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# **Nanomedicine and Voltage-gated sodium channel blockers in pain management : a game changer or a lost cause ?**

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## **Abstract**

Pain, a complex and debilitating condition affecting millions globally, is a significant concern, especially in the context of post-operative recovery. This comprehensive review explores the complexity of pain and its global impact, emphasizing the modulation of voltage-gated sodium channels (VGSC or NaV channels) as a promising avenue for pain management with the aim of reducing reliance on opioids. The article delves into the role of specific NaV isoforms, particularly NaV 1.7, NaV 1.8, and NaV 1.9, in pain process and discusses the development of sodium channel blockers to target these isoforms precisely. Traditional local anesthetics and selective NaV isoform inhibitors, despite showing varying efficacy in pain management, face challenges in systemic distribution and potential side effects. The review highlights the potential of nanomedicine in improving the delivery of local anesthetics, toxins and selective NaV isoform inhibitors for a targeted and sustained release at the site of pain. This innovative strategy seeks to improve drug bioavailability, minimize systemic exposure, and optimize therapeutic outcomes, holding significant promise for secure pain management and enhancing the quality of life for individuals recovering from surgical procedures or suffering from chronic pain.

## **List of abbreviations**

(US) FDA : United States of America's Food and Drug Administration  
AAV : adeno-associated virus (vector)  
CCI : chronic constriction sciatic nerve injury  
CFA : complete Freund's adjuvant  
CNS : central nervous system  
CRISPR : Clustered Regularly Interspaced Short Palindromic Repeats  
DRG : dorsal root ganglion  
GABA : gamma-aminobutyric acid  
GNRs : gold nanorods  
ZFN : zinc finger nucleases  
KRAB-ZFPs: Kruppel-associated box zinc finger proteins  
miRNA : microRNA  
NMDA : N-Methyl-D-Aspartate  
ODN : antisense oligodeoxynucleotide

PNS : peripheral nervous system  
S1SCB : site 1 sodium channel blocker  
SABER : sucrose acetate isobutyrate extended-release  
shRNA : short hairpin RNA  
siRNA small interfering RNAs  
SNL : spinal nerve ligation  
TRPV : Transient Receptor Potential Vanilloid 1  
TTX : tetrodotoxin  
VGSC / NaV channels: Voltage-gated sodium channels

## 1. Introduction

In recent years, there has been a growing interest in exploring blockers of sodium channels as an alternative or complementary approach to pain management, compared to traditional opioid-based therapies. Opioids, while effective in alleviating pain, are associated with a host of severe adverse effects, including the risk of tolerance, dependence, and opioid-related side effects.

Voltage-gated sodium channels (VGSC or NaV channels) play a pivotal role in the initiation of action potential and the transmission of electrical signals in excitable cells, including neurons. In the context of pain, specific NaV channel isoforms like NaV 1.3, NaV 1.8, NaV 1.9 and particularly NaV 1.7, have emerged as key players, influencing nociception and shaping the sensory experience of pain. Several marketed medications with sodium channel-blocking properties are employed for pain control. The most common are local anesthetics, but other classes of drugs are also used, such as class I antiarrhythmics, antiepileptics, and antidepressants. While their primary therapeutic actions may differ, their capacity to inhibit sodium channels contributes their pharmacological effects. However, their potential side effects, due to a lack of selectivity towards sodium channel isoforms, underscore the importance of careful dosage and administration to mitigate adverse reactions and ensure patient safety. Thus, modulating neuronal excitability through the inhibition of specific NaV channel isoforms stands as a central approach in alleviating pain safely. Over the course of the past two decades, selective NaV blockers, including venom-derived peptides, low-molecular-weight compounds, and oligonucleotides, have been developed for this purpose. Nevertheless, only few of them entered clinical trial phase.

The use of nanomedicine to target sodium channels with high precision represents an innovative approach that holds considerable importance in the field of pain relief. Indeed, by engineering nanoscale carriers (ie. liposomes, polymeric or lipid nanoparticles, adeno-associated viral (AAV) vectors), NaV blockers can be transported with unprecedented accuracy to specific nerves or tissues. This approach guarantees a localized analgesic effect, thus maximizing pain relief while reducing systemic exposure and consequently the risk of potential side effects. Nanoformulation also enhance drug solubility and provide protection from fast metabolism in the blood circulation, thus avoiding the administration of high doses of the drug. In addition, using controlled release nanoformulations, pain relief can be sustained over an extended period. This prolonged action reduces the need for frequent dosing and enhances patient's compliance, especially in case of post-operative pain. In addition, the versatility of nanomedicine enables the development of combination therapies that target multiple pain pathways simultaneously [1] [2]. For instance, nanoparticles could be engineered to carry both an analgesic and an anti-inflammatory agent, addressing pain and inflammation in a synergistic manner. This approach holds great potential for enhancing pain relief in complex conditions [2].

This review will give first a general overview on pain mechanism and then will describe the different isoforms of voltage-gated sodium channels as well as their role in nociception. Then, it will outline the diverse clinically available treatments that operate through these sodium channels for pain relief, from the preclinical level to late-stage clinical trials and approved medicines. Finally, it will present innovative approaches and will examine the contributions of nanomedicine in this field.

## **2. Generalities about pain**

Before the 1960s, pain was predominantly perceived as an automatic response to tissue damage, with little consideration for the emotional aspect of this common sensation or for the influence of genetic differences, past experiences, anxiety, or expectations. Over the past few years, significant progress has been achieved in comprehending the mechanisms behind pain and therefore in pain management for individuals experiencing pain.

Pain is probably the most common symptomatic reason for a medical consultation. But what exactly, from a neurobiological perspective, is pain? The term of “pain” actually refers to two quite different pathophysiological events. Initially, there is the sensation of pain, serving as a vital early-warning physiological defense mechanism, crucial for identifying and reducing exposure to harmful or irritating stimuli. It corresponds to a sensation experienced when coming into contact with something excessively hot, cold, or sharp. Since this pain involves the perception of noxious stimuli, it is called “nociceptive pain”, a high-threshold pain only activated by intense stimuli [3]. Nociceptive pain is an alarm mediated by unmyelinated C or thinly myelinated A $\delta$  primary nociceptors allowing to signal impending or actual tissue damage from environmental stimuli. Its protective role demands prompt attention and response, which are initiated through the withdrawal reflex it triggers, the inherent discomfort of the sensation it induces, and the emotional distress it invokes [4]. The second type of pain which is also adaptive and protective is the “inflammatory pain”. This type of pain arises from the immune system's activation due to tissue injury or infection. It can facilitate the healing process by inducing heightened pain sensitivity (dissuading physical contact and movement and reducing further risk of damage) until the recovery is complete. Although this pain is adaptive, in case of persistent inflammation as with rheumatoid arthritis or in cases of severe or extensive injury, it needs to be managed.

Finally, there is the “pathological pain” which is not protective, but maladaptive, arising from nervous system disorders. This pathological pain is a disease state of the nervous system, resulting from its damage (neuropathic pain) or its irregular functioning with no such damage or inflammation (dysfunctional pain) [5]. The dysfunctional pain occurring in the absence of noxious stimuli and with little to no peripheral inflammatory pathology, is often associated with pathologies such as fibromyalgia, irritable bowel syndrome, tension type headache, temporomandibular joint disease, interstitial cystitis.... The lack of specific targets and the complexity of neuropathic pain explains why it remains the most poorly treated pain.

In addition, pain can be multifactorial, as in mixed pain, which possesses a dual nociceptive and neuropathic component acting simultaneously and/or concurrently. This is the case with cancer pain, which results from tumor growth that causes both inflammation, which stimulates the nociceptors (sensory neurons), and alteration of the structure of the surrounding nerves, both through compression and invasion of healthy nerves. Musculoskeletal disorders, such as lumbosciatica, pain diseases with initial inflammatory origin that eventually affect nerve function, such as endometriosis, or post-surgery pain, fall into this category.

### **2.1. Pain pathways**

The transmission of nociceptive information from the site of the noxious stimulus to the brain occurs through a network of specialized nerve fibers and channels, integrated in a system of sensory neurons and interneurons that are part of the nervous system. The process of nociceptive transmission can be divided into several steps (Fig. 1).

#### **2.1.1 Transduction**

Initially, "transduction" involves the transformation of noxious (painful) stimuli, such as heat, damage, or mechanical pressure, into electrical signals within nociceptors, which are specialized sensory neurons designed

to detect noxious stimuli. The activation of these nociceptors occurs through various receptors, including ion channels, G protein-coupled receptors, and transient receptor potential (TRP) channels.

### 2.1.2 Transmission

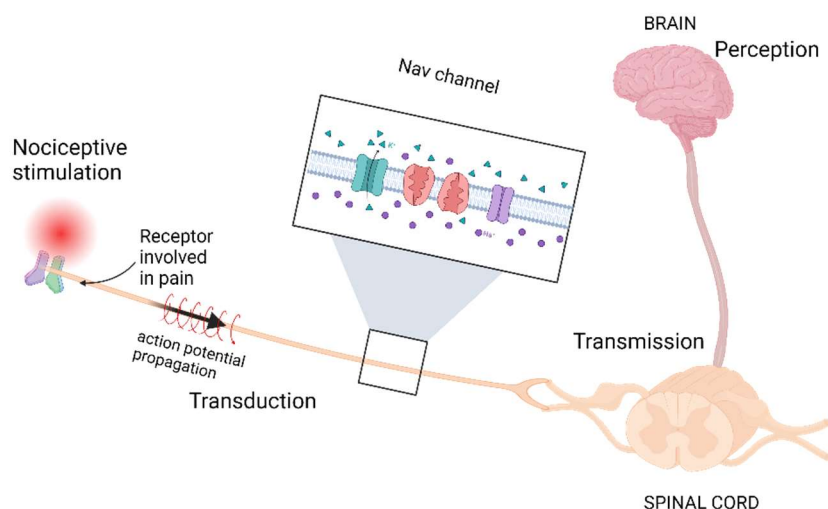
In the second step called “Transmission,” the nociceptors generate action potentials that are transmitted along their axons to the dorsal horn of the spinal cord or to the ganglia of sensory cranial nerves, where they synapse with second-order neurons (Fig. 1). These second-order neurons then send their axons across the midline of the spinal cord (applicable for spinal sensory nerves) and ascend to the brainstem and thalamus.

### 2.1.3 Perception

When nociceptive information reaches higher brain regions, including the somatosensory cortex, it is integrated with other sensory informations to become a conscious perception of the noxious stimulus leading to the “unpleasant sensory and emotional experience.” This phenomenon is called “Perception”.

### 2.1.4 Modulation

Moreover, the nociceptive transmission can be modulated by a plethora of transmitters involved in descending pain control, including opioid peptides and monoamines. In that respect, endogenous opioids such as endorphins and enkephalins, bind to opioid receptors on both nociceptors and second-order neurons and thus reduce nociceptive transmission. This step corresponds to “Modulation” of the pain perception.



**Figure 1 - Overview of ascending pathways of pain perception, from the nociceptive stimulation to the final pain sensation.** NaV channels located in the sensitive neuron fibers are involved in the transduction of the nociceptive signaling, all the way to the central nervous system. As a result, they play a crucial role in nociception and normal pain, and an abnormal function of these channels can lead to pathologic pain.

## 2.2. Pain targets

Pain management encompasses a wide array of physiological targets within the human body, the main ones being receptors, channels, transporters or enzymes.

### **2.2.1 Opioid receptors.**

A key area of emphasis is opioid receptors, which play a central role in alleviating pain [6]. Opioid receptors (OR) belong to the large superfamily of seven transmembrane-spanning (7TM) G protein-coupled receptors (GPCRs). Their mu ( $\mu$ ), delta ( $\delta$ ), and kappa ( $\kappa$ ) subtypes, are extensively distributed within the central and peripheral nervous system and are fundamental in the modulation of pain, mood, and drug addiction. Mu receptors are predominantly located in the central nervous system (CNS) and are primarily responsible for the analgesic effects and physical dependence associated with opioid use. Delta receptors, while also present in the CNS, are found more sparsely and contribute to the modulation of emotional responses and chronic pain. Kappa receptors are distributed both within and outside the CNS and are known to mediate the dysphoric and psychotomimetic effects of certain opioids.

These receptors are activated by endogenous opioids such as endorphins, Leu- and Met-enkephalins, and dynorphins, as well as by exogenous opioids like morphine, heroin, and synthetic opioids. Kappa and delta receptors are of particular interest in drug development due to their potential to produce analgesic effects without the high risk of addiction and respiratory depression typically associated with mu receptor activation, which often leads to severe side effects and high potential for abuse [7].

### **2.2.2 Cannabinoid receptors**

The G-protein coupled cannabinoid (CB) receptors, especially CB1 and CB2, are distributed throughout the nervous system and play a crucial role in pain modulation, inflammation, and immune responses [8]. CB1 receptors are primarily located in the central nervous system (CNS), whereas CB2 receptors are predominantly distributed outside the CNS. These receptors are triggered by both endocannabinoids (2-arachidonylglycerol (*2-AG*) and anandamide) and exogenous cannabinoids (THC ( $\Delta^9$ -tetrahydrocannabinol) and CBD (Cannabidiol) originating from *Cannabis Sativa* plant). CB2 receptors are of particular interest for drug development since they don't elicit the euphoria linked with cannabis, unlike the stimulation of CB1 receptors, which often leads to undesired side effects such as their potential for abuse [9]. Furthermore, various studies have illustrated the involvement of CB2 receptors in the regulation of inflammatory nociception [9].

### **2.2.3 Glutamatergic receptors**

Recently, researchers have discovered the intricate involvement of glutamatergic pathways in nociceptive processes. Glutamatergic receptors include two types of receptors. On one hand the ionotropic glutamate receptors (iGluRs), which are ligand-gated ion channels that facilitate rapid excitatory neurotransmission, and on the other hand, metabotropic glutamate receptors (mGluRs), which are G protein-coupled receptors that regulate synaptic transmission. Both receptors are activated by glutamate neurotransmitter [10]. For instance, one commonly implicated subtype of iGluRs in chronic pain transmission is the NMDA (N-Methyl-D-Aspartate) receptor. Its interaction with glutamate, the primary excitatory neurotransmitter, leads to the opening of the ion channel pore, a process referred to as gating. In this context, NMDA receptor antagonists like ketamine are currently explored for their potential in treating persistent pain poorly managed by opioids.

### **2.2.4 Gamma-aminobutyric acid (GABA) receptors**

For their part, gamma-aminobutyric acid (GABA) receptors, specifically the GABA-A and GABA-B receptor subtypes, are integral components of the pain modulation system. These receptors are activated by GABA, the primary inhibitory neurotransmitter in the central nervous system. Activation of GABA-A receptors results in hyperpolarization of neurons, reducing their excitability and dampening the transmission of pain signals [11]. GABA-B receptors, on the other hand, are G-protein coupled receptors that can also

modulate pain by inhibiting neurotransmitter release [12]. Thus, targeting GABA receptors pharmacologically holds potential for pain management strategies.

### **2.2.5 P2X7 receptors**

P2X7, one of the unique subtypes of the P2X receptor family, are non-selective cation channel which are involved in the perception of pain and inflammation. These ionotropic receptors are activated by high concentrations of extracellular adenosine triphosphate (ATP) that enable the opening of the ion channels (sodium ion, calcium ion influx and potassium ion outflow) on the cell membrane (leading to depolarization), activate multiple intracellular signaling, release multiple inflammatory cytokines, and induce pain [13] [14]. Hence, researchers are exploring P2X7 receptor antagonists as a potential therapeutic avenue in pain management. By blocking P2X7 receptors, it may be possible to mitigate the hyperexcitability of sensory neurons and reduce the inflammatory response associated with pain. Recently, Asahi Kasei Pharma and RaQualia Pharma have signed a licensing agreement for a P2X7 receptor antagonist drug candidate, the AKP-2349454/RQ-00466479, as a new therapeutic agent for neuropathic pain treatment [15].

### **2.2.6 Transient Receptor Potential Vanilloid 1 (TRPV1)**

Transient receptor potential vanilloid subtype 1 (TRPV1), the first member of TRPV family, is a non-selective ion channel (mainly permeable to calcium) and a polymodal receptor for various stimuli. It plays pivotal roles in the detection and modulation of pain, temperature sensation, and inflammation [16]. It is extensively expressed in sensory nerve fibers of peripheral nervous system (PNS) and non-neuronal cells such as certain vascular endothelial cells and smooth muscle cells. This receptor is activated by a variety of physical and chemical factors and inflammatory mediators, such as mechanical stimulation, high temperatures, acidic conditions, capsaicin (the pungent compound found in hot chili peppers), substance P, etc... leading to burning pain or itch [17][18].

Unlike some other pain-related receptors, TRPV1's stimulation does not typically produce euphoric effects but can lead to a burning pain sensation, which limits its potential for abuse.

### **2.2.7 Serotonin and noradrenaline transporters**

The serotonin (SERT) and noradrenaline (norepinephrine) (NAT) transporters represent another interesting target for addressing chronic or neuropathic pain frequently linked to depression. These transporters are tasked with the reuptake of serotonin and noradrenaline neurotransmitters respectively, from the synaptic cleft into the presynaptic nerve terminal [19]. Recent research in pain treatment has focused on the use of dual serotonin noradrenaline reuptake inhibitor antidepressants (SNRIs), with particular attention given to Duloxetine (Cymbalta®) Venlafaxine (Effexor®) and Milnacipran (SAVELLA®).

### **2.2.8 Cyclooxygenase (COX) enzymes**

Other targets for pain relief include the Cyclooxygenase (COX) enzyme, which is responsible for the biosynthesis of prostanoids that contribute to pain, fever, and inflammation. COX exists mainly in two isoforms, COX-1 and COX-2. COX-1 triggers the initial prostanoid response to inflammatory stimuli, whereas COX-2 becomes the predominant contributor to prostanoid biosynthesis as inflammation progresses. Given that an inflammatory response is often associated with pain episode, anti-inflammatory drugs such as NSAIDs (non-steroidal anti-inflammatory drugs) are usually used to relieve pain by inhibiting the **COX-1** (Cyclooxygenase-1) and **COX-2** (Cyclooxygenase-2) **enzymes** [20]. It is important to note that the therapeutic anti-inflammatory action is assigned to COX 2 inhibition while COX-1 inhibition leads to undesirable side effects [21]. This motivates researchers to explore NSAIDs that specifically inhibit COX-2. Celecoxib (marketed as Celebrex®) stands as the sole COX-2 inhibitor accessible in the United States.

## **2.2.9 Acid-Sensing Ion Channel (ASIC)**

Acid sensing ion channels (ASICs) are proton-gated cation channels expressed in both central and peripheral nervous systems. They represent essential acid sensors involved in modulating neural activity in the central nervous system and in detecting tissue acidosis associated with pain in the peripheral system [22]. ASICs are particularly reactive to changes in extracellular hydrogen ion concentrations, enabling them to respond to acidic environments, which commonly occur during ischemic strokes or inflammation. During inflammatory pain episodes, ASIC3 subtype is particularly expressed and activated at the peripheral level by several pain mediators present in the “inflammatory soup” that sensitize nociceptors. As for ASIC1, the most studied subtype, it is mainly found in spinal cord neurons and is involved in the processing of noxious stimuli and in central sensitization especially in conditions of tissue acidosis following injury or disease [23]. ASICs are also sensitive to various toxins and drugs that modulate their activity. This specificity makes ASICs an attractive target for pharmaceutical interventions aimed at treating pain and preventing damage from strokes without the euphoric or addictive effects associated with opioid receptors [22].

## **2.2.10 Voltage-gated sodium channels (VGSC or NaV channels)**

Finally, voltage-gated sodium channels (VGSC), also known as NaV channels, represent an essential target, as they play a particularly important role in the genesis and propagation of action potentials. Indeed, the blockage of these sodium channels leads to inhibition of sodium influx through cell membranes and thus results in a decrease in excitability of neurons. This phenomenon contributes to reduce the sensation of pain. Thus, inhibition of sodium channels represents a fundamental strategy in pain management, aiming at disrupting the transmission of nociceptive signals.

All these *pain* targets can be strategically activated to effectively influence the perception of pain.

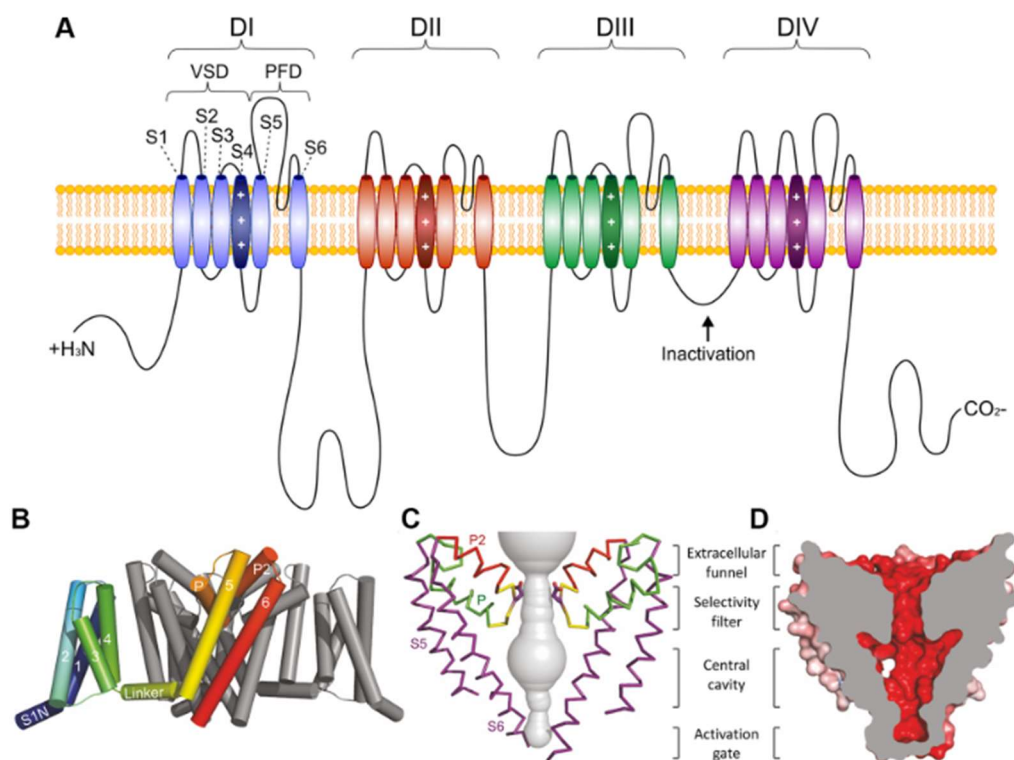
## **3. Focus on voltage-gated sodium channels (VGSC or NaV channels)**

Voltage-gated sodium channels (VGSC or NaV) are pivotal in determining the excitability characteristics of neurons by establishing resting potential and initiating and transmitting action potentials in neurons. Typically, in response to a stimulus, these channels allow the rapid influx of sodium in the nerve cells, a process essential for the depolarizing upstroke. Simultaneously, this phenomenon is accompanied by an efflux of potassium ions, via certain specific potassium ion channels. To generate an action potential, the depolarization must reach a certain transmembrane voltage that activates other voltage-dependent channels such as ionic sodium channels (different from the first), and in particular calcium channels. This results in an influx of large quantities of sodium and calcium ions, in addition to the sodium ions already present. A sufficiently substantial depolarization event, which could result from the convergence of multiple depolarizing inputs occurring simultaneously, can trigger the generation of a nociceptive impulse. The action potential follows an all-or-none principle: it either happens or does not, and when it does occur, it consistently exhibits the same magnitude (independent of the size of the stimulus). However, VGSCs don't stay open for prolonged periods during depolarization. The flow of current through VGSCs can be rapidly terminated, usually within a millisecond, through a process known as fast inactivation.



### 3.1. Structure of voltage-gated sodium channels

NaV channels are transmembrane proteins that consist of a large alpha subunit and one or more smaller beta subunits (Fig. 2). The alpha subunit (transmembrane protein that forms the pore) is the primary functional component of the channel, and contains four homologous domains [24]] (I-IV) that are each composed of six transmembrane segments (S1-S6) [25]. The S5 and S6 segments of each domain form the pore of the channel, while the S4 segment acts as the voltage sensor. Despite their structural and functional similarities, NaV channel subtypes do exhibit some differences in their amino acid sequences and in the structure of their pore-forming regions. These differences are thought to contribute to the unique biophysical properties and gating characteristics of each subtype.



**Figure 2 – Structure of voltage-gated sodium channel.** Voltage-gated sodium channel structure. A: the  $\alpha$ -subunit is a long polypeptide that folds into four homologous domains (DI–DIV), each with six transmembrane spanning regions (S1–S6). S1–S4 comprises the voltage-sensing domain (VSD), with S5 and S6 comprising the pore-forming domain (PFD). S4 (depicted in a darker shade) characteristically contains positively charged arginine and lysine residues. The inactivation gate is found on the intracellular linker between DIII and DIV and is composed of an IFMT (isoleucine, phenylalanine, methionine, threonine) motif. B: structural elements of voltage-gated sodium channels from *Arcobacter butzleri* (Nav Ab). One subunit is highlighted (transmembrane segments S1–S6); the nearest VSD has been removed for clarity. C: architecture of Nav Ab pore module; pore volume is shown in gray. Important structural elements of the pore are colored: S5 (purple), P-helix (green), selectivity filter sequence (yellow), P2-helix (red), and S6 (purple). The pore (P) helices stabilize cations in the central cavity, and a second pore helix (P2) forms an extracellular funnel in Nav Ab. D: electrostatic potential colored from -10 to 10kT (red to blue). Reproduced from [26]

There are nine different types of NaV channels that have been identified in mammals and were typically named according to their alpha subunit, which is the primary pore-forming subunit of the channel. The NaV channel family was divided into two classes, depending on their level of sensitivity to blockade by tetrodotoxin (natural

toxin). Thus, NaV 1.1 to NaV 1.4, NaV 1.6 and NaV 1.7 are considered as tetrodotoxin (TTX)-sensitive channels while NaV 1.5, NaV 1.8 and NaV 1.9 are and TTX-resistant channels. These NaV channel isoforms are each characterized by unique molecular structure, distribution, and physiological function [27].

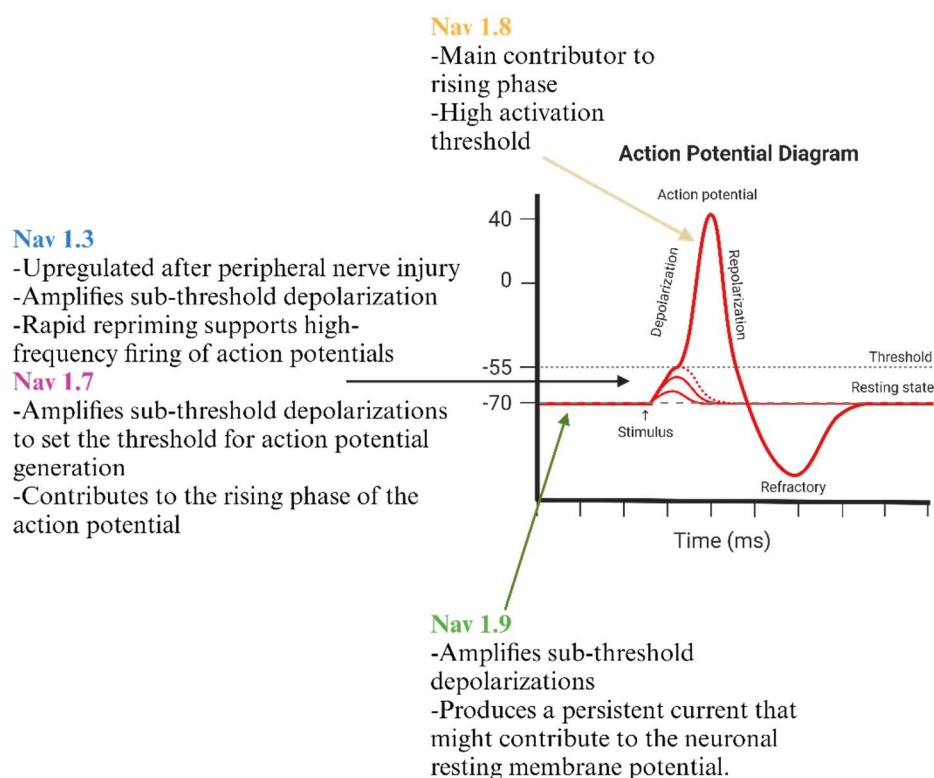
### 3.2. Function of sodium channel isoforms

Each type of NaV channel has a specific role in the nervous system (Tab. 1) and in the depolarization process (Fig. 3), and some of them display very specific functions owing to their localization. NaV 1.1, NaV 1.2, NaV 1.3 and NaV 1.6 channels are primarily expressed in neurons of the central nervous system (CNS) while NaV 1.3 is also found in peripheral nervous system (PNS) [25]. They are all four involved in the initiation and propagation of action potentials. NaV 1.4, for its part, is primarily expressed in skeletal muscle cells and is involved in muscle contraction, while NaV 1.5 is primarily expressed in the heart and plays a crucial role in generating and propagating action potentials in cardiac muscle, thus guaranteeing the normal functioning of the heart. By promoting the rapid influx of sodium ions into the cardiac myocytes, NaV 1.5 channel is responsible for the depolarization phase of the cardiac action potential, and thus triggers the opening of voltage-gated calcium channels and the subsequent release of calcium ions from the sarcoplasmic reticulum. This calcium influx ultimately leads to cardiac muscle contraction. NaV 1.6 is the main NaV channel at nodes of Ranvier and plays important functions in neuronal excitability in both CNS and PNS. NaV 1.7, NaV 1.8 and NaV 1.9 channels are selectively expressed in PNS neurons, especially at the level of sensory neurons [24]. Moreover, investigations involving transgenic/knockout models and studies on mRNA/protein expression in rodents have indicated the significance of NaV 1.8, NaV 1.9, and NaV 1.3 in pain sensation, demonstrating their involvement in nociception. [28] NaV 1.7 is also highly expressed in nociceptive neurons and plays a critical role in the transmission of pain signals [24]. It is encoded by the SCN9A gene and is composed of four homologous domains (DI-DIV), each containing six transmembrane segments (S1-S6). NaV 1.7 channels have unique properties that make them important for nociception. They initiate activity at more negative voltage levels compared to other sodium channels, making them responsive to subthreshold depolarizations and capable of amplifying small signals. They also exhibit slow inactivation kinetics, which enables them to sustain depolarizations and generate longer-lasting action potentials than other sodium channels. NaV 1.7 channels have been associated with several pain-related conditions, including inherited erythromelalgia, paroxysmal extreme pain disorder, and small fiber neuropathy. It was demonstrated that loss-of-function mutations in the SCN9A gene can lead to congenital insensitivity to pain, while gain-of-function mutations can result in hyperexcitability and increased pain sensitivity. Because it is not found to any great extent in vital non-neuronal tissue such as heart or skeletal muscle, NaV 1.7 represents a particularly attractive target for therapeutic purposes. Although it is found in pancreatic alpha and beta cells, it may be inactivated at their normal resting potential (Fig. 3).

**Table 1 - Human voltage-gated sodium (NaV) channels  $\alpha$  subtypes and their function**

NaV isoform	Tissue expression	Implication in pain types	Other therapeutic relevance
NaV1.1	CNS, PNS		Epilepsy
NaV1.2	CNS, embryonic PNS		Epilepsy, autism
NaV1.3	CNS, embryonic PNS	Neuropathic, inflammatory	Epilepsy
NaV1.4	Skeletal muscle		Myotonia
NaV1.5	Cardiac muscle		Cardiac rhythm disorders
NaV1.6	CNS, PNS, glia		Ataxia, motor neuron disease
NaV1.7	PNS (sensory)	Neuropathic, inflammatory, hereditary	
NaV1.8	PNS (sensory)	Neuropathic, inflammatory	

NaV1.9	PNS (sensory)	Inflammatory
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**Figure 3 - Contribution of voltage-gated sodium channels.** NaV channel isoforms differ by their location but also by their function in the generation of the action potential. The role of each isoform is dictated by its kinetics and its activation threshold. The NaV 1.9 isoform exhibits a low activation threshold and slow kinetics, thus contributing to maintaining the neuronal resting membrane potential. NaV 1.3 and 1.7 isoforms exhibit a low activation threshold and fast kinetics, which elucidate their involvement in sub-threshold depolarization and the onset of the action potential's rising phase. Conversely, the NaV 1.8 isoform is characterized by low kinetics and a high activation threshold, defining its contribution to the late rising phase. Immediately after the peak of the action potential – with the membrane potential becoming positive due to Na<sup>+</sup> ions entering the cell—cell repolarization occurs, characterized by a massive efflux of K<sup>+</sup> ions and the inactivation of NaV channels. The final efficacy of a NaV blocker will thus depend on the specific affinity of the drug with each of these isoforms involved in nociception. However, as NaV isoforms share a fair degree of similarity regarding their structure, NaV blockers may lack specificity and block several NaV channels, such as cardiac NaV 1.5 isoform, especially after systemic administration. While some NaV blockers explored in pain alleviation are also used as antiarrhythmic drugs (e.g. lidocaine, class Ib in Vaughan-Williams classification), NaV blockers with higher specificity to NaV 1.3, 1.7, 1.8 or 1.9 isoforms should be privileged to lower side effects. Achieving such specificity requires designing new compounds or enhancing their distribution with the aid of nanomedicine.

Overall, the diversity of functional roles of sodium channel subtypes sodium channels has created the opportunity for high-throughput screening campaigns focused on identifying inhibitors selectively targeting specific subtypes. Over the past decades, numerous research studies have explored the involvement of various ion channels in nociception, hyperexcitability, and heightened sensitivity to pain.

## 4. NaV channel blockers in nociception

The majority of NaV blockers employed in pain management, including local anesthetics (e.g., bupivacaine, tetracaine, lidocaine, ...), class I antiarrhythmics (e.g., mexiletine, lidocaine), antiepileptics (e.g., carbamazepine, lamotrigine, phenytoin), and antidepressants (e.g., mostly tricyclic antidepressants such as amitriptyline, desipramine), generally lack selectivity toward the nine  $\alpha$  subunit subtypes (NaV 1.1 to 1.9). These medications are recognized for their limited therapeutic range and numerous severe side effects resulting from their impact on NaV subtypes in the central nervous system and the heart. These adverse effects may include dizziness, sedation, convulsions, and cardiotoxicity. To overcome this issue, the current approach consists in the discovery of subtype-specific inhibitors that selectively target sodium channel subtypes. Particularly, the voltage-gated sodium (NaV) channel NaV 1.7 has been identified as a potential novel analgesic target due to its involvement in human pain syndromes.

In this paragraph we have decided to present analgesics targeting NaV sodium channels in two categories: “Non-selective NaV inhibitors: marketed analgesics” and “Selective NaV 1.7 inhibitors: analgesic drug candidates”. The first category includes local analgesics, class I antiarrhythmics, antiepileptics, and antidepressants, while the second will present analgesics targeting more specifically NaV 1.7 isoform of these sodium channels. This second category includes venom-derived peptides and toxins, small synthetic molecules undergoing clinical trials, and monoclonal antibodies (Tab. 2).

**Table 2 – Current authorized and experimental NaV channel blockers targeting the different NaV channel isotypes involved in pain.**

Drugs	Mechanism of action	Current status	References
<b>Targeting NaV 1.3</b>			
miR-384-5p miR-30b miR-96	Downregulate SCN3A gene for NaV 1.3	Preclinical	[29],[30], [31]
Diphenylmethyl amide adducts of an aryl sulphonamide series	Channel blocker	Preclinical	[32]
<b>Targeting NaV 1.7</b>			
Lacosamide (antiepileptic)	Inactivated state channel blocker <sup>a</sup>	Clinical trials	[33]
Mepyramine (antihistamine)	Non-selective inactivated state channel blocker (also blocks 1.3, 1.8 and 1.9 isoforms)	Clinical use (topical application in pediatric patients suffering from erythromelalgia)	[34] [35]
PF-05089771 <sup>b</sup>	Inactivated state channel blocker	Clinical trials	[12]
CNV1014802 <sup>b</sup> (vixotrigine or raxatrigine)	Inactivated state channel blocker	Clinical trials (phase 3)	[36],[37]
AM-0466 <sup>b</sup>	NaV 1.7-selective inhibitor <sup>d</sup>	Preclinical	[38]

AZD3161 <sup>b</sup>	NaV 1.7-selective inhibitor <sup>d</sup>	Clinical trials (phase 1)	[39]
Triazine 15 <sup>b</sup>	NaV 1.7 inhibitor with modest NaV 1.5 selectivity	Clinical trials (Phase 2)	[40]
Benzazepinone Pyrrolo-benzo-1,4-diazine	Both compounds displayed comparable NaV 1.7 inhibitory activity, whereas PBD9 exhibited improved selectivity over NaV 1.5	Preclinical	[41]
GX-201 <sup>b</sup> GX-585 <sup>b</sup>	NaV 1.7-selective inhibitors <sup>d</sup>	Preclinical	[42]
Natural and chemically modified toxins such as JNJ63955918 : - JzTx-V - PnTx1 - GpTx-1 - ProTx-11 - μ-conotoxin KIIIA - μ-TRTX-Tp1a - Tap1a - μTap1a-OPT1	Most of these toxins are gating modifiers	Preclinical	[43],[44],[45]
“LATER” (long-lasting analgesia via targeted in vivo epigenetic repression) technology	CRISPR epigenetic technology to suppress NaV1.7 expression	Preclinical	[46],[47]
Carbamazepine (antiepileptic)	Channel block of NaV 1.7 and NaV 1.1	FDA-approved (first line for management of trigeminal neuralgia)	[48]
GDC-0276 <sup>b</sup> GDC-0310 <sup>b</sup>	Selective NaV 1.7 blockers <sup>d</sup>	Clinical trials (phase 1)	[49]
XEN402 <sup>b</sup>	Selective NaV 1.7 blocker <sup>d</sup>	Clinical trials (phase 1)	[50]
<b>Targeting NaV 1.8</b>			
A803467 <sup>b</sup>	Potent NaV 1.8 selective inhibitor <sup>d</sup>	Preclinical	[51]
VX-150 <sup>b</sup>	Highly selective NaV1.8 inhibitor (prodrug) <sup>e</sup> for surgical pain	Clinical trials (phase 2b completed but no published results)	[52]
PF-04531083 <sup>b</sup>	NaV 1.8 blocker <sup>d</sup>	Clinical trials (phase 2)	[50]
PF-01247324 <sup>b</sup>	NaV 1.8 blocker <sup>d</sup>	Preclinical	[53]
Ambroxol	Selective NaV 1.8 and NaV 1.9 channel blocker	Clinical trials (phase 3 completed but no published results)	[54] [55]

(Primarily mucoactive and secretolytic actions but also with anti-inflammatory, antioxidant, and local anesthetic properties)			
<b>Non selective towards NaV isoforms</b>			
Cyclic peptides derived from the structures of natural product channel blockers $\mu$ -conotoxin KIIIA and (PnTx1) Phoneutria nigriventer toxin 1	Channel blockers <sup>d</sup>	Preclinical	[27]
Lidocaine patch (local anesthetics)	Inactivated state blocker	Marketed	[56]
Cationic local anesthetics combined with TRPV1 activators	Local anesthetic effect achieved selectively in TRPV1 expressing neurons by anesthetic permeation of TRPV1 channels	Preclinical	[57],[58],[59]

(a) An "inactivated state blocker" is a compound that specifically binds to voltage-gated sodium channels when they are in their inactivated state. By doing so, it prevents these channels from returning to their activated state and, consequently, inhibits the passage of sodium ions through the cell membrane. This disruption has the effect of impairing the generation and propagation of electrical signals, which can be utilized for therapeutic purposes. (b) refers to experimental drugs designed to block selectively the corresponding NaV channel subtype. (c) A "pore blocker prodrug molecule" refers to a specific type of compound designed to act as a pore blocker when it is metabolized or activated within the body. (d) Undefined or unknown state of activity of NaV channel during inhibition.

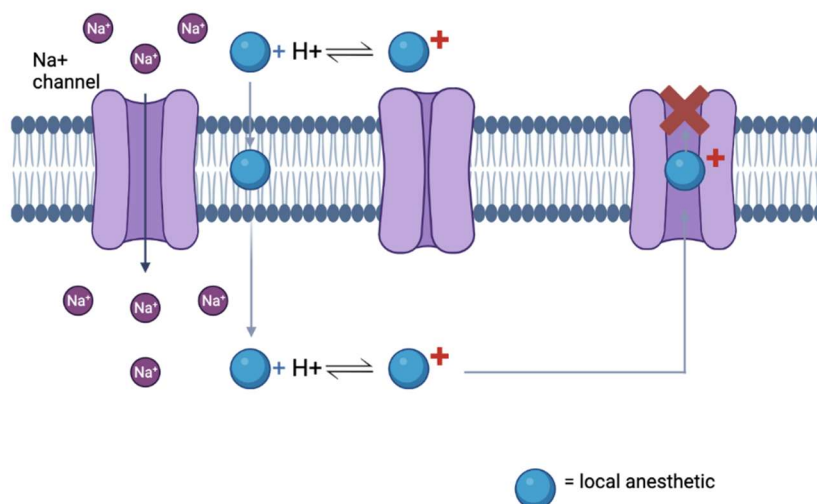
## 4.1. Non-selective NaV inhibitors : marketed analgesics

### 4.1.1. Local anesthetics

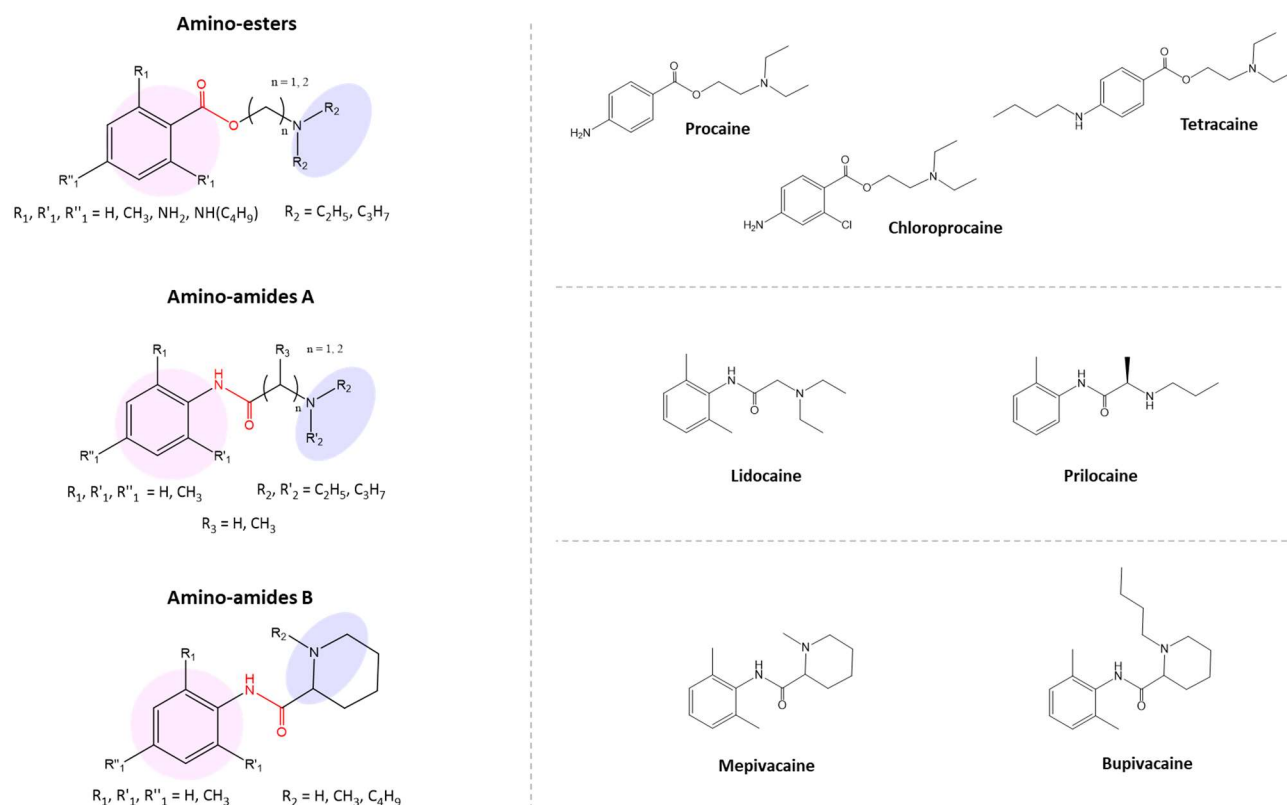
Local anesthetics are commonly used in interventional pain management procedures, such as tissue infiltration, nerve blocks, and neuraxial anesthesia (anesthesia around the spinal cord) [32]. Local infiltration consists in anesthetizing nerve endings in a specific area of tissue by injecting local anesthetics in proximity. In contrast, with peripheral nerve blocks, the target is the nerve axons and the injection may occur at a site that is quite a distance from the actual surgical site. The selection of the most suitable local anesthetic and its concentration relies on the specific procedure at hand, due to variations in systemic absorption and potential toxicity. Infiltration anesthesia is typically employed for minor surgical and dental procedures, while nerve block anesthesia is utilized in a wide range of applications, including surgical, dental, diagnostic procedures, and pain management.

Most local anesthetic agents share a common chemical structure consisting of a lipophilic aromatic ring and a hydrophilic ionizable amine separated by an ester (benzocaine, procaine, tetracaine, chlorprocaine, prilocaine) or amide bond (lidocaine, etidocaine, mepivacaine, ropivacaine, dibucaine) (Fig.5). This ionizable property allows these local anesthetics to exist in both charged and uncharged states, contributing to the mechanism of anesthesia. It has been proposed that while the uncharged form is the main species for transporting the anesthetic across the cell membrane, the protonated form binds to a specific site on the channel,

triggering the anesthetic effect (Fig. 4) [60]. Indeed, while the uncharged form primarily facilitates the anesthetic's transport across the cell membrane, the protonated form binds to and inhibits a specific site of the sodium channel at the level of nerve endings and along the axon, leading to a transient loss of sensation in a limited area [61].



**Figure 4 - Mechanism of action of local anesthetic into NaV channel.** Local anesthetics, which are weak bases, predominantly exist in their ionized form at physiological extracellular pH (pH = 7.4). Only a minor neutral fraction (non-ionized fraction) is able to cross the neuronal membrane. Once inside the axoplasm, the proportion of the local anesthetic in its ionized form increases (pH = 7.2). It is in this ionized state that the local anesthetic blocks the pore of the sodium channel, rendering it non-conductive. This action prevents nociceptive stimuli generated by surgical procedures from initiating action potentials. Nociceptive stimuli produced during surgical interventions are unable to depolarize the axonal membrane, thereby preventing the transduction of the nociceptive impulse.



**Figure 5 – Generic structure of amino-esters and amino-amide local anesthetics.** Local anesthetic agents are categorized either in aminoesters, based on their ester linkage (procaine, chlorprocaine and tetracaine) or in amino-amides, based on their amide linkage (lidocaine, prilocaine, mepivacaine, bupivacaine). Amino-amides A and B are distinguished by the hydrophilic amino group, which is either non-cyclic (A) or cyclic (B)

Amino-esters were found to be more potent than amino-amides, because of the more difficult fitting of the latter in a specific site at the sodium channel due to steric hindrance. However, amino-esters are significantly more likely to induce authentic allergic reactions when compared to amino-amides, owing to their distinct metabolic pathways. As a matter of fact, amino-amides are subject to hepatic metabolism, while amino-esters undergo plasma metabolism. Therefore, the amino-esters metabolism results in the emergence of para-aminobenzoic acid intermediate metabolite, a well-known allergen [62].

Lidocaine known by trade names such as Xylocaine® or Lidoderm®, synthesized in 1944, stands out for its amide linkage, a characteristic shared by the majority of subsequent local anesthetics. Renowned for its adaptability in administration [63]—topical or systemic—and efficacy in a wide range of pain scenarios, from acute to chronic, lidocaine remains a cornerstone in pain management. Its ongoing research, clinical application, and relatively favorable safety profile continually reinforce its significance. Bupivacaine, for its part, known by trade names such as Marcaine® or Sensorcaine®, has long been the preferred choice among long-acting local anesthetics. However, a concerning trend has emerged with an alarming increase in fatalities attributed to accidental intravascular injections of this drug [64] [65]. This alarming trend has prompted the exploration of safer alternatives [66], leading to the emergence of levobupivacaine and ropivacaine as promising candidates. These newer anesthetics are composed exclusively of their pur levo-rotary enantiomer, thus resulting in significantly reduced toxicity profiles within both the central nervous and cardiovascular systems when compared to bupivacaine. This reduction in toxicity can largely be attributed to their distinct three-dimensional molecular structures. Thus, levobupivacaine, typically employed for locoregional conduction anesthesia, exhibits a relatively short duration of action, typically lasting about one and a half hours. It possesses potency comparable to that of lidocaine and is available in injectable forms. Ropivacaine,



commonly available under trade names such as Naropin® and Naropin Polyamp®, finds extensive use across various medical settings, and is commonly used in scenarios such as epidurals for labor and delivery, peripheral nerve blocks for surgical anesthesia, and post-operative pain management [67]. Its popularity arises from its ability to provide effective pain relief while maintaining a favorable safety profile, positioning it as a valuable asset in modern medical practice. Finally, tetracaine stands as another ophthalmologic anesthetic. Recognized for its extended local anesthetic duration, lasting three to four hours, it boasts significantly greater potency than lidocaine, being four times stronger, and is available in the form of an eye drop solution.

Even though they are widely used, the current available local anesthetics often have a relatively short duration of action, primarily due to local pharmacokinetic factors, thus limiting their effectiveness in managing postoperative pain or other long-lasting pain. Indeed, with a relatively small size, local anesthetics molecules easily cross vascular endothelium and are then rapidly removed from the injection site. On the other side, the increase in drug concentration is not a viable solution due to the risk of serious systemic cardiovascular and neurological side effects, originating from the blockage of other NaV isoforms [62][68][69].

### **4.1.2. Antiepileptic drugs**

Lacosamide is an FDA-approved antiepileptic medication. By acting selectively on peripheral NaV 1.7 channels, it is regarded as a promising candidate for neuropathic pain treatment. While it underwent clinical trials for the treatment of diabetic neuropathic pain, it did not receive approval for this specific use [33].

Carbamazepine and oxcarbazepine antiepileptic drugs (AED) are currently used as first-line treatments for trigeminal neuralgia, a notable facial pain disorder resulting in periodic severe pain (carbamazepine being the only drug approved by the United States Food and Drug Administration (FDA) for the management of trigeminal neuralgia). However, these drugs are associated with poor tolerability [70] [71]. The effectiveness of carbamazepine (which is recognized for its ability to block NaV 1.7) and oxcarbazepine provides additional confirmation that NaV 1.7 is a viable target in the context of trigeminal neuralgia.

### **4.1.3. Antidepressants**

Tricyclic antidepressants (TCAs) have been found to inhibit various voltage-gated sodium channels (NaV), including NaV 1.7, which is significant given their role in treating neuropathic pain. A study investigated the effects of antidepressants on sodium currents in *Xenopus* oocytes expressing various NaV channels, such as NaV 1.2, NaV 1.3, NaV 1.6, NaV 1.7, and NaV 1.8, along with a  $\beta 1$  subunit. It was observed that all antidepressants tested inhibited sodium currents in an inactivated state induced by all five  $\alpha$  subunits with  $\beta 1$ . The inhibitory effects were more potent for NaV 1.3, NaV 1.7, and NaV 1.8, which are mainly distributed in dorsal root ganglia, compared to NaV 1.2 and NaV 1.6, predominantly found in the central nervous system. Amitriptyline, specifically, had the most potent inhibitory effect on NaV 1.7 with  $\beta 1$ , with a half-maximal inhibitory concentration (IC<sub>50</sub>) of 4.6  $\mu\text{mol}\cdot\text{L}^{-1}$  [72].

## **4.2. Isoform selective antagonists**

### **4.2.1. Selective NaV 1.7 inhibitors : analgesic drug candidates**

Over the subsequent 20 years, academia and pharmaceutical companies have dedicated their efforts to develop specific inhibitors targeting NaV 1.7 channels [43]. The aim was to create broadly effective analgesics without the addiction and tolerance issues associated with opioids. Despite the fact that numerous highly potent and selective NaV 1.7 channel inhibitors have been discovered (venom-derived peptides, low molecular weight compounds, monoclonal antibodies), there are few examples where their efficacy has been demonstrated in preclinical pain models or in human clinical trials [43]. In the clinical setting, NaV channel inhibitors commonly employed are typically pore-blockers exhibiting a state-dependent mechanism of action, with greater affinity for the open or inactivated state as opposed to the resting state [73].

#### 4.2.1.1. Venoms and toxins

Many venomous animals such as cone snails, spiders, scorpions, anemones, and snakes produce venoms that are complex mixtures of toxins that, among other functions, modulate the activity of NaV channels. These include both polar small molecule toxins and a variety of peptide-based venom toxins.

The non-peptide toxins have played a substantial role in advancing our comprehension of sodium channel physiology, structure, and pharmacology. Most notably, tetrodotoxin (TTX), a cyclic guanidine-containing alkaloid, present in the gonads, liver, intestines, and skin of the puffer fish, was the most extensively studied neurotoxin and is the reference ligand for NaV channels. Indeed, as was already mentioned above (section 3.1), it has played a crucial role in defining the allosteric model of the NaV channel and thus allowed the characterization of the nine  $\alpha$ -subunit isoforms (NaV 1.1 to NaV 1.9) according to their level of sensitivity to it (TTX-sensitive and TTX-resistant subtypes) [74] [75].

Beyond serving as a pharmacological tool, TTX is now acknowledged as a therapeutic agent. Its ongoing development is anticipated to facilitate its application in specific pathologies related to NaV channels, particularly in the field of pain management. Indeed, TTX is a selective site 1 sodium channel blocker and presents a particularly good selectivity for NaV 1.7 channel which is upregulated in chronic pain states [76]. TTX does not cross the blood brain barrier, or very poorly, thus reducing the probability of central system depression and any adverse effects. Hence, TTX has been advanced into clinical trials by Wex Pharmaceuticals and is currently progressing in Phase III trials for cancer and chemotherapy-related pain [77] [78]. Indeed, it was shown that subcutaneous TTX treatment (30  $\mu$ g twice a day) led to sustained cancer pain alleviation during successive treatment cycles lasting over a year, without any signs of tolerance, and with a manageable side effect profile [78],[79],[80]. Nevertheless, these investigations were deemed to lack statistical power because of the limited patient enrollment. The TTX has been also tested in a Phase II trial for chemotherapy-induced neuropathic pain. The results showed that TTX 30  $\mu$ g administered subcutaneously twice a day for 4 days every 3 weeks led to 30% reduction in pain severity and exhibited a long duration analgesic effect among certain patients. Here again, TTX presented good tolerability and safety in a population with limited therapeutic options [81]. In addition, a recent study demonstrated that the oral administration of TTX enhanced its safety profile while maintaining its analgesic effects in a rat model of post-herpetic neuralgia [82]. Elsewhere, toxins also provided extraordinary leads for the development of novel subtype-selective agents with therapeutic potential [83]. For instance, Saxitoxin (STX), a neurotoxic cyclic guanidine-containing alkaloid known as paralytic shellfish poison, has served as the basis for the design of various synthetic derivatives, including ST2530, a highly selective inhibitor of the NaV1.7 isoform. Indeed, ST2530 has proven its effectiveness in alleviating mechanical allodynia in spared nerve injury (SNI) models of neuropathic pain in mice [84]. It is interesting to note that STX and its analogs are practically unexplored in models of neuropathic pain.

In contrast to small molecules, peptides and proteins achieve overall FDA approval at a higher success rate [43]. These large molecules frequently demonstrate increased subtype selectivity, however they have restricted membrane permeability, a poor oral bioavailability, are prone to renal filtration and hepatic metabolism and are potentially immunogenic [85]. Peptides from spider venom stand out as the most potent and selective inhibitors of NaV1.7. They typically range from 3 to 4.5 kDa and bear disulfide bonds that confer to these peptides resistance to proteolytic degradation. Prototoxin II (ProTx-II) and Heteropodatoxin3 (HpTx3, known as inhibiting potassium Kv4.2 channels) are peptidic neurotoxins isolated from spider venom that exhibit potent selectivity for NaV1.7. ProTx-II proved efficient in reducing pain associated to cancer chemotherapy [76] or diabetes [86] after intrathecal administration, while systemic administration of HpTx3 decreased mechanical allodynia in the SNI model in mice [87]. Nevertheless, effectiveness of ProTx-II appears inconsistent when administered intravenously [88]. Pn3a, another tarantula venom peptide, potently inhibited NaV 1.7 channels, however, like for ProTx-II, its *in vivo* analgesic efficacy has been variable depending on animal pain model or the route of administration [89] [90].

Other spider venom peptides such as Hainantoxin-IV (HNTX-IV) and Huwentoxin-IV (HwTx-IV) appear also as potent antagonists of NaV1.7. They have proven to be effective for alleviating acute inflammatory pain and chronic neuropathic pain models in animals after systemic administration [91, 92].

Likewise, a series of compounds obtained after modification of spider toxins exhibited high selectivity for NaV 1.7. One of them, Tap1a derived from the venom of the Venezuelan tarantula *Theraphosa apophysis*, targets multiple voltage-gated sodium and calcium channels, playing a significant role in visceral pain pathways. This was particularly evident in models of irritable bowel syndrome, where Tap1a inhibited visceral mechano-sensing neurons [44,45]. Further development of Tap1a involved the creation of variants like Tap1a-OPT1 and Tap1a-OPT2, which showed increased potency for NaV channels (NaV 1.1, NaV 1.2, NaV 1.3, NaV 1.6, and NaV 1.7). This optimization was achieved through a slowed off-rate mechanism and improved interactions with the voltage-sensor domain II of NaV channels [45].

Ongoing research in this area is exploring structural modifications to Tap1a, with the aim of developing exceptionally potent and effective agents for the treatment of pain. These agents could potentially be used in combination with low-dose opioids, providing a promising strategy for enhancing pain relief. [93].

#### **4.2.1.2. Small fully synthetic molecules undergoing clinical trials**

There is considerable interest in developing inhibitors that target the sodium channel NaV 1.7, which is primarily distributed in the peripheral nervous system. This interest is driven by robust validation from genetic studies regarding their effectiveness in alleviating various types of chronic pain. However, the development of inhibitors for NaV 1.7 faces the challenge of achieving subtype selectivity due to the high sequence homology among NaV isoforms. Indeed, ensuring acceptable selectivity against the cardiac sodium channel NaV 1.5 is crucial to prevent arrhythmic side effects [94] [95]. Vixotrigine (BIIB074), a pyrrolidine carboxamide formerly known as Raxatrigine (CNV-1014802), is a NaV 1.7-selective state-dependent sodium-channel blocker discovered by GlaxoSmithKline (GSK) in 2006 and is currently under phase 3 clinical trial (developed by Convergence Pharmaceuticals) for the treatment of trigeminal neuralgia and presents a very good tolerability profile (150 mg three times per day, orally) [96] [37]. At present, this drug also undergoes Phase 2 study for erythromelalgia and neuropathic pain [97]. Funapide (TV-45070 and formally XEN402) is a spirocyclic oxindole topical selective NaV 1.7 blocker developed by Teva and Xenon that was originally used for the treatment of inherited (primary) erythromelalgia (phase 1 and 2 clinical trial, 400mg twice daily orally). This drug also underwent phase 2 studies (applied topically and twice daily as 4% and 8% w/w ointment) for primary osteoarthritis of the knee and postherpetic neuralgia. The preliminary crossover trial in Phase 2, indicated that topical TV-45070 was safe and well tolerated and indicated potential effectiveness [98]. Following this, phase 2 clinical trials were carried out in a double-blind, placebo-controlled manner and did not find statistically significant improvement in pain. Indeed, variations in clinical response appear to be influenced by individual patient genetics. [98] [99]. According to a statement on Xenon Pharma's official website, the rights for XEN402 were transferred to Flexion Therapeutics in September 2019. Flexion Therapeutics is set to carry on the development under the new name FX301. They are currently working on a novel formulation involving an extended-release thermosensitive hydrogel and have plans to commence clinical trials in 2021 [100]. AZD-3161, a N-chromanilcarboxamide discovered in 2012 by AstraZeneca, has greater than 100-fold selectivity for NaV 1.7 over NaV 1.5 [39]. This molecule displayed very good oral bioavailability and a dose-dependent antinociceptive effect in a formalin rat model of pain. Considering these promising pre-clinical outcomes, there was an expectation that this molecule may be useful in various types of pain, including neuropathic or nociceptive pain. In a Phase 2a study involving chronic osteoarthritis patients experiencing moderate to severe pain, OLP-1002, another selective Nav 1.7 inhibitor developed by OliPass Corporation, exhibited potent analgesic efficacy and prolonged therapeutic duration. The interim findings of this clinical study showed a significant reduction in pain scores for patients receiving OLP-1002 compared to those receiving a placebo, suggesting that a 2 mcg dose of OLP-1002 administered once every two months could be considered an effective therapeutic regimen. The potential of OLP-1002 extends beyond osteoarthritis pain to include a broad range of chronic or refractory pain conditions such as diabetic neuropathic pain, trigeminal neuralgia, chemotherapy-induced pain, fibromyalgia, and cancer pain [101]. However, in a phase-I

clinical study, it has proven ineffective in treating pain sensation and inflammation after intradermal administration in healthy subjects whose forearm skin was irradiated with UV light [102]. AZD3161 was consequently discontinued.

Recently, significant efforts have led to the development of a series of NaV 1.7-selective inhibitors, characterized by either aryl or acyl sulfonamide groups with an emphasis on the latter [102]. PF-05089771 developed by Pfizer, is an aryl sulfonamide compound exhibiting a 1000-fold selectivity for NaV 1.7 (inactivated state) over NaV 1.5 and NaV 1.8 [103] [104]. PF-05089771 showed good tolerance and safety in a phase 1 clinical trial (NCT01529671) concerning patients suffering from osteoarthritis of the knee and is also currently in a phase 2 clinical trial for the treatment of patients with inherited erythromelalgia (single 1600 mg oral dose) [105]. However, PF-05089771 failed in phase 2 trial for the treatment of painful diabetic neuropathy (NCT02215252) (twice daily 150 mg oral dose during 4 weeks) potentially owing to inadequate target coverage and the impractical doses needed for effectiveness [106]. GX-201 and GX-585, two acyl sulfonamides are potent inhibitors of NaV 1.7 and are highly selective against NaV 1.5. These compounds were found to exhibit superior analgesic effectiveness compared to PF-05089771 in mouse models of inflammatory and neuropathic pain. This correlation was associated with their slower dissociation from NaV 1.7 channels, indicating that residency time could be a crucial factor [42]. In addition, it was reported that extended exposure to these inhibitors through repeated dosing increased analgesic efficacy tenfold. Others aryl sulfonamides, namely GDC-0276 and GDC-0310, were developed by Genentech as selective NaV 1.7 blockers. Both were withdrawn following a phase 1 study due to safety concerns and potential off-target effects, likely linked to the compound's elevated lipophilicity [49] [107] [108]. AM-0466, an atropisomeric quinolinone sulfonamide highly NaV 1.7 exhibited in a NaV 1.7-dependent model of histamine-induced pruritus (itch) and also in a capsaicin-induced nociception model of pain [38].

A recent study, unveiled high-resolution structures of NaV 1.7 complexed with three different small molecule inhibitors (XEN907, TCN-1752, and Nav1.7-IN2) and offered comprehensive mechanistic insights into how these three antagonists block and modulate NaV1.7. These findings suggest that future efforts in developing NaV 1.7-selective inhibitors should target the comparatively variable regions of NaV isoforms, such as the voltage-sensing domains [109].

#### **4.2.2 Others selective NaV inhibitors**

A-803467, a furan carboxamide derivative, showed remarkable potency as a selective blocker of NaV 1.8 compared to other NaV isoforms, with up to a 1000-fold higher effectiveness. Intravenous administration of this NaV 1.8 antagonist successfully inhibited both spontaneous and electrically evoked firing in rat DRG neurons and also exhibited a dose-dependent reduction in nociception within spinal nerve ligated rats. A-803467, when administered intraperitoneally, proved most efficient in alleviating pain in models specifically associated with neuropathic and inflammatory pain (spinal nerve ligation, sciatic nerve injury, CFA model of inflammatory pain and capsaicin-induced secondary mechanical allodynia) [51].

## **5. Innovative approaches**

### **5.1. Gene therapy**

More recently, gene therapy has allowed real breakthrough in many therapeutic areas, such as in oncology or to treat some genetic diseases. Even if these approaches have not proved effective yet for pain alleviation in humans, a few noteworthy results regarding NaV blockage deserve to be mentioned.

In gene therapy, several approaches, based on nucleic acids, such as antisense oligodeoxynucleotides (ODNs), small interfering RNAs (siRNAs) or microRNAs (miRNAs) were used to act at the post-transcriptional level by binding complementary target mRNA(s) to selectively stop or decrease the expression of a gene. ODNs function as a single strand while siRNAs and miRNAs are short duplex RNA molecules. The major difference between siRNAs and miRNAs is that the former are highly specific with only one mRNA target, whereas the latter have multiple targets (either on the same mRNA or on different mRNAs) [110]. ODNs and siRNAs became a standard tool for reverse-genetic analysis and thus have been extensively used to elucidate gene functions both in vivo and in vitro. For instance, this approach highlighted the important role of NaV 1.8 channel in the induction and maintenance of persistent hypernociception. In that respect, antisense ODNs targeting specifically NaV 1.8 [111] were intrathecally administered to a rat model of persistent mechanical hyperalgesia induced by intraplantar injections of prostaglandin E2. This inflammatory hypernociception generates up-regulation of mRNA expressing NaV 1.8 in dorsal root ganglia (DRG). Thus, four daily administrations of these antisense ODNs have demonstrated the capability to decrease mRNA expression of NaV 1.8 in DRG. However, the cessation of ODN antisense treatment restored the persistent hypernociception and up-regulation of NaV 1.8 mRNA [112].

MiRNAs, for their part, play pivotal roles in the regulation of numerous biological processes, such as differentiation, development, apoptosis, metabolism and proliferation but have also been linked to several diseases among which viral infections, and the development and progression of cancers [113]. Within the nervous system, miRNAs are emerging as significant regulators of neuronal survival, neurodegeneration, dendritic outgrowth, transcriptional regulation, the regulation of trophic factors. Research also established that they were also associated with several neurological disorders such as Parkinson's disease or pain. Indeed, it was reported that nerve injuries induce changes in miRNA expressions. [114] [115]. Therefore, targeting miRNA expression for pain treatment represents a novel approach for therapeutic intervention. For instance, miR-96 which is present in abundance in the DRG, experiences down-regulation in a spinal nerve ligation (SNL) model of neuropathic pain [116]. These findings suggest that miR-96 might play a crucial role in neuropathic pain in which NaV 1.3 channel is implicated. Using this strategy, Chen *et al.* investigated the potential role of miR-96 in the critical regulation of neuropathic pain behaviors, using a sciatic nerve chronic constriction injury (CCI) rat model. This team not only demonstrated that intrathecal administration of miR-96 Agomir, a chemically modified double-stranded small RNA that mimics the endogenous miR-96, decreased thermal hyperalgesia and mechanical allodynia but also efficiently inhibited NaV 1.3 up-regulation caused by CCI. In addition, they demonstrated that miR-96 specifically inhibited in vitro NaV1.3 expression on embryonic rat dorsal root ganglion (DRG) cultures [31]. Using the same model, that causes neuroinflammation and neuropathic pain, Ye *et al.* reported a significant decrease of miR-384-5p. They also demonstrated in the same model that miR-384-5p Agomir was able to remarkably inhibit mechanical allodynia and thermal hyperalgesia and suppress SCN3A mRNA expression (sodium channel NaV 1.3 expression) [30]. In a spinal nerve ligation (SNL) model (another model of neuropathic pain), using the same approach, Su *et al.* highlighted the role of miR-30b in the regulation of NaV 1.3 expression [29]. All these findings highlight the promising potential of miRNAs in targeting the sodium channels for the treatment of chronic pain.

Although the application of gene therapy based on nucleic acids has shown early promise in pain treatment, a number of issues remain to be overcome. Indeed, the delivery of nucleic acids is often challenging due to potential degradation by serum nucleases, clearance by the kidney, or inappropriate biodistribution. The fact that ODNs binds to serum proteins slow their excretion by the kidney, whereas in the case of siRNAs, all aromatic nucleobases are positioned internally, leaving the duplex's outer surface dominated by highly hydrated phosphates that hinder duplex interactions with cell surfaces thus fostering their metabolism and excretion. In the context of employing miRNA-based therapeutics, ongoing studies are exploring challenges such as insufficient cellular uptake, reduced bioavailability, off-target effects, and potential long-term safety issues in human subjects. Thus, addressing these concerns may involve developing innovative carrier systems, utilizing nanomedicine approach, specifically designed to protect nucleic acids from rapid degradation and to deliver them exclusively to target tissues or cells [117].

## **5.2. SABER Technology**

There is considerable interest in extending the duration of local anesthetic effects for postoperative pain management, to reduce reliance on opioid analgesic medications. In this context, SABER®-bupivacaine (trade name Posimir), a depot formulation of bupivacaine base (12%) developed by DURECT Corporation in collaboration with Sandoz AG, emerges as a promising option. This formulation comprises a semi-viscous solution of a biocompatible and fully bioresorbable matrix constituted of sucrose acetate isobutyrate (SAIB) that contains the bupivacaine and also benzyl alcohol that reduces viscosity and keeps the bupivacaine dissolved. SABER-bupivacaine was designed to provide continuous delivery of bupivacaine during several days when placed in surgical wounds. This innovative technology undergoes clinical studies in orthopedic surgery and soft tissue surgery. Concretely, SABER-Bupivacaine is instilled directly into surgical incisions (open and laparoscopic surgery), or into various exposed tissue layers of the inguinal canal (inguinal hernia repair) and is not intended for intravascular or intra-articular use and should not be employed for regional or neuraxial nerve blocks [98]. In the context of postoperative pain alleviation following arthroscopic subacromial decompression, a randomized, double-blind, placebo-controlled trial was performed with 78 subjects which received SABER-bupivacaine versus SABER-placebo under direct arthroscopic visualization. SABER-bupivacaine was administered as a single 5-mL injection directly into the surgical incision using a needle-free syringe or through an endoscopic port incision into a designated anatomical space (such as the subacromial space)[115]. This study investigated key endpoints such as pain intensity on 90° shoulder flexion and cumulative morphine intake over 72 hours post-surgery. It was demonstrated that SABER-bupivacaine significantly diminished pain during movement compared to placebo and effectively reduced opioid consumption during the critical recovery period [115]. The safety profile remained favorable, and the sustained release mechanism of SABER-bupivacaine supports prolonged analgesic effects without the need for external catheters or pumps. All these observations underscore the potential of SABER-bupivacaine as a valuable and effective component in multimodal pain management strategies for patients undergoing arthroscopic subacromial decompression[113]-

## **5.3. DepoFoam Technology**

DepoFoam technology is a lipid-based depot formulation composed of triglycerides, cholesterol, and phospholipids allowing sustained release of the drugs. DepoFoam consists of micron-sized multivesicular liposomes (MVLs) with an outermost membrane composed of a lipid bilayer, while the inner space is subdivided into numerous non-concentric polyhedral aqueous compartments separated by bilayer septa. It has been proposed that the non-concentric arrangement of lipid layers provides enhanced stability and prolongs the duration of drug release [118] [119]. The sustained release is due to the fact that only ruptures in the outermost membranes of an MVL lead to the release of encapsulated drug into the external medium. Additionally, drug release from the internal vesicles causes the drug to redistribute within the particle, but without being released from the particle itself.

In 2017, in a double-blinded randomized clinical study, Vandepitte *et al.* demonstrated the ability of Exparel® (based on DepoFoam technology) to prolong the postoperative analgesia of standard bupivacaine through interscalene brachial plexus block in patients undergoing major shoulder surgery [120]. Exparel® has gained prominence as the sole FDA-approved extended-release local anesthetic for single infiltration at surgical sites or specific nerve blocks. Thus, 52 adult patients were randomized to receive either 15 mL of 0.25% standard bupivacaine (37.5 mg) or 5 mL of 0.25% bupivacaine (12.5 mg) immediately followed by 10 mL of bupivacaine liposome 4 mg, to establish a rapid onset of peripheral nerve block prior to the release of the active drug from liposomes (the release of bupivacaine from liposomes starts 16 h post-administration and lasts till 72 h). Hence, while primarily indicated for postsurgical analgesia in bunionectomy, hemorrhoidectomy, and interscalene brachial plexus nerve block, Exparel® has found success off-label in various other procedures [121,122]. However, cost considerations may limit its adoption in smaller healthcare facilities compared to more economical standard bupivacaine. Two formulations of Exparel are available, with associated side effects

including nausea, constipation, and pyrexia [123]. Notably, liposomal bupivacaine's inability to cross the blood–brain barrier reduces the risk of central nervous system toxicity. Contraindications include pregnancy, patients under 18, and a history of allergic reactions. Despite variability in recent randomized control trial results, Exparel® has demonstrated efficacy in postoperative pain management, with clinicians urged to carefully choose appropriate techniques and monitor patient outcomes when incorporating Exparel into analgesic regimens. The FDA's approval of liposomal bupivacaine for interscalene blocks in shoulder surgeries in April 2018 further supports its clinical use.

## 5.4. Nanomedicine

By harnessing the unique properties of nanoscale materials and engineered carriers, nanomedicine offers a multitude of promising avenues for enhancing pain relief while minimizing the drawbacks associated with conventional therapies. The main area of investigation in nanoformulations targeting NaV sodium channels has been centered particularly on local anesthetics and to a lesser extent on TTX (Tab. 3) [124] [125].

**Table 3 - Comparative Analysis of Nanomedicine Formulations for Local Analgesic Delivery.**

Drug	Composition/ Functionalization	EE (%)	Benefits of the nanovectorized formulation (therapeutic outcomes)	Inconvenients	Pain model	Ref.
<b>LIPOSOMES</b>						
Mepivacaine	Large multilamellar liposomes composed of egg phosphatidylcholine, cholesterol and alpha-tocopherol (4:3:0.07 molar ratio)	-	Enhanced duration of anesthesia. Reduced injection discomfort (caused by the vasoconstrictor-associated formulations).	Lacks a discussion on potential systemic toxicity.	Evaluation of pain sensitivity in healthy volunteers Buccal maxillary infiltration performed in the upper right canine region Clinical assessment of local anesthesia with electrical stimulation of a selected tooth (electrical pulp tester) and electrical pulp tester) and visual analog scale (VAS). <i>Clinical stage.</i>	[126]
Ropivacaine	Large unilamellar vesicles egg phosphatidylcholine, cholesterol, and α-tocopherol, with a molar ratio of 4:3:0.07	-	Reduced Injection Discomfort. Lower Toxicity. insertion of a needle into the palatal mucosa	Ropivacaine liposomes did not reduce the pain of insertion of a needle into the palatal mucosa and were not effective in reducing pain related to injection of a local anesthetic	Evaluation of topical anesthesia of palatal mucosa in healthy volunteers before needle insertion and after injection of anaesthetic. <i>Clinical stage.</i>	[127]

Saxitoxin (STX) and dexamethasone (DMS)	DSPC,DSPG, cholesterol or DMPC,DMPG, cholesterol	-	Nerve blockage that lasts for about a week	No EE data. Invasive route of administration	In vivo experiments: Rat spared nerve injury model/ Von Frey nociceptive assay Measurement of nerve conduction, gene expression changes and astrocyte activation	[2]
Lidocaine	Gel formulations for transdermal drug delivery containing liposomes (small unilamellar vesicles composed of phosphatidylcholine and cholesterol). The gel is hydroxyethylcellulose-based commercial lubricant.	27 %	Improve the penetration capacity of the substance.	Limited Research on Long-term Effects  Complexity in Formulation.	In vivo: Rat model of inflammatory pain. Topical application. Combination of hot plate, cold plate, and algometer tests.	[128]
Lidocaine	No information concerning excipients	-	Reduced needle insertion pain sensation Reduced canulation procedure time	Minor dermal changes Cost	Evaluation of pain sensitivity in childrens aged 1 month to 17 years undergoing cannulation. Topical application of liposomal lidocaine before canulation <i>Clinical phase 1.</i>	[129]
Bupivacaine	Multilamellar vesicles made from a mixture of egg phosphatidylcholine and cholesterol in a molar ratio of 4:3.	-	Extended duration of postsurgical analgesia. Greater degree of differential block compared with plain bupivacaine. No motor block on the Bromage scale . No neurotoxicity or cardiotoxicity.	The surgical procedures were different within and between groups. The 3-point scale used for quantification of analgesia is less accurate than a visual analogue scale.	Post-surgery human patients Epidural administration. <i>Clinical stage.</i>	[130]
Bupivacaine	Multilamellar vesicles made from a mixture of egg phosphatidylcholine and cholesterol in a molar ratio of 4:3.	-	Threefold extension of analgesia duration No motor block was observed following the liposome-associated drug. No haemodynamic instability was observed		Patients with cancer pain syndrome. Administration via a thoracic epidural catheter suffering from lung cancer of the inferior left pulmonary lobe. <i>Clinical stage.</i>	[131]



			the liposomal bupivacaine.			
Tetracaine	Multilamellar phospholipid vesicles (soya phosphatidyl choline, stearic acid, cholesterol with a molar ratio of 10:1:1)	-	Extended duration of action. Long-lasting anesthesia lasting up to 1 hour. Liposomes promoting the cutaneous absorption. Deepest anesthesia with shortest on set.	Depth anesthesia was studied on a limited number of volunteers (insertion of needle and removal of superficial skin lesion) Lacks a discussion on long-term effects. The impact of the lipidic excipients on release and penetration of drugs has to be studied.	Evaluation of pain sensitivity in volunteers. Topical application on forearm and covered by tape. Pin-prick test. <i>Clinical stage.</i>	[132] [133]
TTX	NIR-triggered liposomes encapsulating NIR-triggerable photosensitizer (DSPC, DLPC, DSPG, cholesterol)	90 % (loading efficiency)	Adjustable timing, intensity, and duration of nerve blockade. No sign of skin injury	Depth and location of the target nerve could significantly affect the effectiveness of NIR light penetration, potentially limiting the applicability of the technology in different clinical scenarios.	In vivo experiments : Evaluation of pain sensitivity in rats Injection in sciatic nerve. NIR-irradiation at 730 nm. Modified hotplate test.	[134]
TTX and dexmedetomidine (Lip-GNR-TD)	Gold nanorods (GNRs) attached to liposomes (DPPC, DPPG, cholesterol and HS-PEG-DSPE)	-	Adjustable timing, intensity, and duration of nerve blockade. On-demand drug release. Enhanced patient compliance by reducing need for frequent dosing or medical interventions and with the non-invasive modality.	No EE data. Cost of implementation. No long-term safety and biocompatibility studies. Invasive route of administration.	In vivo experiments : Evaluation of pain sensitivity in rats footpad. Subcutaneous injection in the rat footpad followed infiltration anesthesia. Continuous NIR-irradiation at 808 nm produced on-demand local analgesia in the rat footpad in	[1]

				Scalability and Patient Variability (differences in skin type, body composition, and individual responses to nanomaterials)	proportion to the irradiance, with minimal toxicity. Mechanical pressure Test on rat's footpad. Modified hotplate test.	
				High irradiances can cause thermal injury		
<b>POLYMER NANOPARTICLES</b>						
TTX	Poly(triol dicarboxylic acid)-co-poly(ethylene glycol) (TDP)	-	Reduced mortality, myotoxicity and tissue inflammation vs. free TTX Duration of effect changed depending on TDP backbone's hydrophilicity	No EE data	In vivo: Evaluation of pain sensitivity in rats. Injection in rat sciatic nerve. Motor nerve blockade. Hotplate test.	[137]
Benzocaine	Benzocaine-loaded polymeric nanocapsules (polymers poly(D,L-lactide-co-glycolide)poly(L-lactide) (PLGA), and poly( $\epsilon$ -caprolactone))	70 %	Slower release profiles, prolonged its anesthetic action	Invasive route of administration Model of pain : no preexisting increased levels of pain in mice	In vivo experiments : Evaluation of pain sensitivity in mice. Sciatic nerve blockade Injection in popliteal space (near the sciatic nerve) Paw pressure test. In vitro : release assay through a cellulose membrane.	[138]
Articaine	Poly(ethylene glycol)-poly( $\epsilon$ -caprolactone) (PEG-PCL) nanocapsules and alginate/chitosan nanospheres	45-60 %	Reduced cell toxicity	No pain alleviation assay	In vitro experiments : MTT assay on mouse Balb-c 3T3 cells.	[139]
Lidocaine	Poly(caprolactone) (PCL) nanospheres	93 %	Reduced cell toxicity Prolonged anesthetic action	Invasive route of administration Model of pain : no preexisting increased levels of pain in mice	In vivo experiments : Evaluation of pain sensitivity in mice Drug injection near the sciatic nerve for sciatic nerve blockade Paw pressure test In vitro : MTT assay on mouse Balb-c 3T3	[140]

Lidocaine	PCL-PEG-PCL nanospheres Pluronic F-127 hydrogel	98 %	Rapid onset (both) Longer-lasting (hydrogel)	Safety of formulation based on visual inspection only No histological studies Tail flick test does not mimic wound pain	In vivo experiments : Evaluation of pain sensitivity in rat. Subcutaneous injection. Tail flick test. [141]
Lidocaine	Lipid-polymer hybrid nanoparticles (core-shell nanoparticles) with polymer cores (chitosan) and lipid shells (DLPC, DSPE-PEG <sub>2000</sub> )	78 % 85 %	Higher EE, sustained release, better skin permeation and longer-lasting anesthesia using hybrid nanoparticles vs. conventional liposomal form	No biodistribution assay	In vivo experiments : Topical application on rat's tail Tail-flick test Ex vivo experiments : Skin permeation assays using shaved rat skins [163]
Crotoxin	Crotoxin conjugated to SBA-15 nanostructured mesoporous silica	-	Longer-lasting analgesic effect and increased anti-inflammatory effect	No EE data. No biodistribution assay.	In vivo experiments : Partial sciatic nerve ligation in mice. Subcutaneous or oral drug administration. Von Frey nociceptive assay. Evaluation of LD50 and glia cell expression. [142]
Lamotrigine	PLGA functionalized with transferrin or lactoferrin	70 %	Enhanced blood-brain barrier passage and antinociception	No sham group in Hargreaves test, paw withdrawal latencies may be too high	In vivo experiments : Partial sciatic nerve ligation rat model. Intravenous drug administration Hargreaves test Pharmacokinetic and biodistribution study of labeled lamotrigine. [143]
Bupivacaine	Alginate nanoparticles with anionic surfactant sodium bis(2-ethylhexyl) sulfosuccinate (AOT). Alginate nanoparticles with chitosan.	75 %	Long-lasting anesthesia. Prolonged duration of motor and sensory blockades. Longer lasting anesthesia with alginate-AOT nanoparticles versus alginate-chitosan nanoparticles and even.	Similar cell toxicity of encapsulated bupivacaine in comparison with bupivacaine solution. No biodistribution assay	In vivo experiments : Sciatic nerve blockade model Drug injection into the popliteal space near the sciatic nerve Paw pressure test In vitro experiments: MTT assay on mouse Balb-c 3T3 cells [164]

SOLIDE LIPIDE NANOPARTICLES						
Lidocaine	Composed of different lipids— monostearin (MS), glyceryl palmitostearate (GP), and stearic acid (SA).	-	Longer-lasting effects than free lidocaine with more effective sensory and motor blocks.	No EE data. Lacking long-term biocompatibility and potential toxicity studies	In vivo experiments: Administration through the catheter. Analgesic tests on Wistar rat model. Hot plate test Tail Withdrawal Latency test and motor function test.	[145]
Lidocaine and prilocaine	Solid lipid nanoparticles (soya lecithin and glycerol monostearate) Nanostructured lipid carriers (Compritol® 888 ATO, Precirol® ATO 5 and glycerol monostearate)	22 %	Enhanced efficacy Improved safety.	No long-term safety profiles or no exploring of the metabolic pathways involved in the degradation of these nanoparticles.	In vivo experiments: Evaluation of pain sensitivity in rat. Topical application. Tail-flick test.	[146]
VIRAL NANOPARTICLES						
CRISPR-dCas9-KRAB and ZFP-KRAB targeting NaV 1.7.	AAV construct encapsulating CRISPR-dCas9 or ZFP-KRAB for targeted gene repression.	-	Targeted repression of NaV 1.7 in lumbar dorsal root ganglia, resulting in reduced thermal hyperalgesia in the inflammatory state and tactile allodynia in neuropathic state with long-lasting effects.	Potential for off-target effects, challenges in genetic payload capacity, and scalability.	In vivo experiments: Carrageenan-induced inflammatory pain model, paclitaxel-induced neuropathic pain model, and BzATP-induced pain in rat models. Administration by lumbar intrathecal route Measurement of thermal hyperalgesia and tactile allodynia.	[149] [150] [151]
DNA plasmid targeting and down-regulating SCN9A gene.	Synthetic DNA-based delivery platform (SMARTmid™).	-	Potential 50% reduction in SCN9A gene expression, corresponding to a decrease in NaV 1.7 protein expression.	Unclear potential for immunogenicity, given that the platform targets immune system pathways	In vitro experiments: Cell line (specific animal models not described).	[152]

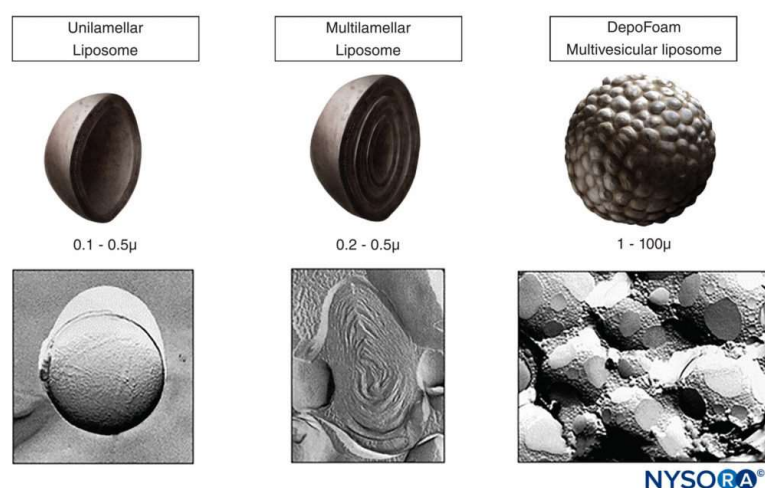
Abbreviations - EE : encapsulation efficiency, DPPC : 1,2-dipalmitoylphosphatidylcholine, DPPG : 1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol, DSPC : 1,2-distearoyl-sn-glycero-3-phosphocholine, DSPG : 1,2-distearoyl-sn-glycero-3-phosphoglycerol, DSPE : 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, PLGA :

poly(lactic-co-glycolic) acid, PEG : polyethylene glycol, PCL : polycaprolactone, MTT : 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, LD50 : median lethal dose, AAV : adeno-associated virus

### 5.4.1. Liposomes

Liposomes are nanometric or micrometric (20 nm to many microns) unilamellar vesicles or multilamellar vesicles, constituted of amphiphilic lipid molecules with a polar head and two lipophilic hydrocarbon tails forming one or more lipid bilayer(s). While unilamellar vesicles (small SUVs or large LUVs) are liposomes constituted of a single bilayer enclosing an aqueous compartment, multilamellar vesicles (MLVs) are liposomes with concentric lipid bilayers (Fig.6). Other micron-sized liposomes, characterized by numerous smaller vesicles encapsulated within larger vesicles, are referred to as multivesicular liposomes (MVLs) such as DepoFoam. As the DepoFoam formulation does not fall within the nanometric size range, it has not been classified in this chapter dedicated to nanometric sizes (DepoFoam technology see chapter 5.3).

Liposomes can incorporate lipophilic or hydrophilic drugs into their lipid or aqueous phases, respectively. Liposomes' size, structure and composition determine their *in vivo* behavior, of which release kinetics of encapsulated drugs and biodistribution. For instance, after subcutaneous administration, large liposomes (more than 120 nm in diameter) are prone to remain at the injection site, while smaller ones tend to reach capillaries. It's interesting to note that drugs exhibit a quicker release from liposomes with a single lipid bilayer, while the release is delayed when multiple bilayers separate the encapsulated drug from the extraliposomal environment. The permeability of the liposomal membrane is significantly influenced by factors like the lipid type and the inclusion of additional components in the bilayer, such as cholesterol [125].



**Figure 6 - Classification of liposomes** (source : Nysora©)

Due to limited duration of action of local anesthetics and severe systemic toxicity, drug delivery systems such as liposomes appear as very attractive option to circumvent these limitations. The two essential criteria for an effective drug delivery system for local anesthetics are residence time at the injection site and drug release rate. Furthermore, slow and sustained drug release from the carrier vehicle is needed to produce significant prolongation of analgesia. Indeed, this approach is very useful especially in the clinics for prolonged intraoperative anesthesia and extended postoperative analgesia.

The first reported *in vivo* application of a liposomal local anesthetic to enhance analgesic duration was reported in 1980 using dibucaine [155]. In 1988, the initial human trial of a liposomal local anesthetic formulation was documented, evaluating the ability of liposomal tetracaine to provide skin analgesia upon topical application [133]. In that respect, the application of these liposomes at a very low concentration (0.5%) to a 10 cm<sup>2</sup> area of the forearm under occlusion for one hour, produced at least 4 h of anesthesia (evaluated by the pinprick method). It is interesting to note that the mechanism by which liposomes enhance local drug penetration into

the skin is not fully understood [125, 132, 133]. Another topical formulation of liposomes carrying lidocaine (4%) developed by Taddio et al. reached clinical phase. In this double-blind randomized controlled trial, children aged 1 month to 17 years received topical liposomal lidocaine before intravenous cannulation. This non-invasive formulation offers the benefits of rapid onset of action with minor dermal changes [129].

Liposomal formulations of other local anesthetics such as bupivacaine with sustained release properties were also tested in humans for the management of postoperative and cancer pain. Indeed, in patients who had undergone major surgery (abdominal, vascular, urologic, thoracic, orthopedic), it was demonstrated, using Bromage scale, that in comparison with conventional bupivacaine (0.5%), epidural administration of liposomal bupivacaine (0.5%) extended the duration of analgesia without inducing motor block. Furthermore, this nanoformulation did not cause adverse side effects such as neurotoxicity or cardiotoxicity [130]. Patients suffering from pain associated with lung cancer were also evaluated for the efficacy of bupivacaine-loaded liposomes (0.25%) administered via the same route. Here again, a prolonged duration of analgesia (11 h compared to 4 h for plain bupivacaine) was observed without inducing motor block [131]. Regarding surgical site infiltration, the analgesic effectiveness and safety profile of liposomal bupivacaine have been extensively studied across various surgical procedures, such as inguinal hernia repair, total knee arthroplasty, hemorrhoidectomy, breast augmentation, and corrective osteotomy for hallux valgus repair. These studies have been conducted in numerous randomized, multicenter, double-blind trials with active-agent and placebo controls, spanning phases 2 and 3 [121, 156]. Nevertheless, certain studies have reported no discernible difference in analgesic outcomes between liposome-encapsulated bupivacaine and bupivacaine hydrochloride in many of these surgical procedures [157]. Another team developed liposomal formulation of ropivacaine for topical anesthesia of the palatal mucosa and found no significant advantage over placebo in reducing needle insertion pain in phase 1 clinical trial, suggesting limitations in the effectiveness of liposomes for certain types of mucosal anesthesia [127].

To address some limitations of liposomal formulations such as early partial release of the latter which limits the efficacy of the dosage, and to increase their duration of action, innovative strategies have emerged [1, 135, 158]. Thus, with the aim of enhancing the quality of life of patients suffering from postoperative or even chronic pain, Rwei et al. developed a phototriggerable nanoformulation that produces repeated on-demand analgesia over extended periods with limited toxicity [134]. In that respect, near-infrared (NIR) light-triggered liposomes encapsulating TTX and a NIR-triggerable photosensitizer, the 1,4,8,11,15,18,22,25-octabutoxyphthalocyaninatopalladium(II), (subsequently abbreviated "PS") were tested. Irradiation at 730 nm of these phototriggerable liposomes produced reactive oxygen species (ROS) and subsequent per-oxidation of these liposomes destabilized them, leading to the release of TTX. Thus, the injection of PS-TTX liposomes at the rat sciatic nerve, initially induced nerve block for nearly 14 h (due to passive release of TTX) and then, an additional period of nerve block was induced by irradiation at 730 nm, 24 h post-injection. In addition, this approach demonstrated precise timing control, intensity, and duration of nerve blockade by adjusting the parameters of light exposure [134]. Based on the same approach, the same team developed NIR-triggered nanosystems by conjugating liposomes encapsulating TTX and dexmedetomidine (DMED), to gold nanorods (GNRs). These GNRs are nanoscale solid objects with a rod-like morphology capable of converting near-infrared (NIR) light into heat. This configuration was designed to enable the light-triggered phase transition of liposomal lipid bilayers, resulting in the controlled release of the encapsulated drugs. These liposome gold nanosystems (Lip-GNRs) were injected subcutaneously in the rat footpad and a 10-min 808 nm NIR laser irradiation was performed at the injection site 24 h post-injection [1]. The local anesthetic effect of Lip-GNRs was assessed on the basis of the vocal or motor (foot withdrawal) response following mechanical stimulation to the rat footpad using Touch Test sensory evaluators. It was shown in this study that the irradiation generated on-demand and repeated infiltration anesthesia within the rat footpad and the intensity and duration of nerve blockade was able to be tuned by adjusting the parameters of light exposure. It is noteworthy that the efficacy of phototriggered systems is influenced by the tissue's depth of penetration, with high irradiances potentially causing thermal injury. Indeed, elevated irradiances and/or prolonged exposure times to NIR light may result in burns [1, 136, 158]. These findings underscore the need for careful consideration of parameters to optimize the therapeutic effects of phototriggered formulations while minimizing potential risks associated with light exposure.

### 5.4.2. Polymeric nanoparticles

The use of polymers as the main ingredient of nanoparticles represents a fascinating avenue in drug delivery. Polymeric nanoparticles (nanocapsules or nanospheres) are designed to increase drug half-life and bioavailability, and to control its release. In that respect, the polymers constituting these NPs can be biodegradable and/or biocompatible (natural, e.g. chitosan, or synthetic polymers, e.g. polyglycolic acid). They can be associated to the active pharmaceutical ingredient either chemically using bioconjugation approach or physically (ie. hydrogels). They can also be combined with metals to form nanocomposites. All these characteristics allow the design of finely tuned polymeric NPs, that allow improved time- and space-controlled drug delivery as well as reduced toxicity [159, 160].

One notable application of polymer nanoparticles in pain management involves investigating site 1 sodium channel blockers (S1SCBs) like TTX, which demonstrated exceptional potency and provided extended nerve blocks after injection in rat sciatic nerve. A prime example involves covalently attaching tetrodotoxin (TTX) to poly(triol dicarboxylic acid)-co-poly(ethylene glycol) polymer backbone through hydrolyzable ester linkages. This approach, explored by Zhao et al., not only prevented the initial burst release but also facilitated the gradual, controlled release of TTX in its native, physiologically active form [137]. The hydrophilicity of polymers played a crucial role in the hydrolysis rate of ester linkages, thus influencing the release of TTX from these polymers. Hence, modulating the hydrophilicity of the polymer backbone emerges as a potential avenue to tailor the release profile of TTX [161].

Local anesthetics were also incorporated into polymer nanoparticles, leading to encouraging results. With the aim at providing long-lasting infiltration anesthesia using lidocaine devoid of severe toxicities, Yin et al. used a new formulation based on biodegradable polymeric nanoparticles incorporating lidocaine in a thermo-sensitive Pluronic F-127 hydrogel, known for its reversible sol-gel transition property. The polymeric nanoparticles were composed of PCL-PEG-PCL (poly( $\epsilon$ -caprolactone)-poly(ethylene glycol)-poly( $\epsilon$ -caprolactone)) copolymers. Thus, this lidocaine nanogel formulation was administered by subcutaneous injection into Sprague-Dawley rats and was compared, using tail flick test, with lidocaine-loaded PCL-PEG-PCL nanoparticles (lido-nano) and lidocaine base containing thermo-sensitive Pluronic F-127 hydrogel (lidogel) [141]. Their findings revealed that this lido-nanogel formulation produced both rapid-onset and long-lasting infiltration anesthesia while the two other formulations only generated quickonset. Although these findings suggest a potential avenue for developing long-lasting local anesthetics, it's important to note that only visual inspection was used to assess the safety of the formulation, and no histological studies were conducted. Additionally, the tail flick test may not accurately mimic wound pain and thus may not be a relevant model [141].

From another perspective, *in vitro* studies conducted by Melo et al. focused on evaluating the viability of mouse Balb/c 3T3 cells (a cell line commonly used in cell viability tests, derived from disaggregated albino mice embryos) in the presence of articaine loaded polymeric nanocapsules [139]. Their research demonstrated a notable decrease in articaine toxicity when it was encapsulated into PCL-PEG (poly( $\epsilon$ -caprolactone)-poly(ethylene glycol)) nanocapsules compared to free articaine in solution. Additional *in vivo* studies were also performed by the same team with PCL nanospheres of lidocaine on one hand, and PLGA (poly(D, L-lactide-co-glycolide)), PLA (poly(L-lactide)), and PCL nanocapsules of benzocaine on the other hand [138, 140]. The use of polymer nanoparticles enabled to reduce toxicity and allowed a sustained drug release and subsequent prolonged analgesic activity when injected near the sciatic nerve in mice, similarly to the outcomes of Exparel nanoformulation. However, these positive results did not lead to further studies. Moreover, the route of administration is very restrictive, as it is limited to post-surgery applications.

In a double-blinded, randomized clinical study, biodegradable microcapsules of polylactide glycolic acid (PLGA) polymer incorporating bupivacaine were administered as subcutaneous infiltrations. The analgesic effect of bupivacaine formulation was evaluated using von Frey hairs and contact thermodes in order to analyse pain responses to mechanical and heat stimuli and sensory thresholds (touch, warm, and cold detection thresholds). From 24 to 96 h period post-injections, these microcapsules proved to be more efficient than aqueous solution of bupivacaine, with a maximum effect at 96 h. The safety profile of these bupivacaine microcapsules was studied and did not evidenced serious side effects up to six months [162].

As alternatives to commercial formulations such as lidocaine and prilocaine cream EMLA®, AstraZeneca), lidocaine tape (Penles®, Wyeth) or lidocaine gel patch (Lidoderm®, Endo Pharmaceuticals), Wang et al. [163] developed lipid polymer hybrid nanoparticles (LPHNPs) carrying lidocaine to produce a fast acting and long lasting topical formulation. These LPHNPs consist of core-shell nanoparticle structures encompassing polymer cores (chitosan) and lipid shells (DLPC and DSPE-PEG<sub>2000</sub>). The local anesthetic effect of these hybrid nanoparticles encapsulating lidocaine was evaluated in rats by tail flick latency test and was compared to conventional liposomes (DLPC, DSPE-PEG<sub>2000</sub>, and cholesterol). Remarkably, the lipid-polymer hybrid nanoparticles exhibited superior encapsulation efficiency over liposomes, combined with a more prolonged release profile of lidocaine. Additional investigations have explored the potential of alginate nanoparticles loaded with bupivacaine to mitigate local anesthetics toxicity and extend the anesthetic effect in murine models [164].

To expand the application of nanoparticles to a broader range of NaV-dependent pain conditions, systemic routes of administration are frequently considered the most suitable option. Among the few examples in literature, the use of lamotrigine (originally used as an antiepileptic drug) encapsulated in surface-functionalized PLGA NPs conjugated with the lactoferrin and transferrin as ligands is intriguing. Lamotrigine which acts as a blocker of sodium and calcium channels, has already shown effectiveness in treating neuropathic pain across various randomized, controlled trials. These protein-functionalized PLGA nanoparticles of lamotrigine were administered intravenously in a partial sciatic nerve injury model using Hargreaves test based on thermal stimuli. In this work, carried out by Lalani et al., a striking three- to four-fold increase of paw withdrawal latency was achieved in rats, owing to (i) the nanoformulation itself and (ii) the specific transferrin or lactoferrin coating that ensures a better passage of the blood-brain barrier and better targeting of brain tissue, and indirectly, brain NaV channels. Pharmacokinetic studies showed an improved blood-brain barrier passage, while off-target distribution remained negligible [143]. Nonetheless, as the central nervous system (CNS) is more heavily targeted, the chronic use of such NPs may raise concerns regarding neurotoxicity and CNS serious side effects. Another noteworthy example is the use of crotoxin, by Sant'Anna et al., vectorized in nanostructured mesoporous silica NPs. Subcutaneous injection and even oral administration of these nanoparticles enabled to alleviate sciatic nerve ligation pain along with local inflammation [142]. This recent work also showed that the NP formulation lowered crotoxin toxicity, but new evidence of innocuity on off-target organs or biodistribution studies are still lacking.

In summary, polymer-based approaches offer a multifaceted solution to the challenges of local anesthesia, showcasing their potential to revolutionize pain management. By leveraging polymer properties to modulate drug release and enhance drug targeting, these innovations hold promise for enhancing patient care, optimizing recovery, and reducing opioid reliance in the pursuit of effective and tailored pain relief. In practice, combining optimal release kinetics, long-term stability and preventing burst release, remains a difficult task, while targeted delivery is still a very rare application, explored in the unique case of functionalized PLGA NPs containing lamotrigine. As a result, polymer NPs still did not stand out of other types of NPs and none of them went beyond preclinical stage. The only noteworthy application of polymer formulation aimed at blocking NaV at clinical stage is the specific case of bupivacaine- and dexamethasone-loaded PLGA microsized (25–125 µm) capsules. In this double-blinded, randomized phase I study, the microcapsules formulation was compared to the bupivacaine in solution, both injected subcutaneously in the calf skin, using Von Frey, hot plate and verbal ranking scale nociceptive tests [153]. Despite a significant prolongation of analgesia granted by the microcapsule formulation, there is no mention of further clinical studies on this drug.

### **5.4.3. Solid lipid nanoparticles**

Derived from oil-in-water emulsions, lipid nanoparticles with solid particle matrices are created by replacing the liquid lipid (oil) with a solid lipid that remains solid at body temperature. These lipidic NPs integrate the beneficial properties of conventional colloidal carriers such as liposomes and emulsions while mitigating some of their critical shortcomings. Specifically, solid lipid nanoparticles (SLNs) address the issue of rapid and uncontrolled drug release observed in liposomes, as well as the stability problems commonly associated with emulsions [163]. The first generation of these SLNs nanoparticles, was developed in the early 1990s using



only a solid lipid. In the second generation technology, known as nanostructured lipid carriers (NLCs), particles are formed by blending a solid lipid with a liquid lipid, resulting in a blend that solidifies at body temperature. The second generation technology offers the advantage of enhanced loading with active ingredients compared to SLNs, as well as a more secure incorporation of these components within the particle matrix throughout the shelf life. This latest technology was applied to anesthetics and enabled their controlled release, thus prolonging the anesthetic effect. In addition, they exhibit strong adherence to the skin when topically applied, and enhance skin permeation [154].

In a study, conducted by You et al., solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) co-loaded with lidocaine and prilocaine were prepared to enable combination anesthesia and overcome the respective drawbacks of these active ingredients. Preparation of SLNs involved the use of soya lecithin and glycerol monostearate (GMS) as lipid excipients while in NLCs, lipid excipients corresponded to Compritol® 888 ATO, Precirol® ATO 5 and GMS. The notably superior anesthesia analgesic effect observed with the carriers co-loaded with dual drugs compared to those loaded with a single drug highlighted the synergistic effect of delivering the active ingredients together via the same carrier. SLN systems exhibited superior *in vivo* skin permeation capability compared to NLCs due to their smaller size, while the latter nanosystems demonstrated a more potent *in vivo* analgesic effect with faster release during the first several hours. The anesthesia analgesic effect was assessed in rats through the tail-flick test. These benefits collectively contributed to a significant reduction in systemic exposure and potential side effects associated with these local anesthetics. Despite these positive outcomes, the study presented certain limitations. It predominantly concentrated on the short-term efficacy of the lipidic NPs and did not encompass aspects such as long-term safety profiles or the metabolic pathways involved in the biodegradation of these nanoparticles. These concerns underscore the necessity for additional research to comprehensively ascertain the therapeutic benefits and safety of SLN-encapsulated lidocaine and prilocaine for topical application [146].

In another study directed by Leng et al., solid lipid nanoparticles (SLNs) were utilized to encapsulate lidocaine to enhance its analgesic efficacy for epidural anesthesia. The SLNs were engineered using a trio of lipids (monostearin, glyceryl palmitostearate, and stearic acid) chosen specifically to tailor the release profile of lidocaine, aiming for a sustained and controlled release that surpasses the duration of traditional aqueous solutions. The SLNs' primary efficacy assessment was conducted on male Wistar rats, chosen for their physiological consistency and suitability for epidural administration, to simulate the human response, reflecting clinical application in treating surgical pain. The effectiveness of the SLNs was determined using the tail withdrawal latency test to gauge sensory block and a modified Bromage scale test to evaluate motor impairment. The findings demonstrated that lidocaine-loaded SLNs significantly extended both sensory and motor block duration, achieving up to 12 h of sensory block, markedly longer than that achieved with conventional aqueous solutions of this anesthetic. Despite yielding promising results, the study acknowledged certain limitations, particularly the absence of a thorough investigation into the long-term biocompatibility and potential toxicity of the lipid components used in the SLNs [145].

Ongoing research efforts aimed at optimizing nanoparticle-based epidural delivery seek to minimize motor weakness, reduce systemic absorption, optimize controlled release kinetics, and decrease the required analgesic dosage [144].

#### **5.4.4. Adeno-associated virus (AAV) vectors**

Adeno-associated viral (AAVs) vectors stand as the primary platform for delivering genes in the treatment of various human diseases. AAVs share similarities with adenoviruses (AVs), but are considered safer due to their limited replication and reduced pathogenicity. They are based on the adeno-associated virus, a non-pathogenic, single-stranded DNA parvovirus, composed of an icosahedral protein capsid of ~ 26 nm in diameter in which viral DNA was replaced with new DNA. Using this strategy, Samad et al. used a recombinant AAV vector encoding short hairpin RNA (shRNA) to knockdown expression of NaV 1.3. The shRNA is an artificial RNA molecule, which consists of two complementary 19–22 bp RNA sequences linked by a short loop of 4–11 nt. Thus, the sequence encoding the shRNA is introduced via viral transduction into the genome of host cells. After transcription, the shRNA sequence is transported to the cytosol where it is

identified by Dicer, an endogenous enzyme, that processes the shRNA into the siRNA duplexes. Then, these endogenous siRNAs bind to the target mRNA and induce mRNA degradation following incorporation into RISC complex, thus generating long-term knockdown expression of protein. In that respect, AAV vectors expressing shRNA against NaV 1.3 were injected into DRG of rats with spared nerve injury (SNI) leading to a notable decrease in NaV 1.3 expression and reduced nerve injury-induced neuropathic tactile allodynia [147]. Based on these results, the same team demonstrated that viral delivery of AAV- shRNA-NaV 1.3 in a rat model of painful diabetic neuropathy using intrathecal administration, a less invasive route, led to a targeted knockdown of NaV 1.3 expression thus resulting in a reduced tactile allodynia [148]. This approach holds promise in addressing pain-related disorders by targeting the underlying molecular mechanisms. Nevertheless, AAVs still pose certain challenges, particularly in terms of genetic payload capacity and scalability of production.

Another approach dealing with epigenetic modulation of NaV 1.7 expression based on AAV mediated gene delivery was proposed to cause a down regulation of SCN9A leading to the repression of NaV 1.7. This approach uses two major generations of genome editing tools with programmable nucleases such as “zinc-finger nucleases”, and the more recent “clustered regularly interspaced short palindromic repeats-Cas9” (CRISPR-associated Cas9) endonucleases. These systems, that share the same mechanism of action, were designed and produced by molecular biology methods to modify the genome in eucaryote cells specifically. Indeed, their function consists in targeting a selected DNA sequence in order to cleave the DNA double-strand that triggers endogenous DNA repair systems and enables genome editing. However, since these systems permanently edits gene, in the context of pain, off-target editing could lead to a permanent alteration of pain perception. This phenomenon is undesirable given that pain sensation is important to alert and protect the body from harm. Thus, to avoid permanent gene repression, CRISPR-Cas9 and Zinc-finger nucleases (ZFN) systems were fused to a repressor domain (Krüppel-associated box or KRAB). The KRAB domain which consists of about 75 amino acid residues serves as a powerful transcriptional repression module that enables nonpermanent gene repression of NaV 1.7. Thus, KRAB interacts with the scaffold protein KAP-1, which has the capability to recruit diverse transcription factors, resulting in the repression of genes to which CRISPR-Cas9-KRAB and ZFN-KRAB bind.

Hence, ZFN are composed of three to four zinc-finger proteins (DNA-binding motif) fused with the FokI nuclease (DNA-recognition and cleavage motifs). Zinc-finger proteins (ZFP) comprise 30 amino acid residues with Cys<sup>2</sup>His<sup>2</sup> amino-acid patterns coordinated to zinc ions. The fusion of ZFP with KRAB led to ZFP-KRAB system in which zinc finger motifs are found on the C-terminal side of the protein, while KRAB domain is located near its N-terminal side.

As for CRISPR, which emerges from bacterial immune systems, it utilizes a Cas9 nuclease along with guided RNA (RNA<sub>g</sub>) that selectively bind to DNA sequence within a gene, to enable permanent gene repression of NaV1.7. CRISPR-CAS system can be engineered to specifically modify the nucleotide sequence of a gene, either to render it non-functional, or to eliminate or introduce from one to several thousand nucleotides in order to correct a mutation responsible for hereditary disease. CRISPR-Cas9 has already demonstrated therapeutic effectiveness in various animal models of human diseases [149, 150]. However, since CRISPR-Cas system permanently edits gene, its fusion through guide RNA (gRNA) to KRAB domain resulted in the catalytically inactivation of Cas9 (dCas9), leading to CRISPR-dCas9 system also known as CRISPRi [151].

By encapsulating these epigenetic tools (CRISPR-dCas9 or ZFP-KRAB) in an adeno-associated virus (AAV) construct, Moreno et al. investigated targeted repression of NaV1.7 at the spinal level for the treatment of chronic pain. In that respect, these AAVs were administered by the lumbar intrathecal route in three mice pain models: (i) carrageenan induced inflammatory pain, (ii) paclitaxel-induced neuropathic pain, and (iii) BzATP-induced pain (BzATP being a P2X receptor agonist). Thus, by this way, these researchers expect to minimize the possibility of off-target biodistribution and reduce the viral load required to get transduction. The results demonstrated the effectiveness of these AAVs in repression of NaV1.7 in lumbar dorsal root ganglia. This reduced thermal hyperalgesia in the inflammatory state, decreased tactile allodynia in the neuropathic state with long-lasting effect, suggesting their use in management of persistent and chronic pain [22]. This approach offers multiple benefits, allowing for the utilization of minimal viral loads thus decreasing the likelihood of systemic immunogenicity [165]. Nevertheless, AAVs still pose certain challenges, particularly in terms of genetic payload capacity and scalability of production [166, 167].

An alternative technology was proposed by Dormer et al. from R&D department of PepVax, Inc. [152]. By taking advantage of immune system's potential, this early-stage biotech company has developed a DNA-based delivery platform to target the specific antigen protein MAGE A (expressed by many tumors), with the goal of developing a treatment for triple-negative breast cancer (TNBC). This drug delivery system called SMARTmid™ uses a completely synthetic approach to produce plasmids, aiming to enhance the efficiency and reduce the discomfort associated with cell therapies. In a review published in 2023, Dormer et al. proposed to apply SMARTmid™ to target and down regulated SCN9A gene for the treatment of chronic pain [152]. They claimed that initial results indicated a 50% reduction in the expression of the SCN9A gene, aligning with a corresponding decrease in NaV 1.7 protein expression in the in vitro cell line (data not presented). The authors suggest that compared to AAVs, this technology offers better genetic payload capacity, replication competence, and the absence of permanent integration. However, no more explanations were provided concerning this approach, and it's hard to imagine that this system, which is based on immunotherapy, will not develop immunogenicity against NaV 1.7.

## General discussion

Blocking VGSCs channels emerges as a relevant therapeutic strategy for addressing pain. By not interacting with the opioid signaling pathway, VGSCs blockers provide a potential alternative to opioids. Furthermore, due to the abundant presence of NaV in nociceptors, NaV channel blockers tend to more precisely target certain types of neuropathic pain, which are frequently inadequately managed by traditional analgesics. These unique characteristics prompted the identification VGSC blocking properties of existing medications and the development of new drugs, often inspired by natural toxins, to more effectively target NaV isoforms located in the peripheral nervous system. However, pharmacokinetics and biodistribution are still far from optimal to ensure a safe use, and greatly restrains the therapeutic potential of these NaV blockers, essentially to local infiltrations.

Nanomedicine, harnessing the unique properties of nanoscale materials, offers significant advancements in VGSC blockade-based pain relief, particularly in the encapsulation of local anesthetics. A key advantage is the precision in delivering therapeutic agents to targeted sites, such as specific nerves or tissues, which maximizes pain relief while reducing systemic exposure and potential side effects. This targeted approach is showcased in various nanoscale carriers, including liposomes, polymeric and lipidic nanoparticles, which have mainly demonstrated efficacy in reducing myo-, neuro- and cardiotoxicity [168, 169]. Furthermore, the nanomedicine approach facilitated a sustained release of the encapsulated NaV blockers, thereby significantly prolonging their duration of action.

Numerous studies have progressed beyond the preclinical stage, mainly employing liposomes, yet only one, Exparel, has received FDA approval. This formulation comprises micron-sized multivesicular liposomes containing bupivacaine and is primarily employed for postoperative pain relief in bunionectomy and interscalene brachial plexus nerve block procedures. It's worth mentioning that the application of nanomedicines targeting VGSCs was mainly limited to local infiltration analgesia and sciatic nerve block for local or regional anesthesia during surgical procedures and post-operative pain management. It is surprising that researchers haven't explored leveraging the nanometric size of nanoparticles for addressing cancer pain or inflammatory pain via intravenous administration. Indeed, it was established that VGSCs are overexpressed in various types of tumors, while they exhibit minimal expression in corresponding normal tissues. Furthermore, VGSCs overexpression enhances cancer cell metastasis and invasion and is associated with the metastatic behavior of different cancers [170]. Thus, by taking advantage of EPR (enhanced permeability and retention) effect, we can imagine that nanomedicines can extravasate from tumor vascular lumen into the tumor tissues and release VGSC blockers within the overexpressed VGSCs for an effective cancer pain relief and inhibition of tumor growth. Following the same approach, we can envisage to address inflammatory pain, by administering intravenously VGSC blocker nanomedicines which can reach inflamed tissues through altered vascular endothelium by EPR-like effect, where VGSCs (particularly Nav 1.7) are overexpressed within membrane of peripheral nociceptor terminals [171].

Overall, the advancements in nanomedicine underscore its potential to improve pain management significantly. Beyond alleviating patient discomfort, effective postoperative pain management can mitigate cardiorespiratory morbidity and promote swift recovery. The associated positive impact on hospital costs makes early discharge a valuable consideration. Thus, nanomedicine offers groundbreaking possibilities that could pave the way to fill the gap between narcotic and non-narcotic drugs, revolutionizing pain management approaches.

Despite these advantages, nanomedicine faces several challenges. First and foremost, achieving optimal pharmacokinetic and pharmacodynamic properties is sometimes challenging. On one hand, despite the highly tunable physicochemical properties of nanovectors, optimal stability is not always possible, and some outcomes such as burst release are common concerns. On the other hand, neuropathic pain, often involving NaV channels, is not always caused by a well-defined or easily accessible target. While local anesthesia, either in post-surgery or for superficial wounds, can benefit from sustained drug release properties of nanoparticles injected near the involved nerve or nociceptor, reaching central nervous system or other areas— if any—not associated with favorable targeting properties (e.g. EPR or EPR-like effect, overexpression of membrane proteins...) remain arduous. Furthermore, the complexity and expense involved in developing and manufacturing nanoparticle-based therapies at an industrial scale remain high, thereby restricting their accessibility. Regulatory challenges also arise, as the approval process for nanomedicine products can be lengthy and complicated, owing to a lack of standardized regulation specific to nanomedicine, delaying their availability to patients who need them [172]. Another crucial challenge is about safety concerns. The unique properties of nanoparticles that make them so valuable in industry and medicine also necessitate a careful examination of their long-term adverse effects on both human health and the environment, due to their ability to easily penetrate cells and tissues. Unfortunately, long-term toxicity remains poorly explored in the experimental work mentioned in this review, despite the use of heavy metals or some non-biodegradable functional moieties. As regards the environmental impact of nanomedicine, there is evidence suggesting that nanoparticles can interact with ecosystems in ways that may be concerning—such as potential accumulation in water bodies and soil, and interactions with aquatic and terrestrial life [173, 174], making it essential to frame these findings within a broader context of research and innovation.

Finally, while nanomedicine offers a promising new direction in pain management, especially in the encapsulation of local anesthetics, addressing the current challenges is crucial for its widespread adoption and efficacy. Future research should focus on improving the safety and stability of nanoparticle formulations, reducing costs, and streamlining regulatory pathways.

## Conclusion

The application of nanomedicine in targeting sodium channels represents a cutting-edge approach with significant relevance in the realm of pain relief. Nanoparticles, owing to their unique physicochemical properties and tailored drug delivery capabilities, offer a platform for precision medicine in the treatment of pain conditions associated with aberrant sodium channel activity. By encapsulating or conjugating sodium channel blockers within nanocarriers, it becomes possible to enhance drug solubility, bioavailability, and target-specific delivery, thereby optimizing therapeutic outcomes. This nanoscale precision could be a game changer for minimizing off-target effects and improving the overall safety profile of sodium channel-targeted therapies. Furthermore, nanomedicine allows for the customization of drug release kinetics, ensuring sustained and controlled delivery at the site of action. In the context of pain management, where achieving localized and prolonged efficacy is crucial, the utilization of nanomedicine for sodium channel targeting opens new avenues for the development of more efficient and patient-friendly pain relief strategies. This interdisciplinary approach underscores the evolving landscape of pain therapeutics, as nanomedicine continues to bridge the gap between targeted molecular interventions and enhanced clinical outcomes in the pursuit of effective and well-tolerated pain relief. In the evolving landscape of pain management, the synergy between nanomedicine, local anesthetics, and advanced delivery systems holds the potential to revolutionize how we approach pain relief, improving patient comfort, reducing reliance on opioids, and enhancing the overall quality of medical care.

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