Narrative Review

Receptor and Molecular Targets for the Development of Novel Opioid and Non-Opioid Analgesic Therapies

Rosalyn Chen, BA[1], O.J. Michael Coppes, MD[2], and Richard D. Urman, MD[3]

From: [1]Boston University School of Medicine, Boston, MA; [2]Department of Anesthesiology, University of Chicago Medical Center, Chicago, IL; [3]Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

Address Correspondence:
Richard D. Urman, MD
Department of Anesthesiology
Perioperative and Pain Medicine
Brigham and Women's Hospital
Harvard Medical School
75 Francis St.
Boston, MA 02115
E-mail:
rurman@bwh.harvard.edu

Background: Although conventional pain relief therapeutics have centered around μ-opioid agonists, these drugs are limited by adverse side effects, including respiratory depression and addiction potential. The ongoing opioid epidemic has galvanized research into novel analgesic therapies with more favorable profiles. New pharmacologic agents have been developed to target neuronal pathways involved in pain sensation. Certain receptors have been recognized to mediate nociceptive transmission, central sensitization, and the development of chronic pain states.

Objectives: We conducted a literature review to identify potential targets for novel analgesic therapies.

Study Design: This study is a narrative review of potential analgesic targets. We characterize their antinociceptive mechanisms of action and evaluate their therapeutic potential.

Methods: A systemized search of available literature on novel analgesics was performed. A search was performed through the PubMed database to identify articles with key words of "novel analgesics," "novel non-opioid analgesics," "novel pain targets," and "non-opioid analgesics." Potential drug classes were identified and researched through corresponding keywords, with an emphasis on publications from 2018 to 2020. Older articles were included if frequently referenced by current literature.

Results: Potential novel analgesic targets include Nav1.7, Nav1.8, CaV2.2, and transient receptor potential vanilloid-1 (TRPV1) cation channel receptors in the peripheral nervous system. Other approaches disrupt the synthesis of pronociceptive signaling molecules such as nitric oxide, prostaglandin E2, and interleukin-6 (IL-6). Within central pain pathways, modification of κ-opioid, δ-opioid, N-methyl-D-aspartate, and cannabinoid receptors have been investigated in chronic pain and hyperalgesia models. Recent advances in molecular technology have also presented opportunities to modify protein expression or the cellular genome altogether.

Limitations: Several analgesic targets have only demonstrated efficacy in preclinical trials. There are limited data evaluating the long-term safety profiles of therapies further on in development.

Conclusions: We provide an overview of potential analgesic therapies in various stages of development, which may become clinically relevant in the near future. Some drugs such as TRPV1 agonists, anti-IL-6, and anti-nerve growth factor antibodies have demonstrated analgesic effect in specific clinical pain states.

Key words: Nav1.7, Cav2.2, TRPV1, mPGES-1, IL-6, FAAH, NGF, gene therapy

Pain Physician 2021: 24:153-163

Pain poses a substantial burden on patient morbidity and population health, and in the United States alone costs the economy an estimated $600 billion annually in medical costs and lost productivity (1). Chronic pain conditions are leading causes of disability worldwide, yet pain management

options remain limited (2). Although μ-receptor opioids remain a mainstay of pain control, the ongoing opioid epidemic has spurred the investigation of alternative therapies. There are current efforts to reformulate drugs so as to limit adverse effects and addiction risk, along with other research that has focused on the endogenous pain pathways to guide the development of novel analgesic compounds (3).

Novel targets for analgesic therapies have been identified throughout the nervous system. Several ion channel receptors are involved in pain transmission through peripheral neurons, including isoforms of voltage-dependent sodium channels (Nav1.7, Nav1.8), voltage-dependent calcium channels (Cav2.2), and transient receptor potential vanilloid-1 (TRPV1) receptors (4-6). Other molecules involved in multisystem signaling, such as nitric oxide, prostaglandin E2 (PGE2), and interleukin-6 (IL-6), mediate inflammation and pronociceptive neuroplastic remodeling (7-9). Within the central nervous system, pain sensation is recognized to involve μ-opioid, κ-opioid, δ-opioid, N-methyl-D-aspartate (NMDA), and cannabinoid receptor activity (10-12). With the realization of new technologies, other approaches are now aimed at modifying neurotrophin signaling, epigenetic acetylation, or the pain genome altogether (13-15). This narrative review of the existing literature aims to characterize several receptors and mechanisms that hold promise as potential targets for novel analgesic therapies, including primary afferents, voltage-gated sodium channel inhibitors, voltage-gated calcium channel blockers, TRPV1 targets, systemic mediators, nitric oxide synthase inhibitors, microsomal prostaglandin E synthase 1 (mPGES-1) inhibitors, IL-6 inhibitors, central pain pathways, κ-opioid agonists, δ-opioid agonists, NMDA receptor antagonists, cannabinoids, fatty acid amide hydrolase (FAAH) inhibitors, anti-nerve growth factor (NGF) antibodies, and various genetic interventions.

Methods

A literature search was conducted in PubMed and Medline databases to identify articles with key words of "novel analgesics," "novel non-opioid analgesics," "novel pain targets," and "non-opioid analgesics." Potential drug classes were identified through peer-reviewed primary studies and literature reviews. Specific drug targets were further subject to searches in PubMed and Medline in combination with "pain," "analgesia," "analgesic," or "efficacy." This review

emphasizes publications from 2018 to 2020, although older articles were included if frequently referenced by current literature.

Primary Afferents

The sensation of pain begins with activation of primary afferents within the peripheral nervous system; noxious stimuli are communicated by nociceptors to the dorsal root ganglion (DRG). Many receptors have been characterized in the small-fiber nerves that are associated with pain sensations. Numerous natural compounds—often discovered as toxins—have been found to inhibit ion channels and depolarization of these afferent pain neurons and subsequently interfere with the transmission of painful stimuli in first-order neurons (5). Several studies have demonstrated the analgesic effects of specific channel blockades, which include voltage-gated sodium channels, voltage-gated potassium channels, and TRPV1 receptors. A summary of all novel analgesic targets discussed in this review is listed in Table 1.

Voltage-Gated Sodium Channel Inhibitors

Voltage-gated sodium channels are expressed throughout the nervous system, but only certain classes are involved in the transmission of nociception in peripheral nerves. The Nav1.7 and Nav1.8 isoforms are concentrated at DRGs and modulate the transmission of pain sensation (4). Nav1.7 appears to play a critical role in the initiation and transmission of action potentials (16). Nav1.8 shares a similar role in pain transmission and further appears involved in cardiac conduction activity (17). Abnormalities in these voltage-gated sodium channels have been linked to a variety of painful neuropathies, likely owing to aberrant hyperactivity (4). Specifically, gain-of-function mutations in Nav1.7 and Nav1.8 have been observed in patients with idiopathic painful neuropathy. Follow-up voltage-clamp experimentation in mouse models in which incremental application of electrical stimuli resulted in increased spontaneous firing and depolarized resting potential demonstrated the hyperexcitability of nociceptive neurons with Nav1.8 gain-of-function mutations (18). Loss-of-function Nav1.7 mutations have been conversely associated with congenital pain indifference (19). Extrapolated from these observations, the analgesic potential of Nav blockade has galvanized research into targeted inhibitors. Natural small toxins such as tetrodotoxin and saxitoxin have been shown to inhibit pain in pre-

clinical inflammatory and neuropathic pain models (4). Synthetic acyl sulfonamide Nav1.7 inhibitors have also demonstrated robust antinociceptive effects in mouse pain models (20). Although the significance of Nav activity is recognized in inflammatory pain conditions, such as inherited erythromelalgia, it is uncertain whether the sodium channels play an important role in noninflammatory pain. As such, further research is needed to evaluate Nav activity across different pain mechanisms.

Voltage-Gated Calcium Channel Blockers

Voltage-gated calcium channels are involved in a variety of physiological functions. Aside from inducing presynaptic neurotransmitter release, neuronal calcium signaling is also recognized to activate calcium-dependent enzymes and downstream gene expression (21). There are several subtypes of voltage-gated calcium channels, which are variably expressed by different neuron subtypes. N-type (Cav2.2) channels are associated with nociception and expressed densely in presynaptic nerve terminals. On depolarization and activation of N-type channels, the influx of calcium induces the release of glutamate, calcitonin gene-related peptide, and substance P (5). Gabapentin and pregabalin are recognized to exert their analgesic effects not through GABAergic activity, but through the inhibition of the Cavα2-δ subunit of these N-type calcium channels (22). The analgesic potential of Cav2.2 has been further investigated through specific inhibitors. Naturally occurring ω-conotoxins, peptides produced by predator marine snails, occlude Cav2.2 channel pores with poor reversibility. The disrupted release of nociceptive neurotransmitters results in potent pain suppression, as seen with initial intrathecal administration in rodent models (23). A synthetic form of the ω-conotoxin, ziconotide, has been developed as an atypical analgesic for chronic pain patients. Although ziconotide is effective for severe pain refractory to other systemic treatments, the compound requires intrathecal administration because of its inability to cross the blood–brain barrier. Complications include a narrow therapeutic range and dose-related neurologic effects, including dizziness, ataxia, and cognitive impairment (24). Current research on ziconotide explores less invasive and titrated methods of delivery, such as intranasal administration into the cerebrospinal fluid (25). There are yet other investigations into small organic compounds, such as CNCB-2 (cationic N-type calcium channel blocker) and physalin F, that inhibit Cav2.2 channels (26,27).

Table 1. *Novel analgesic targets under development.*

Drug Targets/Mechanism of Action	Examples
Nav1.7, Nav1.8 inhibitors	tetrodotoxin, saxitoxin, synthetic acyl sulfonamides
Cav2.2 inhibitors	ziconotide, CNCB-2, physalin F
TRPV1 agonist	capsaicin, resiniferatoxin
NOS inhibitors	cindunistat
mPGES-1 inhibitors	AF3485, MF63 (35)
IL-6 inhibitors	tocilizumab, sarilumab
κ-opioid agonist	butanorphanol, TRK-820, CR845 (42)
δ-opioid agonist	SNC-80, BU-48 (47)
Biased μ-opioid agonist	oliceridine (TRV130)
NMDA antagonist	ketamine
CB1, CB2 agonists	dronabinol, nabilone, APD371
FAAH inhibitors	BIA 10-2474
Anti-NGF antibodies	tanezumab

TRPV1 Targets

Transient receptor potential cation channels are a superfamily of receptors involved in thermoregulation and nociception. The channels are nonselectively activated by a variety of stimuli, including noxious heat, acidic pH, and molecular activators (28). The vanilloid-1 subfamily (TRPV1) has been implicated in inflammatory pain and postinjury hyperalgesia. TRPV1 channels are expressed in DRG neurons and are notably activated by capsaicin, noxious heat, and acidic conditions (29). Activation results in the influx of calcium and sodium ions, which induces predominantly calcium-dependent intracellular activity (30). Although the exact mechanism of action remains unclear, capsaicin activation of TRPV1 channels appears to modulate phosphoinositide signaling and promote vasodilation, vascular leakage, and accumulation of proinflammatory molecules (6). These factors subsequently sensitize primary afferent neurons and produce hyperalgesia. Prolonged exposure to capsaicin conversely "defunctionalizes" TRPV1 channels, in which downstream mitochondrial dysfunction is theorized to disrupt nociception (30). Although topical high-dose capsaicin has demonstrated some analgesic efficacy in postherpetic neuralgia, there has been further development of selective TRPV1 capsaicin analogues. Resiniferatoxin is an ultrapotent TRPV1 agonist with substantially greater selectivity and efficacy than capsaicin. Topical application of resiniferatoxin induces reversible analgesia, and injection produces irreversible analgesia through highly selective neuronal cytotoxic-

ity (31). Intraarticular administration of resiniferatoxin has demonstrated long-lasting analgesic effect and functional improvement in canine osteoarthritis models (32). Intrathecal injection has induced long-lasting pain suppression in animal models, and at this time there is an ongoing clinical trial on intrathecal administration of resiniferatoxin in severe cancer pain (33). The irreversible, neurolytic effects of resiniferatoxin likely limits its clinical analgesia to progressive or terminal pain conditions. TRPV1 antagonists have also been evaluated as potential analgesic agents with ongoing clinical trials, but side effects have included induced hyperthermia and impaired thermosensation (34).

Systemic Mediators

Several molecules involved in multisystem signaling also play endogenous roles in inflammation or neuroplasticity in pain pathways. Increased afferent activity may stimulate the expression of systemic mediators, which consequently modulate neuronal excitability and promote central sensitization. The inhibition of such mediators is regarded as a potential target for analgesia, with the knockdown of specific prostaglandins and other biological messengers being evaluated for their analgesic potential.

Nitric Oxide Synthase Inhibitors

Nitric oxide synthases (NOS) convert l-arginine into nitric oxide, a molecule involved in multisystem physiological processes. Overactivity of NOS has been associated with neuropathic pain, specifically the neuronal isoform expressed throughout the nervous system, which regulates blood pressure, vasodilation, and synaptic plasticity (7). This inducible isoform is present in neurons, glial cells, and macrophages and plays a role in immunologic defense mechanisms (35). In these processes, nitric oxide acts through the cGMP (cyclic guanosine monophosphate) signaling pathways to activate protein kinases and transcriptional factors (36). Within nociception increased nitric oxide production induces neuronal hyperexcitability by modulating DRG Ca2+ and Na+ ion channels. Excess nitric oxide also produces reactive superoxide anions that phosphorylate NMDA receptors and promote central hypersensitization (7). Nitric oxide has also been shown to stimulate the production of proinflammatory cytokines such as tumor necrosis factor α and IL-1β. These cytokines in turn promote the expression of NOS resulting in further generation of nitric oxide (37). To disrupt this positive feedback loop, NOS inhibitors have been investigated

as a potential treatment for neuropathic pain. Challenges include refining selectivity for the neuronal or inducible isoforms as endothelial NOS plays a vital role in regulating cardiovascular tone. Selective neuronal NOS inhibitors have been investigated in pain associated with migraines and chronic pancreatitis (35,38). There is also some experimental evidence that inducible NOS inhibitors reduce neuropathic pain in rat models (39). However, clinical trials of cindunistat, an inducible NOS inhibitor, did not report improvement in osteoarthritic joint pain (40). Other inhibitors have produced promising results in animal models and remain under development.

mPGES-1 Inhibitors

mPGES-1 is a terminal prostanoid pathway enzyme that catalyzes the synthesis of PGE2, a potent mediator of pain and inflammation. PGE2 binds to prostanoid receptors throughout the nervous system and sensitizes primary afferent nociceptors. Downstream cyclic adenosine monophosphate (cAMP) signaling promotes sensitization of various ligand and voltage-gated ion channels in nociceptive afferents (41). Although PGE2 promotes inflammatory and pain processes, it is also involved in mucosal protection of the gastrointestinal tract. Nonsteroidal antiinflammatory drugs exert analgesic and antiinflammatory effects by inhibiting PGE2 synthesis further upstream but are complicated by gastrointestinal side effects. Selective cyclooxygenase-2 (COX-2) inhibitors do not prevent mucosal injury but are associated with increased incidence of cardiovascular complications thought to arise from a deficiency of vasoprotective, antithrombotic prostacyclin (42). mPGES-1 has become an attractive target for inhibition because of its selective production of PGE2 without implication of other prostaglandins. An mPGES1 inhibitor has demonstrated improved pain in a canine osteoarthritis model without increased adverse events (43). Other inhibitors have revealed moderate efficacy by inhibiting thermal hyperalgesia response in guinea pig models (44). Early trials of mPGES-1 inhibitors in humans were complicated by hepatic injury, possibly secondary to the toxicity of metabolites. The development of other inhibitors continues at preclinical stages (8).

IL-6 Inhibitors

IL-6 is a T-cell-derived cytokine well-known for its role in immunologic defense and inflammatory disorders. Elevated levels of IL-6 are present throughout the DRG and spinal cord in several pathological pain mod-

els (45). The production of IL-6 through the inflammatory cascade results in a formation of a complex with the IL-6 receptor and gp130 transmembrane proteins. The gp130 subunit is responsible for subsequent intracellular JAK/STAT, MAPK, PI3K/AKT signal transduction, through which IL-6 promotes cell growth, modulates release of neurotrophic factors, and sensitizes nociceptive neurons (9). As such, IL-6 inhibitors have been examined in inflammatory pain conditions. Some anti-IL-6 antibodies such as tocilizumab and sarilumab have demonstrated reduction of patient-reported pain along with disease-modifying antirheumatic effects in moderate-to-severe rheumatoid arthritis (46). Tocilizumab has also been noted to improve weight-bearing and reduce mechanical allodynia in rat osteoarthritis models (47). The efficacy of IL-6 inhibition continues to be explored in neuropathic, inflammatory, and cancer pain (45).

Central Pain Pathways

Pain sensation integrates signaling through the ascending and descending pathways, and the central nervous system is a critical component of both. Within the ascending pathway, peripheral DRG activation leads to signal propagation through the spinal cord to thalamic processing, and ultimately higher brain centers. The descending pathway conveys the supraspinal, brainstem, and cortical feedback on nociceptive transmission (48). In chronic pain states, a barrage of primary afferent activity may result in sensitization of central pathways. Hyperalgesia is associated with the overexcitability of second-order neurons, modulated through receptor trafficking and intracellular signaling (49). Some currently available analgesics are recognized to act on central pain transmission, but not without the risk of inducing psychotropic side effects. Selective central inhibition has been explored so as to preserve analgesia while minimizing potential adverse effects.

κ-Opioid Agonists

κ-opioid receptors (KORs) are involved in opioidergic pain control throughout the neuroaxis. Endogenous ligands such as dynorphin activate the inhibitory G-protein coupled receptor, through which downstream kinase signaling modulates protein scaffolding (50). At the presynaptic terminal of DRGs, activation of KOR inhibits calcium influx and stimulates potassium efflux. The consequent hyperpolarization of nociceptive neurons provides analgesic effects similar to conventional μ-receptor agonists (10). Activation of KOR in the dor-

sal raphe nucleus also appears to modulate descending antinociceptive pathways. The central effects of KORs are mediated through its interactions with dynorphin to act in the regulation of stress, aversion, and addiction (51). Although κ-agonists produce less respiratory depression compared with μ-agonists, previous trials have been complicated by adverse effects including dysphoria and psychotomimesis (10). Development has since shifted to κ-agonists with lower incidence of significant central effects. Peripherally selective κ-agonists have undergone clinical trials for postsurgical and osteoarthritic pain, although results have not yet been released (52). An alternative approach has been the combined administration of κ-receptor and μ-receptor agonists. In rat models, combined κ-receptor and μ-receptor agonism induces additive analgesia for somatic pain and synergistic analgesia for visceral pain (53). The dysphoria associated with κ-receptor agonists may be useful in decreasing the reinforcing euphoric effects of μ-receptor opioids (50). Butorphanol is a partial μ-opioid receptor (MOR) agonist that also exhibits 20-fold higher affinity for KOR. This synthetic opioid demonstrates higher potency and lower dependence when compared with morphine (54). Butorphanol is available as a narcotic analgesic in parenteral and transnasal formulations, although tolerability may be limited by central nervous effects including somnolence, nausea, and vomiting (55). Administration may also precipitate withdrawal in opioid-dependent patients. Dezocine is another partial μ-opioid agonist, originally believed to be a κ-receptor agonist structurally similar to pentazocine (56). Recent in vitro studies have, however, revealed the compound to be a mixed μ-opioid agonist and κ-receptor antagonist, with additional inhibitory action at norepinephrine and serotonin reuptake transporters (56,57). Dezocine has been observed to present minimal addiction potential, augment the analgesic effect of morphine, and attenuate morphine withdrawal symptoms (57). Drugs with mixed opioid receptor actions may prove clinical usefulness as supplemental analgesia for postoperative pain management.

δ-Opioid Agonists

The δ-opioid receptors (DORs) are endogenously activated by enkephalin and modulate pain transmission. DORs are distributed throughout the peripheral and central nervous system, most densely within the dorsal horn, basal ganglia, and neocortex (58). Similar to other subclasses of opioid receptors, DORs are coupled to inhibitory G-protein activity that regulates

intracellular cAMP signaling and ion channel function. The inhibition of presynaptic Ca2+ influx and stimulation of K+ efflux additionally hyperpolarizes neurons and inhibits pronociceptive neurotransmitter release (59). In the central nervous system, DORs have been implicated in the regulation of pain, anxiety, and depression (60). δ-opioid agonists have thus been pursued for their analgesic effect and desirable risk profile. In several animal models, δ-agonists inhibit hyperalgesia associated with chronic inflammation (58). Agonism of DORs was observed to produce effective analgesia without respiratory depression and constipation, although some report increased convulsive effects in rat models (61). Recent efforts have examined ligands with combined μ-receptor and δ-receptor agonism to maximize analgesia while minimizing side effects. The combination of μ/δ-agonism has been demonstrated to reduce opioid tolerance while maintaining antihyperalgesic effects in a rat neuropathic pain model (61). DORs remain a promising target for the treatment of chronic pain through its modulation of ascending and descending pathways.

Biased μ-Opioid Agonists

Conventional opioid analgesics have largely targeted MORs. These receptors have high affinity for endogenous enkephalins and β-dynorphin, which act through an inhibitory G-protein pathway to reduce neuronal excitability in pain transmission (48). Although MOR-activated guanosine triphosphatase activity produces profound analgesia and subjective "liking," the simultaneous β-arrestin recruitment leads to respiratory depression and gastrointestinal distress (62). Several research compounds have been pursued as biased MOR agonists. These drugs preferentially activate guanosine triphosphatase activity as opposed to β-dynorphin, thereby producing analgesia while minimizing adverse effects. Oliceridine (TRV130) is a novel biased agonist that is under development for moderate-to-severe acute pain (63). Several studies have evaluated the analgesic effect and risk profile of oliceridine versus conventional morphine. In a postsurgical pain mouse model, oliceridine was observed to be 4-fold more potent than morphine. Repeated dosing of oliceridine was also associated with less tolerance and opioid-induced hyperalgesia, although physical dependence risk was similar to morphine (64). A safety profile study between oliceridine and morphine in healthy male volunteers revealed similar analgesic potency at clinical concentrations. At equal analgesic levels, oliceridine was noted to produce less respiratory depression than morphine (63). The postoperative utility of oliceridine has specifically been evaluated for bunionectomies and abdominoplasties. The results from Phase III clinical trials conclude effective relief of moderate-to-severe pain, with less respiratory depression and nausea compared with morphine (65,66). Although oliceridine is emerging as a viable intravenous analgesic for postoperative pain, concerns remain over its abuse potential (64). The current data of oliceridine abuse liability remains inconclusive and warrants further investigation.

NMDA Receptor Antagonists

NMDA receptors are glutamatergic ionotropic channels involved in a variety of physiological functions. Located on both pre- and postsynaptic terminals, NMDA receptors are recognized to mediate synaptic transmission and neuronal plasticity (67). Activation of the receptor requires the binding of glutamate resulting in the voltage-gated release of an Mg2+ block, which in turn stimulates calcium-dependent signaling pathways that increase neuroexcitability and promote central sensitization (68). Excessive glutamatergic NMDA activity enhances nociceptive transmission; therefore several NMDA antagonists have been evaluated for analgesic effect. Ketamine is a well-established NMDA antagonist that is used clinically for various neuropathic pain conditions and in preoperative pain management (69). Although other compounds with varying levels of NMDA agonism have been evaluated, a 2019 meta-analysis concluded that low-dose ketamine most reliably confers analgesia and reduces hyperalgesia (11). Ketamine infusions have been clinically effective in the management of acute pain, but not without risk of cardiovascular, hepatotoxic, and psychomimetic events. Intravenous ketamine infusions have demonstrated short-term analgesia for chronic pain syndromes, but long-term benefits remain unclear (70). Consensus guidelines for ketamine in both chronic and acute pain conditions have been released through a collaboration between the American Society of Regional Anesthesia and Pain Medicine, the American Academy of Pain Medicine, and the American Society of Anesthesiologists to help guide clinical use.

Cannabinoids

The endocannabinoid system is involved in numerous physiological processes including pain and inflammation. The cannabinoids tetrahydrocan-

nabinol (THC) and cannabidiol (CBD) from Cannabis sativa have long been used for mood and pain (71). Recent research has investigated the endogenous pathways of cannabinoids. Endocannabinoids bind to various receptors throughout the nervous system, most notably cannabinoid receptor types 1 and 2 (CB1 and CB2). Both CB1 and CB2 are coupled to inhibitory G-proteins that inhibit cAMP secondary signaling (12). Activation of CB1 stimulates mitogen-activated protein kinases and regulates potassium and calcium channels, which inhibit presynaptic neurotransmitter release in nociceptive signaling (12). The psychotropic effects of cannabinoids have been attributed to dense CB1 activity in the central nervous pathways (71). Conversely, CB2 receptors are expressed chiefly in the peripheral nervous system and immune response cells. CB2 activation appears to have antinociceptive effects through the inhibition of mast cell degranulation and neutrophil accumulation, and also indirectly simulates peripheral opioid receptors by promoting the release of β-endorphin. The antiinflammatory analgesic effects of cannabinoids may have applications in inflammatory and neuropathic pain states (72). Although there is no formally approved use of cannabis in the United States, synthetic cannabinoids dronabinol and nabilone, which are structurally analogous to THC and exhibit partial agonism toward the CB1 and CB2 receptors, have been approved to treat nausea and anorexia in chemotherapy patients. There has been investigation into their analgesic potential (73). A 2017 meta-analysis concluded that an extended regimen of dronabinol, but not nabilone, was efficacious in relieving neuropathic pain. Limitations, however, included variable bioavailability, the development of tolerance, and unclear adverse effects including gastrointestinal distress and in rare instances psychosis. Selective cannabinoids were, however, associated with improved quality of life and increased patient satisfaction (74). To minimize psychotropic adverse effects, drug design has focused on peripherally selective CB1 and CB2 agonists. The peripheral CB2 agonist APD371 (Olorinab) is in clinical trials for abdominal pain associated with Crohn disease and irritable bowel syndrome (12). Several other drugs targeting the endocannabinoid system are in earlier phases of development.

FAAH Inhibitors

The degradation of endocannabinoids is primarily carried out by FAAH. These integral membrane enzymes cleave endogenous anandamide and oleamide, thereby terminating their activation of cannabinoid signaling (75). In contrast to direct cannabinoid agonists, which may produce undesirable psychotropic and motor effects, inhibition of catalytic pathways is theorized to enhance endogenous endocannabinoid activity. Inhibition of FAAH has been demonstrated to attenuate neuropathic pain, acute inflammatory pain, and mechanical hyperalgesia in mouse and rat models (76). Early clinical trials of FAAH inhibitors were complicated by serious adverse effects. A 2016 Phase I study of BIA 10-2474, an irreversible FAAH inhibitor, demonstrated that high doses were capable of inducing a rapidly progressive, severe neurologic syndrome characterized by headache, altered consciousness, and microhemorrhages in the pons and hippocampus (77). Although the mechanism of toxicity remains unclear, the BIA 10-2474 trial illustrates the dangers of nonselective endocannabinoid activation. Although a later US Food and Drug Administration (FDA) investigation concluded that the neurotoxicity was specific to BIA 10-2474, other FAAH inhibitors have failed to demonstrate analgesic efficacy or safety beyond Phase II. As anandamide is recognized to stimulate pronociceptive prostaglandin production and TRPV1 activation, there is new research into combining FAAH with other TRPV1 or COX-2 inhibitors (12).

Alternative Modalities

The development of new technologies has expanded the possibilities of new analgesic compounds. In contrast to conventional agonists and antagonists, it is now possible to sequester signaling molecules or to edit protein expression altogether. Although early in development, such strategies may prove valuable in pain research and ultimately in clinical applications.

Anti-NGF Antibodies

NGF is a neurotrophin, proteins involved in the growth and regulation of peripheral neurons. Within neurotrophic signaling, NGF is highly selective for tyrosine receptor kinase trkA expressed on nociceptors (78). Activated trkA stimulates PI3K (phosphatidylinositol-4,5-bisphosphate 3-kinase)–Akt (protein kinase B), ERK (extracellular signal-regulated kinases), and PLCγ (phospholipase Cγ) secondary signaling that promotes neuronal growth and survival (13). In A-delta and unmyelinated C-fiber neurons, internalization of NGF-trkA complex may furthermore increase the expression of pronociceptive bradykinin receptors, TRPV1 receptors,

and voltage-gated ion channels (79). More recently, NGF has also been reported to bind p75, a nonselective neurotrophic receptor. The low-affinity interaction of NGF and p75 may lead to heterodimerization with other receptors and reduce neuronal growth (13). Nevertheless, chronic pain is associated with increased NGF expression. Although administration of exogenous NGF induced hyperalgesia, antagonism of NGF activity decreased pain behaviors in animal models (80). In preclinical models, monoclonal antibodies against NGF produced significant analgesia without antiinflammatory effects (79). The anti-NGF antibody tanezumab has since entered clinical trials for osteoarthritis and chronic back pain. A pooled analysis of Phase III trials found tanezumab to improve joint pain and physical function in patients with moderate-to-severe osteoarthritis (81). Tanezumab produced similarly promising analgesic effects in patients with chronic lower back pain, diabetic neuropathy, and interstitial cystitis (79). Abnormal peripheral sensation was reported as the most common adverse event, although at low incidence across all subgroups (81). There were reports of osteonecrosis and rapidly progressive osteoarthritis associated with anti-NGF therapy. A later FDA committee concluded that although the mechanism remains uncertain, bone destruction is more likely with prolonged use of anti-NGF and concurrent nonsteroidal antiinflammatory drug use (79). Although anti-NGF antibody trials have since resumed, long-term safety studies are still necessary.

Genetic Interventions

Various genetic interventions target the DRG, and modified expression of specific proteins can impair their nociceptive function. Some genetic therapies have involved both viral transduction and nonviral transfection of endogenous, antinociceptive proteins. The delivery of opioid peptides, antiinflammatory cytokines, and hyperpolarizing ion channels has demonstrated some analgesic and antihyperalgesic effects (49). Conversely, transfection has also been utilized to block the synthesis of particular proteins. Transfection of antisense-RNA reduced expression of nociceptive receptors, including NMDA, TRPV1, and Nav1.8, resulting in reduced inflammatory hyperalgesia in animal models (49). The clinical potential of such techniques may be limited as viral vectors induce strong immune responses and have a limited duration of effect. Nonviral vectors, however, have not been able to be delivered efficiently for a therapeutic effect (82).

An alternative approach has been the modification of epigenetic profiles, which stabilize transcriptional abnormalities in chronic pain. Inhibition of histone deacetylase (HDAC) and histone acetyltransferases has been recognized to attenuate neuropathic and inflammatory pain in several animal models (14). Concerns have arisen regarding the nonselectivity of HDAC inhibitors, which may induce systemic adverse effects. More specific epigenetic silencing remains under development in preclinical models (83).

The development of Clustered Regulatory Interspaced Short Palindromic Repeats-associated Cas9 (CRISPR/Cas9) realized the possibility of targeted genome editing. The CRISPR/Cas9 system consists of a bacterial endonuclease, which is guided by a short, synthetic RNA targeted against a genomic sequence. Double-stranded cleavage of the target DNA induces endogenous repair mechanisms. By disrupting a gene or introducing a synthetic template, CRISPR/Cas9 is capable of editing the genome (15). The system has become a valuable tool in pain research and there are investigations into potential clinical applications. An in vitro model of degenerative intervertebral disc disease demonstrated that CRISPR-induced knockdown of a pronociceptive mediator reduced pathologic DRG activity (84). In rat osteoarthritis models, CRISPR/Cas9 ablation of NGF, IL-1β, and matrix metalloproteinase 13 palliated joint pain and attenuated structural damage (85). The CRISPR/Cas9 system has vast therapeutic potential for pain therapies, but several challenges remain. There are ongoing efforts to reduce nonspecific DNA binding and develop efficient, safe delivery methods (86). In the meantime, CRISPR/Cas9 editing will likely aid in the characterization of pain pathways and novel analgesic targets.

Conclusions

This review has attempted to characterize novel targets for analgesic drug development. Chronic pain continues to present a substantial burden on patient health, but the ongoing opioid epidemic imposes long-term limits on conventional therapies. Some compounds, such as resiniferatoxin, tocilizumab, and tanezumab have demonstrated positive analgesic effects in clinical trials. Other classes of potential targets remain in preclinical stages. Further trials in safety and efficacy are required to realize the analgesic potential of these drug classes.

REFERENCES

1. Institute of Medicine. Relieving Pain in America: A Blueprint for Transforming Prevention, Care, Education, and Research. Washington, DC, *The National Academies Press*, 2011.

2. Vos T, Flaxman AD, Naghavi M, et al. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: A systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 2012; 380:2163-2196.

3. Woolf CJ. Capturing novel non-opioid pain targets. *Biol Psychiatry* 2020; 87:74-81.

4. Cardoso FC, Lewis RJ. Sodium channels and pain: From toxins to therapies. *Br J Pharmacol* 2018; 175:2138-2157.

5. Bourinet E, Altier C, Hildebrand ME, Trang T, Salter MW, Zamponi GW. Calcium-permeable ion channels in pain signaling. *Physiol Rev* 2014; 94:81-140.

6. Julius D. TRP channels and pain. *Ann Rev Cell Dev Biol* 2013; 29:355-384.

7. Ahlawat A, Rana A, Goyal N, Sharma S. Potential role of nitric oxide synthase isoforms in pathophysiology of neuropathic pain. *Inflammopharmacology* 2014; 22:269-278.

8. Bergqvist F, Morgenstern R, Jakobsson PJ. A review on mPGES-1 inhibitors: From preclinical studies to clinical applications. Prostaglandins *Other Lipid Mediat* 2020; 147:106383.

9. Kelleher JH, Tewari D, McMahon SB. Neurotrophic factors and their inhibitors in chronic pain treatment. *Neurobiol Dis* 2017; 97:127-138.

10. Vanderah TW. Delta and kappa opioid receptors as suitable drug targets for pain. *Clin J Pain* 2010; 26:S10-S15.

11. Thompson T, Whiter F, Gallop K, et al. NMDA receptor antagonists and pain relief: A meta-analysis of experimental trials. *Neurology* 2019; 92:e1652-e1662.

12. Stasiulewicz A, Znajdek K, Grudzień M, Pawiński T, Sulkowska JI. A guide to targeting the endocannabinoid system in drug design. *Int J Mol Sci* 2020; 21:2778.

13. Denk F, Bennet DL, McMahon SB. Nerve growth factor and pain mechanisms. *Ann Rev Neurosci* 2017; 40:307-325.

14. Niederberger E, Resch E, Parnham MJ, Geisslinger G. Drugging the pain epigenome. *Nat Rev Neurol* 2017; 13:434-447.

15. Krupkova O, Cambria E, Besse L, Besse A, Bowles R, Wuertz-Kozak K. The potential of CRISPR/Cas9 genome editing for the study and treatment of intervertebral disc pathologies. *JOR Spine* 2018; 1:e1003.

16. Hoffmann T, Sharon O, Wittmann J, et al. NaV1.7 and pain: Contribution of peripheral nerves. *Pain* 2018; 159:496-506.

17. Verkerk AO, Remme CA, Schumacher CA, et al. Functional NaV1.8 channels in intracardiac neurons: The link between SCN10A and cardiac electrophysiology. *Circ Res* 2012; 111:333-343.

18. Faber CG, Lauria G, Merkies ISJ, et al. Gain-of-function Nav1.8 mutations in painful neuropathy. *Proc Natl Acad Sci USA* 2012; 109:19444-19449.

19. Hameed S. Nav1.7 and Nav1.8: Role in the pathophysiology of pain. *Mol Pain* 2019; 15:1-11.

20. Shields SD, Deng L, Reese RM, et al. Insensitivity to pain upon adult-onset deletion of Nav1.7 or its blockade with selective inhibitors. *J Neurosci* 2018; 38:10180-10201.

21. Lee S. Pharmacological inhibition of voltage-gated Ca2+ channels for chronic pain relief. *Curr Neuropharmacol* 2013; 11:606-620.

22. Taylor CP. Mechanisms of analgesia by gabapentin and pregabalin -- Calcium channel $\alpha 2-\delta$ (Cav$\alpha 2-\delta$) ligands. *Pain* 2009; 142:13-16.

23. Scott DA, Wright CE, Angus JA. Actions of intrathecal ω-conotoxins CVID, GVIA, MVIIA, and morphine in acute and neuropathic pain in the rat. *Eur J Pharmacol* 2002; 451:279-286.

24. Schmidtko A, Lötsch J, Freynhagen R, Geisslinger G. Ziconotide for treatment of severe chronic pain. *Lancet* 2010; 375:1569-1577.

25. Manda P, Kushwaha AS, Kundu S, Shivakumar HN, Jo SB, Murthy SN. Delivery of ziconotide to cerebrospinal fluid via intranasal pathway for the treatment of chronic pain. *J Control Release* 2016; 224:69-76.

26. Lee S, Jo S, Talbot S, et al. Novel charged sodium and calcium channel inhibitor active against neurogenic inflammation. *Elife* 2019; 8:e48118.

27. Shan Z, Cai S, Yu J, et al. Reversal of peripheral neuropathic pain by the small-molecule natural product physalin F via block of CaV2.3 (R-type) and CaV2.2 (N-type) voltage-gated calcium channels. *ACS Chem Neurosci* 2019; 10:2939-2955.

28. Wang H, Siemens J. TRP ion channels in thermosensation, thermoregulation and metabolism. *Temperature* 2015; 2:178-187.

29. Rosenbaum T, Simon S. *TRPV1 Receptors and Signal Transduction*. Boca Raton, FL, CRC Press/Taylor & Francis, 2007.

30. Anand P, Bley K. Topical capsaicin for pain management: Therapeutic potential and mechanisms of action of the new high-concentration capsaicin 8 patch. *Br J Anaesth* 2011; 107:490-502.

31. Pecze L, Viskolcz B, Oláh Z. Molecular surgery concept from bench to bedside: A focus on TRPV1+ pain-sensing neurons. *Front Physiol* 2017; 8:378.

32. Iadarola MJ, Sapio MR, Raithel SJ, Mannes AJ, Brown DC. Long-term pain relief in canine osteoarthritis by a single intra-articular injection of resiniferatoxin, a potent TRPV1 agonist. *Pain* 2018; 159:2105-2114.

33. Heiss J, Iadarola M, Cantor F, Oughourli A, Smith R, Mannes A. A Phase I study of the intrathecal administration of resiniferatoxin for treating severe refractory pain associated with advanced cancer. *J Pain* 2014; 15:S67.

34. Trevisani M, Raffaele G. TRPV1 antagonists as analgesic agents. *Open Pain J* 2013; 6:108-118.

35. Pradhan AA, Bertels Z, Akerman S. Targeted nitric oxide synthase inhibitors for migraine. *Neurotherapeutics* 2018; 15:391-401.

36. Förstermann U, Sessa WC. Nitric oxide synthases: Regulation and function. *Eur Heart J* 2012; 33:829-837.

37. Hartung JE, Ciszek BP, Nackley AG. $\beta 2$- and $\beta 3$-adrenergic receptors drive COMT-dependent pain by increasing production of nitric oxide and cytokines. *Pain* 2014; 155:1346-1355.

38. Demir IE, Heinrich T, Carty DG, et al. Targeting nNOS ameliorates the severe neuropathic pain due to chronic pancreatitis. *EBioMedicine* 2019; 46:431-443.

39. Staunton CA, Barrett-Jolley R, Djouhri L, Thippeswamy T. Inducible nitric oxide synthase inhibition by 1400W limits pain hypersensitivity in a neuropathic pain rat model. *Exp Physiol* 2018; 103:535-544.

40. Le Graverand MPH, Clemmer RS, Redifer P, et al. A 2-year randomised, double-blind, placebo-controlled, multicentre study of oral selective iNOS inhibitor, cindunistat (SD-

6010), in patients with symptomatic osteoarthritis of the knee. *Ann Rheum Dis* 2013; 72:187-195.

41. Sauer RS, Rittner HL, Roewer N, et al. A novel approach for the control of inflammatory pain: Prostaglandin E2 complexation by randomly methylated β-cyclodextrins. *Anesth Analg* 2017; 124:675-685.

42. Koeberle A, Laufer SA, Werz O. Design and development of microsomal prostaglandin E2 synthase-1 inhibitors: Challenges and future directions. *J Med Chem* 2016; 59:5970-5986.

43. Robertson-Plouch C, Stille JR, Liu P, et al. A randomized clinical efficacy study targeting mPGES1 or EP4 in dogs with spontaneous osteoarthritis. *Sci Transl Med* 2019; 11:1-13.

44. Muthukaman N, Tambe M, Shaikh M, et al. Tricyclic 4,4-dimethyl-3,4-dihydrochromeno(3,4-d)imidazole derivatives as microsomal prostaglandin E2 synthase-1 (mPGES-1) inhibitors: SAR and in vivo efficacy in hyperalgesia pain model. *Bioorganic Med Chem Lett* 2017; 27:2594-2601.

45. Zhou YQ, Liu Z, Liu ZH, et al. Interleukin-6: An emerging regulator of pathological pain. *J Neuroinflammation* 2016; 13:1-9.

46. Choy EHS, Calabrese LH. Neuroendocrine and neurophysiological effects of interleukin 6 in rheumatoid arthritis. *Rheumatology* 2018; 57:1885-1895.

47. Lin Y, Liu L, Jiang H, Zhou J, Tang Y. Inhibition of interleukin-6 function attenuates the central sensitization and pain behavior induced by osteoarthritis. *Eur J Pharmacol* 2017; 811:260-267.

48. Yam MF, Loh YC, Tan CS, Adam SK, Manan NA, Basir R. General pathways of pain sensation and the major neurotransmitters involved in pain regulation. *Int J Mol Sci* 2018; 19:2164.

49. Yaksh T, Woller S, Ramachandran R, Sorkin L. The search for novel analgesics: Targets and mechanisms. *F1000Prime Rep* 2015; 7:56.

50. Beck TC, Hapstack MA, Beck KR, Dix TA. Therapeutic potential of kappa opioid agonists. *Pharmaceuticals* 2019; 12:95.

51. Corder G, Castro DC, Bruchas MR, Scherrer G. Endogenous and exogenous opioids in pain. *Annu Rev Neurosci* 2018; 41:453-473.

52. Burch R, Atkins T. Inside the potential of peripheral kappa opioid receptor agonists. *Pract Pain Manag* 2020; 20:23-27.

53. Rech RH, Mokler DJ, Briggs SL. Effects of combined opioids on pain and mood in mammals. *Pain Res Treat* 2012; 2012:145965.

54. Ji J, Lin W, Vrudhula A, et al. Molecular interaction between butorphanol and κ-opioid receptor. *Anesth Analg* 2020; 131:935-942.

55. Gillis J, Benfield P, Goa K. Transnasal butorphanol. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in acute pain management. *Drugs* 1995; 50:157-175.

56. Liu R, Huang X, Yeliseev A, Xi J, Roth B. Novel molecular targets of dezocine and their clinical implications. *Anesthesiology* 2014; 120:714-723.

57. Wu F, Babazada H, Gao H, et al. Dezocine alleviates morphine-induced dependence in rats. *Anesth Analg* 2019; 128:1328-1335.

58. Peppin JF, Raffa RB. Delta opioid agonists: A concise update on potential therapeutic applications. *J Clin Pharm Ther* 2015; 40:155-166.

59. François A, Scherrer G. Delta opioid receptor expression and function in primary afferent somatosensory neurons. *Handb Exp Pharmacol* 2018; 247:87-114.

60. Quirion B, Bergeron F, Blais V, Gendron L. The delta-opioid receptor; A target for the treatment of pain. *Front Mol Neurosci* 2020; 13:1-52.

61. Günther T, Dasgupta P, Mann A, et al. Targeting multiple opioid receptors – Improved analgesics with reduced side effects? *Br J Pharmacol* 2018; 175:2857-2868.

62. Urits I, Viswanath O, Orhurhu V, et al. The utilization of mu-opioid receptor biased agonists: Oliceridine, an opioid analgesic with reduced adverse effects. *Curr Pain Headache Rep* 2019; 23:31.

63. Dahan A, van Dam CJ, Niesters M, et al. Benefit and risk evaluation of biased µ-receptor agonist oliceridine versus morphine. *Anesthesiology* 2020; 133:559-568.

64. Liang DY, Li WW, Nwaneshiudu C, Irvine KA, Clark JD. Pharmacological characters of oliceridine, a µ-opioid receptor G-protein-biased ligand in mice. *Anesth Analg* 2019; 129:1414-1421.

65. Viscusi ER, Skobieranda F, Soergel DG, Cook E, Burt DA, Singla N. APOLLO-1: A randomized placebo and active-controlled phase III study investigating oliceridine (TRV130), a G protein-biased ligand at the µ-opioid receptor, for management of moderate-to-severe acute pain following bunionectomy. *J Pain Res* 2019; 12:927-943.

66. Singla NK, Skobieranda F, Soergel DG, et al. APOLLO-2: A randomized, placebo and active-controlled Phase III study investigating Oliceridine (TRV130), a G protein–biased ligand at the µ-opioid receptor, for management of moderate to severe acute pain following abdominoplasty. *Pain Pract* 2019; 19:715-731.

67. Deng M, Chen SR, Pan HL. Presynaptic NMDA receptors control nociceptive transmission at the spinal cord level in neuropathic pain. *Cell Mol Life Sci* 2019; 76:1889-1899.

68. Basbaum AI, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain. *Cell* 2009; 139:267-284.

69. Aiyer R, Mehta N, Gungor S, Gulati A. A systematic review of NMDA receptor antagonists for treatment of neuropathic pain in clinical practice. *Clin J Pain* 2018; 34:450-467.

70. Orhurhu V, Orhurhu MS, Bhatia A, Cohen SP. Ketamine infusions for chronic pain: A systematic review and meta-analysis of randomized controlled trials. *Anesth Analg* 2019; 129:241-254.

71. Baron EP. Medicinal properties of cannabinoids, terpenes, and flavonoids in cannabis, and benefits in migraine, headache, and pain: An update on current evidence and cannabis science. *Headache Curr* 2018; 58:1139-1186.

72. Manzanares J, Julian M, Carrascosa A. Role of the cannabinoid system in pain control and therapeutic implications for the management of acute and chronic pain episodes. *Curr Neuropharmacol* 2006; 4:239-257.

73. Vučkovic S, Srebro D, Vujovic KS, Vučetic Č, Prostran M. Cannabinoids and pain: New insights from old molecules. *Front Pharmacol* 2018; 9:1259.

74. Meng H, Johnston B, Englesakis M, Moulin DE, Bhatia A. Selective cannabinoids for chronic neuropathic pain: A systematic review and meta-analysis. *Anesth Analg* 2017; 125:1638-1652.

75. Brindisi M, Borrelli G, Brogi S, et al. Development of potent inhibitors of fatty acid amide hydrolase useful for the treatment of neuropathic pain. *Chem Med Chem* 2018; 13:2090-2103.

76. Donvito G, Nass SR, Wilkerson JL, et al. The endogenous cannabinoid system: A budding source of targets for treating inflammatory and neuropathic

pain. *Neuropsychopharmacology* 2018; 43:52-79.

77.	Kerbrat A, Ferré JC, Fillatre P, et al. Acute neurologic disorder from an inhibitor of fatty acid amide hydrolase. *N Engl J Med* 2016; 375:1717-1725.

78.	Miller R, Block J, Malfait A. Nerve growth factor (NGF) blockade for the management of osteoarthritis pain: What can we learn from clinical trials and preclinical models? *Curr Opin Rheumatol* 2018; 29:110-118.

79.	Chang DS, Hsu E, Hottinger DG, Cohen SP. Anti-nerve growth factor in pain management: Current evidence. *J Pain Res* 2016; 9:373-383.

80.	Knezevic NN, Mandalia S, Raasch J,

Knezevic I, Candido KD. Treatment of chronic low back pain -- New approaches on the horizon. *J Pain Res* 2017; 10:1111-1123.

81.	Tive L, Bello AE, Radin D, et al. Pooled analysis of tanezumab efficacy and safety with subgroup analyses of Phase III clinical trials in patients with osteoarthritis pain of the knee or hip. *J Pain Res* 2019; 12:975-995.

82.	Yamano S, Viet CT, Dang D, et al. Ex vivo nonviral gene delivery of μ-opioid receptor to attenuate cancer-induced pain. *Pain* 2017; 158:240-251.

83.	Manion J, Waller MA, Clark T, Massingham JN, Neely GG. Developing modern pain therapies. *Front Neurosci*

2019; 13:1370.

84.	Stover JD, Farhang N, Berrett KC, Gertz J, Lawrence B, Bowles RD. CRISPR epigenome editing of AKAP150 in DRG neurons abolishes degenerative IVD-induced neuronal activation. *Mol Ther* 2017; 25:2014-2027.

85.	Zhao L, Huang J, Fan Y, et al. Exploration of CRISPR/Cas9-based gene editing as therapy for osteoarthritis. *Ann Rheum Dis* 2019; 78:676-682.

86.	Lino CA, Harper JC, Carney JP, Timlin JA. Delivering CRISPR: A review of the challenges and approaches. *Drug Deliv* 2018; 25:1234-1257.