are widely used as antiseizure agents to manage SE. However, there is significant evidence that some seizures are refractory to diazepam and midazolam, which limits their efficacy (McDonough et al., 2010; Reddy and Reddy, 2015). The mechanistic basis of benzodiazepine-refractory seizures following OPNA intoxication has been investigated in multiple studies (Apland et al., 2014; Kuruba et al., 2018; Wu et al., 2018). Animals were exposed to OPNAs (e.g., DFP, soman) and treated with diazepam or midazolam to evaluate their ability to suppress seizures and prevent SE, as well as their ability to protect against brain damage. These studies demonstrated that when administered 10 min after OPNA exposure, both diazepam and midazolam effectively controlled seizures, reduced neurodegeneration, and mitigated neuroinflammation. However, when administered at 60 or 120 min after exposure, both medications were completely ineffective. Delayed therapy at 40 min post-OPNA exposure, simulating the therapeutic window for first responders or hospital admission, reduced seizure control and neuroprotection (Kuruba et al., 2018; Wu et al., 2018), strongly suggesting that OPNA-induced seizures and brain damage become progressively resistant to delayed diazepam or midazolam treatment. This condition, known as "benzodiazepine RSE," is triggered by the cholinergic crisis induced by OPNA intoxication (de Araujo et al., 2012; Wu et al., 2018).

The exact molecular mechanism underlying benzodiazepine resistant SE is not well understood. Benzodiazepines are positive allosteric agonists of synaptic GABA-ARs (Reddy et al., 2015), and multiple mechanisms may contribute to their reduced efficacy, including pharmacokinetic factors (Reddy et al., 2015; Goodkin et al., 2005; Naylor et al., 2005; Deeb et al., 2012; Sankar, 2023), as well as pharmacodynamic factors, e.g. receptor loss or internalization. OPNA-induced SE rapidly becomes self-sustaining, often leading to pharmacoresistance to benzodiazepines and other antiseizure drugs. Numerous studies have focused on neurophysiological changes during SE, particularly changes in GABA-AR trafficking (Niquet et al., 2016). Time-dependent internalization of post-synaptic GABA-ARs is a significant observation in early stages of refractory SE (Naquet et al., 2023). Within 10 to 20 min of OPNA-induced seizure onset, over half of post-synaptic receptors that contain the benzodiazepine site disappear in neurons (Naylor et al., 2005). Therefore, when benzodiazepines are administered 40 min following OPNA exposure, less than half of the receptors are still present or functional at neuronal membrane targets. Although benzodiazepines can bind to the remaining receptors, their maximum effect is more limited by the number of available receptors, than by the dose administered. Further, increased N-methyl-D-aspartate (NMDA) type glutamate receptors may contribute to the excessive excitation and excitotoxicity observed in refractory SE. These changes have significant therapeutic implications, as repeated diazepam doses are required for partial seizure control, leading to undesirable side effects such as sedation, respiratory depression, and tolerance in affected individuals.

Neuroinflammation and neurodegeneration are prominently observed in acute OPNA intoxication (Fig. 2). Substances such as DFP and nerve agents induce widespread neuroinflammation, leading to neuronal damage and neurodegeneration of both principal neurons and interneurons (Apland et al., 2010; Guignet et al., 2020). Moreover,
OPNA poisoning results in the death of neurons that host benzodiazepine receptors, thereby exacerbating receptor deficiency. This is consistent with extensive neurodegeneration in principal and interneurons (Kuruba et al., 2018; Wu et al., 2018). Neuron survival is crucial for drug binding to its target receptors. Loss of inhibitory interneurons, which play a role in preventing excessive neuronal excitation and synchronization leading to seizures, leads to the establishment of a self-sustaining seizure circuit. Additionally, OPNA poisoning induces persistent brain inflammation, characterized by astrogliosis and microgliosis (Kuruba et al., 2018; Wu et al., 2018), further contributing to cell death and receptor loss. Collectively, this evidence establishes the mechanistic basis of diminished benzodiazepine efficacy when administered "late" in a field setting.

Overall, studies have shown that benzodiazepines are inadequate to control SE beyond 40 min after exposure, not due to insufficient brain penetration, but more likely due to the loss of target receptors, neuronal loss, and inflammation induced by OPNA exposure. OPNAs may affect the receptor targets, preventing diazepam and midazolam from binding to their receptors and disrupting seizure circuits. These findings highlight the need to develop next-generation anticonvulsants that surpass benzodiazepines. Notably, recent discoveries identified a new GABA-AR type at perisynaptic and extrasynaptic sites (Fig. 4), which are not affected by OPNA molecules (Chuang et al., 2018). These extrasynaptic receptors are promising targets for new drugs as they remain intact even 40 or 50 min after OPNA exposure. Preferential activation of these receptors by new drugs is likely to effectively control RSE and prevent neuronal loss, thereby breaking the seizure circuit.

**Benzodiazepine-Resistant Status Epilepticus and its Management**

SE is characterized by continuous or repeated seizures without regaining consciousness for more than 5 min. It is a life-threatening, medical emergency that, if not controlled promptly, can lead to brain damage and death. SE is a hallmark of cholinergic crisis following OPNA intoxication and nerve agent exposures. SE can elicit permanent neuronal damage due to persistent seizures and excitotoxicity. There are two types of SE, generalized convulsive SE and non-convulsive SE, with the former being the most common and severe type of SE. Treatment of SE begins when the seizure duration reaches 5 min, and treatments are generally classified into successive lines of therapy (Shorvon and Ferlisi, 2011). First-line therapy is intravenous benzodiazepines (lorazepam or diazepam); second-line therapy is intravenous ASMs (e.g. phenytoin, valproate, or levetiracetam); and third-line therapy is general anesthetics to induce pharmacologic coma (propofol, phenobarbital). Treatment responsive SE occurs in 60% of all SE cases. If SE continues despite administering a benzodiazepine and one ASM, it is classified as RSE, which occurs in 40% of all SE cases. SE that resists the first course of general anesthetics for 24 hrs is referred to as super-refractory SE (SRSE), and occurs in 4% of all RSE cases. These resistant SE forms are very complex and challenging for clinical management due to increased morbidity and mortality (Delaj et al., 2017). The first occurrence of refractory SE in a patient without active epilepsy and without a clear acute or active structural, toxic, or metabolic cause, is referred to as new-onset RSE (NORSE). The related febrile infection-related epilepsy syndrome (FIRES) is a subgroup of NORSE preceded by a febrile illness from 2 weeks to 24 hr prior to RSE onset. There is no specific treatment for RSE. Anesthesia using either propofol or midazolam is the only option. However, the risk of prolonged...
anesthesia creates risks including prolonged immobility, immunosuppression, homeostatic plasticity, and neurological deficits.

New and effective lifesaving anticonvulsants are needed to effectively manage RSE and neurologic outcomes. Benzodiazepines are usually able to terminate SE attacks and provide short-term control. Intravenous ASM is usually used for prolonged therapy because they are effective and less sedative than benzodiazepines. Fosphenytoin, a safer, water-soluble prodrug form of phenytoin, is used intravenously. In a trial of three ASMs for SE, levetiracetam, fosphenytoin, and valproate had equivalent overall efficacy and adverse side effects in children and adults with benzodiazepine-refractory SE (Kapur et al., 2019). These three ASMs produced a 45-47% seizure cessation and increased alertness after 60 min. The recent ESETT study confirmed that patients with established SE respond similarly to levetiracetam, fosphenytoin, and valproate, with efficacy in 49-52% of patients (Chamberlain et al., 2020). Thus, any of the three ASMs can be used as a second-line drug for benzodiazepine-refractory SE. Intramuscular midazolam is as safe and effective as intravenous lorazepam for prehospital management of SE in community settings (Silbergleit et al., 2012). Benzodiazepine efficacy dramatically decreases with increasing duration of SE (Mayer et al., 2002). In RSE cases, the therapeutic efficacy of benzodiazepines and ASMs is completely lost, and more drastic third-line therapies (propofol or phenobarbital) must be tried, but are not always successful (Wheless and Treiman, 2008). These pharmacoresistant forms of RSE remain a management challenge due to poor prognosis. Overproduction of pro-inflammatory cytokines is a common response in SE patients. Serum neurofilament light (NfL) levels are also increased in SE and correlate with treatment response therefore, NfL has been suggested as a potential biomarker of seizure-related neuronal damage. (Giovannini et al., 2022).

Nerve agents cause a severe cholinergic crisis that can induce RSE. In animal studies, nerve agents and OPNA intoxication with pesticides have been shown to elicit benzodiazepine RSE (Morgan et al., 2021; Reddy et al., 2021). Second-line ASMs were not able to terminate SE and no current drug therapy is effective against RSE and SRSE. RSE requires prompt antiseizure intervention and increased treatment delays are associated with a worsened prognosis (Gainza-Lein et al., 2018a,b). This time dependency is critical in cases of mass nerve agent exposure, where treatment delays could occur due to decontamination procedures, and limited medical staff to administer medications. There are many differences between normal SE and nerve agent SE, which typically occurs with signs of massive cholinergic crisis. New-onset SE and RSE, including those caused by nerve agents, are associated with severe morbidity and permanent long-term neurological dysfunction. Although recent approval of midazolam is an advance for rapid control of SE and an important treatment for nerve agent exposures, novel MCs are needed for to improve RSE outcomes after nerve agent exposure and its long-lasting impact on neurological function. Hence, there is an urgent unmet need for therapeutic management of RSE. An improved understanding of RSE mechanisms, animal models, and neuropathology will be essential to develop new RSE treatments.

**Neurosteroids as Novel Anticonvulsants for Nerve Agents and RSE**

Neurosteroids are endogenous steroids that are synthesized in the brain and rapidly modulate neuronal excitability (Kulkarni and Reddy, 1995; Rupprecht and Holsboer, 1999; Reddy, 2003; 2010). Neurosteroids promote
inhibition of neuronal excitability by allosterically modulating GABA-ARs in the brain (Harrison and Simmonds, 1984). Neurosteroids are steroids that are endogenously produced de novo in neurons, microglia, and astrocytes (Haraguchi and Tsutsui, 2020; MacLusky et al., 1994). Steroids that are found in neurons, microglia, and astrocytes but are produced at other sites are called neuroactive steroids (Paul and Purdy, 1992). A variety of neurosteroids exist in the brain, such as allopregnanolone (AP) (bexanolone), pregnanolone, allotetrahydro-deoxycorticosterone (THDOC), and androstane diol (Fig. 3). Although their precise mechanisms are not well understood, neurosteroids are thought to modulate GABA-ARs by directly binding to ‘neurosteroid-binding sites’ on the receptor channel and increasing channel conductance (Akk et al., 2007; Reddy and Rogawski, 2010). These neurosteroid-binding sites’ are distinct from the benzodiazepine and GABA binding sites (Carver et al., 2013). Because neurosteroids do not interact directly with steroid hormone receptors, they do not exhibit the typical hormonal effects associated with them. Neurosteroids can be classified into three structural groups: pregnanes (e.g. AP), androstanes e.g. androstane diol), and sulfated neurosteroids (Reddy, 2010). Pregnanes and androstanes positively modulate GABA-ARs (Fig. 4), whereas sulfated neurosteroids act as negative modulators. Neurosteroids that are positive modulators, play critical roles in modulating neuronal excitability and neuroplasticity (Reddy, 2010). Neurosteroids that enhance GABAergic inhibition have anticonvulsant properties (Table 1). Unlike benzodiazepines, neurosteroids can directly open receptor chloride channels at low micromolar concentrations. Moreover, neurosteroids act on all GABA-AR isoforms in the brain and hence can produce broad-spectrum anticonvulsant activity with promising clinical potential for treating seizure disorders (Reddy et al., 2016). We are among the first to design and implement neurosteroid-based therapies to treat seizure disorders (Reddy, 2003; 2010; 2016; 2022).

**Neurosteroid Interactions with Synaptic and Extrasynaptic GABA-ARs**

Neurosteroids have a unique mechanism of action, acting as positive allosteric modulators and direct activators of synaptic and extrasynaptic GABA-ARs, which mediate primary inhibition in the brain (Harney et al., 2003; Seighart et al., 2002). Synaptic GABA-ARs exist within the synaptic cleft, while extrasynaptic GABA-ARs are located outside of the synaptic cleft (Goetz et al., 2017). Structurally, GABA-ARs are pentamers formed from a possible combination of 19 different subunits and are categorized into two groups based on their location on postsynaptic sites. Synaptic GABA-ARs contain the γ-subunit and extrasynaptic GABA-ARs contain the δ-subunit (Goetz et al., 2007). While synaptic GABA-ARs have rapid and transient inhibitory effects, extrasynaptic, δ-containing GABA-ARs produce long-lasting tonic currents that shunt inhibition and modulate excitatory waveforms (Brickley and Mody, 2012; Carver and Reddy, 2013). Shunting inhibition utilizes a two-pronged approach, the first being hyperpolarization from chloride influx to subtract from EPSPs, and the second being a drop in membrane resistance to reduce the overall amplitude of excitatory currents through current leakage from the neuron. Combined action of these receptor subtypes produces strong inhibitory effects by hyperpolarizing the cell membrane and reducing the amplitude of excitatory impulses (Fig. 4).

Neurosteroids bind to all GABA-AR isoforms but have a strong binding preference for δ-containing GABA-AR isoforms, although their binding affinity varies depending on the specific steroid molecule (Mortensen et al., 2011;
This binding preference is unique to neurosteroids, unlike benzodiazepines, which are selective based on subunit composition (Hájos et al., 2000; Herd et al., 2008). Certain GABA-AR isoforms, such as those containing the α4, α6, or β2 subunits, or those lacking the γ2 subunit, are not sensitive to benzodiazepines (BZDs) (Hájos et al., 2000; Herd et al., 2008). Neurosteroids with high affinity for the BZD-sensitive GABA-AR γ-subunit may elicit a lower response at δ-containing receptors than other neurosteroids (Stórustovu and Ebert, 2006) and deficient expression of δ-containing GABA-ARs reduces sensitivity to neurosteroids (Mihalek et al., 1999; Spigelman et al., 2003; Stell et al., 2003; Pandit et al., 2013). Crystal structure studies have identified neurosteroid-binding sites within GABA-ARs that influence allosteric activation and direct interactions, particularly within helices lining the chloride channel pore that regulate desensitization-gate confirmation (Miller et al., 2017; Laverty et al., 2017; Chen et al., 2018).

Extrasynaptic GABA-ARs, which are located outside of the synaptic cleft, play a specific role in mediating tonic inhibition and modulating neural network activity through their distinct functional properties and regulation. They respond to ambient GABA from ‘spillover’ that occurs when synaptic receptors are saturated during an abundant release of GABA. Unlike their synaptic counterparts that transiently modulate excitatory signaling, extrasynaptic receptors provide sustained inhibition known as tonic inhibition (Brickley and Mody, 2012; Farrant and Nusser, 2005; Chuang and Reddy, 2018a). Tonic inhibition regulates baseline neuronal excitability and increases the action potential threshold, thereby influencing overall neural network activity in limbic regions and various cortical regions (Connelly et al., 2013). Mutations in the Gabrd gene lead to dysfunctional tonic currents and are associated with clinical cases of generalized epilepsy (Dibbens et al., 2004; Feng et al., 2006), which highlights the importance of the Gabrd gene in normal GABA-AR function and suggests a potential genetic basis for certain forms of epilepsy.

Interestingly, the functional properties and regulation of synaptic and extrasynaptic GABA-ARs differ. Synaptic receptors are regulated by intracellular calcium and calmodulin-dependent protein kinase II, while extrasynaptic receptors are regulated by protein kinase A activity. These independent receptor modulation pathways allow precise and flexible control over neural circuit activity and signal processing in various cortical regions (Abramian et al., 2014; Joo et al., 2014; Reddy et al., 2017). The interplay between synaptic and extrasynaptic receptors provides a dynamic inhibitory system that is crucial to maintaining proper neural circuit balance and information processing in the brain. Consequently, neurosteroids have a unique mechanism of activating these receptors that is not evident with other GABA-AR pharmacological modulators.

Binding and activation of GABA-Rs by neurosteroids involves a complex interplay of concentration, stereochemistry, and specific ligand-receptor interactions that ultimately determine the efficacy and potency of neurosteroid action on GABA-ARs (Reddy, 2018 Belelli et al., 2022). At low concentrations (10-300 nM), neurosteroids can act as positive allosteric modulators to enhance the affinity of GABA for GABA-ARs and promote chloride influx. At higher concentrations (3-10 μM), neurosteroids can directly open GABA-AR channels in the absence of GABA, promoting chloride influx (Reddy and Rogawski, 2002). Accumulation of endogenous
neurosteroids in the plasma membrane can also directly activate GABA-ARs, albeit at a slower rate (Akk et al., 2009). These neurosteroid-binding and activation mechanisms result in two distinct binding sites. Allosteric modulation occurs at the α-subunit M3/M4 domains, while direct activation occurs at the receptor α/β interface (Hosie et al., 2006; 2007). Key neurosteroid binding sites include the C3α-hydroxy and C20 ketone groups, as well as the C17 ketone group in androstane-derived neurosteroids. Binding occurs through interaction of the hydrophobic sterol structure with a hydrophobic pocket on the receptor, forming hydrogen bonds with polar or charged amino acids (Mitchell et al., 2008). Binding potency and specificity are determined by these interactions, with the C17 and C20 ketone groups and the C5α hydrogen group playing crucial roles (Harrison et al., 1987; Kokate et al., 1994; Reddy and Jian, 2010). Additionally, attachment of polar functional groups to the C11/21 sterol regions attenuated neurosteroid-mediated potentiation of tonic inhibition (Qian et al., 2014).

We identified a consensus neurosteroid pharmacophore model that targets extrasynaptic δ-GABA-ARs, providing key insights into tonic current activation (Carver and Reddy, 2016). Patch-clamp studies have extensively shown that modifications of the C17 or C20 regions of the neurosteroid molecule significantly impact tonic current activation (Modgil et al., 2017; Chuang and Reddy, 2018b; Althaus et al., 2020). Notably, C3β-OH epimers do not activate tonic currents, highlighting the critical role of the C3α-OH group in functional activity at extrasynaptic receptors. Among the tested analogs, AP and related pregnane analogs have the highest potency and maximal efficacy in promoting tonic currents, while androstane analogs have the weakest modulatory response. The functional significance of δ-subunit receptors and neurosteroid sensitivity in tonic inhibition is supported by experimental findings. Complete (~95%) elimination of tonic inhibition has been observed in hippocampal granule cells from δ-knockout mice, underscoring the essential role of δ-subunit receptors in mediating neurosteroid potentiation of tonic inhibition. These findings are consistent with the known functional role of δGABA-ARs in tonic inhibition and their sensitivity to neurosteroids (Hosie et al., 2007; Jensen et al., 2013; Wu et al., 2013; Carver et al., 2014; Carver and Reddy, 2016). These unique neurosteroid characteristics contribute to maximal inhibitory tone and have the potential to effectively counteract hypersynchronous and focal brain discharges. Therefore, neurosteroids hold promise for protecting against seizures and present a potential avenue for treatment of seizure disorders in patients.

**Anticonvulsant Neurosteroid Activity**

Neurosteroids have been extensively studied in preclinical epilepsy models over the past three decades (Reddy, 2002; Reddy and Estes, 2017). As potent GABAergic agonists, neurosteroids exhibit broad-spectrum anticonvulsant activity in various seizure models (Reddy and Kulkarni, 2000; Reddy, 2010; 2011; 2022). In acute seizure models, neurosteroids protect against seizures induced by GABA-AR antagonists such as pentylentetrazol and bicuculline, as well as the chemoconvulsants, flurothyl and butylbicycloorthobenzoate (Gasior et al., 2000; Reddy and Rogawski, 2002; Kokate et al., 1994; Mares et al., 2010). Neurosteroids are also highly efficacious in the amygdala kindling model with evoked focal and generalized seizures (Reddy et al., 2004; Reddy et al., 2010), in the mouse hippocampus kindling model of focal complex seizures (Chuang and Reddy, 2018b), and effectively mitigate pilocarpine-induced convulsive seizures (Reddy et al., 2019). The pharmacological potency of neurosteroids can vary...
across different seizure models (Table 1). Notably, several neurosteroids are highly active in the 6-Hz model of psychomotor seizures (Kaminski et al., 2004; Carver and Reddy, 2016), in which seizures are triggered by abrupt withdrawal of GABAergic agents, including neurosteroids and benzodiazepines, as well as substances like ethanol and cocaine, and have been shown to protect against such withdrawal seizures (Devaud et al., 1996; Tsuda et al., 1997; Reddy and Rogawski, 2000a; 2001; Kaminiski et al., 2003). Neurosteroids also suppress neonatal seizures (Miller et al., 2022) and seizures in pediatric neurogenetic models (Ciarlone et al., 2017). However, they are less active in generalized seizure models involving maximal electroshock and excitatory agents such as NMDA agonists, kainic acid, and 4-aminopyridine.

The ability of neurosteroids to protect against seizures differs between sexes (Reddy, 2017), with neurosteroids such as AP and androstanediol having more seizure protection in females than males (Reddy et al., 2004; 2019; Singh et al., 2024a). Consequently, neurosteroids have been investigated extensively in animal models of catamenial epilepsy (Reddy, 2009; 2016), a type of refractory epilepsy characterized by seizure exacerbations in a cyclical pattern during particular phases of the menstrual cycle, mostly around the perimenstrual or periovulatory periods. Perimenstrual catamenial seizures are attributed to withdrawal of progesterone-derived neurosteroids due to reduced progesterone levels at the time of menstruation (Reddy et al., 2001; 2012). Neurosteroids such as AP, THDOC, and GX exhibit enhanced protection against catamenial seizures in rat and mouse models of catamenial epilepsy (Reddy and Reddy, 2000a; 2001; Reddy et al., 2012; Carver et al., 2014; Reddy and Clossen, 2017; Reddy et al., 2019). Although the mechanism of such enhanced neurosteroid antiseizure activity in catamenial epilepsy is poorly understood, we found that δGABA-ARs are upregulated in the perimenstrual-like neuroendocrine milieu (Gangisetty and Reddy, 2010; Reddy et al., 2012; Carver et al., 2014; Reddy, 2016), providing an extrasynaptic receptor mechanism of neurosteroid sensitivity that may explain antiseizure protection against catamenial seizures. As expected, benzodiazepines and other ASMs do not effectively control catamenial seizures.

Unlike benzodiazepines, which can develop tolerance with repeated administration, neurosteroids do not induce anticonvulsant tolerance, even after repeated or chronic use (Kokate et al., 1998; Reddy and Rogawski, 2000b). Although this lack of tolerance was also seen in neurosteroid clinical trials (Sperling et al., 2017; Knight et al., 2022), there is limited research on the effects of neurosteroids in chronic epilepsy models with spontaneous seizures. Nevertheless, strong evidence suggests that neurosteroids may modulate epileptogenesis, the process leading to development of epilepsy (Biagini et al., 2006; Reddy and Mohan, 2011; Joshi et al., 2017). For instance, progesterone, a precursor for biosynthesis of neurosteroids such as AP and pregnanolone, has disease-modifying effects in the kindling model (Reddy et al., 2010). AP significantly retards kindling epileptogenesis in a mouse model of temporal lobe epilepsy, with a greater effect in females than in males (Reddy et al., 2019). Inhibition of epileptogenesis by neurosteroids is an area of intense research, with the goal of advancing synthetic neurosteroids as disease-modifying therapeutics to prevent or treat epilepsy (Reddy and Clossen, 2017b).

The efficacy of neurosteroids in suppressing seizures is closely related to their ability to activate extrasynaptic tonic currents (Table 3). Among the neurosteroids, ganaxolone is more potent than AP and other neurosteroids. The
correlation between neurosteroid plasma levels and seizure protection, likely mediated by tonic inhibition, was analyzed in structure-activity studies, (Carver and Reddy, 2016; Reddy et al., 2019). Estimated threshold concentrations for 50% seizure protection (3.3–3.6 μM) exceed the potentiation of extrasynaptic receptors by allosteric modulation or direct neurosteroid activation. This pharmacokinetic-pharmacodynamic relationship aligns with neurosteroid anticonvulsant properties (Table 2). Neurosteroids have enhanced protection against seizures when δ-GABA-ARs are upregulated (Reddy and Rogawski, 2000a; 2001; Reddy et al., 2012; Carver et al., 2014; Clossen and Reddy, 2017b) and potentiation of tonic currents by neurosteroids was abolished in neurons from δ-knockout mice, indicating that δ-GABA-ARs are required to mediate neurosteroid effects (Mihalek et al., 1999; Carver and Reddy, 2016). Consistently, female δ-knockout mice, lacking δ-GABA-AR expression, were less responsive to the antiseizure effects of neurosteroids (Reddy et al., 2019), further supporting the role of extrasynaptic δ-GABA-ARs in neurosteroid antiseizure mechanisms.

Recently, we further evaluated the anticonvulsant potential of neurosteroids in combination regimens with other ASMs using the 6-Hz model of refractory epilepsy (Chuang and Reddy, 2020). Neurosteroids AP and GX worked synergistically with tiagabine (TG) combinations to potentiate tonic inhibition in the hippocampus, resulting in better protection against acute seizures. This synergistic effect is due to greater potentiation at extrasynaptic δGABA-ARs by neurosteroids through TG-induced elevation of extracellular GABA levels. Furthermore, combinations of GX and the benzodiazepine, midazolam, had synergistic antiseizure effects. This pharmacodynamic synergism between neurosteroids and benzodiazepines improves protection against acute seizures (Chuang and Reddy, 2020). Thus, neurosteroids have potential as add-on medications with other ASMs at lower doses and may reduce side effects.

Brexanolone (BX), an intravenous AP formulation, was evaluated as adjunctive therapy in patients with super-refractory SE (Vaitkevicius et al., 2013; Broomall et al., 2014; Rosenthal et al., 2017). Initial studies showed that BX was well tolerated and moderately successful at weaning from anesthetic third-line agents. Despite its promise as a therapeutic anticonvulsant, further evaluation of BX in SRSE trials did not meet the primary endpoint, primarily due to its lack of efficacy in controlling seizures and achieving satisfactory outcomes in a clinical setting (Rosetti, 2018). Other contributing factors included the poor prognosis of SRS, high mortality, and morbidity rates, as well as challenges with study design and inclusion of heterogeneous patient populations.

Natural neurosteroids like AP (brexanolone) have limitations including short half-lives, first-pass metabolism, and poor oral absorption. AP can also induce hormonal side effects by metabolizing into C3-keto steroids, which can bind to steroid hormone receptors including the progesterone receptor (Rupprecht et al., 1996). Synthetic neurosteroids, such as ganaxolone, were developed to address these limitations (Upasani et al., 1997; Blanco et al., 2018; Reddy, 2023). Ganaxolone incorporates a 3β-methyl substitution to minimize metabolic conversion to hormonally-active C3-keto forms. This modification makes ganaxolone orally active and extends its half-life 4-6 times longer that of AP. Various synthetic compounds have also been designed using structure-activity principles to improve biopharmaceutical properties (**Fig. 3**). Molecular studies investigating the potentiation of GABA-ARs by neurosteroids provided insights into creating novel neurosteroid analogs to optimize treatment of seizures, including
OPNA-induced seizures (Robichaud and Doherty, 2017; Hogenkamp et al., 2014; Reddy, 2023). These advances offer new opportunities to develop synthetic neurosteroids with improved therapeutic profiles.

Based on their ability to interact with GABA-ARs without extrasynaptic receptor internalization gives neurosteroids like AP, THDOC, and ganaxolone more potential than benzodiazepines to effectively and safely halt RSE (Reddy et al., 2008; Kuruba et al., 2011; Briyal et al., 2008), making neurosteroids more suitable for treating RSE (Reddy et al., 2019). Experimental studies in rodent models of cholinergic SE induced by pilocarpine (Rogawski, 2013), DFP (Reddy et al., 2015; 2019a; 2020), and soman (Reddy et al., 2019a; Althaus et al., 2017) demonstrated neurosteroid efficacy. Even when administered 40 min or later after OPNA exposure, these neurosteroids were able to rapidly control SE and mitigate neuronal damage (Reddy et al., 2015; Barker et al., 2020). Neurosteroids were tested as potential treatments for OPNA seizures and SE induced by tetramethylenedisulfotetramine, a toxic rodenticide with GABA-AR antagonistic activity. Intramuscular AP and GX suppressed SE and prevented mortality. Pharmacokinetic analysis showed that brain exposure was approximately 3-fold higher than plasma exposure for both steroids (Zolkowska et al., 2018). In summary, neurosteroids are more effective as anticonvulsants and neuroprotectants than benzodiazepines and ASMs in OPNA intoxication seizures.

### Preclinical Profile of Ganaxolone in OPNA and RSE Models

Ganaxolone (GX), the 3β-methylated analog of AP (Fig. 3), (3α-hydroxy-3β-methyl-5α-pregnan-20-one) was first synthesized in 1995 to have modulatory activity comparable to that of AP (Carter et al., 1997). The synthetic 3β-substitution provides a more favorable pharmacokinetic profile as an anticonvulsant drug, overcoming the limitations of AP by preventing oxidation of the 3α-hydroxyl group (Reddy and Woodward, 2004). In radioligand binding and electrophysiological studies, GX has characteristics of a positive allosteric modulator of GABA-ARs with affinity in the low nM range (Carter et al., 1997; Carver and Reddy, 2016). Unlike AP, the 3α-OH group of GX is sterically hindered from oxidation into the hormonally-active 3-keto steroid, making GX inactive (IC$_{50}$ >10 μM) at many off-target receptors. In essence, the pharmacological effects of GX are similar to AP, but with slightly less potency.

In preclinical PK studies, GX had a large volume distribution, indicating extensive tissue distribution, including the brain (Ram et al., 2000; Reddy and Rogawski, 2000b). In humans, GX had a short half-life of 4 hrs in plasma after a single 300 mg oral dose (Fitch et al., 2023). GX binds strongly to plasma proteins and is metabolized, producing multiple metabolites, the primary being 16α-hydroxyganaxolone, which binds to GABA-ARs with less potency than does GX. In humans, GX had a complex, multi-step metabolic pathway, involving: 1) hydroxylation at the 16α-hydroxy position, 2) stereoselective reduction of the 20-ketone, producing a corresponding 20α-hydroxysterol, and 3) sulfation of the 3α-hydroxy group (Fitch et al., 2023). GX is a CYP3A4 autoinducer in rodents but not in dogs or humans that is eliminated primarily through urine (20%) and feces (80%). Orally administered GX has poor bioavailability due to limited absorption, aqueous insolubility, and rapid hepatic inactivation. In preclinical safety studies, whether administered in single or multiple doses, GX, did not have target organ or systemic toxicity. GX is known to be safe for pre- and post-natal development in mice, rats, and dogs, with no negative effects on fetal
implantation, viability, or growth, and no teratogenic or mutagenic properties. In dogs, oral administration of GX at a dose of 10 mg/kg did not cause affect cardiovascular hemodynamics (Reddy and Rogawski, 2012).

GX has been extensively evaluated in experimental epilepsy models (Table 1), with potent antiseizure effects in the hippocampus kindling model of complex partial seizures (Albertson et al., 1980; Sutula, 1990; Reddy et al., 2010). Like AP, GX is very effective against seizures induced by triggers, including chemoconvulsants, electrical kindling, and chemical kindling (Gasier et al., 2000; Kaminski et al., 2003; Reddy and Woodward, 2004; Reddy and Rogawski, 2010). GX also effectively suppresses behavioral and electrographic seizures in amygdala kindled mice with an ED$_{50}$ of 6.6 mg/kg (Reddy and Rogawski, 2010), but exacerbates seizures in animal models of absence epilepsy (Snead, 1998). GX is active in the 6-Hz model, which is very responsive to GABA-AR positive modulators (Kaminski et al., 2004; Reddy et al., 2015). Because GX has enhanced antiseizure effects in a model of catamenial epilepsy induced by neurosteroid withdrawal (Reddy and Rogawski, 2000a; Reddy et al., 2012) it was evaluated in the kindling seizure test in male and female mice (Reddy et al., 2019), where it induced dose-dependent protection against acute partial seizures with females being more sensitive to the antiseizure activity. Mice lacking extrasynaptic δGABA-ARs did not exhibit such sex differences in GX protection, indicating that extrasynaptic δGABA-ARs mediate seizure protection. GX has a unique advantage over midazolam in that tolerance does not appear to occur with extended use (Reddy and Rogawski, 2000b). In the above preclinical models, GX causes mild side effects such as sedation and hypoactivity, which are comparable to the benzodiazepine, midazolam. GX also has higher antiseizure potency in the presence of increased extrasynaptic δ-GABA-ARs (Rogawski and Reddy, 2000a; Reddy et al., 2019). This GX sensitivity was reduced in mice lacking δ-GABA-ARs, indicating that these receptors contribute to mediating its antiseizure effects (Reddy et al., 2019).

**Mechanism of Ganaxolone Action**

Mechanistic studies using patch-clamp electrophysiology play a crucial role in assessing the functional impact of neurosteroids on tonic inhibition (Chuang and Reddy, 2018a). GX was developed as a potent modulator of GABA-ARs, aiming to overcome the limitations of AP (Carter et al., 1997; Carver and Reddy, 2016). Although GX and AP have similar abilities to modulate the activity of GABA-ARs expressed in *Xenopus oocytes* (Carter et al., 1997; Carver and Reddy, 2016), the precise mechanism of GX action on native neurons remains elusive. However, our recent research shed light on the mechanism of GX at native GABA-ARs (Fig. 4) (Chuang and Reddy, 2018b). To investigate the mode of GX action, we examined extrasynaptic receptor-mediated tonic currents and synaptic receptor-mediated phasic currents in native hippocampal neurons (Chuang and Reddy, 2018) and found significantly more GX potentiation of GABA-AR-activated currents in dentate gyrus granule cells (DGGCs) containing the δ-subunit, than in CA1 pyramidal cells (CA1PCs) containing the γ2-subunit (Chuang and Reddy, 2018). Like AP, GX was selective for δGABA-ARs, with significantly less effect in mice lacking extrasynaptic δGABA-ARs (Chuang and Reddy, 2018) and a preferential ability to enhance δGABA-AR-mediated tonic inhibition. The relative potency and efficacy of GX in enhancing allosteric and direct activation of tonic conductance are similar to AP (Table 2). At
nanomolar concentrations, GX potentiated allosteric tonic currents, while at micromolar levels, it directly promoted tonic currents, providing a mechanistic rationale for its clinical use in seizure disorders (Chuang and Reddy, 2018).

Protein kinases play a role in regulating the function of various proteins by phosphorylating hydroxyl groups on target proteins (Moss and Smart, 1996; Brandon et al., 2000). In the context of GABAergic neurotransmission, protein kinases can impact GABA-AR surface expression, trafficking, conductance, and sensitivity to neurosteroids (Abramian et al., 2010; 2014; Chuang and Reddy, 2018a). Because modulation of tonic currents by GX depends on the physiological state and trafficking of GABA-ARs at neuronal membranes (Chuang and Reddy, 2018b), protein phosphorylation, has emerged as a significant factor in neurosteroid action. To investigate the involvement of protein kinase C (PKC) in the allosteric potentiation of tonic currents by ganaxolone, we used native hippocampal neurons (Chuang and Reddy, 2018) pretreated with the PKC inhibitor GF109203X, which completely reduced GABA-evoked tonic currents. The inhibitory effect of the PKC inhibitor on GX-mediated allosteric potentiation was time-dependent, indicating that the extent of PKC activity in neurons correlates with the ability of GX to potentiate tonic currents (Chuang and Reddy, 2018). Certain GABA-AR subunits, such as α4 and β, can be phosphorylated by PKC (Abramian et al., 2010; Adams, 2015), thus PKC inhibition is likely to diminish receptor phosphorylation and subsequent internalization, thereby influencing the ability of GX to inhibit through GABA-ARs (Chuang and Reddy, 2018). Taken together, these studies shed light on the intricate interplay between neurosteroids, protein kinases, and GABA-AR function.

Of note, the specificity of GX is regulated by zinc, an abundant trace metal in the hippocampus that can completely block GX inhibitory current responses (Carver et al., 2016; Chuang and Reddy, 2019). Our previous studies showed zinc's ability to block neurosteroid-sensitive extrasynaptic GABA-ARs (Carver et al., 2016). We also recently confirmed that zinc interferes with the efficacy of GX in activating tonic currents and preventing seizures (Chuang and Reddy, 2019). Zinc selectively blocks extrasynaptic δGABA-ARs, impeding GX activity, which primarily targets extrasynaptic receptors. Zinc also has an impact at the systems level, counteracting the protective effects of GX in an experimental epilepsy model (Chuang and Reddy, 2019). In summary, GX activates both synaptic and extrasynaptic GABA-ARs, leading to enhanced phasic and tonic inhibition in neuronal networks responsible for inhibitory transmission (Chuang and Reddy, 2018). However, the presence of zinc can modulate GX function by blocking neurosteroid-sensitive extrasynaptic receptors (Carver et al., 2016; Chuang and Reddy, 2019).

**Evaluation of Ganaxolone in OPNA Exposure Models of RSE**

Our research focuses on developing neurosteroids as novel anticonvulsants to treat RSE, a hallmark of OPNA intoxication. During SE, there is a rapid decline in synaptic GABA-ARs and reduced hippocampal phasic inhibition, leading to benzodiazepine resistance (Goodkin et al., 2005; Naylor and Wasterlain, 2005; Deeb et al., 2012). Thus, we propose to use neurosteroids, which activate both extrasynaptic and synaptic receptors, to more effectively treat SE (Reddy, 2015). Our neurosteroid strategy is based on the concept that extrasynaptic δGABA-ARs, which generate tonic inhibition, do not internalize during SE. Therefore, neurosteroids, which enhance both extrasynaptic and
synaptic inhibition, have the potential to counteract sustained seizure activity more effectively than benzodiazepines. In 2008, we first proposed "tonic inhibition (neurosteroid)" therapy for refractory SE, and in 2010 we received the first NIH project to test this concept. Since then, we have conducted a series of studies to investigate neurosteroid efficacy in various SE models. Initially, we determined the effectiveness of GX in treating SE in epilepsy rats (Briyal and Reddy, 2008). Subsequently, we demonstrated the neuroprotective effects of the neurosteroids AP and androstanediol in the pilocarpine SE model (Reddy, 2009). We further confirmed the protective effects of four other neurosteroids in a pilocarpine model of refractory SE (Kuruba and Reddy, 2011). Building on these findings, we advanced the exploration of neurosteroids as anticonvulsants for OPNA-induced refractory SE (Reddy, 2016; 2019a).

Over the past decade, we evaluated several neurosteroids, including GX, AP, and related compounds, in rodent models of SE induced by cholinergic agents, OP chemicals (such as DFP), and nerve agents (such as soman and VX). GX, and other neurosteroids terminated RSE rapidly and completely when administered early (10 min) or late (60 min) after SE onset. Even with delayed therapy, GX effectively aborted seizures with minimal recurrence, surpassing the efficacy of diazepam. GX therapy was also neuroprotective by reducing neuronal cell death and neurodegeneration associated with refractory SE (Reddy, 2015; 2019b; Barker et al., 2020). We further characterized the efficacy of GX and novel neurosteroid analogs in OP exposure models, including DFP, soman, and VX (Reddy, 2019b). Neurosteroids were effective when administered 40 min or more after OPNA exposure, resulting in rapid and efficient control of SE that mitigated neuronal damage (Table 4). These studies, supported by multiple preclinical findings, established GX as a highly effective anticonvulsant and neuroprotectant for OPNA intoxication. Among synthetic neurosteroids, GX has been extensively studied, with well-documented mechanisms of action, anticonvulsant profiles, pharmacokinetics, and safety profiles (Bailer et al., 2015). As a result, we suggested to the NIH CounterACT program and BARDA that GX is an excellent candidate to be developed as a medical countermeasure for OPNA intoxication and RSE treatment. These studies are outlined below in detail.

**Formulation, Pharmacokinetic and Toxicokinetics of Injectable Ganaxolone Products**

Our research has led to the development of both intravenous (IV) and intramuscular (IM) formulations of GX that use aqueous complexes with beta-cyclodextrin (Reddy, 2015; 2019b), a chemically inert molecule that forms inclusion complexes with hydrophobic neurosteroid compounds like GX. This process of making complexes has several advantages, including improved solubility, stability, and bioavailability of GX. To form inclusion complexes with beta-cyclodextrin, GX molecules are encapsulated within the complex, resulting in a stable solution that can be stored up to several weeks. This formulation ensures the integrity and availability of GX for administration. A key advantage of the GX-beta-cyclodextrin complex is its efficient absorption and rapid distribution to the brain. These properties are crucial for achieving a swift and effective response when treating conditions such as SE. These formulations have enhanced the pharmaceutical properties of GX, making it more suitable for clinical use.

Using IV and IM products, we demonstrated that GX has desirable features of efficient absorption and rapid distribution to the brain. In rat PK studies, plasma and brain levels of GX increased proportionately with increasing
dosage. Following IV administration of GX, the concentration at the first time point (2 min) was 4,813 ng/ml and the C0 was 7,632 ng/ml. The elimination phase $t_{1/2}$ was 4.6 hrs, total clearance was high at 3,883 ml/hr/kg, and the distribution volume of 25,712 ml/kg indicated extensive tissue distribution. After IM administration of GX (6 mg/kg), plasma distribution was rapid, with a $C_{max}$ of 603 ng/ml at the $T_{max}$ (0.167 hrs). The bioavailability was >95% after IM administration with a $t_{1/2}$ of 2.4 hr.

The IM GX injectable product was evaluated in pharmacokinetic (PK) and toxicokinetic (TK) studies in two species (rodent and non-rodent), including rats exposed to GD and DFP (Reddy, 2015; 2019b). In control rats, GX rapidly distributed to both plasma and brain after IM injection (15-20-min). The peak plasma level was ~1,280 ng/ml and ~1,570 ng/ml to brain, with a $t_{1/2}$ of 3.3 hr for plasma and 2.6 hr for the brain. The brain to plasma ratio was 2.6, indicating a consistently higher brain distribution of GX. In GD-exposed rats, distribution to plasma and brain was rapid, with peak plasma and brain drug levels of 1,010 ng/ml and 2,130 ng/g, respectively. Brain levels were higher than plasma, with a brain to plasma exposure ratio of 3.4. In a TK study in rats and non-rodent species, GX-IM was safe and well tolerated with normal clinical observations. Neither the no-adverse-effect level (NOAEL) nor maximum tolerated dose (MTD) could be determined in the TK studies as both are greater than the highest dose studied (10 mg/kg), indicating that GX-IM may have a greater safety and therapeutic index.

### Ganaxolone Efficacy in the DFP Model of RSE

We tested GX in the rat DFP model, as a widely used chemical for studying OPNA intoxication that is considered a surrogate for nerve agents (Reddy, 2015; 2019b; 2020). Induction of persistent RSE is characteristic of DFP intoxication (Kuruba et al., 2018; Wu et al., 2018). The goal of these studies was to define the optimal dose of IM GX as an anticonvulsant to suppress seizures, control SE, and reduce lethality when administered 40 min or more after DFP exposure. To evaluate GX efficacy in the DFP model, we performed a series of experiments using a delayed post-exposure protocol (Wu et al., 2018; Reddy et al., 2021). GX was given at 40, 60, or 120 min after DFP. This timeline was chosen to model refractory SE due to resistance observed with diazepam and midazolam treatments (Kuruba et al., 2018; Wu et al., 2018). We found that GX effectively terminated electrographic and behavioral SE activity within 45 min after treatment, resulting in minimal seizure recurrences (Table 4). In untreated animals, a significant mortality rate of 50% was observed following DFP exposure. However, all animals that received GX 40 min or later survived. Further, over 90% of the animals survived when GX was administered 120 min after DFP exposure, highlighting the remarkable protective effect of GX in the DFP model. To determine the dose-responsive ability of GX (1.5-10 mg/kg) to protect against DFP-induced SE, we estimated that $ED_{50}$ was 4.8 mg/kg, further confirming the efficacy of GX in the DFP model.

To compare the efficacy of combination therapy using GX and midazolam (MDZ), with MDZ alone in the rat model of DFP (Reddy, 2015; 2019b), we asked whether combination therapy would enhance anticonvulsant effects when administered 40 min or later after DFP exposure. When administered alone, MDZ (2 mg/kg) was partially effective when given 40 min after DFP exposure. However, when GX and MDZ were combined, anticonvulsant
efficacy was significantly improved over MDZ alone (Table 4). GX was effective at a dose lower than its ED$_{50}$ in the DFP model, indicating a synergistic response when combined with MDZ. We also investigated the neuroprotective ability of GX to reduce acute neuronal injury, neuronal cell death, and chronic neurodegeneration when administered 40 min after DFP exposure. In control rats, DFP exposure resulted in extensive neuronal injury in the hippocampus and other brain regions, as observed by staining with Fluoro-Jade B, a fluorophore that stains necrotizing neurons (Reddy and Abegunarte, 2022). However, GX treatment significantly (>80%) reduced neuronal injury by completely preventing cell death of principal neurons, as shown by NeuN staining and stereology counts. GX also markedly decreased interneuron cell death. The neuroprotective effects of GX were further supported by significantly improved neurological and memory function, along with a striking reduction in neurodegeneration 3-months after DFP exposure, indicating long-term neuroprotective activity of GX.

**Ganaxolone Efficacy in Soman Exposure Models of RSE**

Using the soman (GD) model, we evaluated the efficacy of GX, a common G-type nerve agent with challenging neurotoxic effects (Reddy, 2015; 2019b). Soman produces RSE as a salient neurotoxic manifestation (McDonough et al., 2010; Reddy et al., 2020; 2021), binds irreversibly to AChE, and rapidly ‘ages’ within min, making it difficult to counteract its neurotoxicity with antidotes. We administered GX intramuscularly at 40, 60, or 120 min after soman exposure using a delayed post-exposure protocol (Reddy et al., 2020; 2021) and found that GX provided dose-dependent protection against soman-induced RSE, with an ED$_{50}$ of 5.5 mg/kg (Table 4). In untreated animals, soman exposure induced 50% mortality, animals that received GX 40 min or more post-soman exposure survived, and those that received MDZ (2 mg/kg) at 40 min post-soman exposure were not protected against soman-induced seizures and SE. However, the combination of GX and MDZ had significantly more effective anticonvulsant effects, with rapidly terminated seizures and electrographic SE, suggesting exceptional anticonvulsant efficacy in the soman model. We also assessed the neuroprotective effects of GX in the rat soman model. In control rats, soman exposure led to extensive neuronal injury, particularly in the hippocampus and various brain regions. GX treatment significantly reduced neuronal injury (Table 4) and significantly prevented cell death of principal neurons and interneurons. In a cellular neuroinflammation assay, we observed >80% protection against chronic inflammation of microglia and astrocytes. T$_2$-weighted MRI images from rats 3 months post-soman exposure further confirmed the neuroprotective potential of GX, as soman-exposed rats had significant hippocampal atrophy indicating severe damage and neuronal loss, that was prevented with GX therapy (Reddy et al., 2021). These outcomes confirm the efficacy of GX in the soman model and highlight its chronic neuroprotectant potential, even with delayed treatment.

**Ganaxolone Efficacy in VX Exposure Model**

In the context of nerve agents, VX belongs to the V-class category with properties distinct from G-class nerve agents. Like GD (soman), VX exposure produces persistent SE in rats (Shih and McDonough, 1999). In a rat model specifically designed to study VX, GX was administered intramuscularly 40 min after VX exposure (Reddy, 2015; 2019b). Similar to soman, VX exposure led to prolonged seizures and SE. GX treatment effectively and completely suppressed both electrographic and behavioral seizures within 40 to 60 min after administration, and significantly
reduced the duration of SE (Table 4). GX-treated animals also had improved survival rates with minimal seizure recurrences.

**Efficacy of Ganaxolone in Mitigating Long-term Neurological Deficits**

OPNA exposure is associated with long-term neuronal damage and devastating neurobehavioral deficits. In neuroimaging studies, long-term structural and neuronal lesion abnormalities were observed in the hippocampus, ventricles, and cortical regions of soman-exposed rats (Reddy et al., 2020). Hippocampal atrophy with neuronal loss correlated positively with histological markers of neurodegeneration and neuroinflammation. Significant memory deficits were seen in rats 3 months after soman exposure. These chronic deficits were significantly reduced in GX-treated groups. Untreated rats had epileptic seizures 30 days after soman exposure, which were significantly reduced in GX-treated animals. We also investigated the ability of GX to mitigate long-term (10-month) neuropsychiatric impairments, chronic neurodegeneration, and neuroinflammation in a pediatric model of acute DFP exposure (Singh et al., 2024b). GX has neuroprotective effects against long-term memory dysfunction, neurodegeneration, and neuroinflammation in this OPNA model (Neff and Reddy, 2024), underscoring the potential use of neurosteroid therapy to mitigate chronic neuropsychiatric sequelae following acute OPNA exposure.

**Clinical Profile of Ganaxolone in RSE**

**General Safety and Efficacy Profile of Ganaxolone**

Clinical studies involving GX have increased over the last decade, with GX being tested in over 2000 patients with various types of seizures and other conditions (Table 5). In most cases, GX was tested for efficacy and safety as adjunctive therapy in children and adults with uncontrolled partial-onset seizures or epileptic syndromes (Reddy and Woodward, 2004, Nohria and Giller, 2007; Lattanzi et al., 2021). After oral administration of single doses (50-500 mg) in humans, GX levels increased in a dose-dependent fashion with $C_{\text{max}}$ of 27 to 130 ng/ml and $T_{\text{max}}$ of 1.2-2.5 hr (Monaghan et al., 1997; Ram et al., 2001). With multiple oral doses of GX (600-1000 mg, twice daily), steady-state levels occurred after 7 days of dosing (Monaghan et al., 1997). Overall, GX has been studied in more than 1,900 pediatric and adult subjects across various indications at therapeutically relevant doses and treatment regimens for more than two years.

In a total of 1,844 patients involved in placebo-controlled studies, 743 patients received placebo while 1,101 patients received GX. The frequency of adverse events in these trials was 62.9% for GX and 53.8% for placebo. The serious adverse event rate was similar for GX (2.8%) and placebo (3.8%) with the most common adverse effects of GX being somnolence, dizziness, fatigue, and headache. GX has been evaluated in adult patients who are medically refractory for complex partial seizures (Laxer et al., 2000), adult epilepsy patients with uncontrolled partial-onset seizures (Sperling et al., 2017), drug-resistant partial-onset seizures in adults (NCT01963208), girls with PCDH19-clustering epilepsy (Lappalainen et al., 2017; Sullivan et al., 2023), and children with complex epileptic seizure conditions (Kerrigan et al., 2000; Pieribone et al., 2007; Ligsay et al., 2017). In these trials, oral GX (~1,500 mg/day) was generally safe and well tolerated with no serious adverse events. In most of these trials, GX did not
meet the primary outcome for efficacy. Reasons for not meeting the efficacy endpoints include limited drug absorption from oral formulations and insufficient brain levels for target receptor interactions. While the success of clinical efficacy in adult partial epilepsy and pediatric infantile spasms were limited, GX had significant efficacy in children with CDKL5-deficient epilepsy (Knight et al., 2022). In 2022, GX was approved by the FDA to treat seizures associated with CDKL5-deficiency. An overview of the GX clinical profile for this pediatric epilepsy is shown in Table 6. Based on the latest meta-analysis of outcomes from four randomized controlled trials in a total of 659 patients (Meng et al., 2022), despite a ≥50% reduction in mean seizure frequency, the percentage of seizure-free days in these patients did not differ significantly from placebo (p = 0.36). Future trials will determine the effectiveness of GX in managing refractory epilepsy.

**Pilot Ganaxolone Efficacy Trials in RSE Patients**

GX is a potential anticonvulsant for SE. Based on preclinical GX datasets in OPNA models of RSE from the NIH CounterACT program (Reddy, 2016), GX has been redirected to treat SE using an injectable route. GX has advanced to clinical trials in patients with SE and RSE using an intravenous product (2 mg/ml) formulated in a β-cyclodextrin mixture (Captisol, betadex sulfobutyl ether sodium), with a strict intake limit of 50 g/day. Due to its limited bioavailability and rapid clearance, GX is given as a bolus injection followed by a 24-90 hr maintenance infusion protocol and an 18 hr taper. At these levels, pharmacological actions of GX stem from its allosteric modulation and direct activation of extrasynaptic and synaptic GABA-ARs (Fig. 4). Manufacturing, formulation, and clinical development, including phase 3 GX trials in patients with RSE, were initiated with the support of a BARDA contract to develop GX as an MC for nerve agent exposures (see BARDA press release, September 14, 2020). GX is the first anticonvulsant MC that stemmed from the NIH CounterACT program and successfully advanced to BARDA-supported clinical development.

The PK features of GX appear suitable for RSE treatment. After intravenous GX bolus doses of 30 mg (over 5 min), C\text{max} values were 1240 ng/ml with T\text{max} of 5 min. Following infusions of 10 or 30 mg over 1 hr, C\text{max} values were 80.2 ng/ml and 257 ng/ml. The levels follow a triphasic decline after ceasing drug administration. To evaluate the effect of IV GX in a phase 1 trial with 36 healthy volunteers, PK-PD correlation analysis showed rapid changes in EEG bispectral indices that likely result from brain distribution of GX (Hussain et al., 2019).

An open-label, dose-finding, phase 2 study evaluated the efficacy and safety of IV GX when added to the standard-of-care ASMs in 17 patients (8 males, 9 females) with RSE (Vaitkevicuis et al., 2022). Patients (age range 23-88 years) with convulsive or non-convulsive SE and who did not respond to at least one second-line ASM were enrolled in this trial (NCT03350035). GX infusion started with an IV bolus administered over 3 min, followed by continuous infusion at decreasing rates for 2-4 days, and finally by an 18-hr taper. The study included three GX cohorts: low (500 mg/day, n=5), medium (650 mg/day, n=4), and high (713 mg/day, n=8) doses. The primary endpoint was prevention of treatment escalation to IV anesthesia 24 hrs after GX initiation. This criterion was met in all patients in all three cohorts, with somnolence as the main treatment-emergent adverse effect (12%). No patients required third-line intravenous anesthetics within 24 hrs after starting GX. The median time to cessation of SE after
GX initiation was 5 min. The initial bolus of IV GX resulted in rapid plasma GX levels (~900 ng/ml). Cohorts receiving high-dose GX, achieved and maintained plasma levels >500 ng/ml for ~8 hrs with sustained reduction in seizure burden (>88%) throughout entire analysis window. Cohorts that received medium-dose GX reached plasma levels >400 ng/ml with a reduced seizure burden (>75%) and low-dose GX achieved and maintained plasma levels >500 ng/ml for ~4 hrs with reduced seizure burden (>60%) (Vaitkevicuis et al., 2022). Factors affecting these therapeutic outcomes include patient heterogeneity, enrollment criteria, and intubation status. A total of 23 related adverse events (AEs) were reported with 16 mild, 5 moderate, and 2 severe AEs. However, many patients discontinued therapy (n=3 each in low- and medium-dose cohorts and 1 in the high-dose cohort) due to lack of efficacy or adverse sedation effects. The antiseizure response in patients who completed the trial was not dose-related despite increased plasma levels. Doses higher than 713 mg/day were not tested due to FDA limitations on daily Captisol intake (50 g/d).

IV GX was also tested in two pediatric patients (ages 7 and 17) with super RSE (Singh et al., 2022). These patients received an initial bolus of IV GX followed by maintenance infusion for up to 4.5 days. Intermittent IV boluses were given as needed, and on day 5, a taper was initiated. Subsequently, patients were transitioned to oral treatment using a GX suspension. Adjunctive GX effectively terminated SRSE in both patients, allowing for safe reduction of IV anesthetics. Seizure control was maintained after transitioning to enteric GX.

**Pivotal Ganaxolone Trials for Efficacy and Safety in RSE Patients**

Favorable results from the phase 2 study prompted the advancement of pivotal GX trials in patients with RSE. A phase 3 double-blind, placebo-controlled study (RAISE, NCT04391569), funded by a BARDA contract, is currently underway to evaluate the efficacy and safety of IV GX in RSE. The goal of this pivotal trial is to establish the efficacy and safety of IV GX in SE patients 12 years of age or older who failed treatment with benzodiazepines and two common second-line SE treatments, fosphenytoin (or phenytoin), and levetiracetam (or valproate), and who have not yet received IV sedation. Patients receive placebo or a GX bolus dose, followed by continuous infusion for 36 hrs, then a 12-hr taper. Primary outcomes are the proportion of patients with SE cessation within 30 min of drug initiation, determined by clinical and EEG findings, and lack of progression to IV anesthesia for 36 hrs. Secondary outcomes are the proportion of patients with SE cessation within 48 min, as determined by clinical and EEG findings, lack of progression to IV anesthesia for 72 hrs, and time to SE cessation following GX initiation. Patients are excluded if they had SRSE with >18 hr of high-dose IV anesthesia and patients with anoxic brain injury.

In addition to the RAISE trial at 80 centers in North America and Australia, IV GX is being evaluated in two other pivotal trials. RAISE II is a phase 3 study in Europe with key criteria being failure of benzodiazepines and at least one IV ASM. Patients receive placebo or GX with concurrent IV ASM initiation. The primary outcome focuses on responder analysis of SE cessation within 30 min with no escalation of care within 36 hrs. In the RESET trial, GX is evaluated in established SE patients who failed a first-line benzodiazepine. Patients receive placebo or GX with concurrent second-line ASM initiation with the primary endpoint being SE cessation within 30 min.
Novel Synthetic Neurosteroids (Super-ganaxolones) for RSE

Despite their potential clinical applications for SE and seizure conditions, GX and other synthetic neurosteroids contend with numerous significant limitations that present serious challenges for therapeutic development (Table 7). Based on clinical experience, GX has several drawbacks, including lack of water solubility, poor oral bioavailability, short plasma half-life, low patient adherence due to multiple daily dosing, limited correlations between PK and PD, and the necessity for complex formulations (such as β-cyclodextrin) that have potential side effects. β-Cyclodextrin is not absorbed, its excessive oral consumption can lead to gastrointestinal symptoms like bloating and diarrhea, it interferes with nutrient absorption (Braga, 2019), and can be fermented by human gut bacteria. Although β-cyclodextrin is designated as GRAS (“generally recognized as safe”), its maximal daily oral intake is limited. Injectable β-cyclodextrin formulations may pose a risk of renal toxicity, especially in persons with kidney disease, prompting the FDA to establish safe daily β-cyclodextrin intake limits (Table 5). The antiseizure effects of GX are diminished when associated with elevated zinc in the brain (Chuang and Reddy, 2019). Therefore, novel neurosteroid analogs with improved biopharmaceutical properties are needed to overcome the limitations of GX and related neurosteroids highlighted above.

Many analogs of brexanolone and ganaxolone structure have been designed and tested for anticonvulsant efficacy (Reddy and Kulkarni, 2000; Qian et al., 2014; Hogenkamp et al., 2014; Branco et al., 2018; Zorumski et al., 2019; Reddy, 2023). Several water-soluble analogs were synthesized (super-ganaxolones) with improved hydrophilicity, an essential feature for intravenous and injectable formulations (Reddy, 2023; 2024). These novel analogs are designed to exhibit subunit selectivity and preferential interaction with extrasynaptic GABA-ARs to achieve greater therapeutic outcomes than GX (Fig. 3). We recently synthesized over 20 new synthetic GX analogs with significantly improved potency, antiseizure efficacy, greater water solubility, and a strong preference for acting on extrasynaptic receptors (Chuang and Reddy, 2018b, Reddy, 2023). Development of these new analogs was guided by three key factors: 1) ability to cross the blood-brain barrier, which is crucial for their effectiveness in the brain; 2) ability to dissolve effectively in water, ensuring stable injection products and proper tissue distribution; and 3) a slow metabolism rate, enabling longer plasma half-lives and improved therapeutic results. GX analogs at the C-21 position (e.g. hydroganaxolone) preferentially interact with and selectively increase extrasynaptic δGABA-AR-mediated tonic currents, producing greater antiseizure activity against focal seizures than GX (Chuang and Reddy, 2018b), indicating their potential for preferential allosteric and direct activation of extrasynaptic δGABA-ARs that effectively regulate network discharges and seizures. When tested in animal models of OPNA-induced RSE, these lead analogs effectively blocked RSE when given intramuscularly, 40 min after DFP administration, indicating their efficacy in the DFP model. The lead analogs significantly reduced neuronal injury, neurodegeneration, and inflammation in the hippocampus and other regions (Reddy, 2024; Ramakrishnan et al., 2024), suggesting their neuroprotectant potential to mitigate OPNA-induced brain damage. The most promising drug-like compounds are in advanced development as anticonvulsants and neuroprotectants for SE indications. Future studies will determine if these innovative molecules provide superior clinical outcomes for treating OPNA poisoning and RSE.
Development of new GX extended-release and advanced delivery formulations could enhance neurosteroid use for RSE therapy by offering advantages such as prolonged and consistent therapeutic effects that will potentially improve seizure control and patient compliance. These innovations may also reduce the healthcare burden associated with frequent dosing and emergency interventions. Ultimately, these formulations could optimize the use of neurosteroid therapy to treat RSE. Instead, the development of synthetic neurosteroids with improved biopharmaceutical and therapeutic attributes can circumvent the constraints associated with formulation.

Developing novel extrasynaptic-targeted neurosteroids for RSE and other nerve agent applications is an efficient approach to rapidly translate neurosteroid therapeutics to clinic. Novel synthetic neurosteroids have many advantages over benzodiazepines and other GABAergic anticonvulsants for therapeutic use (Reddy, 2021), including: 1) Neurosteroids can be effective in benzodiazepine-refractory conditions because they activate most GABA-AR isoforms; 2) Unlike benzodiazepines, neurosteroids do not induce tolerance upon repeated use and do not have drug interaction issues; 3) Neurosteroids have a rapid action onset and intermediate duration; 4) Maximal efficacy is expected even in resistant seizures due to direct, non-allosteric actions; 5) Neurosteroids promote tonic inhibition that does not require interneurons to be beneficial; 6) Neurosteroids are readily available and FDA-approved (BX and GX) for clinical use; 7) Neurosteroids are anti-inflammatory and neuroprotectant in many neuronal injuries; 8) Neurosteroids are lipophilic for brain distribution; 9) New hydrophilic synthetic analogs can surpass oral GX bioavailability; and 10) New water-soluble analogs would allow superior injectable products. Neurosteroid treatment is considered safe and well tolerated in clinical trials. The most common neurosteroid side effect is transient sedation, an extension of their therapeutic effect at GABA-ARs (Meltzer-Brody et al., 2018; Knight et al., 2022). Some patients report adverse events including dizziness, fatigue, and somnolence that can be reversed when therapy is discontinued. The half-life of synthetic neurosteroids is inadequate for once daily administration as monotherapy or adjunct therapy. Although they lack pharmacokinetic drug interactions, neurosteroids can potentially interact with other GABAergic drugs and zinc, affecting their overall efficacy and safety. Administering GX with other ASMs, such as tiagabine and midazolam, can have synergistic effects but allows adjunct utility in polytherapy (Chuang and Reddy, 2020). In contrast, zinc interactions can prevent neurosteroid protective effects by blocking extrasynaptic receptors (Chuang and Reddy, 20019). These drug interactions have clinical implications in GX therapy for brain conditions associated with zinc fluctuations, including OPNA intoxication, SE, stroke, and meningitis.

Medical Gaps and Challenges in Developing Anticonvulsants for RSE

The process of developing new MCs for RSE is complex, tedious, and uncertain, mainly due to scientific and regulatory challenges. Lack of a standard validated preclinical model is hampering efforts to identify new RSE anticonvulsants. In most RSE animal models, acute persistent seizures are induced by nerve agents that are often benzodiazepine resistant. These models mimic refractory SE but lack actual human etiology underlying SE. Such key differences may contribute to discrepancies in the efficacy or potential value of these animal model outcomes.
With the exception of PB, there is no record of FDA approving a MC via the Animal Rule pathway, which is a very complex and nearly implausible regulatory strategy for new nerve agent MCs. Nerve agent-induced RSE is very resistant to current and new anticonvulsants, as seen in experimental rodent model outcomes showing minimal responses to second-line antiseizure medications such as valproate, levetiracetam, and phenytoin (Morgan et al., 2021). There may be a disparity between PK results of medications in animal RSE models and those in patients with RSE, which may appear as poor efficacy or suboptimal protection if the PK-PD relationship is not clear.

SE is a dynamic and highly complex condition involving multiple pathophysiological processes that are not necessarily mutually exclusive, and occur at a rapid pace. Pro-convulsant cascades may co-occur during SE development. Stopping SE is an immediate, but not sufficient, goal because the longer a seizure continues, the more difficult it is to stop with ASMs. Seizure activity can exhaust neuronal networks, triggering secondary signaling cascades, and pro-inflammatory cytokines are commonly overproduced in NORSE. Thus, while neuroprotection therapies are necessary to prevent long-term effects of RSE, multiple combination therapies are complex and may have serious side effects, including sedation and respiratory depression. Ambulatory or field settings are a key concern because with combination treatments, lack of intubation facilities could harm the victim. Also, while nasal benzodiazepine rescue treatments are vital to limit brain damage from repeated acute seizures, such therapies should be used with medical supervision and be carefully monitored by a physician to spot drug resistance trends.

Current RSE clinical trial designs and clinical outcomes are not optimal due to variability between hospitals or facilities providing emergency care. Overall outcomes are good in 56%, and poor in 42% of patients. Predictors of poor outcome include electrographic seizures, non-convulsive seizures, diffuse cortical edema, and multifocal abnormality on imaging with no obvious relation to etiology or treatment in some cases. We need to improve this therapeutic management issue. While stroke has well-established guidelines for acute emergency treatment, RSE lacks such clear clinical protocols. Risks of prolonged anesthesia are scrutinized in some patients with RSE, including infectious complications, severe hypotension, need for vasopressor treatment, and mechanical ventilation.

Optimal RSE clinical study designs involve rigorous methods to assess an interventions’ effectiveness and safety. A randomized controlled trial (RCT) with an adaptive design can provide dynamic insights into treatment efficacy by adjusting sample sizes based on interim results, while observational longitudinal studies, complemented by propensity score matching, enable the evaluation of real-world treatment outcomes while controlling for confounding factors. By combining RCTs with adaptive designs and observational studies with propensity score matching, researchers can obtain comprehensive data that informs evidence-based care for RSE patients, potentially leading to improved clinical outcomes. Nonetheless, successful implementation of these designs requires meticulous planning and consideration of budgetary constraints, particularly related to drug development.

The definition of SE and RSE are often confused. According to the ILAE, SE is defined as "a condition resulting either from failure of the mechanisms responsible for seizure termination or from initiation of mechanisms, which lead to abnormally prolonged seizures (after time point t1). This condition can have long-term consequences (after time point t2), constituting a neurological emergency." Per this definition, time point t1 is usually defined as 5 min,
and time point $t_2$ represents the time beyond which there is an increased likelihood of long-term consequences. In essence, SE is characterized by a prolonged seizure or a series of seizures that occur without recovery between them, and it is considered a medical emergency due to the potential for serious neurological and systemic complications. There is no clear definition of RSE, which is interpreted as a specific subtype of SE characterized by seizures that persist and do not respond to initial or second-line treatments. In contrast to SE, RSE poses significant management and treatment challenges. More aggressive therapies, such as anesthetic agents or other advanced or combination interventions are often required to achieve seizure control. Because of the diverse range of patient conditions and potential long-term risk factors, RSE outcomes often lack a standardized parametric measure.

There are different perspectives on how and what to communicate with patients and family members about a patient’s condition. Families are often unprepared for final decisions, outcomes, and rehabilitation requirements, and in most cases, physicians are not fully aware of long-term patient outcomes from therapeutic management of RSE. Patient advocates recommend using common language and measures with unified, candid evaluations, providing therapy information, discharge planning guide, printable brochure, discussing long-term outcomes and collaborative infrastructure linking acute and post-acute stages in patient management. Many questions need to be addressed by further research and refinement of models and clinical evaluation, including: 1) does race/ethnicity/ gender affect response to specific treatments; 2) are there better approaches to personalized therapy; 3) are there validated biomarkers of SE-induced neuronal injury; 4) can we prevent SE recurrence; and 5) what are better ways to clinically evaluate new medications?

Further research is essential to enhance our understanding of OPNA exposure and the effectiveness of RSE anticonvulsant treatments in vulnerable populations, including children, elderly, and military personnel. Future research should focus on addressing gaps in modeling of RSE and studying the long-term neurological outcomes associated with OPNA intoxication. By addressing these gaps, we can advance the development of targeted interventions and improve national preparedness to manage mass casualties in the event of chemical exposures.

**Conclusions and Future Directions**

Chemical warfare agents including nerve agents and OPs are highly neurotoxic substances that cause acute and long-term neurotoxicity and are used as weapons of mass destruction. Acute exposure to OPNAs causes predictable toxic signs, such as hypersecretion, tremors, convulsions, respiratory distress, and even death. RSE induced by these agents can lead to long-term neuronal damage and severe neurologic dysfunction. Prompt and effective control of RSE is crucial for survival and preventing long-term brain injury. Benzodiazepines are the first-line therapy for SE but are significantly limited in controlling RSE seizures. Presently, no FDA-approved post-exposure MCs are available to mitigate the effects of OPNA intoxication, specifically RSE (Younus et al., 2017), creating an urgent unmet medical need for novel and innovative anticonvulsants to protect civilians and soldiers against neurotoxic nerve agent effects. Effective anticonvulsants should ideally be administered within 30 min to protect against seizures and neurological damage, which is not always feasible in emergency situations. Therefore, OPNA-induced seizures and neurotoxicity can produce enduring brain injury and significant neuropsychiatric dysfunction in
chemical attack survivors and in animal models. Standard-of-care MCs for pre-exposure (pyridostigmine bromide), and post-exposure (atropine and 2-PAM) regimens including the anticonvulsant benzodiazepine (midazolam) do not effectively prevent or mitigate all nerve agent intoxication symptoms, especially RSE and its devastating effects on neurons and microglia. Rapid and effective RSE suppression is crucial to improve short- and long-term outcomes.

We and others have worked for 10 years to develop neurosteroid-based MCs for OPNA intoxication and these bench to clinic efforts were primarily supported by the NIH CounterACT program (Fig. 5). While benzodiazepines are currently standard-of-care MCs for post-exposure treatment, they have limited ability to prevent or mitigate seizures associated with nerve agent intoxication. Neurosteroids, including GX, are highly effective anticonvulsants for RSE. Our mechanistic hypothesis on extrasynaptic tonic inhibition translated to a robust neurosteroid therapeutic efficacy when we discovered the potential of neurosteroids to treat nerve agent-induced SE. GX, a lead synthetic neurosteroid, acts on both extrasynaptic and synaptic GABA-ARs and surpasses benzodiazepines in suppressing SE. GX has broad-spectrum anticonvulsant activity in animal seizure models and OPNA-induced RSE. Specifically, GX and related neurosteroids that activate extrasynaptic GABA-ARs are more powerful anticonvulsants and improve overall neurological outcomes after OPNA exposure and RSE. Neurosteroids are more effective anticonvulsant antidotes than benzodiazepines because even when administered very late after OPNA exposure, they produce greater protection alone or together with midazolam, making them practical MCs for OPNA attacks and RSE treatment. In preclinical studies, GX strongly protected against DFP- and soman-induced seizures and against SE even when administered 40-120 min after agent exposure. In OPNA models, GX not only provides strong neuroprotection by reducing neuronal damage and neuroinflammation, but also helps to alleviate long-term neuropsychiatric impairments. The strong synergistic protection GX provides in combination regimens with benzodiazepines, formed the basis for moving it into advanced, BARDA-supported phase 3 RSE and nerve agent seizure trials. In pilot trials, intravenous GX rapidly suppressed RSE, avoiding escalation to intravenous anesthesia.

With the backing of a BARDA contract to develop GX as an MC for nerve agent exposures, manufacturing and clinical development stages, including pivotal phase 3 trials, have been initiated for patients with RSE. Notably, GX is the first anticonvulsant MC to emerge from the NIH CounterACT program. Neurosteroid therapy using GX has promising potential as a practical anticonvulsant antidote for both military and civilian individuals affected by OPNA intoxication. GX offers advantages over benzodiazepines, including broad-spectrum effectiveness, absence of tolerance with repeated use, quick onset and intermediate duration of action, a well-understood mechanism of action, proven safety from clinical trials, and suitability for rapid deployment using autoinjector formulations by first responders. Ketamine and glutamate receptor antagonists are other agents in development that show promise in OPNA models. However, many complexities, challenges, and regulatory uncertainties are involved in developing safe and effective RSE anticonvulsants. Further research is needed to address key gaps in modeling of OPNA exposure and anticonvulsant treatments for OPNA intoxication in vulnerable populations, as well as military, including long-term neurological outcomes.
Despite its revolutionary clinical potential, GX faces significant limitations of drug delivery, including poor water solubility, low oral bioavailability, short plasma half-life, and complex formulations with β-cyclodextrin that carry potential side effects and dosing limitations. These limitations create an unmet medical need to develop novel formulations and new analogs with improved biopharmaceutical properties to overcome these challenges. Recently, we developed novel water-soluble neurosteroids with improved biopharmaceutical properties, offering enhanced options to develop injectable MCs for OP intoxication and RSE management. Unlike GX that requires complex formulations with limits on daily administration, these newer hydrophilic neurosteroid analogs, known as super-ganaxolones, have been specifically designed to maintain their GABA-AR activity while increasing hydrophilicity and improving drug delivery.
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Data Availability Statement

The authors declare that all the data supporting the findings of this study are contained within the paper.

Conflict of Interest

The authors declare no competing financial interests.

Author Contributions

Wrote or contributed to the writing of the manuscript: Reddy.
References


Table 1.
Comparative anticonvulsant profile of ganaxolone (GX) and brexanolone (AP) in experimental models.
Values in parentheses are 95% confidence limits.

<table>
<thead>
<tr>
<th>Model</th>
<th>AP (mg/kg)</th>
<th>GX (mg/kg)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kindling Models</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampus kindling</td>
<td>3.5</td>
<td>3.5</td>
<td>(Carver et al., 2014; Reddy et al., 2012)</td>
</tr>
<tr>
<td>Amygdala kindling</td>
<td>14 (8–23)</td>
<td>6.6 (5.1–9.7)</td>
<td>(Reddy and Rogawski, 2010)</td>
</tr>
<tr>
<td>Cocaine kindling</td>
<td>17.0 (ND)</td>
<td>17.0 (ND)</td>
<td>(Kaminski et al., 2003)</td>
</tr>
<tr>
<td>Pentyleneetetrazol kindling</td>
<td>ND</td>
<td>3.5 (2.4–5.1)</td>
<td>(Gasior et al., 2000)</td>
</tr>
<tr>
<td>Corneal kindling</td>
<td>ND</td>
<td>4.5 (4.0–5.1)</td>
<td>(Carter et al., 1997)</td>
</tr>
<tr>
<td><strong>Chemocorvulsant Models</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentyleneetetrazol (mice)</td>
<td>13.7 (10.1–18.7)</td>
<td>3.5 (2.1–5.8)</td>
<td>(Carter et al., 1997; Kokate et al., 1994)</td>
</tr>
<tr>
<td>Pentyleneetetrazol (rats)</td>
<td>2.14 (1.10–4.15)</td>
<td>4.3 (2.8–6.9)</td>
<td>(Reddy and Rogawski, 2000; 2001)</td>
</tr>
<tr>
<td>Bicuculline</td>
<td>12 (10–15)</td>
<td>4.6 (3.2–6.8)</td>
<td>(Carter et al., 1997)</td>
</tr>
<tr>
<td>Picrotoxin</td>
<td>10 (5–19)</td>
<td>ND</td>
<td>(Belelli et al., 1989)</td>
</tr>
<tr>
<td>t-Butylbicycloorthobenzoate</td>
<td>ND</td>
<td>11.7 (8.8–15.7)</td>
<td>(Carter et al., 1997)</td>
</tr>
<tr>
<td>Flurothyl (rats)</td>
<td>ND</td>
<td>5.0 (ND)</td>
<td>(Liptakova et al., 2000)</td>
</tr>
<tr>
<td>N-Methyl-D-aspartate</td>
<td>&gt;40</td>
<td>&gt;30</td>
<td>(Carter et al., 1997)</td>
</tr>
<tr>
<td>Kainic acid</td>
<td>&gt;40</td>
<td>&gt;30</td>
<td>(Carter et al., 1997)</td>
</tr>
<tr>
<td>4-Aminopyridine</td>
<td>&gt;40</td>
<td>11.5 (8.1–16.3)</td>
<td>(Carter et al., 1997)</td>
</tr>
<tr>
<td>Strychnine</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>(Carter et al., 1997)</td>
</tr>
<tr>
<td><strong>Electroshock Models</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal electroshock</td>
<td>29 (19–44)</td>
<td>29.7 (25.3–34.8)</td>
<td>(Carter et al., 1997)</td>
</tr>
<tr>
<td>6-Hz stimulation</td>
<td>4.2 (2.7–5.8)</td>
<td>1.5 (1.3–1.7)</td>
<td>(Carver and Reddy, 2016)</td>
</tr>
<tr>
<td><strong>Status Epilepticus Models</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pilocarpine</td>
<td>7 (4–11)</td>
<td>~6</td>
<td>(Briyal &amp; Reddy, 2008; Kokate et al., 1996)</td>
</tr>
<tr>
<td>Kainic acid</td>
<td>~20</td>
<td>ND</td>
<td>(Rogawski et al., 2013)</td>
</tr>
<tr>
<td><strong>OP Models</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFP model (rats)</td>
<td>~5</td>
<td>~4.8</td>
<td>(Reddy, 2015; 2019)</td>
</tr>
<tr>
<td>Soman model (rats)</td>
<td>~6</td>
<td>~5.5</td>
<td>(Reddy, 2015; 2019)</td>
</tr>
<tr>
<td>VX model (rats)</td>
<td>ND</td>
<td>~6</td>
<td>(Reddy, 2015; Reddy, 2019)</td>
</tr>
<tr>
<td>TETS model (mice)</td>
<td>~3</td>
<td>~3</td>
<td>(Zolkowska et al., 2018)</td>
</tr>
</tbody>
</table>

ND, not determined.
### Table 2.
Comparative mechanistic assessment of GX with AP for relative potency and efficacy for allosteric and direct-gating effect at GABA-A receptors in hippocampus dentate gyrus neurons.

<table>
<thead>
<tr>
<th>Compound</th>
<th>GABA-gated whole-cell current</th>
<th>Tonic current</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Allosteric effect</td>
<td>Direct effect</td>
</tr>
<tr>
<td></td>
<td>EF\textsubscript{(2-fold GABA)} (nM)\textsuperscript{b}</td>
<td>E\textsubscript{30}μM (pA)\textsuperscript{d}</td>
</tr>
<tr>
<td>AP</td>
<td>474</td>
<td>273.5</td>
</tr>
<tr>
<td>GX</td>
<td>389</td>
<td>336.1</td>
</tr>
<tr>
<td>GABA\textsuperscript{a}</td>
<td>-</td>
<td>1426.3</td>
</tr>
</tbody>
</table>

\textsuperscript{a}GABA \textsubscript{EC\textsubscript{50}}: 18.6 μM. \textsuperscript{b}EF values represent the effective functional drug concentration (nM) required to double the 3 μM GABA (EC\textsubscript{10}) response. \textsuperscript{c}GABA 1 μM tonic current response: 0.66 ± 0.22 pA/pF, 19.6 pA. (Chuang and Reddy, 2018b).
Table 3.
Correlation between neurosteroid activation of extrasynaptic tonic inhibition and seizure protection in mouse 6-Hz model.

<table>
<thead>
<tr>
<th>Neurosteroid</th>
<th>Tonic Currents</th>
<th>Seizure Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$E_1 \mu M$ (pA)*</td>
<td>EF (2-fold GABA) (nM)**</td>
</tr>
<tr>
<td>Ganaxolone</td>
<td>64.0</td>
<td>290</td>
</tr>
<tr>
<td>Brexanolone (AP)</td>
<td>100.6</td>
<td>80</td>
</tr>
<tr>
<td>Pregnanolone</td>
<td>44.4</td>
<td>780</td>
</tr>
<tr>
<td>Isopregnanolone</td>
<td>15.3</td>
<td>&gt;10000</td>
</tr>
<tr>
<td>THDOC</td>
<td>66.6</td>
<td>410</td>
</tr>
<tr>
<td>Alfaxolone</td>
<td>40.9</td>
<td>990</td>
</tr>
<tr>
<td>ORG-20599</td>
<td>86.4</td>
<td>120</td>
</tr>
<tr>
<td>Androstanediol</td>
<td>33.2</td>
<td>1710</td>
</tr>
</tbody>
</table>

*$E_1 \mu M$ values represent the mean normalized tonic current drug responses at 1 $\mu M$ concentration co-applied with 1 $\mu M$ GABA. GABA 1 $\mu M$ tonic current: 0.66 ± 0.22 pA/pF, 19.6 pA. **EF values represent the effective functional concentration drug (nM) required to double or triple the 1 $\mu M$ GABA response (Carver and Reddy, 2016).
Table 4.
Summary of ganaxolone experimental efficacy studies in OPNA and RSE rat models.

<table>
<thead>
<tr>
<th>Study Type and protocol</th>
<th>DFP Model</th>
<th>Soman Model</th>
</tr>
</thead>
</table>
| (a) Anticonvulsant efficacy (monotherapy) in adult rats: GX given @ 40, 60, or 120 min after OP | - Stopped electrographic SE  
- Stopped behavioral SE  
- Significantly decreased seizure activity  
- Significantly decreased SE duration  
- 100% survival rate | - Stopped electrographic SE  
- Stopped behavioral SE  
- Significantly decreased seizure activity  
- Significantly decreased SE  
- 100% survival rate |
| (b) Acute neuroprotective efficacy (monotherapy) in adult rats: GX given @ 40 min after OP | - Significantly reduced neuronal injury  
- Significantly prevented loss of principal neurons  
- Significantly decreased cell death of inhibitory interneurons | - Significantly reduced neuronal injury  
- Significantly prevented principal neuron loss  
- Significantly decreased inhibitory interneuron loss |
| (c) Combination anticonvulsant efficacy (with midazolam) in adult rats: GX given @ 40 min after OP exposure along with midazolam | - Combination regimen was more effective in rapidly terminating SE than midazolam alone  
- Extent of neuroprotection was greater better than midazolam alone  
- 100% survival rate | - Combination regimen was more effective in terminating SE than midazolam alone  
- Extent of neuroprotection was better than midazolam alone  
- 100% survival rate |
| (d) Combination neuroprotectant efficacy (with midazolam) in adult rats: GX given @ 40 min after OP exposure along with midazolam | - Significantly reduced neuronal injury  
- Combination regimen was more effective neuroprotectant than midazolam alone | - Significantly reduced neuronal injury  
- Combination regimen was more effective neuroprotectant than midazolam alone |
| (e) Chronic protective efficacy in adult rats: Animals tested 3 months after OP exposure. GX given @ 40 min after OP | - Significant decrease in frequency and severity of epileptic seizures and other electrographic ictal abnormalities.  
- Attenuation of chronic behavioral anxiety, depression, and memory deficits | - Significantly reduced incidence of epilepsy and seizure frequency and EEG-based ictal abnormalities  
- Attenuated chronic behavioral anxiety, depression, and memory deficits |
| (f) Chronic neuroprotective efficacy in adult rats: Animals tested 3 months after OP exposure. GX given @ 40 min after OP exposure | - Significant reduction in neurodegeneration of principal neurons and inhibitory interneurons  
- Significantly reduced cellular neuroinflammation of astrogliosis and microgliosis.  
- Reduced mossy fiber sprouting | - Significantly reduced neurodegeneration of principal cells and inhibitory interneurons  
- Significantly reduced cellular neuroinflammation of astrogliosis and microgliosis  
- Reduced mossy fiber sprouting |
| (g) Anticonvulsant efficacy in pediatric rats: GX given @ 40 min after OP exposure | - Rapidly stopped electrographic SE  
- Effectively stopped behavioral SE  
- Significantly decreased seizure activity and SE duration  
- 100% survival rate | - Rapidly stopped electrographic SE  
- Effectively stopped behavioral SE  
- Significantly decreased seizure activity and SE duration  
- 100% survival rate |
| (h) Neuroprotectant efficacy in pediatric rats: GX given @ 40 min after OP exposure | - Significantly reduced neuronal injury  
- Significantly reduced loss of principal neurons  
- Significantly prevented loss of inhibitory interneurons  
- Significantly reduced neuroinflammation | - Significantly reduced neuronal injury  
- Significantly reduced principal neuron loss  
- Significantly prevented inhibitory interneuron loss  
- Significantly reduced neuroinflammation |
| (h) Chronic protective efficacy in pediatric rat models: Animals tested 3, 5, and 10 months after OP exposure GX given @ 40 min after OP | - Reduced mood deficits, anxiety, and aggressive traits  
- Attenuation of memory deficits  
- Reduced epileptic seizures and ictal abnormalities | - Decreased chronic anxiety, depression, and memory deficits  
- Attenuated epileptic seizures and ictal abnormalities |
<table>
<thead>
<tr>
<th>Study Type and protocol</th>
<th>Overall Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DFP Model</strong></td>
<td><strong>Soman Model</strong></td>
</tr>
<tr>
<td>(i) Chronic neuroprotective efficacy in pediatric rat models: Animals tested 3 and 10 months after OP exposure. GX given @ 40 min after OP exposure</td>
<td>- Reduced neurodegeneration of principal neurons and interneurons</td>
</tr>
<tr>
<td></td>
<td>- Significantly reduced cellular neuroinflammation (astrogliosis and microgliosis)</td>
</tr>
<tr>
<td></td>
<td>- Reduced mossy fiber sprouting</td>
</tr>
<tr>
<td>(j) Anticonvulsant efficacy in aged rats: GX given @ 40 min after OP exposure</td>
<td>- Stopped electrographic SE</td>
</tr>
<tr>
<td></td>
<td>- Effectively terminated behavioral SE</td>
</tr>
<tr>
<td></td>
<td>- Reduced seizure activity and SE duration</td>
</tr>
<tr>
<td></td>
<td>- 100% survival rate</td>
</tr>
<tr>
<td>(k) Neuroprotectant efficacy in aged rats: GX given @ 40 min after OP exposure</td>
<td>- Reduced neuronal injury</td>
</tr>
<tr>
<td></td>
<td>- Reduced loss of principal neurons and inhibitory interneurons</td>
</tr>
<tr>
<td></td>
<td>- Significantly reduced cellular neuroinflammation</td>
</tr>
<tr>
<td>ND, not determined</td>
<td>ND</td>
</tr>
</tbody>
</table>
### Table 5.
Summary of past and ongoing ganaxolone clinical trials in brain disorders.

<table>
<thead>
<tr>
<th>Reference or NCT ID</th>
<th>Indication</th>
<th>Phase</th>
<th>Population &amp; Number</th>
<th>Study Design (Dose and duration)</th>
<th>Safety outcomes</th>
<th>Efficacy outcomes</th>
<th>Study dates</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data et al. 1998</td>
<td>Treatment of migraine</td>
<td>2</td>
<td>Adult female (N=45)</td>
<td>Double-blind, placebo-controlled; Placebo (N=25); GX (N=30). Daily oral GX (200 mg) for 12 weeks</td>
<td>Tolerable</td>
<td>Reduced migraine frequency</td>
<td>1996-1998</td>
<td>Completed, endpoint Not met</td>
</tr>
<tr>
<td>Pieribone et al. 2007</td>
<td>Refractory Epilepsy</td>
<td>2</td>
<td>Children and adolescent aged 5-15 (N=15)</td>
<td>Single group assignment: Dose titration ranged between IV GX 1 mg/kg b.i.d. to 12 mg/kg, t.i.d. Dose escalation phase took up to 16 days. Following dose escalation, subjects remained on a ganaxolone maintenance dose for 8 weeks</td>
<td>Tolerable at low doses, mild side effects at high doses: - Somnolence (20%) - Convulsion (13%)</td>
<td>58% reduction of seizure frequency by week 4 compared to baseline</td>
<td>1995-1999</td>
<td>Completed, end point partially met</td>
</tr>
<tr>
<td>Monaghan et al. 1997</td>
<td>Generalized Seizures</td>
<td>2</td>
<td>Adults (N=96)</td>
<td>96 healthy male and female volunteers received ganaxolone in a variety of formulations, doses, and dosing regimens</td>
<td>Tolerable: - Headache, dizziness, somnolence (82%) - Gastrointestinal disturbances (14%)</td>
<td>Baseline safety, tolerability, and PK of GX were established</td>
<td>1997</td>
<td>Completed, endpoint met</td>
</tr>
<tr>
<td>Laxer et al. 2000</td>
<td>Complex partial seizures</td>
<td>2</td>
<td>Adults aged 18 to 65 (N=52)</td>
<td>Randomized, double-blind, placebo-controlled: GX oral suspension 500 mg TID one day 1, GX 625 mg TID on days 2-8 (N=24). Placebo was parallel matched (N=28)</td>
<td>Tolerable: - Agitation (8%) - Depression (8%) - Anxiety (8%) - Postictal psychosis (8%) - Seizures (13%)</td>
<td>60% of placebo group discontinue d due to seizures, whereas 38% of GX group discontinue d due to seizures</td>
<td>1996-1998</td>
<td>Completed, endpoint met</td>
</tr>
<tr>
<td>Kerrigan et al. 2000</td>
<td>Intractable infantile spasms</td>
<td>N/A</td>
<td>Children aged 7 months to 7 years (N=20)</td>
<td>Multicenter, open-label, add-on trial: GX oral suspension 4.5 mg/kg/d (all total daily doses divided into three doses per day) and was increased to 9 mg/kg/d after 3 days. At week 2, the dose was increased to 18 mg/kg/d and progressed through weekly increases of 9 mg/kg/d to a maximum allowable dose of 36 mg/kg/d</td>
<td>Tolerable: - Diarrhea (20%) - Somnolence (25%) - Nervousness (15%) - Vomiting (15)</td>
<td>33% of study subjects with active spasms showed at least a 50% improvement in spasm frequency</td>
<td>2000</td>
<td>Completed, endpoint met 33%</td>
</tr>
<tr>
<td>NCT00465517 Sperling et al. 2017</td>
<td>Uncontrolled Partial-onset Seizures</td>
<td>2</td>
<td>Adults aged 18-69 (N=147)</td>
<td>Double-blind, Randomized, placebo-controlled. Daily oral GX suspension 1,500 mg/day (N=98) or Placebo (N=49) TID for 10 weeks</td>
<td>Tolerable with mild side effects: - Fatigue (16%) - Dizziness (16%) - Headache (8%)</td>
<td>Reduced seizure frequency by 50% compared to placebo group</td>
<td>2007-2008</td>
<td>Completed, primary endpoint partially met</td>
</tr>
<tr>
<td>Reference or NCT ID</td>
<td>Indication</td>
<td>Phase</td>
<td>Population &amp; Number</td>
<td>Study Design (Dose and duration)</td>
<td>Safety outcomes</td>
<td>Efficacy outcomes</td>
<td>Study dates</td>
<td>Status</td>
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<tr>
<td>---------------------</td>
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</tr>
<tr>
<td>NCT004418 96</td>
<td>Infantile Spasm</td>
<td>2</td>
<td>Children aged 4 months to 24 months (N=57)</td>
<td>A Double-blind, Placebo-controlled, Dose-ranging. Daily oral suspension GX/placebo/GX (N=36) or placebo/GX/placebo (N=18) with ascending range for 20 days (12mg/kg TID to 18 mg/kg TID)</td>
<td>Somnolence (13%)</td>
<td>Some participants exhibited spasm free days but not statistically significant; spasms reduced by 1.78% compared to baseline</td>
<td>2007-2008</td>
<td>Completed, endpoint not met</td>
</tr>
<tr>
<td>NCT013396 89</td>
<td>PTSD</td>
<td>2</td>
<td>Adults aged 18-55 (N=112)</td>
<td>Double-blind, Randomized, Placebo-controlled. GX 200-600 mg oral capsules BID (N=59) Placebo capsules BID (N=53) daily for 12 weeks</td>
<td>Tolerable; subjects in GX group experienced: - Fever (2%) - Confusion (2%) - Suicidal ideation (2%)</td>
<td>GX treatment had 6 points lower CAPS score compared to placebo group each week</td>
<td>2011-2014</td>
<td>Completed, endpoint not met</td>
</tr>
<tr>
<td>NCT017251 52</td>
<td>Children with Fragile X Syndrome</td>
<td>2</td>
<td>Children aged 6-17 (N=59)</td>
<td>Controlled, Double-blind, Crossover trial: group A (N=30): GX oral suspension 12 mg/kg TID x 6 weeks, 2 weeks washout period, placebo TID x 6 weeks. Group B (N=29): placebo TID x 6 weeks, 2 weeks washout period, GX 12 mg/kg TID for 6 weeks</td>
<td>Tolerable: - Rash (8%) - Somnolence (34%) - Decrease appetite (14%) - URI (15%) - Fatigue (49%)</td>
<td>GX group on average had lower CGI-I score (3.4) compared to placebo group (3.5) at week 14</td>
<td>2012-2016</td>
<td>Completed, endpoint not met</td>
</tr>
<tr>
<td>NCT018575 31</td>
<td>Smoking cessation</td>
<td>2</td>
<td>Adults aged 18-65 (N=36)</td>
<td>Single group assignment study: Pre-quit period: GX oral suspension 200 mg BID for 3 days, 400 mg BID for next 3 days, 600 mg BID for remaining 2 weeks; nicotine patches 21 mg/day daily for week 3 and 4. Post quit period: GX 600 mg BID for week 5, 400 mg BID for 3 days, and 200 mg BID for 3 days; nicotine patches 21 mg/day for 4 weeks, 14mg/day for 1 week, and 7mg/day for 1 week</td>
<td>Tolerable: - Thirst (19%) - Dry mouth (6%) - Headache (13%) - Fatigue (69%) - Dizziness (25%) - Anxiety (13%)</td>
<td>There was a 0.52% decrease in expired air carbon monoxide at 2 weeks compared to baseline</td>
<td>2013-2014</td>
<td>Completed, primary endpoint not met</td>
</tr>
<tr>
<td>NCT019632 08</td>
<td>Drug-resistant Partial-onset seizures</td>
<td>3</td>
<td>Adults (N=405)</td>
<td>Double-blind, Randomized: oral GX capsules 1200 mg/day and 1800 mg/day +AED (N=24), Placebo +AED (N=22), GX 1800 mg/day +AED (N=179) for 14 weeks</td>
<td>Tolerable: - Convulsion (2%) - Gait disturbances (1%) - Falls (1%) - Suicidal Ideation (1%)</td>
<td>GX group had on average 10 fewer seizures per 28 days compared to placebo group</td>
<td>2013-2016</td>
<td>Completed, endpoint not met</td>
</tr>
<tr>
<td>NCT025194 39</td>
<td>Drug-resistant Partial-onset</td>
<td>3</td>
<td>Adults (N=26)</td>
<td>Single Group Assignment: GX 900 mg oral capsules BID (N=26) for 104 weeks</td>
<td>Tolerable: - Headache (12%) - Dizziness</td>
<td>Decrease in seizure frequency by 42%</td>
<td>2015-2016</td>
<td>Terminated, endpoint not met</td>
</tr>
<tr>
<td>Reference or NCT ID</td>
<td>Indication</td>
<td>Phase</td>
<td>Population &amp; Number</td>
<td>Study Design (Dose and duration)</td>
<td>Safety outcomes</td>
<td>Efficacy outcomes</td>
<td>Study dates</td>
<td>Status</td>
</tr>
<tr>
<td>---------------------</td>
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</tr>
<tr>
<td>NCT023585-38</td>
<td>Status Epilepticus (SE)</td>
<td>2</td>
<td>12 years or older (N=17)</td>
<td>Double-blind Randomized, Placebo-controlled Study: Low IV GX infusion 500 mg/day (N=5), Medium IV GX 650 mg/day (N=4), High IV GX 713 mg/day (N=8) for 24 hrs and 4-week follow-up</td>
<td>Tolerable: - Sedation (25%) - Falls (25%)</td>
<td>100% of all patients in all dose levels did not require IV anesthetic drug for SE</td>
<td>2018-2019</td>
<td>Completed, endpoint met 100%</td>
</tr>
<tr>
<td>NCT029000-92</td>
<td>Treatment resistant depression</td>
<td>N/A</td>
<td>Post-menopausal women aged 50-75 (N=10)</td>
<td>Open-label, uncontrolled pilot study: oral GX 225 mg capsules BID, increased to 450 mg BID if tolerated, adjunctive therapy with current SSRI or SNRI regimen for 8 weeks</td>
<td>Tolerable with sedative effects with twice daily dosing: - Sleepiness (100%) - Fatigue (100%) - Dizziness (60%)</td>
<td>Exerts antidepressant effects; average MADRS score was 12.8 (scale of 0-60)</td>
<td>2016-2018</td>
<td>Completed, endpoint partially met</td>
</tr>
<tr>
<td>NCT034607-56</td>
<td>Postpartum depression</td>
<td>2</td>
<td>Adult women aged 18-48 experiencing postpartum depression (N=84)</td>
<td>Double-blind, Placebo-controlled, Multicenter Study: group 1: GX 300 mg oral capsules TID for 2 weeks (N=2), group 2: GX 675 mg/day QHS for 2 weeks (N=14), group 3: GX 675 mg/day QHS for 4 weeks (N=25), GX 1125 mg/day Q7PM for 4 weeks (N=43)</td>
<td>Tolerable for lower doses, higher doses experienced: - Dry mouth (7%) - Dizziness (16%) - Headache (26%) - Sedation (16%) - Somnolence (21%)</td>
<td>All groups had lower HAMD17 scores compared to baseline (ranging from -0.8 to -8.0)</td>
<td>2017-2019</td>
<td>Completed, endpoints met for 2 weeks, not for 4 weeks</td>
</tr>
<tr>
<td>NCT032283-94</td>
<td>Postpartum depression</td>
<td>2</td>
<td>Adult women aged 18-45 experiencing postpartum depression (N=91)</td>
<td>Double-blind, Placebo-controlled, Multiple-dose Escalation Study: cohort 1: IV infusion of GX at rate of 4 mg/h for 48 hrs (N=5), cohort 2: IV infusion of GX at rate of 8 mg/hr for 48 hrs (N=15), cohort 3: IV bolus of 12 mg GX over 2 min; then GX at 12 mg/h for 48 hrs (N=10), cohort 4: IV infusion of GX at rate of 20 mg/hr for 6 hrs followed by 900 mg capsules orally at dinner for 28 days (N=16). Each cohort has a matched placebo arm</td>
<td>Tolerable: - Dry mouth (20%) - Headache (20%) - Dizziness (40%) - Somnolence (25%) - Sedation (60%)</td>
<td>All GX groups had lower HAMD17 compared to their baseline and the placebo group (ranging from -11.3 to -14)</td>
<td>2017-2020</td>
<td>Completed, endpoints partially met</td>
</tr>
<tr>
<td>NCT033500-35</td>
<td>PCDH19 and genetic related epilepsies</td>
<td>2</td>
<td>Children (age 2-18) with PCDH19</td>
<td>Open-label Proof-of-concept Trial: GX oral suspension 63 mg/kg/day with maximum 1800 mg/day for 6 months. CDKL5 (N=7), CSWS (N=2), Lennox Gastaut (N=10), PCDH19 (N=11)</td>
<td>(4%) - Neck &amp; Back pain (4%)</td>
<td>Decrease in seizure frequency by 20% at 52 weeks compared to baseline</td>
<td>2015-2019</td>
<td>Completed, endpoint not met for PCDH19 and Lennox Gastaut groups</td>
</tr>
</tbody>
</table>

**Reference**
Dichtel et al. 2022
Marinus Press Release 2016
Vaitkevicius et al. 2022

**Population**
- Children (age 2-18) with PCDH19
- Female Pediatric Epilepsy (N=30)
- Post-menopausal women aged 50-75 (N=10)
- Adult women aged 18-48 experiencing postpartum depression (N=84)
- Adult women aged 18-45 experiencing postpartum depression (N=91)
- Children (age 2-18) with PCDH19

**Study Design**
- Double-blind, Placebo-controlled, Multicenter Study
- Double-blind, Placebo-controlled, Multiple-dose Escalation Study
- Open-label Proof-of-concept Trial

**Safety Outcomes**
- Tolerable: - Neck & Back pain (4%)
- Tolerable: - Somnolence (50%)
- Tolerable: - Somnolence (100%)
- Tolerable: - Sleepiness (100%)
- Tolerable: - Dry mouth (7%)
- Tolerable: - Dizziness (16%)
- Tolerable: - Headache (26%)
- Tolerable: - Sedation (16%)
- Tolerable: - Somnolence (21%)
- Tolerable: - Dry mouth (20%)
- Tolerable: - Headache (20%)
- Tolerable: - Dizziness (40%)
- Tolerable: - Somnolence (25%)
- Tolerable: - Sedation (60%)

**Efficacy Outcomes**
- Decrease in seizure frequency by 20% at 52 weeks compared to baseline
- Exerts antidepressant effects; average MADRS score was 12.8 (scale of 0-60)
- All groups had lower HAMD17 scores compared to baseline (ranging from -0.8 to -8.0)
- All GX groups had lower HAMD17 compared to their baseline and the placebo group (ranging from -11.3 to -14)

**Study Dates**
- 2015-2019
- 2016-2018
- 2017-2019
- 2017-2020
- 2018-2019

**Status**
- Completed, endpoint met
- Completed, endpoint partially met
- Completed, endpoints met for 2 weeks, not for 4 weeks
- Completed, endpoint met 100%
<table>
<thead>
<tr>
<th>Reference or NCT ID</th>
<th>Indication</th>
<th>Phase</th>
<th>Population &amp; Number</th>
<th>Study Design (Dose and duration)</th>
<th>Safety outcomes</th>
<th>Efficacy outcomes</th>
<th>Study dates</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT035729 33</td>
<td>CDKL5 deficiency disorder epilepsy</td>
<td>3</td>
<td>Children, young adults aged 2-21 (N=101)</td>
<td>Double-blind, Randomized, Placebo-controlled: GX oral suspension (50 mg/ml) TID for 17 weeks (N=50), Placebo suspension TID for 17 weeks (N=51)</td>
<td>Tolerable: - Bronchitis (2%) - UTI (2%)</td>
<td>At week 17, placebo group had increase seizure frequency (5 more) and GX group had decrease seizure frequency (9 less) compared to baseline over a 28 day period</td>
<td>2018-2021</td>
<td>Completed, endpoint met 100%</td>
</tr>
<tr>
<td>NCT038657 32</td>
<td>PCDH19 related epilepsy</td>
<td>2</td>
<td>Female children aged 1-17 (N=21)</td>
<td>Double-blind, Randomized, Placebo-controlled: GX oral suspension (50 mg/ml) TID for 17 weeks (N=10), placebo suspension TID for 17 weeks (N=11)</td>
<td>Tolerable: - Somnolence (40%) - Decrease appetite (20%) - URI (10%) - Fatigue (20%) - Constipation (10%) - Diarrhea (10%)</td>
<td>GX group had 52% lower seizure frequency at week 17 compared to baseline</td>
<td>2019-2022</td>
<td>Completed, endpoint partially met</td>
</tr>
<tr>
<td>NCT042853 46</td>
<td>Tuberous sclerosis complex</td>
<td>2</td>
<td>2 Years to 65 Years (N=23)</td>
<td>Open-label Trial: GX 1800 mg/day oral suspension for 12-week Treatment period, participants with a seizure reduction of &gt;=35 percent compared to the Baseline continues to open-label period</td>
<td>Tolerable: - Fatigue (13%) - Somnolence (43%) - Sedation (13%) - Dizziness (9%)</td>
<td>17% reduced seizure frequency compared to baseline</td>
<td>2020-2022</td>
<td>Completed, endpoint partially met</td>
</tr>
<tr>
<td>Singh et al. 2022</td>
<td>Pediatric super-refractory Status Epilepticus</td>
<td>N/A</td>
<td>Pediatric female patients (age 7 and 17) (N=2)</td>
<td>A single hospital case report: IV GX administered as an initial bolus and a maintenance infusion for up to 4.5 days with intermittent IV boluses as needed followed by taper on day 5. 17-year-old patient was given maximal adult dose of 1800 mg/day divided three times a day. 7-year-old patient given 63 mg/kg/day divided three times a day</td>
<td>Tolerable: - Recurrent SE</td>
<td>Adjuvant GX was effective in terminating SRSE in both patients, safely permitting IV anesthetics to be weaned. Seizure control was maintained after transitioning to oral GX</td>
<td>2022</td>
<td>Completed, Case reports</td>
</tr>
<tr>
<td>NCT043915 69</td>
<td>Status Epilepticus</td>
<td>3</td>
<td>12 Years and older (N=124)</td>
<td>A double-blind, Randomized, Placebo-controlled: GX IV bolus dose followed by continuous infusion for 36 hrs, followed by 12 hr taper</td>
<td>Not yet reported</td>
<td>Not yet reported</td>
<td>2020-2023</td>
<td>Ongoing, recruiting</td>
</tr>
<tr>
<td>Reference or NCT ID</td>
<td>Indication</td>
<td>Phase</td>
<td>Population &amp; Number</td>
<td>Study Design (Dose and duration)</td>
<td>Safety outcomes</td>
<td>Efficacy outcomes</td>
<td>Study dates</td>
<td>Status</td>
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<tr>
<td>NCT05604170</td>
<td>Tuberous sclerosis complex</td>
<td>3</td>
<td>1 Year to 65 Years (N=169)</td>
<td>Open-label single arm study with no blinding: GX oral suspension (50mg/mL) will be administered TID for 52 weeks</td>
<td>Not yet reported</td>
<td>Not yet reported</td>
<td>2022-2024</td>
<td>Ongoing, enrolling by invitation</td>
</tr>
<tr>
<td>NCT05323734</td>
<td>Tuberous sclerosis complex</td>
<td>3</td>
<td>1 Year to 65 Years (N=162)</td>
<td>Double-blind, Randomized, Placebo-controlled: GX oral suspension 50 mg/mL will be administered TID for 16 weeks, placebo will be matched</td>
<td>Not yet reported</td>
<td>Not yet reported</td>
<td>2022-2025</td>
<td>Ongoing, recruiting</td>
</tr>
<tr>
<td>NCT05249556</td>
<td>CDKL5 deficiency disorder epilepsy</td>
<td>3</td>
<td>6 months to 2 years (N=20)</td>
<td>Double-blind, Randomized, Placebo-controlled: GX oral suspension 50 mg/mL for 12 weeks, matched placebo (study details limited)</td>
<td>Not yet reported</td>
<td>Not yet reported</td>
<td>2023-2024</td>
<td>Ongoing, not yet recruiting</td>
</tr>
<tr>
<td>NCT05814523</td>
<td>Refractory Status Epilepticus</td>
<td>3</td>
<td>Adults aged 18 years or older (N=70)</td>
<td>Double-blind, Randomized, Placebo-controlled: GX IV infusion + standard-of-care (SOC) IV AED or matched placebo (study details limited)</td>
<td>Not yet reported</td>
<td>Not yet reported</td>
<td>2023-2025</td>
<td>Ongoing, not yet recruiting</td>
</tr>
<tr>
<td>NCT05757544</td>
<td>Established Status Epilepticus</td>
<td>2</td>
<td>Adults aged 18 years or older (N=120)</td>
<td>Dose optimization phase (open-label): IV GX bolus (variable) followed by infusion (variable), Experimental group: Double-blind phase: IV GX+ SOC, Comparator: IV Placebo + SOC (study details limited)</td>
<td>Not yet reported</td>
<td>Not yet reported</td>
<td>2023-2026</td>
<td>Ongoing, recruiting</td>
</tr>
</tbody>
</table>

Total = ~2000 (including completed and ongoing trials)
Table 6.
Pharmacological and clinical profile of ganaxolone oral suspension in pediatric (CDKL5) epilepsy.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Profile</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Indications</strong></td>
<td>Treatment of seizures associated with CDKL5-deficiency disorder in patients 2 years of age and older</td>
</tr>
<tr>
<td><strong>Product</strong></td>
<td>Oral suspension: 50 mg/ml. Size: 110 ml bottle</td>
</tr>
<tr>
<td><strong>Product composition</strong></td>
<td>Each ml contains 50 mg GX and 100 mg beta-cyclodextrin</td>
</tr>
<tr>
<td><strong>Dosage</strong></td>
<td>For patients weighing 28 kg or less: starting dose 6 mg/kg three times daily (18 mg/kg/day); max dosage 21 mg/kg three times daily (63 mg/kg/daily). For patients weighing over 28 kg: starting dose 150 mg three times daily (450 mg daily); max dose 600 mg three times daily (1800 mg daily)</td>
</tr>
<tr>
<td><strong>Administration instruction</strong></td>
<td>Shake the bottle vigorously before measuring and administering each dose. High-fat meal increases absorption by 2-3-fold than fasted condition</td>
</tr>
<tr>
<td><strong>Storage</strong></td>
<td>Store at room temperature</td>
</tr>
<tr>
<td><strong>Product expiry</strong></td>
<td>24 months from date of manufacture. Medication expiry 30 days after the first opening of the bottle. Discard it after 30 days</td>
</tr>
<tr>
<td><strong>Oral bioavailability</strong></td>
<td>Bioavailability has not been evaluated (estimated &lt;10%, as per Oral PK studies)</td>
</tr>
<tr>
<td><strong>Volume of distribution (Vd)</strong></td>
<td>Vd has not been evaluated</td>
</tr>
<tr>
<td><strong>Plasma half-life (t1/2)</strong></td>
<td>T1/2 has not been evaluated</td>
</tr>
<tr>
<td><strong>Therapeutic levels</strong></td>
<td>Effective therapeutic level has not been evaluated</td>
</tr>
<tr>
<td><strong>Metabolism</strong></td>
<td>Hepatic metabolism by metabolized by CYP3A4/5, CYP2B6, CYP2C19, and CYP2D6. Eliminated (55%) in feces and urine (18%)</td>
</tr>
<tr>
<td><strong>Tmax</strong></td>
<td>2-3 hrs following oral administration</td>
</tr>
<tr>
<td><strong>Clearance (CL)</strong></td>
<td>Terminal half-life is 34 hrs. Inducers of CYP2C19, CYP3A4 and CYP2B6 can decrease drug levels. Strong CYP3A4 inhibitor can increase drug levels</td>
</tr>
<tr>
<td><strong>Protein binding</strong></td>
<td>About 99% protein binding in serum</td>
</tr>
<tr>
<td><strong>Drug-drug interactions</strong></td>
<td>Cytochrome P450 inducers will decrease ganaxolone levels. Avoid concomitant use with strong or moderate CYP3A4 inducers</td>
</tr>
<tr>
<td><strong>Hepatic effects</strong></td>
<td>The impact of hepatic impairment on the disposition of ganaxolone has not been evaluated. Since it undergoes clearance via the hepatic route, hepatic impairment can increase drug levels</td>
</tr>
<tr>
<td><strong>Renal effects</strong></td>
<td>The impact of renal impairment on ganaxolone pharmacokinetics has not been studied</td>
</tr>
<tr>
<td><strong>Cardiac effects</strong></td>
<td>The impact of ganaxolone on the cardiac QTc interval has not been studied</td>
</tr>
<tr>
<td><strong>Adverse effects</strong></td>
<td>Somnolence, pyrexia, salivary hypersecretion, and seasonal allergy</td>
</tr>
<tr>
<td><strong>Warning and precautions</strong></td>
<td>Monitor for somnolence and sedation. Patients should not drive or operate machinery until they have gained sufficient experience with medication. Concomitant use with other CNS depressants or alcohol could increase side effects. Monitor patients for suicidal behavior and thoughts</td>
</tr>
<tr>
<td><strong>Medication discontinuation</strong></td>
<td>Withdraw gradually to minimize the risk of increased seizure frequency and status epilepticus</td>
</tr>
<tr>
<td><strong>Long-term safety</strong></td>
<td>Long-term safety has not been evaluated</td>
</tr>
<tr>
<td><strong>Drug abuse and dependence</strong></td>
<td>It has potential for abuse. Physical dependence has not been evaluated.</td>
</tr>
<tr>
<td><strong>Regulatory instructions</strong></td>
<td>Controlled substance (Schedule V).</td>
</tr>
</tbody>
</table>
Table 7.
Limitations of ganaxolone (GX) as therapeutic medication in epilepsy.

<table>
<thead>
<tr>
<th>Core issue</th>
<th>Clinical impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lack of water solubility</td>
<td>GX is insoluble in water making it harder to make oral and injectable products. Cyclodextrin complexation is used to make oral suspension and injectable products.</td>
</tr>
<tr>
<td>Poorly absorbed from oral route</td>
<td>GX is very poorly and often erratically absorbed after oral administration; &lt;10% reaches the plasma and brain tissues due to hepatic inactivation. Injectable products are made for delivering GX.</td>
</tr>
<tr>
<td>Short plasma half-life and distribution</td>
<td>GX has a short effective plasma half-life (&lt;2 h) requiring repeated administration every 4-6 hrs by oral route and continuous IV infusion for injectable route. Due to high lipophilicity, it shows a high brain to plasma ratio.</td>
</tr>
<tr>
<td>Reduced patient compliance</td>
<td>The requirement to administer the GX three times daily may pose a big disadvantage and hinder treatment adherence, especially in cases involving multiple medications and children.</td>
</tr>
<tr>
<td>Extrasynaptic selectivity</td>
<td>Activates both extrasynaptic and synaptic receptors; extrasynaptic selectivity is less than newer neurosteroid analogs.</td>
</tr>
<tr>
<td>Lack of established PK profile</td>
<td>Details of PK values of oral GX in patients are not well established.</td>
</tr>
<tr>
<td>Lack of PD-PD correlations</td>
<td>Despite completion of multiple clinical trials in children, adults and women with epilepsy, there is no data or evidence of pharmacokinetic and pharmacodynamic correlation, a key biomarker for drug therapy optimization and therapy titration for better outcomes.</td>
</tr>
<tr>
<td>Complex (organic) formulation</td>
<td>Clinical GX products are prepared using cyclodextrin mixtures. Such organic formulations, although help delivering the drug, pose issues including renal toxicity due to elevated cyclodextrin levels.</td>
</tr>
<tr>
<td>Age- and sex differences</td>
<td>Like other neurosteroids, GX exhibits sex differences in potency and efficacy but there is limited data on sex-specific dosing patterns. GX has not been tested in vulnerable and aged patients for differences in safety, efficacy, and drug interaction outcomes. Potential for hormone interactions in women was not tested.</td>
</tr>
<tr>
<td>Side effects, drug interactions and synergism</td>
<td>Like other GABAergic drugs, GX causes CNS side effects such as somnolence, dizziness, and fatigue. GX can synergistically increase these events in combination with other medications (tiagabine, midazolam and barbiturates) and increase adverse events.</td>
</tr>
<tr>
<td>Pharmacodynamic interactions</td>
<td>GX can exacerbate absence seizures, and hence should be given to patients with active or history of absence epilepsy.</td>
</tr>
<tr>
<td>Interaction with zinc</td>
<td>The potential interaction of GX with zinc, a metal ion blocker of extrasynaptic GABA-ARs, can affect the extent of its seizure protection. Human conditions that enhance brain zinc levels, such as brain injury, SE, and meningitis, can diminish the antiseizure effects of GX.</td>
</tr>
<tr>
<td>Regulatory and sponsor issues</td>
<td>Controlled substance with Schedule V classification. Limits on access to or availability of drug substances. Real-world data and independent trials are needed to confirm long-term safety and efficacy.</td>
</tr>
</tbody>
</table>
Figure Legends

**Fig. 1. Chemical structures of organophosphate chemicals and nerve agents.** Organophosphate chemical threat agents include parathion, paraoxon, chlorpyrifos, diazinon, and diisopropylfluorophosphate (DFP). Nerve agents include G-class chemicals tabun (GA), sarin (GB), tabun (TA), soman (GD), and cyclosarin (GF), and V-class chemicals VX and Russian VX.

**Fig. 2. Schematic illustration of potential mechanistic basis of organophosphate (OP) and nerve agent neurotoxicity after acute exposure.** OP compounds and nerve agents produce acute lethal neurotoxicity and long-term neurological effects in survivors. The primary mechanism of action of both classes is irreversible inhibition of acetylcholinesterase (AChE) resulting in accumulation of toxic levels of acetylcholine (ACh) at synaptic junctions, which induces muscarinic and nicotinic receptor stimulation, and many other pathways. OPs can also rapidly cross the blood–brain barrier (BBB) and induce severe seizures, initially by overstimulating cholinergic pathways. Seizures can reversibly permeabilize the BBB and trigger a massive inflammation response in the brain. As status epilepticus (SE) progresses, glutamatergic networks are recruited, and several other changes may occur. The secondary events of SE and non-seizure activity, such as neuronal necrosis, cell death, and axonal degeneration can potentially result in severe brain damage.

**Fig. 3. Chemical structures of ganaxolone and other anticonvulsant neurosteroids.** The naturally occurring neurosteroid aallopregnanolone has been renamed brexanolone. Ganaxolone is a 3β-methyl analog of brexanolone. Hydroganaxolone is an orally active synthetic neurosteroid with powerful anticonvulsant properties.

**Fig. 4. Mechanism of ganaxolone action at synaptic and extrasynaptic GABA-A receptors.** Ganaxolone (GX) is a preferential allosteric modulator and direct activator of extrasynaptic δGABA-AR receptors (δGABA-ARs). Like other neurosteroids, GX enhances the function of extrasynaptic and synaptic GABA-ARs by binding to “neurosteroid-binding” sites, which are distinct from sites for GABA, benzodiazepines, and barbiturates. Based on the location, two categories of GABA-ARs are present on neurons. Synaptic receptors (composed of α12β2γ2 subunits), are localized on post-synaptic sites within the synaptic cleft and conduct chloride influx in response to GABA released from presynaptic buttons to generate phasic currents. Extrasynaptic receptors (composed of α4β2δ subunits), are located at peri- and extrasynaptic sites and primarily contribute to tonic currents. Recently, we established the molecular mechanism of GX action at GABA-ARs in native hippocampal dentate gyrus granule cell (DGGC) neurons. GX produces significantly greater potentiation of phasic currents (mIPSC) in neurons that express δGABA-ARs. At extrasynaptic sites, GX potentiates and directly activates tonic currents in neurons with δGABA-ARs in hippocampal slices, in which synapses and dendritic connections remain functional. These responses are reduced in neurons lacking δGABA-ARs, confirming GX's selectivity for δGABA-ARs. It enhances the tonic current over the entire duration of its application with little rundown (Fig. 2C) as evident from the persistent tonic current measured as the shift in mean conductance before and after applying the GABA-AR antagonist gabazine (GBZ).
Thus, GX can promote maximal inhibition by simultaneously enhancing both phasic and tonic inhibition in the brain (Reddy and Chuang, 2018b).

**Fig. 5. Overview of a 10 year effort to develop ganaxolone for status epilepticus.** Preclinical ganaxolone development projects were supported by the National Institutes of health (NIH) CounterACT Program (2011-2023) and the NIH Chemical Countermeasures Research Program (2012-2023). Based on promising findings from these NIH-supported research projects at Texas A&M (Dr. Reddy lab), the U.S. Biomedical Advanced Research and Development Authority (BARDA) extended project support (2020-2024) to advance clinical development and launch ganaxolone as an RSE anticonvulsant and chemical nerve agent, including domestic manufacturing and supplying ganaxolone for stockpiling. Ganaxolone development in RSE trials is funded in part under a BARDA contract to supply ganaxolone injection for field-based rapid response treatment in the event of a nerve gas attack.
Figure-1

Organophosphates

- Parathion
- Paraoxon
- Chloropyrifos
- Diazinon

Nerve Agents

- phosphoramidocyanidate
- phosphonofluoridates
- phosphonothioate

- Tabun (GA)
- Sarin (GB)
- Soman (GD)
- Cyclosarin (GF)
- VX
Organophosphates (pesticides/nerve agents)

Muscarinic AChR overstimulation → Glutamate Receptors Activation → Nicotinic AChR overstimulation

Cholinergic neuronal excitotoxicity & dysfunction

Cholinergic Crisis (from minutes to hours)
- Hypersecretion
- Miosis
- Headache
- Fasciculations
- Tremors

Cellular Edema (Cytotoxicity, ion imbalance, Ga^2+ influx)

Neuroinflammatory Response (from hours to days)
- Astrogliosis
- Microgliosis
- Cytokine release
- Interleukin release
- Proinflammatory factors etc.

Apoptosis, neuronal cell death, neuronal loss, axonal degeneration

Secondary neuronal damage / re-organization

Long-term neuropsychiatric and neurological disorders (from months to years)

Neuropsychiatric disorders
- Memory impairment
- Forgetfulness
- Learning disabilities
- Concentration difficulties etc.

Persistent memory & Cognitive deficits
- Mental and emotional symptoms
- Anxiety
- Psychomotor depression
- Reduced psychomotor function
- Somatic complaints etc.

Neurologic disorders
- Excitability
- Epileptogenic process
- Interictal spikes
- Epileptiform discharges
- Mossy fiber sprouting etc.

Potential secondary damage effects
- Posttraumatic stress disorder
- Inflammation
- Brain cancer etc.
Figure-3

Brexanolone  Ganaxolone  Hydroganaxolone
Figure-5

NIH

CounterACT Program

Texas A&M Facility

Neurosteroid Therapy

US Patent

GX Efficacy Testing in OP Models in Adult Animals

♦ DFP Model ♦ Soman Model ♦ VX Model

Formulation, PK & Safety

IND

BARDA