Grant Report

Controlling the "Opioid Epidemic": A Novel Chemical Entity (NCE) to Reduce or Supplant Opiate Use for Chronic Pain †

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ABSTRACT

We report on the ongoing project "A Novel Therapeutic to Ameliorate Chronic Pain and Reduce Opiate Use." Over 100 million adults in the U.S. suffer from intermittent or constant chronic pain, and chronic pain affects at least 10% of the world's population. The primary pharmaceuticals for treatment of chronic pain have been natural or synthetic opioids and the use of opioids for pain treatment has resulted in what has been called an "epidemic" of opioid abuse, addiction and lethal overdoses. We have, through a process of rational drug design, generated a novel chemical entity (NCE) and have given it the name Kindolor. Kindolor is a non-opiate, non-addicting molecule that was developed specifically to simultaneously control the aberrant activity of three targets on the peripheral sensory system that are integral in the development and propagation of chronic pain. In our initial preclinical studies, we demonstrated the efficacy of Kindolor to reduce or eliminate chronic pain in five animal models. The overall goal of the project is to complete the investigational new drug (IND)-enabling preclinical studies of Kindolor, and once IND approval is gained, we will proceed to the clinical Phase Ia and 1b safety studies and a Phase 2a efficacy study. The work is in its second year, and the present report describes progress toward our overall goal of bringing our compound to a full Phase 2 ready stage.

KEYWORDS: chronic pain; non-opiate medication; novel chemical entity; multi-target action

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INTRODUCTION

Chronic Pain and Magnitude of Problem

Acute pain is an important component of our sensory systems for maintaining survival and reducing the extent of harm to our body. However, the peripheral sensory system, as well as the spinal cord and brain, has the capacity to (mal) adapt to tissue or neuron injury [1], such that pain is perceived well beyond the time (months/years) that the injury is healed. Pain can then be generated by stimuli that are normally innocuous (allodynia), or the response to a noxious stimulus is greatly exaggerated (hyperalgesia). In some cases pain can arise spontaneously, and without provocation (this type of pain can be continuous or paroxysmal). If pain persists for longer than three months after an injury is healed, it is referred to as chronic pain. The recent version of the ICD-11 has developed a new and pragmatic classification of chronic pain [2]. Chronic neuropathic (neuronal damage) pain, as exemplified by the pain arising from diabetic neuropathy or osteoarthritis, is many times treated with opiate medications. However, typical opiates have low efficacy in these types of chronic pain, and dose escalation is common [3–7]. Our aim is to produce a medication that supplants the use of opiates for chronic pain treatment and/or our medication can be used in conjunction with opiates to reduce the doses of opiates to levels incompatible with development of addiction. Another feature of the medication we are developing is the ability to block the development of chronic pain, when given soon after tissue/nerve damage.

Chronic pain can be considered the major public health problem in the U.S. The Institute of Medicine report “Relieving Pain in America…” released in 2011 [4] stated that chronic pain affects at least 100 million adults in the U.S., costs society $560–$635 billion annually and significantly reduces the quality of life for the individuals suffering from chronic pain [5]. The Centers for Disease Control and Prevention estimated in 2014 that 29.1 million people (9.3% of the US population) have diabetes, and that 30–50% of these individuals will eventually develop diabetic peripheral neuropathy (DPN) [8,9], which is caused by decreased blood flow and hyperglycemia [10]. DPN consists of several symptoms, has a wide tissue distribution and is usually chronic and progressive [11]. DPN is defined as “pain initiated or caused by a primary lesion or dysfunction in the nervous system” [12]. The pain associated with DPN is described as burning, stabbing, numbness, or pins-and-needles sensations [10,13]. Untreated DPN often leads to foot ulceration and lower extremity amputation.

Therapy for Chronic Pain and Opiate Use. Current pharmacotherapy for DPN incudes anticonvulsants, non-steroidal anti-inflammatory drugs (NSAIDs), cyclooxygenase 2 inhibitors, tricyclic antidepressants, benzodiazepines and opioids [14,15]. Only duloxetine, a selective serotonin norepinephrine reuptake inhibitor (SSNRI), and pregabalin, an anticonvulsant, are currently approved by the FDA for treating DPN [16].

There is no good evidence that typical opiates are effective for treatment of DPN [6,7], although atypical opiates (e.g., tramadol, which has SSNRI activity) were reported to be moderately effective (but with low strength of evidence) [17]. Nevertheless, a study of DPN patients receiving pharmacotherapy found that 53% had DPN-related opiate use, and 33% received opioids as first line treatment [10]. Only 1% and 6%, respectively, received duloxetine or pregabalin. A review of DPN pharmacotherapy studies revealed that while duloxetine, pregabalin and some anticonvulsants and tricyclic antidepressants, as well as atypical opioids, were more effective than placebo in reducing DPN pain, most of these drugs had low to moderate effect sizes and low strength of evidence [17]. In addition, all of the treatments had substantial risks of adverse effects. In particular, for the atypical opiates, the duration of studies was short, and most guidelines recommend against the use of opioids for chronic pain, given the lack of evidence for long-term benefits and evidence of risk for abuse and overdose [18]. Volkow and McLellan [19] have recently provided an overview of the problems associated with opiate use to treat chronic pain and stressed several points. A major point made for mitigating risk associated with opiate use was "common strategies that can help mitigate all risks, include limiting the prescribed opioid to the lowest effective dose for the shortest effective duration (for acute and chronic pain) without compromising effective analgesia." The concern over the significant increases in the use of opioids to treat pain, and accompanying problems of overdose, misuse and diversion, led to the CDC "Guidelines for Prescribing Opioids for Chronic Pain" [20]. These "Guidelines" presented two principles: (1) Non-opioid therapy is preferred for chronic pain outside of active cancer, palliative, and end of life care; (2) When opioids are used, the lowest possible effective dosage should be prescribed to reduce risk of opioid use disorder and overdose. The recommendation was not to exceed a dose of opiate equivalent to 90 mg of morphine. This brought a plethora of concerns from both physicians and patients ([21] and [22] (e.g., Editorial: Have we gone too far? Can we get back?)) that these guidelines will stifle the optimum treatment of chronic pain. The one area in which pain experts agree is that pain needs to be controlled and the best option is the development of new non-opioid medications based on scientific knowledge of chronic pain etiology, including persistence, initiation, conduction, transduction and perception [23,24].

In fact, there is no shortage of attempts to develop novel medications for treatment of chronic pain and some of the more advanced agents are listed in Yekkirala et al. [25] and Worley [26], but the pain medication development field has been likened to the state of cancer medication research sixty years ago [26]. One hopes that the missteps in the cancer medication development are not repeated in developing new medications for chronic pain. Target selection is the key feature of the initiation of a program of chronic pain drug development, and the lesson learned from cancer has been that agents highly selective for a single target/site are most

times less effective than a therapy engaging two or more relevant targets mediating cancer survival and progression [27]. When we began the design of our multi-target agent for chronic pain treatment in 1998 [28], the pharmaceutical industry was (and is still) wedded to the concept that acceptable medications had one high affinity on-target site and any functional interactions with other (off-target) sites were undesirable and predictive of adverse events [29]. In the interim, perceptions have changed due to carefully designed (with some serendipity) successful multi-target medications for treatment of schizophrenia, viral infections, neurodegenerative disease and cancer [29], and a multitude of recent publications [29–32] are touting and refining the multi-target approach for complex disease treatment.

Novel Drug Design Strategy

Our initial strategy was to focus on targets that conduct sensory information from nociceptors (i.e., sodium channels of the peripheral nervous system) and the systems that transduce information within and between sensory neurons. We particularly wanted to focus on systems that show evidence of (mal) adaptation coincident with the development of chronic pain. The systems/targets that gained our attention were the glutamatergic (NMDA receptors) and voltage sensitive sodium channels, VSNaCs (Nav 1.7 and 1.8). We used "rational drug design" to create a single molecule ("Kindolor") with affinity and inhibitory function at these glutamatergic receptors and VSNaCs. As will be explained below, the function of the molecule we created is confined primarily to the peripheral sensory neurons. The expectation is that actions at multiple sites within the same system/network should result in additive or synergistic effects [33]. The location of our multiple targets to the peripheral sensory system also allows for synergistic effects with agents that act on the extended elements of the initiating and integrating (CNS) features of chronic pain (i.e., opioids, gabapentinoids, antidepressants, anti-inflammatory agents).

Molecular Targets that Initiate and Maintain Chronic Pain—*Voltage Sensitive Sodium Channels.* Once initial damage or insult occurs to peripheral sensory neurons, there are some notable similarities in the adaptive consequences evident in these neurons. One of the most investigated molecular mechanisms leading to neuropathic pain syndromes is an upregulation of the activity of peripheral VSNaCs [34–38]. Outside of the CNS, and within sensory neurons, Nav 1.3, Nav 1.6, and Nav 1.7 isoforms comprise the primary TTX-sensitive VSNaCs (Nav 1.3 is a VSNaC primarily expressed during fetal development but which can reappear after nerve injury). Nav 1.8 and Nav 1.9 are the TTX-insensitive isoforms present in sensory neurons. The Nav 1.7 channel is located along the projections of and cell bodies of the slowly conducting nociceptive neurons [36,39]. The role of the Nav 1.7 channel in both acute and chronic pain in humans and other animals has been well demonstrated by genetic manipulation of this channel in mice and through identified loss and gain

of function mutations in humans (see references in [40]). The Nav 1.7 channel has been particularly linked to pain resulting from inflammation [41]. The upregulation of Nav 1.7 during inflammation contributes to the increased generation and conduction of action potentials in chronic pain syndromes. The contribution of the Nav 1.7 channel to initiation of action potentials is related not only to its own activation characteristics [42], but also to its ability to amplify generator potentials and promote the activation of other sensory neuron VSNaCs such as the Nav 1.8 channel [38,42]. The TTX-resistant VSNaC, Nav 1.8, which interacts with Nav 1.7, is implicated in the early, developmental, stages of chronic pain syndromes [43]. The Nav 1.8 channel has been linked to development of both inflammatory and neuropathic pain conditions. The expression of Nav 1.8 channels increases significantly in both myelinated and unmyelinated sensory axons after nerve damage in animals [37]. In our appraisal of the literature, the upregulation of the activity of Nav 1.7 and Nav 1.8 channels in peripheral sensory neurons constitutes a common component of induction and maintenance of chronic pain syndromes [39,44,45]. The targeting of the Nav 1.7 channel for treatment of chronic pain has not escaped the attention of the pharmaceutical industry [1] and even a recent article authored by Skolnick and Volkow [46] presents the Nav 1.7 channel as a worthy target to supplant the use of opiates. In the development of VSNaC blockers as pain therapeutics the mantra of target specificity has consumed the pharmaceutical industry. Given the presence of the Nav 1.8 channels, as well as the Nav 1.7 (and Nav 1.9) on adult sensory neurons, particularly the small diameter, unmyelinated C fibers, medicinal chemists have targeted either the Nav 1.7, or the Nav 1.8, channels to generate molecules that inhibit one channel type with minimal effect on other channels. Thus, A-803467 is a compound developed by Abbott Laboratories [47] that in vitro showed at least a 1000-fold selectivity for Nav 1.8, but when used in vivo, the doses needed were in ranges that produced blood concentrations that could affect other Nav channels, such as Nav 1.5, which maintains pacemaker function in the heart [48]. Several Nav 1.7 highly selective compounds have reached the clinical stage of development [49], but the indications for these compounds have been restricted to those known to result from Nav 1.7 gain of function mutations [50] (with hope of extensive off-label use). The strategy of selectivity is definitively important, given the role of Nav channels in both the CNS and periphery (heart, kidney, etc.), and the desire to prevent untoward effects. One can, however, contemplate a compound affecting more than one Nav channel (two) while leaving others unaffected. This can be accomplished both by endowing the compound with high anatomical selectivity (e.g., restricted to the periphery) as well as molecular structure characteristics that allow some overlap in concentration necessary for action at the two chosen channels, but significant separation from concentrations necessary to affect channels important for functions other than conduction of pain impulses.

If one were to choose two Nav channels to simultaneously inhibit, one would choose the Nav 1.8 and Nav 1.7 channels. This choice is based on the

desire to affect several pain modalities, and the fact that these two channels can appear on the same sensory neuron and thus both participate in conduction of acute pain information and/or become upregulated in chronic pain syndromes [42,51]. Two types of interactions of Nav 1.7 and 1.8 are particularly important. A recent study by Klein et al. [52] demonstrated an anatomical difference in distribution of Nav 1.7 and 1.8 channels along the length of the axon of the C fibers, with the Nav 1.8 channels primarily occupying the peripheral end of the fiber closest to the nociceptor and Nav 1.7 being located along the sensory neuron projections and particularly along the portion of the axon that enters the spinal cord [52]. A drug affecting both Nav 1.7 and 1.8 could, theoretically, be more advantageous by diminishing action potentials through the whole length of a sensory neuron. The other aspect is the already mentioned electrophysiologic interaction between Nav 1.7 and 1.8 channels [42]. The slow rectification of Nav 1.7 in the vicinity of Nav 1.8 maintains a train of Nav 1.8 mediated action potentials [42]. Blocking either one of the Nav 1.7 or 1.8 channels extensively could generate a positive effect, but reducing the activity of both could provide a broader spectrum of action (across a variety of nociceptors) and additive effects within a particular neuron.

Although Kindolor acts on both Nav 1.7 and Nav 1.8 channels, Kindolor's action can easily be distinguished from "non-selective" agents such as phenytoin, lamotrigine, carbamazepine or lidocaine, by the fact that Kindolor is more potent against the tetrodotoxin-resistant Nav 1.8 channel than the tetrododoxin- sensitive channels (e.g., Nav 1.7). Kindolor is even less potent against the brain Nav 1.2 channel [53]. The "non-selective" sodium channel blockers, mentioned above, have the opposite order of potency [54]. Kindolor, unlike the "non- selective" agents [55], has little, or no, effect on Nav 1.5, a channel with intermediate sensitivity to tetrodotoxin [56]. Kindolor has no effect on acute pain as witnessed in the early stage of the formalin test in mice (i.e., it is not an analgesic), but reduces the later (chronic) pain to pre-formalin injection levels (acts as an antihyperalgesic) [4,7]. Shannon et al. [57] tested twelve anticonvulsants in the same formalin test. Most were known Nav channel blockers but all had equivalent effects on both the early and late phase of the formalin test. Thus, these sodium channel blockers affected acute and chronic pain in a similar manner, while Kindolor targets the chronic pain component. Finally, most of the tested anticonvulsants produced locomotor impairment at the same or lower dose compared with doses necessary to produce a significant effect in the formalin test [57]. Ten times or more than the therapeutic dose of Kindolor is necessary to produce any effect on locomotor activity or coordination (unpublished data).

NMDA Subtype of Glutamate Receptors. The role of excitatory amino acids, particularly glutamate, in the physiology of normal pain sensing and transmission and in chronic pain phenomena was originally established in the late 1980s [58–60] and recent reviews [61] further emphasize the role of glutamate and its receptors in chronic pain. Mechanically induced

(constriction, transection) chronic pain syndromes have been demonstrated to involve alterations in the quantity and/or activity of ionotropic glutamate receptors (AMPA, kainate and NMDA) in the peripheral projections of nociceptive neurons, in their soma within the dorsal root ganglia (DRG), and in synapses of the primary and second order neurons in the substantia gelatinosa (lamina 1 and 2) of the spinal cord [60,62–66]. What needs to be emphasized is that sensory neuron activation or damage produces increased release of glutamate from both the "peripheral" and "central" branches of the primary afferents [61] and the released glutamate can act on nearby NMDA receptors (for instance in nociceptor regions] to activate sensory nociceptors (e.g., TRPV1 receptors, see below) and in the long run, contribute to peripheral sensitization [67]. The release and actions of glutamate within the dorsal root ganglia (DRG) and its interaction with NMDA receptors has also gained prominence as a mechanism of amplification of sensory signals [68–70]. Thus, NMDA receptors are intimately involved in both the initiation and amplification of a pain sensation and its transmission into the CNS, as well as in the phenomenon termed "wind up", wherein the transmission of signal between primary and second order sensory neurons is amplified in conditions of repetitive sensory input as seen after nerve injury [58,71]. Changes in the expression levels of NMDA receptor subunit proteins are seen in animal models of mechanically- induced sensory nerve damage. Such changes are evident both in the DRG neurons and the second order neuron soma in the spinal cord. Both the peripheral and spinal cord NMDA receptor upregulation is thought to contribute to tactile allodynia and thermal hyperalgesia seen in neuropathic (mechanically-induced) chronic pain syndromes [66]. In examining the literature it becomes obvious that changes in the quantity of GluN2B (NR2B) subunits may play the most important role in the hypersensitivity to glutamate in the DRG and the dorsal horn neurons [72–75]. The persistent increase in NR2B subunit expression in chronic pain syndromes has led to proposals that antagonists selective for NR2B-containing NMDA receptors (e.g., ifenprodil or conantoxin-G) may be particularly effective treatments for chronic pain syndromes [66,76,77]. Mention also needs to be made of the upregulation of NMDA receptors during chronic, high dose treatment with opioids [78]. The opiate-dependent increase in NMDA receptors in the DRG cell bodies has been considered a significant component of the development of hyperalgesia during chronic treatment with opioids [79]. Thus, NMDA receptor antagonists may counteract the hyperalgesia and may also prevent the development of tolerance to opiates [80]. The effect of inhibiting the NMDA receptor on the development of tolerance to opiates was initially demonstrated by Trujillo and Akil [81]. They administered dizocilpine during chronic treatment with morphine, to block the development of tolerance to the analgesic effects of morphine. On the other hand, using competitive NMDA antagonists, including the glycine B site antagonist, HA-966, together with a single, acute dose of morphine,

Fischer et al. [82] demonstrated a significant potentiation of morphine's analgesic actions by HA-966 and the other competitive NMDA antagonists. The NMDA receptor antagonists tested in the studies of Fischer et al. [82] produced no analgesic effects on their own. An important question is whether one needs to have an NMDA receptor antagonist enter the CNS to produce effects on either acute analgesia produced by opiates or to produce a block in the development of tolerance to opiates? The answer to this question is no, and peripherally acting NMDA receptor/glycine B site antagonists can block the development of tolerance to morphine [83]. Our own studies demonstrate that Kindolor, which is confined to the periphery, synergistically potentiates opiate effects in animals pre-treated with CFA to produce chronic pain. Additionally, it has been demonstrated that peripherally restricted opiates (e.g., loperamide) generate tolerance to their own analgesic effects when used to ameliorate pain in a chronic pain model (spinal cord ligation), and NMDA receptor antagonists can block the development of tolerance to loperamide [84]. The point that we are stressing here, is that tolerance can develop to opiates which do not enter the CNS, and opiate tolerance can be blocked by NMDA antagonists which do not enter the CNS (but this is not to say that there are no CNS mechanisms contributing to opiate tolerance [85]). Another important feature of NMDA receptors containing the NR2B subunits in the region of nociceptors is their interaction with TRPV1 receptors [86]. TRPV1 receptors and NR1 and NR2B proteins physically interact, and activation of the NMDA receptor leads to phosphorylation and sensitization of the TRPV1 receptor [86,87]. The clinical importance of targeting peripheral NMDA receptors has recently been well emphasized [88,89].

In summary, there are several ways in which NMDA receptors can modulate the analgesic actions of opiate receptor activation, both directly and indirectly by reducing or eliminating development of tolerance. Directly, there is evidence that the mu opiate receptor (MOR) can interact with the NR1 subunit of the NMDA receptor in brain [90] and possibly in the peripheral nervous system in Nav 1.8-containing neurons. In the NR1 receptor associated state, the opiate receptor functions to generate analgesia and does not display acute tolerance [91]. This NMDAR-MOR protein-protein interaction can be eliminated by association of the opiate receptor with an agonist and subsequent internalization of the MOR and development of acute tolerance. NMDA receptor antagonists can maintain the association of the mu opiate receptor with NR1 to maintain analgesic activity of opiates and prevent acute tolerance. With regard to the chronic administration of opiates, the development of tolerance during extended treatment is well known [92] and as mentioned above, compounds with NMDA receptor antagonist action, such as Kindolor, can prevent chronic tolerance development and maintain opioid efficacy.

Hypothesis and Goals. It would be of some interest to develop a multifaceted molecule with a good safety profile that could engage multiple sites on the pain sensory system. We believe that we have through

design and serendipity produced one of the first of such molecules, Kindolor. Our goal is to develop this chronic pain medication to the point that it can be tested in human trials.

SPECIFIC AIMS

This grant was awarded as part of the NIH effort to ameliorate the national opioid crisis, helping to End Addiction Long-term Initiative, the NIH-HEAL Initiative. The award mechanism is a cooperative UG3/UH3 from the National Institute on Drug Abuse (NIDA). The UG3 cooperative agreement is part of the bi-phasic approach to funding exploratory and developmental research. The UG3 provides support for the first phase of establishing feasibility. After achieving a milestone, the UH3 cooperative award provides support for the second phase of exploratory and developmental research activity. The duration of the UG3 award is 2 years, and of the UH3 award is 3 years.

The aims for years 1 and 2 were to develop the synthesis and scale-up of cGMP Kindolor to produce multi-kilogram amounts of Kindolor for pre-clinical studies, and to produce a formulation for oral drug administration to humans. In addition, pre-clinical IND-enabling studies were to be performed in years one and two. These studies include single-dose pharmacokinetic studies and metabolite profiling in two species, completion of in vitro metabolism studies, and escalating dose and repeat dose toxicology and toxicokinetic studies of Kindolor in two species. In addition, safety (cardiovascular, respiratory and CNS) studies, and mutagenicity and genotoxicity studies will be completed in the first two years.

End of Year 2 Milestone: Complete a Pre-IND Meeting with the FDA with a Resultant Positive Response to Completed Studies and Written Responses to Questions regarding Completion of Data for an IND Application

The aims for year 3 are to obtain an IND and IRB approval for the Phase 1a clinical trial, to generate the needed quantities of cGMP Kindolor and its formulation for completion of non-clinical studies (13-week toxicology/toxicokinetic studies in two species) and for the Phase 1a clinical trial (placebo-controlled, single dose escalating study of safety, tolerability and pharmacokinetics of Kindolor). The aims for year 4 are to complete the Phase 1a study, obtain IRB approval for and complete the Phase 1b clinical trial (placebo-controlled, multiple dose escalating study of safety, tolerability and pharmacokinetics of Kindolor), and to complete non-clinical reproductive toxicology studies.

End of Year 4 Milestone: Establish Safety for Use of Kindolor in Humans

The aims for Year 5 are to obtain IRB approval and complete a Phase 2a placebo-controlled clinical trial to evaluate safety, tolerability and efficacy of multiple daily (14 days) dosing of Kindolor for moderate to severe pain

of diabetic neuropathy, and to complete non-clinical studies of phototoxicity of Kindolor.

APPROACH AND RESULTS TO DATE

Kindolor Efficacy. The effect of Kindolor to ameliorate pain in five animal models is described in our publication [56], and was provided as preliminary data on efficacy in our grant application. In addition we showed in our preliminary data a synergistic effect of Kindolor in combination with morphine or aspirin to reduce inflammatory pain (caused by Complete Freund's Adjuvant) or diabetic neuropathic pain (caused by streptozotocin treatment), respectively. In these experiments, Kindolor and the opiate or NSAID were administered either alone, at doses that were ineffective at reducing pain, or in combination. The combination of the drugs completely reversed the chronic pain. We have since expanded these studies to test the effects of Kindolor in combination with synthetic and semisynthetic opioids, as well as other NSAIDs. These studies were performed using animal models of inflammatory (Complete Freund's Adjuvant, CFA) and arthritic (monoiodoacetate-induced) chronic pain. The effect of Kindolor was found to be additive or synergistic with oxycodone (semsynthetic opioid) and methadone (synthetic opioid), as well as with the NSAID, diclofenac, in the CFA model, in which mechanical pain was measured. Kindolor also potentiated the effect of the synthetic atypical opioid tramadol when mechanical pain was measured in the monoioidoacetate model of arthritic pain. These results demonstrate that Kindolor has the potential to reduce the use of many classes of opioids for treating chronic pain, as well as to reduce the need for high doses of NSAIDs (which can produce severe GI disturbances). The combination of Kindolor (once it has been approved for use in humans) with opioids can help to reduce opioid doses below the level where addiction is an issue, and to reduce the doses of NSAIDs, such that adverse effects can be avoided, while still allowing for optimal control of chronic pain.

Kindolor Synthesis and Formulation. We worked with a CRO which has assisted us in developing a method for the cCGMP synthesis of the tosylate salt of Kindolor that we have used in non-clinical studies, and that can be used for clinical studies in humans. We demonstrated that this formulation, administered as a suspension to animals, provides higher circulating levels of Kindolor than other formulations that we have previously used (Tabakoff, B and Hoffman, PL, unpublished results). A solid formulation of Kindolor tosylate (capsule, tablet) is being developed for use in human trials.

Non-clinical Studies. We also worked with another CRO to perform single-dose pharmacokinetic studies, escalating dose and repeat dose toxicology and toxicokinetic studies in rodents and non-rodent species. These studies to date have provided pharmacokinetic parameters and indications of dose levels that are well tolerated after a single administration or after repeated dosing for 7 days. The pharmacokinetic

and toxicokinetic data from these studies allows us to determine appropriate dose or plasma levels and dosing protocols to be used for longer-term non-clinical toxicology studies, which are currently planned to be performed in rats and minipigs under GLP conditions. The results of all of these studies will provide the data needed to determine the doses that will be used in the first in human studies that are planned for year 3.

We have also completed cardiovascular, respiratory and CNS safety studies, as well as genotoxicity studies, all of which have provided no evidence for adverse effects.

We are in the process of compiling all results needed for the pre-IND meeting with the FDA. We are on track to meet the milestone for proceeding to the UH3 grant.

DISCUSSION

The pharmaceutical industry has, since the introduction of the term “Magic Bullet”, focused attention on drugs with a high affinity for a single site of action. In many cases, and particularly in complex pathological conditions, this focus has not been fruitful. Chronic pain is such a condition. In the search for alternatives to opiates, with their attendant adverse side effects, high affinity ligands for just one of the many targets that contribute to chronic pain have not provided broad spectrum, highly efficacious products. In fact, the high level of inhibition of certain individual targets has led to untoward effects (e.g., the insensitivity to heat by blocking the TRPV1 receptor has resulted in patients acquiring burns, and the high level of inhibition of sodium channels (Nav 1.7) may have effects similar to genetic polymorphisms which inactivate the Nav 1.7 channel and result in self-mutilation as a result of analgesia).

Nowadays, creating molecules that can simultaneously and selectively interact with two or more targets along a biological pathway has become an accepted concept in drug discovery, and this approach has been determined to be more effective than a single target approach. Calculations demonstrate that partial inhibition of more than one target in a pathway can produce a more effective modulation of a pathway than almost complete inhibition of a single target.

Kindolor is an NCE that has been engineered to address the overactivity of three generators and conductors of chronic pain in the peripheral sensory system. Kindolor is an anti-hyperalgesic and not an analgesic. It inhibits NMDA receptors of the glutamate excitatory system and inhibits the function of Nav 1.8 and Nav 1.7 voltage sensitive sodium channels that conduct pain information from the periphery to the spinal cord. Figure 1 illustrates the three (peripheral) targets in the chronic pain pathway which are upregulated in chronic pain conditions, and are inhibited simultaneously by Kindolor. The illustration details the three sites at which Kindolor modulates the activity of the peripheral sensory neurons. The boxes in the Figure enlarge the schematic rendition of the actions of Kindolor. The illustrated actions of Kindolor are extrapolated from its

measured actions on receptors and ion channels in model systems and demonstrations in the literature on the participation of the receptors/channels in generation and conduction of pain formation in sensory systems. It remains to be directly demonstrated that the antihyperalgesic actions of Kindolor are specifically mediated by the illustrated events [56].

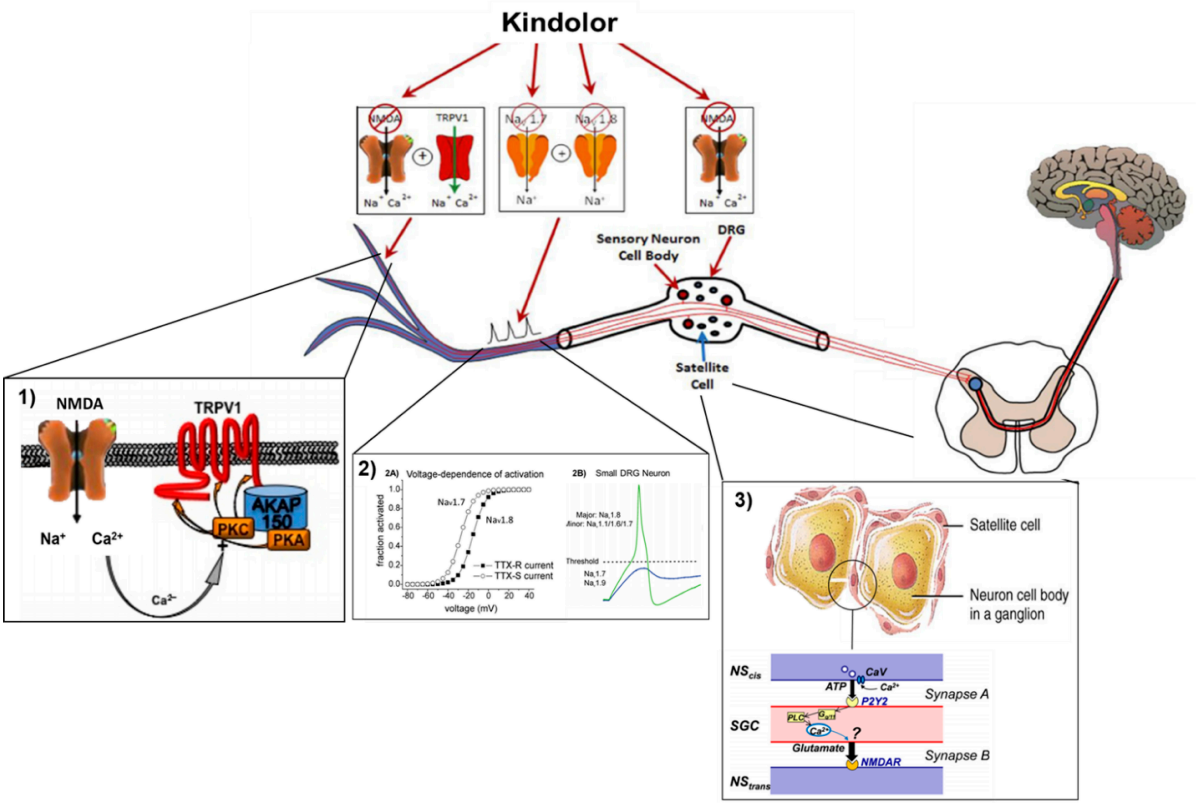

Figure 1. Three (peripheral) targets in the chronic pain pathway that are upregulated in chronic pain conditions, and are inhibited simultaneously by Kindolor. The illustrated actions of Kindolor are extrapolated from its measured actions on receptors and ion channels in model systems and demonstrations in the literature on the participation of the receptors/channels in generation and conduction of pain in sensory systems. It remains to be directly demonstrated that the actions of Kindolor are specifically mediated by the illustrated events [56].

In Figure 1,

- **Box 1:** Illustrates the interaction of NMDA receptors with TRPV1 receptors in the sensory terminals of nociceptors. NMDA receptors are co-localized with the TRPV1 receptors, and the activation of the NMDA receptors by glutamate released into the surrounding milieu upon local irritation, generates a phosphorylation cascade via PKC and PKA. The end-product of this cascade is the phosphorylation of TRPV1 and an increase in its activity in generating pain signals [69]. Kindolor inhibits NMDA receptor function and significantly dampens this enhanced excitability.
- **Box 2:** Focuses attention on the conduction of pain information from

the nociceptor terminals. Nav 1.7 channels have a low (hyperpolarized) activation threshold, and this activation initially (prior to responding with an action potential) produces a change in membrane potential which can activate the Nav 1.8 channels, which quickly respond with an action potential [90]. Both the Nav 1.8 and Nav 1.7 action potentials are propagated to the dorsal root ganglia where the cell bodies of the sensory neurons reside [91]. Kindolor inhibits both the Nav 1.8 (more potently) and Nav 1.7 channels and thus depresses both the excessive signal generation by Nav 1.7 and increased conduction by both Nav 1.8 and Nav 1.7 in patients suffering from chronic pain.

- **Box 3:** Illustrates events happening within the sensory dorsal root ganglia. Within the dorsal root ganglia, a close juxtaposition is evident between two cell bodies of the sensory neurons and satellite glial cells. The satellite glial cells are "sandwiched" between the two cell bodies of the sensory neurons, and this structure is called a "sandwich synapse". One of the important functions of the sandwich synapse is to amplify the signals reaching the dorsal root ganglia. As illustrated, this occurs via release of ATP by the cell body of the initially activated neuron. The ATP activates the purinergic receptors (P2Y2) on the satellite glial cells, and the satellite glial cells respond by releasing glutamate. The glutamate activates NMDA receptors on the neighboring (juxtaposed) sensory neuron cell body and instigates depolarization and conduction of signal to the spinal cord [92]. Kindolor, by inhibiting the NMDA receptor on cell bodies of the sensory neurons prevents the amplification of the signal mediated by the sandwich synapses [56,69,90–92].

Prior to submitting our grant application, Lohocla Research Corporation had pursued studies of Kindolor designed to demonstrate the non-clinical efficacy of the drug to ameliorate chronic pain, using several different animal models. Some of these studies were carried out in our own laboratories and others at other sites, providing evidence for the robust effect of the drug in various models, as well as the replicability of the efficacy data. We also performed preliminary pharmacokinetic studies showing that Kindolor does not enter the brain to any significant extent, and we contracted with CROs to determine the pathways of Kindolor metabolism, interaction with drug transporters and plasma protein binding, as well as some genotoxicity studies. The transporter studies, in particular, provided us with the information needed to understand the absorption characteristics of the drug and its peripheral (vs CNS) site of action. We also performed some toxicology and toxicokinetic studies, also with CROs, to demonstrate the safety of the drug and its lack of addictive potential in a conditioned place preference model. In terms of efficacy, we performed further studies of the effect of Kindolor to reduce chronic pain when administered in combination with different classes of opioids and

NSAIDs, which showed that Kindolor can have an "opiate (or NSAID)-sparing" effect. All of these studies, as well as the work completed in the initial period of our grant support, are shown in the Gantt chart (Table 1) below.

Table 1. Gantt Chart Listing the Work and Documents Being Prepared for the Kindolor IND submission to the FDA. The Gantt chart illustrates the studies that are needed for an IND application for Kindolor, the progress that has been made to date, and the studies that are currently underway.

(a) Module 1: Regional Administrative Information.

Study	Status	June				June/July					August			
		1–5	8–12	15–19	22–26	29–3	6–10	13–17	20–24	27–31	3–7	10–14	17–21	24–28
Forms														
Form FDA 1571	**In progress** (Lohocla)													
Form FDA 3456	**In progress** (Lohocla)													
Cover Letter-Initial IND Application	**In progress** (Lohocla)													
Administrative Information	**In progress** (Lohocla)													
References														
Letter of Authorization, Statement of Right of Reference, List of Authorized Persons to Incorporate by Reference, Cross-Reference to Previously Submitted Information	**In Progress** (FastTrack)													
Meetings														
Meeting Request, Meeting Background Materials, Correspondence Regarding Meetings	**In Progress** (FastTrack)													
Other Correspondence														
Pre-IND Correspondence	Complete													
Request for Comments and Advice	Complete													
Environmental Assessment–Categorical Exclusion	**In progress** (FastTrack)													
General Investigational Plan for Initial IND	**In progress** (Lohocla)													
Labeling														
Investigators Brochure	**In progress** (Lohocla)													
Investigational Drug Labeling	**In progress** (Lohocla)													

Table 1. *Cont.*

(b) Module 2: Common Technical Document Summaries.

Study	Status	June				June/July					August			
		1–5	8–12	15–19	22–26	29–3	6–10	13–17	20–24	27–31	3–7	10–14	17–21	24–28
Introduction														
Investigation New Drug Summary Introduction	**In progress** (Lohocla)													
Nonclinical Overview														
Nonclinical Testing Strategy	**In Progress** (Lohocla)													
Pharmacology	**In Progress** (Lohocla)													
Pharmacokinetics	**In Progress** (Lohocla)													
Toxicology	**In Progress** (Dr. McLain)													
Overview and Conclusions	**In Progress** (Lohocla)													
Clinical Overview														
Phase I Clinical Trial Protocol	**In Progress** (FastTrack)													

(c) Module 3: Quality.

Study	Status	June				June/July					August			
		1–5	8–12	15–19	22–26	29–3	6–10	13–17	20–24	27–31	3–7	10–14	17–21	24–28
Drug Substance														
General Information	Complete													
Manufacture														
Final Procedure of Kindolor Synthesis Route	Complete (Hangzhou Co.)													
Preliminary Report for Kindolor Synthetic Optimization	Complete (Enantiotech)													
Process Research and Development for the Synthesis of Kindolor	Complete (AMRI)													
Characterization														
Mass Spectrometric Analysis of Kindolor	Complete (UCD)													
Characterization of Kindolor (XRPD, DSC, TGA, pKa, HPLC)	Complete (SSCI)													
Control of Drug Substance	Complete (AMRI)													

Table 1. *Cont.*

(c) Module 3: Quality.

Study	Status	June				June/July					August			
		1–5	8–12	15–19	22–26	29–3	6–10	13–17	20–24	27–31	3–7	10–14	17–21	24–28
Reference Standards or Materials														
Reference Standard Development of Kindolor by NMR, FTIR, Mass Spectroscopy, UPLC, DSC, and XRPD	Complete (AMRI)													
Container Closure System	**TBD**													
Stability														
Drug Product														
Description and Composition of Drug Product	Complete													
Pharmaceutical Development														
Salt Selection for Kindolor	Complete (Catalent)													
Manufacture														
Scale up of Kindolor Tosylate Salt	Complete (Catalent)													
Manufacture of Kindolor Tosylate Salt	Complete (AMRI)													
Batch analysis and process specification for Kindolor Tosylate Salt manufacture	Complete (AMRI)													
Control of Excipients	Complete													
Control of Drug Product														
Physical characterization of Kindolor Tosylate salt by appearance, XPRD, DSC, NMR, FTIR, Mass Spec., UPLC	Complete (AMRI)													
Impurity characterization by UPLC (area %)	Complete (AMRI)													
Analysis of residual solvents and water content	Complete (AMRI)													
Reference Standards or Materials	Complete													
Container Closure System	**TBD**													
Stability														
Evaluation of Kindolor Tosylate salt Stability for 2 years	**In Progress** (AMRI)													

Table 1. *Cont.*

(c) Module 3: Quality.

Study	Status	June				July					August			
		1–5	8–12	15–19	22–26	29–3	6–10	13–17	20–24	27–31	3–7	10–14	17–21	24–28
Placebo														
Description and Composition of Placebo														
Pharmaceutical Development														
Manufacture														
Control of Excipients														
Control of Investigational Medicinal Product														
Container Closure System														
Stability														

(d) Module 4: Nonclinical Study Reports.

Study	Status	June				July					August			
		1–5	8–12	15–19	22–26	29–3	6–10	13–17	20–24	27–31	3–7	10–14	17–21	24–28
Pharmacology														
Pharmacodynamics														
Kindolor Radioligand Receptor Binding and Functional Assays	Complete (PDSP)													
Kindolor Computerized Molecular Modeling	Complete (Backos, UCD)													
Effect of Kindolor on Displacement of [3H] Kainate from Rat Cortical Membranes	Complete (Lohocla)													
Effect of Kindolor on Displacement of [3H] SR95531 from Rat Cortical Membranes	Complete (Lohocla)													
Effect of Kindolor on Displacement of [3H] MK-801 from Rat Cortical Membranes	Complete (Lohocla)													
Effect of Kindolor on Displacement of [3H] Ifenprodil from Rat Cortical Membranes	Complete (Lohocla)													
Effect of Kindolor on Displacement of [3H] Flunitrazepam from Rat Cortical Membranes	Complete (Lohocla)													

Table 1. *Cont.*

(d) Module 4: Nonclinical Study Reports.

Study	Status	June				June/July					August			
		1–5	8–12	15–19	22–26	29–3	6–10	13–17	20–24	27–31	3–7	10–14	17–21	24–28
Pharmacology														
Pharmacodynamics														
Effect of Kindolor on Displacement of [3H] Batrachotoxinin from Rat Cortical Membranes	Complete (Lohocla)													
Effect of Kindolor on Displacement of [3H] AMPA from Rat Cortical Membranes	Complete (Lohocla)													
Effect of Kindolor on Displacement of [3H] 5,7-dichlorokynurenate from Rat Cortical Membranes	Complete (Lohocla)													
Effect of Kindolor on Displacement of [3H] CGP 39653 from Rat Cortical Membranes	Complete (Lohocla)													
Effect of Kindolor on Displacement of [3H] RO 15-1788 from Rat Cortical Membranes	Complete (Lohocla)													
Kindolor Inhibition of NaV 1.7 and 1.2 Channels	Complete (Lohocla)													
Nav1.8 Neursolutions Report	Complete (Neurosolutions)													
Further electrophysiological investigation of the effects of Kindolor on tetrodotoxin-resistant sodium currents (Nav 1.8 use dependence at 10 Hz)	Complete (Neurosolutions)													
Screening of Lohocla Compounds Kindolor against human Nav 1.5 using QPatch Automated Electrophysiology	Complete (Neurosolutions)													

Table 1. *Cont.*

(d) Module 4: Nonclinical Study Reports.

Study	Status	June				June/July					August			
		1–5	8–12	15–19	22–26	29–3	6–10	13–17	20–24	27–31	3–7	10–14	17–21	24–28
Pharmacology														
Pharmacodynamics														
Effect of Kindolor on the Electrophysiology of Recombinant NMDA Receptors in Transfected HEK293 Cells	Complete (Lovinger, NIAAA)													
The effects of Kindolor on recombinant NMDA receptors expressed in cultured HEK293 cells	Complete (Woodward, MUSC)													
The Effects of Kindolor on recombinant NMDA receptors containing NR3 subunits expressed in cultured HEK293 cells	Complete (Woodward, MUSC)													
Effect of Kindolor on voltage-gated calcium channels	Complete (Lohocla)													
Efficacy														
Effect of Kindolor in the rat CFA model of inflammatory pain	Complete (Lohocla)													
Effect of Kindolor in the rat STZ model of diabetes-induced neuropathic pain	Complete (Lohocla)													
Effect of Kindolor in the rat SNL model of Neuropathic pain	Complete (NINDS)													
Effect of Kindolor in the Mouse Formalin Inflammatory Pain Model	Complete (NINDS)													
Effect of Kindolor on Cisplatin Induced Neuropathic Pain	Complete (Lohocla)													
Lack of Tolerance to the effect of Kindolor in the Complete Freund's Adjuvant inflammatory pain model	Complete (Lohocla)													

Table 1. *Cont.*

(d) Module 4: Nonclinical Study Reports.

Study	Status	June				June/July					August			
		1–5	8–12	15–19	22–26	29–3	6–10	13–17	20–24	27–31	3–7	10–14	17–21	24–28
Pharmacology														
Efficacy														
Effects of Kindolor Administered PO Twice in a 15-Day Mono-iodoacetate Induced Knee Pain in Male Sprague Dawley Rats	Complete (Bolder Biopath)													
Effects of Kindolor in an 8-Day Model of Adjuvant-Induced Monoarthritis in Sprague Dawley Rats	Complete (Bolder Biopath)													
Secondary Pharmacodynamics														
Effects of Kindolor on dopamine stimulated Adenylyl Cyclase activity in cells transfected with opiate receptors and AC isoforms	Complete (Yoshimura, LSU)													
Safety Pharmacology														
Kindolor receptor binding hERG Assay Assessment	Complete (PDSP)													
Kindolor Tosylate: A Respiratory Assessment Following Oral Gavage Dosing to Plethysmograph-Restrained Sprague Dawley Rats	Complete (CRL)													
Kindolor Tosylate: An Irwin Test Assessment Following Oral Gavage Administration to Sprague Dawley Rat	Complete (CRL)													
Kindolor Tosylate: Cardiovascular Safety Assessment in Minipigs	Complete (CRL)													
Kindolor Tosylate electrophysiological hERG Assay Assessment	**In progress**													

Table 1. *Cont.*

(d) Module 4: Nonclinical Study Reports.

Study	Status	June				June/July					August			
		1–5	8–12	15–19	22–26	29–3	6–10	13–17	20–24	27–31	3–7	10–14	17–21	24–28
Pharmacology														
Pharmacodynamic Drug Interactions														
Effects of Kindolor Alone or in Combination with Oxycodone in 10-day Model of Adjuvant-Induced Monoarthritis in Sprague Dawley Rats	Complete (Bolder Biopath)													
Effects of Kindolor Alone or in Combination with Methadone in a 7-day Model of Adjuvant Induced Monoarthritis in Sprague Dawley Rats	Complete (Bolder Biopath)													
Effects of Kindolor Alone or in Combination with Diclofenac in a 4-day Model of Adjuvant-Induced Monoarthritis in Sprague Dawley Rats	Complete (Bolder Biopath)													
Two Stage Study of the Effects of Kindolor Alone or in Combination with Tramadol Administered PO in a 29-Day Mono-iodoacetate Induced Knee Pain in Male Sprague Dawley Rats	Complete (Bolder Biopath)													
Pharmacokinetics														
Analytical Methods and Validation Reports														
Validation of a Liquid Chromatographic Method for the Determination of Kindolor in Dose Formulations	Complete (CRL)													
Note: Other validation reports are included in the CRL reports														

Table 1. *Cont.*

(d) Module 4: Nonclinical Study Reports.

Study	Status	June				June/July					August			
		1–5	8–12	15–19	22–26	29–3	6–10	13–17	20–24	27–31	3–7	10–14	17–21	24–28
Pharmacokinetics														
Absorption														
In Vitro Interaction Studies of Kindolor with human ABC (efflux) Transporters and with human Uptake Transporters	Complete (Xenotech)													
Single Dose Study of Kindolor Tosylate by Oral Gavage in Rats	**In Progress** (CRL)													
Single Dose Study of Kindolor Tosylate by Oral Gavage in Rabbits	Complete (CRL)													
Single Dose Study of Kindolor Tosylate by Oral Gavage in Minipigs	Complete (CRL)													
Rising Dose, Single Dose, and Multiple Dose Tolerance Study of Kindolor Tosylate by Oral Gavage in Minipigs	Complete (CRL)													
Distribution														
Plasma Protein Binding of Kindolor	Complete (Xenotech)													
Radiolabeled Distribution Study of Kindolor Tosylate in Rats	**Planned**													
Metabolism														
Kindolor: Cytochrome P450 Induction in Cultured Human Hepatocytes	**In Progress** (Xenotech)													
In Vitro Phase I and II Metabolism of Kindolor	Complete (Eurofins)													
Metabolite Characterization of Kindolor in Rat, Dog, and Human Hepatocytes	Complete (Xenotech)													
In Vitro Evaluation of Kindolor as an Inhibitor of Cytochrome P450 Enzymes	Complete (Xenotech)													

Table 1. *Cont.*

(d) Module 4: Nonclinical Study Reports.

Study	Status	June				June/July					August			
		1–5	8–12	15–19	22–26	29–3	6–10	13–17	20–24	27–31	3–7	10–14	17–21	24–28
Pharmacokinetics														
Excretion														
Single Dose Study of Kindolor Tosylate by Oral Gavage in Rats	**In Progress** (CRL)													
Single Dose Study of Kindolor Tosylate by Oral Gavage in Rabbits	Complete (CRL)													
Single Dose Study of Kindolor Tosylate by Oral Gavage in Minipigs	Complete (CRL)													
Rising Dose, Single Dose, and Multiple Dose Tolerance Study of Kindolor Tosylate by Oral Gavage in Minipigs	Complete (CRL)													
Toxicology														
Single Dose Toxicity														
Rising Dose Study of Kindolor Tosylate by Oral Gavage in Rats	**In Progress** (CRL)													
Rising Dose Study of Kindolor Tosylate by Oral Gavage in Rabbits	Complete (CRL)													
Rising Dose Study of Kindolor Tosylate by Oral Gavage in Minipigs	Complete (CRL)													
Kindolor: Maximum Tolerated Dose (MTD) Study in Sprague Dawley Rats	Complete (Advinus)													
Repeat Dose Toxicity														
Kindolor Tosylate 14-Day Repeat Dose Study in Rat	**In Progress** (CRL)													
Kindolor Tosylate 14-Day Repeat Dose Study in Minipig	**In Progress** (CRL)													
Multiple-Dose Tolerance Study of Kindolor Tosylate by Oral Gavage in Rabbits (7-days)	Complete (CRL)													
Multiple-Dose Tolerance Study of Kindolor Tosylate by Oral Gavage in Minipigs (7-day)	Complete (CRL)													

Table 1. *Cont.*

(d) Module 4: Nonclinical Study Reports.

Study	Status	June				June/July					August			
		1–5	8–12	15–19	22–26	29–3	6–10	13–17	20–24	27–31	3–7	10–14	17–21	24–28
Pharmacokinetics														
Repeat Dose Toxicity														
Kindolor: 7-Day Repeated Dose (Oral) Toxicity and Toxicokinetic Study in Sprague Dawley Rats	Complete (Advinus)													
Kindolor: 28-Day Oral (Gavage) Toxicity and Toxicokinetics Study in Sprague-Dawley Rats with Two Week Recovery Period	Complete (Advinus)													
Genotoxicity														
Kindolor Bacterial Reverse Mutation Assay	Complete (CRL)													
An In Vivo Micronucleus Assay of Kindolor Tosylate by Oral Gavage in Sprague Dawley Rats	Complete (CRL)													
Behavioral Toxicology														
Kindolor Conditioned Place Preference	Complete (Roberts, Scripps)													
Kindolor Effects on Mouse Rotarod Performance and Clonic Seizure Protection	Complete (NINDS)													
Kindolor Effects on Rat Rotarod Performance	Complete (Lohocla)													

The simple listing of the completed work and work in progress may suggest that the path to an IND can be easily traversed in a few years, however, there are in fact many obstacles that needed to be overcome once we set out to develop the drug for eventual use in human studies. The first challenge was the scale-up of drug synthesis. There is an early step in our original Kindolor synthesis that requires the use of high temperatures (250 °C) in the presence of phenyl ether. While this step was feasible for making small amounts of the Kindolor precursor, it was too dangerous when large amounts of Kindolor needed to be synthesized. The resolution to this problem required the use of a quite novel method of synthesis, termed “flow chemistry”, to overcome the most dangerous step, and modification of multiple other steps in the process to make the synthetic route for producing multi-kilogram amounts of Kindolor safe and reliable. We also tested numerous salts of Kindolor for ability to improve bio-

availability, and the synthesis of the tosylate salt, which proved to be most useful, had to be incorporated into the Kindolor synthetic route. At every step of the way, regulatory requirements have to be met and processes documented (including assays for residual solvents, heavy metals, etc.) in order to produce a drug product that can be utilized in clinical trials.

Choosing the species for pharmacokinetic and toxicology studies has its own challenges. The FDA requires that a rodent and non-rodent species be used for these studies. The overall goal of these non-clinical studies is to choose doses for the first in human clinical trials of drug safety (Phase 1 trials), and the desired outcome of the non-clinical toxicology studies is to determine a "no adverse effect level (NOAEL)" of the drug, which can then be translated into a human dose, using available methods. However, there are numerous other considerations for choosing appropriate species. Drug metabolism is an important issue, since the FDA requires that if a metabolite is present at a level of more than 10% of the parent drug in human, the toxicology of the metabolite must be established. However, if the species chosen for toxicology studies produces the metabolite at a level consistent with that found in human, it is considered that the exposure of the species to the metabolite is sufficient to allow for human studies without extra toxicology being performed. Therefore, both in vitro and in vivo metabolism needs to be ascertained in the chosen species, as well as the route of metabolism (e.g., enzymes involved). It is also necessary to determine (in vitro) the function of human influx and efflux transporters that can be responsible for the candidate drug absorption and distribution, so that a species expressing the appropriate drug transporters can be utilized. Furthermore, the intestinal anatomy and physiology of the species needs to be similar to that of human, as far as possible, such that, for example, when drug is administered orally, it will be processed similarly in humans and the chosen species. The routes of excretion of drug and metabolites need to be established with a mass balance study. Once the species have been chosen, appropriate drug doses need to be decided upon which will generate plasma levels of drugs and metabolites thought to be appropriate for drug efficacy at the drug targets. The studies that go into making the decision to use a particular species, and to arrive at appropriate doses for the first in human studies in the IND application, are outlined (pharmacokinetic and toxicology studies) in the Gantt chart (Table 1).

CONCLUSIONS

Overall, we are on track to complete the studies needed for the IND application by the end of the second year of the grant, and to then move on to compiling and submitting the application and initiating the first in human studies during the third year of the grant (first year of the UH3 grant).

DATA AVAILABILITY STATEMENT

The unpublished data is proprietary in nature, but can be discussed with the authors on an individual basis under a non-disclosure agreement.

AUTHOR CONTRIBUTIONS

BT and PLH are co-PIs on the grant “A Novel Therapeutic to Ameliorate Chronic Pain and Reduce Opiate Use” and both contributed equally to the writing of this manuscript.

CONFLICT OF INTEREST

Both BT and PLH are employed by Lohocla Research Corporation.

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