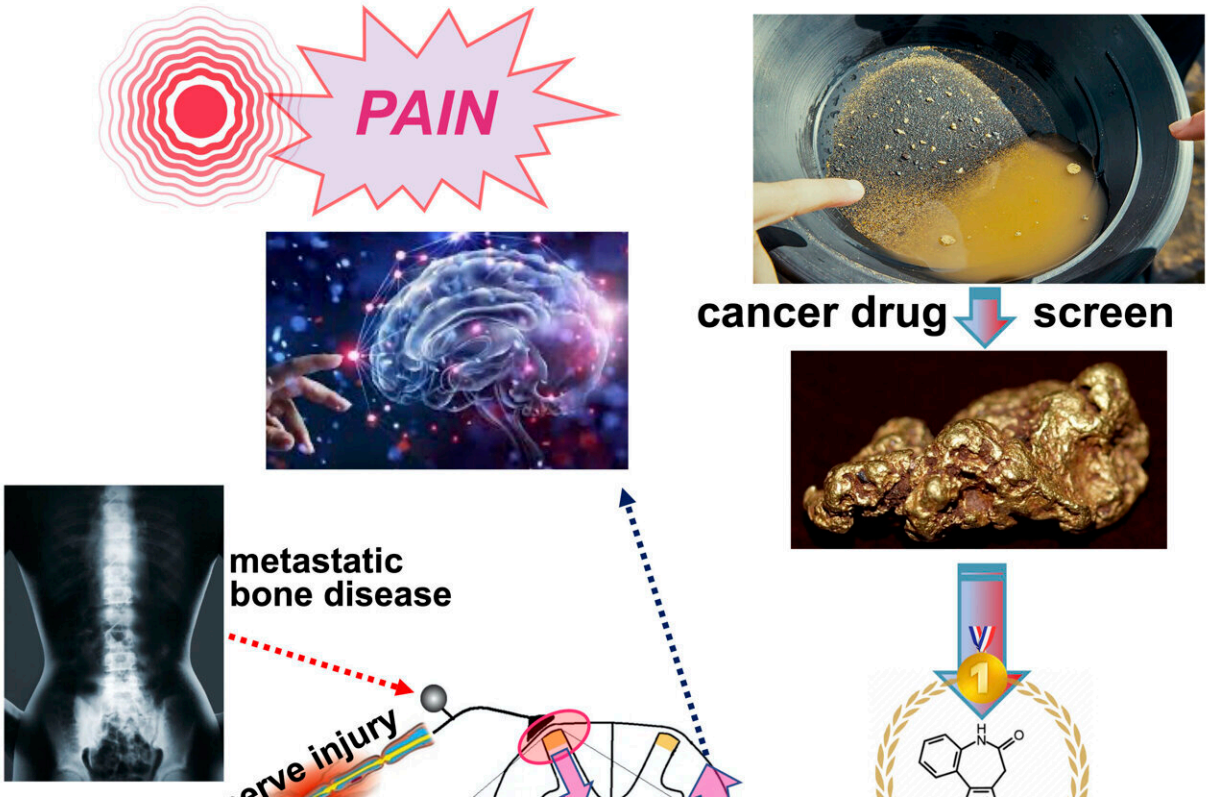
New life for a cancer drug that reprograms pain pathways to treat chronic pain

October 27 2021



Upper right: Screening compounds in the "Junkyard of cancer drugs", akin to sieving through sand, looking for gold nuggets. Kenpaullone was identified as a promising candidate owing to its ability to switch on the Kcc2 gene, which has been predicted to alleviate chronic pain. Upper left: Intractable chronic pain is a serious and pressing unmet medical need. Kenpaullone was shown to be highly effective in preclinical models of nerve injury pain and bone cancer pain. Left box (untreated pain): Pain-causing events such as constriction nerve injury or cancer cell expansion in bone activate GSK3ß, an enzyme that tags proteins with

phosphate. In pain-relay neurons in the spinal cord, GSK3ß tags β-catenin (β-CAT), routing β-CAT to the cellular garbage bin. Without β-CAT in the cell's nucleus, Kcc2 remains switched off, thereby allowing maintenance of high chloride levels in the cell, which makes the cell electrically more jittery and thus results in refractory chronic pain. Right box (treatment): Kenpaullone treatment inhibits GSK3β phosphate-tagging, allowing untagged β-CAT to enter the nucleus of a pain-relaying neuron. There it binds the region of DNA in the Kcc2 gene that switches it on and off, the promoter. By binding the promoter, β-CAT switches on expression of Kcc2, thereby inducing production of KCC2 protein. KCC2 pumps chloride ions out of pain-relay neurons, making them less jittery. This stability enables circuit repair and pain relief, based on resetting of genetic switches. In this way, Kenpaullone, or β-CAT delivered via gene therapy, can upregulate KCC2, thereby abating pain transmission in the spinal cord. Credit: Wolfgang Liedtke, MD, PhD

Chronic pain associated with nerve injury and chronic bone pain from metastatic cancer are unmet medical needs. This sober assertion does not begin to capture the crushing and devastating impact of these pain conditions on the lives of people affected by them, nor the effects on their families. Indeed, people's social and professional lives can be upended by these conditions.

One patient with cancer metastasis-induced bone pain described it as follows:

"I just cannot sleep anymore because turning in bed hurts, my spine hurts laying down, and sitting up to sleep hurts even more. During daytime, I have constant brain fog, interrupted by pain that within minutes gets worse 10-out-of-10, against a background of constant burning pain which gets worse toward the afternoon and evening. I hurt more when I go to the bathroom. The pain medication makes my brain fog worse. I feel like a zombie. I am badly constipated and itch all over."

Similarly, patients with chronic nerve injury pain due to peripheral nerve damage caused by diabetes, adverse secondary effects of medication, or severe shingles describe their lives as being upended by intractable pain.

Thus, there is clearly a need for new pain alleviating treatments. Regarding the desired profile of potential new pain medications, "New drugs and other therapies against chronic pain need to be safe, i.e., the fewer side effects the better. It is especially important that they be non-addictive and non-sedative, while being effective against nerve injury pain and cancer pain, preferably with a minimal time to official approval. Because chronic pain, like many chronic diseases, has an important root in genetic switches being reprogrammed in a bad way, a disease modifying treatment for chronic pain should reset the genetic switches, not just cover up the pain, as with opioid and aspirin/Tylenol-like painkillers." says Wolfgang Liedtke, who practiced pain medicine for the last 17 years at Duke University Medical Center and directed the former Liedtke-Lab to elucidate basic pain mechanisms (now an executive at Regeneron Pharmaceuticals, since April 2021).

Liedtke's Duke team, jointly with colleagues from UC Irvine, tackled this pain problem by starting with a survey of the "junkyard of cancer drugs" that might have the potential to be repurposed. The tested 1,057 compounds contained in two Compound Libraries of the National Cancer Institute. Liedtke was particularly interested in examining cancer drugs because a sizeable number of them influence epigenetic regulation of genes. In addition to stopping rapidly dividing cancer cells from multiplying, such epigenetic effects can reset maladaptive genetic switches in non-dividing nerve cells.

To identify useful candidate anti-pain drugs from this starting pool, Liedtke's team devised a screening method that relies on neurons (nerve cells) from genetically-engineered mice. These cells had a "knock-in" modification that enables them to serve as a convenient reporter gene

system. Specifically, compounds that enhance expression of an anti-pain target gene trigger these cells to generate a measurable bio-luminescent signal. The selected anti-pain target gene triggered in these cells is *Kcc2*, which encodes a chloride extruding transporter molecule, KCC2. KCC2 expels chloride from neurons. Low chloride levels within neurons inhibit neurotransmission. When inhibitory neurotransmission is robust and strong in pain pathways, pain signals are silenced. In essentially all forms of chronic pain studied in experimental animals and also in human spinal cord models, KCC2 disappears from the neurons making up the primary pain gate in the dorsal spinal cord.

Upon testing the aforementioned 1,057 compounds in their reporter system, Liedtke's team identified 137 that enhanced expression of Kcc2. Iterative retesting pointed to four highly promising candidates. Among them, Kenpaullone was selected for in-depth work-up owing to its strong record of protecting neurons in multiple experimental models.

In mice, Liedtke's team found that Kenpaullone functioned effectively against pain caused by nerve constriction injury and pain caused by cancer cell seeding in the femur. The pain relief was profound, long-lasting, and with a protracted onset, consistent with the drug having an impact on gene regulation. Liedtke remarked,

"At this stage, we knew we had met the basic requirement of our screen of shelved cancer drugs, namely identified *Kcc2* gene expression-enhancers, and demonstrated that they are analgesics in valid preclinical pain models."

Thus encouraged, Liedtke's team assessed whether Kenpaullone affects spinal cord processing of pain and, subsequently, whether Kenpaullone treatment can reduce nerve injury-induced elevation of chloride levels in pain-relaying neurons. Both sets of experiments yielded resoundingly affirmative results.

Having obtained these auspicious findings, the investigators sought to clarify how exactly Kenpaullone augments Kcc2 gene expression. They discovered the underlying signaling mechanism, a key element of which had not been described previously. Briefly, they found that Kenpaullone inhibits GSK3-beta, an enzyme that adds phosphate tags to proteins; phosphate tags have a potent function-switching effect. They found that GSK3beta adds phosphate tags to delta-catenin (delta-CAT), which, when tagged in this way, is fated for destruction by the cell. Hence, in the context of chronic pain, activation of GSK3-beta leads to loss of delta-CAT in pain relaying neurons. Liedtke's team demonstrated an original function of delta-CAT in relation to Kcc2 expression and the relaying of pain signals. That is, they showed that non-phosphorylated delta-CAT is transported into the cell's nucleus where it binds directly to the Kcc2 gene, in its promoter region, which switches on the expression of a switched-off Kcc2 gene.

To probe the relevance of this pathway for pain, Liedtke and colleagues devised a gene-therapeutic approach wherein they loaded a virus, known as an AAV9 gene-therapy viral vector, with phosphorylation-resistant delta-CAT. To infect spinal cord dorsal horn neurons with AAV9 driving phosphorylation-resistant delta-CAT , they injected it into the cerebrospinal fluid of mice. Remarkably, they found that this experimental gene therapy had analgesic effects similar to those of Kenpaullone. These findings suggest that Kenpaullone and similarly-acting kinase-inhibitory compounds, as well as delta-CAT gene therapy, have the potential to become new tools in our toolbox against chronic refractory pain, including nerve injury pain and cancer bone pain, and likely against other forms of chronic pain (trigeminal pain) associated with low Kcc2 expression. This approach may also be effective against other neurologic and psychiatric disorders in which this mechanism appears to contribute to the disease.

The findings are published in *Nature Communications*.

More information: Repurposing cancer drugs identifies kenpaullone which ameliorates pathologic pain in preclinical models via normalization of inhibitory neurotransmission, *Nature Communications* (2021). doi.org/10.1038/s41467-021-26270-3

Provided by Duke University