>> Good morning, everybody. It's a pleasure to introduce our first session of today, Cancer Symptom/Treatment Side Effect Management. And this is going to be preclinical research. And we've got an excellent array of speakers this morning and topics. We're going to start actually with my presentation first, followed by Dr. Piomelli, who will be speaking about cannabis, the endocannabinoid system and cancer pain. Followed by Dr. Sara Ward from Temple University, who will be talking about cannabinoid-based treatment strategies for pain associated with cancer. And then finally, Dr. Linda Parker from Guelph University, who will be speaking about CBDA or CBD acid and some other analogues and how they are excellent treatments for nausea and vomiting. And she'll be describing this work in her elegant preclinical models. So I'll begin the session with my presentation. And here we are. So we could just advance to the next slide. And just very quick overview of the endogenous cannabinoids that are modulated through different regulatory enzymes. The first one that was discovered was anandamide. And anandamide is a partial CB1 receptor agonist. And this was discovered about 25 years ago in the lab of Raphael Mechoulam by his postdoc at the time, William Devane. And a few years after the discovery of anandamide, 2-AG was discovered simultaneously by the Mechoulam group and Sugiura from Japan. And I'm going to be focusing my presentation on enzymes that regulate 2-AG. And this is the most highly expressed endocannabinoid in the brain. And it is also very notable that it's a precursor for free arachidonic acid in the nervous system. So we can move to the next slide. I'll just briefly show the enzymatic regulation of anandamide on the right and focusing on 2-AG on the left for the purposes of this presentation. So 2-AG is produced by two biosynthetic enzymes that are very similar in structure, DAG lipase alpha and beta, although they are expressed on different types of cells within the nervous system and the periphery. And there are several enzymes that degrade to 2-AG. And the most notable is monoacylglycerol lipase or MAG lipase for short. And a major product of 2-AG is arachidonic acid, which was a very bioactive lipid that's also -- it's also attacked by a variety of enzymes too that's involved in inflammatory processes. So we could move to our next slide. And again, really just described DAG lipase alpha. And it's largely expressed on presynaptic neurons in the central nervous system as well as in astrocytes. When DAG lipase alpha is deleted or blocked, endocannabinoid mediated short-term synaptic plasticity is disrupted. So this plays an important role in learning and memory. It also plays a role in neurogenesis. In contract, DAG lipase beta is expressed on microglia and macrophages in the periphery. It contributes to inflammatory responses. And its inhibition has been shown to decrease pain and inflammation in a variety of models. On the right of this slide, you could MAG lipase, a degradative enzyme. This is largely expressed on presynaptic neurons as well as other cell types. As I already said, it is a major catabolic enzyme for 2-AG as well as other arachidonoylglycerols. And it's also a rate limiting enzyme of arachidonic acid, as I said, in brain, liver, and lung, but not in gut as well as a few other organs. So if we could move to the next slide. This shows first the expression of the two enzymes that I'll be focusing my presentation on. And as you can see, in the postsynaptic neuron, DAG lipase alpha is highly expressed. And it reduces 2-AG, which then travels retrogradely to the presynaptic neuron, where it binds CB1 receptors. And then it's ultimately uptaken into the cell and degraded by MAG lipase. And if we could advance to the next slide. This shows that both alpha, DAG lipase alpha and MAG lipase expressed on astrocytes. And the next slide will show that a different pattern of enzymes exist on microglia. And that is we have DAG lipase beta now instead of alpha. And we still have the expression of MAG lipase, the degradative enzyme. So if we could move to the next slide, I'm now going to transition and talk about the purpose of examining these enzymes in a mouse model of chemotherapy-induced peripheral neuropathy. And it's very well described that the taxanes, platinum derivatives, vinca alkaloids produce neuropathy. This is a major side effect of these chemotherapeutic agencies through a variety of different mechanisms. These side effects could be dose limiting and have very negative consequences on quality of life in the patients. And in fact, the neuropathy could continue for quite some time, including years, after the cancer is cured or in remission. Next slide, please. So paclitaxel is the chemo agent I'll be focusing on in my talk. It's used to treat solid cancers of the breast, the lung, the ovary. And again, a major dose limiting side effect is this sensory neuropathy. And it could occur in approximately half of patients even years after treatment has ended. Next slide, please. So this chemotherapy-induced peripheral neuropathy, it is characterized by numbness, particularly of the feet and hands. But it could also be accompanied by severe pain in the hands or fingers, in the feet, and the toes. And this slide from Patrick Dougherty shows a patient who colored in where they feel the most severe pain. And this can be very much exacerbated by touch, by temperature, particularly cold or heat. And again, this is very rate limiting in terms of cancer treatment and ultimately as far as quality of life of the patients as well. Next slide, please. So here, I'm going to show two different approaches that paradoxically produce antinociceptive effects in a mouse model of chemotherapy-induced peripheral neuropathy. And what this slide shows is 2-AG in the middle. This is rather endocannabinoid centric. And we've got our degradative enzyme down in red, MAG lipase. And upstream, we've got DAG lipase. And that's responsible for the biosynthesis of 2-AG. So this hypothesis is an inhibition of 2-AG biosynthesis that is inhibiting DAG lipase beta as well as inhibiting the degradative enzyme MAG lipase will reverse nociceptive behavior in a mouse model of chemotherapy-induced pain. And this is believed to occur through distinct mechanisms of action. May I have the next slide? So the way this model is set up is that mice are given a series of four injections of paclitaxel, eight milligrams per kilogram every other day. This is considered a cycle of paclitaxel. And following this treatment, the animals will show disturbances and nociceptive behavior. In fact, increased sensitivity to touch, to heat that persists for many months. Next slide, please. So in the models that I'll be showing, we used von Frey filaments to assess mechanical allodynia. And that is just a non-noxious mechanical stimulation of the bottom of the paw. And this results in a rather robust withdraw response and often a paw shake from the mice. So this is our nociceptive endpoint. And the animals that are given paclitaxel will show rather severe decreases in withdrawal thresholds to this relatively innocuous stimulus. Next slide, please. So again, on the first series of studies, I'm going to show you the consequences of inhibiting MAG lipase. And this slide just shows you very quickly that when you inhibit MAG lipase, 2-AG levels go up. And you get increased stimulation of CB1 receptors that are present presynaptically as well as you'll get stimulation on CB2 receptors, which are expressed on microglia and macrophages throughout the periphery. We also get a decrease of arachidonic acid and prostaglandins. So there are really three different pathways that could be mediating antinociceptive and anti-inflammatory effects of a MAG lipase inhibitor. Next slide, please. So the two MAG lipase inhibitors that we tested in this model are JZL184. This is relatively preferential for inhibiting 2-AG degradation compared to anandamide. And MJN110, which is much more selective than JZL184. And MJN110 is also more potent than JZL184. So it requires lower doses. Next slide, please. So this slide shows the basic behavioral endpoint that we've examined in these series of studies. On our Y-axis, it shows withdrawal thresholds. So the larger the threshold, that means the animals are really not responding to that tactile stimulus. On the X-axis, it shows the treatment. The two furthest to your left, it shows the control vehicle animals. And those animals will withdraw their paw with about two grams of pressure or so. So they're basically really not responsive to the von Frey filaments. But after these four treatments of paclitaxel and one week later, the animals will show a very significant decrease in the threshold to withdraw their paw to these von Frey filaments. And you can see the symbols. The circles show JZL184 and the upside-down triangle shows the MAG lipase inhibitor MJN110. And they each in a dose dependent fashion reverse this paclitaxel-induced nociception. And these closed symbols signify that there's a significant difference from the paclitaxel vehicle treatment. This is all within subject. So the mice are treated in a random order with the different drugs. So we do get a nice reversal of this antinociceptive effect. And if we can move to the next slide, our next question -- and that just shows the ED50s. So MJN110 is considerably more potent than JZL184. So our next question is what is a mechanism of action for these MAG lipase inhibitors. And as I already presented earlier, potential sites of actions would be a stimulation of a CB1 receptor or the CB2 receptor, as well as downstream by reducing free arachidonic acid and prostaglandin and some other inflammatory mediators. So to address this question, we used the two complementary approaches using pharmacological antagonists for these receptors as well as global knockout mice, a CB1 and CB2 knockout mouse. And the next slide shows a table of these results. So we have drug. Each drug is listed here on your left. JZL184 and MJN110. And we've got the two different treatments for the CB1 receptor and CB2 receptor. Pharmacological antagonist as well as knockout animals. And in each case, the antinociceptive effects of these MAG lipase inhibitors were completely blocked or prevented, either by CB1 or CB2 deletion as well as the antagonist treatment of the selected CB1 and CB2 antagonists. So this shows that both CB1 and CB2 receptors are required for this response. So they're both necessary for the full antinociceptive effect of these MAG lipase inhibitors. Now, they don't preclude the possibility of a contribution of downstream effects of reducing arachidonic acid. Next slide, please. Okay. So also the MAG lipase inhibitor that was tested here, the selective MJN110 was also tested against a series of different cytokines and chemokines. And this slide shows the effects of MCP-1. And this was taken from a study that we conducted immunohistochemistry on dorsal root ganglia. And these were from mice that were either treated with a course of paclitaxel or with the vehicle. And then right before sacrifice, an hour or two before sacrifice, the animals were treated with vehicle or MJN110. And as you can see, under the paclitaxel treatment with vehicle pretreatment, there's a significant increase of MCP-1. And this is completely prevented or blocked by MJN110. On the right, we show phoso-P38 MAP kinase in the dorsal root ganglia. And again, we show the same pattern of effects right here. This was all in DRG. We also looked at dorsal horn. And paclitaxel also induced MCP-1 in dorsal horn of a spinal cord. And this was at the L5 to the S1 region of the spinal cord. But there was no effect of paclitaxel on phoso-P38 MAP kinase. Next slide, please. This slide shows that MJN blocked the co-localization of paclitaxel-induced MCP-1 and phoso-P38 MAP kinase in DRGs. And as you can see on the slide, the first column shows MCP-1 alone. Second column shows phoso-P38 MAP kinase. And the third column shows the merge with DAPI. And as you can see, there's very little signal in the control animals. Paclitaxel treatment caused a significant fluorescence of these mediators. And the MAG lipase inhibitor on the bottom decreased this expression of both mediators. Next slide, please. So because the CIPN and nociception is really a chronic effect and patients often experience chronic pain, any potential treatment would be given repeatedly. So here, we tested whether the paclitaxel course of CIPN, which produces this chronic nociception, would we get sustained antinociceptive effects with repeated dosing of a MAG lipase inhibitor. So we treated mice for six days with JZL184. And then we evaluated their withdrawal thresholds. Next slide, please. So what this shows right here is after paclitaxel and just with vehicle treatment, the second bar, we see a increase of nociception. So there's a decrease in paw withdrawal thresholds. So this is a very severe nociceptive response. Next slide, please. And the next slide over here in the middle, this shows a low dose of the MAG lipase inhibitor JZL184. And note that this formula gram per kilogram dose given acutely really doesn't have any effect. It's a threshold dose. It does increase 2-AG levels within the nervous system about two- to threefold. But we see very marginal effects. However, after six days of treatment, we do see a full antinociceptive effect. We contrast that with a high dose of JZL184. When given acutely, we get full reversal of this nociceptive effect of paclitaxel. But with repeated dosing of JZL184, this effect had undergone tolerance. And this is consistent with work that Steve Kinsey and others have shown previously in which high dose of MAG lipase inhibitors, given repeatedly, causes a downregulation and desensitization of a CB1 receptor. So this shows that there really is a sweet spot for MAG lipase inhibition. We can't give high doses of inhibitor or these antinociceptive effects will be short lived, they'll undergone tolerance, where they're sustained with a low dose. Next slide, please. So here I'd like to now switch gears and look upstream. And let's look at the consequences of inhibiting diacylglycerol lipase. In this case, it's going to be beta. And this will result in a decrease of 2-AG. But recall, this 2-AG will be decreased in macrophages in the periphery as well as microglia within the central nervous system. And concomitantly, there'll be an increase of diacylglycerols. And these DAGs are also playing a role in signaling of protein kinases and could actually produce antinociceptive effects either upstream as a consequence of this increase of DAGs or potentially downstream by reducing arachidonic acid from the macrophages and microglia. Next slide, please. So in these studies, we used a selective inhibitor for DAG lipase beta, KT109. Shows very nice selectivity for DAG lipase beta over alpha. However, this drug has very poor accessibility across the blood-brain barrier. So when given peripherally, it has trouble passing the blood-brain barrier. And as a consequence, does not inhibit DAG lipase beta within the nervous system. But it does inhibit DAG lipase beta that's expressed on macrophages in the periphery. And KT109 has also been shown to reduce LPS-induced pro-inflammatory cytokines, TNF alpha release from macrophages. And has also been shown to inhibit other pro-inflammatory responses in the periphery. Next slide, please. So in this study, we treated animals after they were given paclitaxel or vehicle with KT109. And again, on our Y axis, this just shows a stimulus intensity of the von Frey filaments. And V is for vehicle. So compared to the no paclitaxel treatment, we did get, once again, a very severe hypersensitivity to touch, to this mechanical pain. And in a dose related fashion, KT109 reversed this nociceptive response from the MAG lipase inhibitor. Next slide, please. So we next asked whether these antinociceptive effects of this DAG lipase beta inhibitor would be sustained after repeated dosing. So once again, just like the previous study that I showed you, animals were treated with paclitaxel, whole course. So that was four injections over the course of eight days followed by an injection of either high dose KT109 or vehicle. And then they were tested with von Frey filaments 2 hours and 24 hours after the last injection of KT109. Next slide, please. So over on your left, this just shows the controls that did not receive paclitaxel. So those animals are basically not responding to the von Frey filaments. The white bars at the 2-hour and 24-hour time points show paclitaxel treated animals that were then treated with vehicle. And then the striped bars show the effects of KT109 given acutely. And the dark bars show KT109 given repeatedly. So as you can see with the striped bars, KT109, given acutely, produced antinociceptive effects 2 hours later, but these were completely resolved by 24 hours. Once again, the animals were showing allodynia. Repeated dosing of KT109, and again that's the black bars, we had an immediate antinociceptive effect at two hours. And with a single injection, this effect was -- or rather, with repeated dosing, this effect was sustained for at least up to 24 hours. It's very likely that this could be due to a pharmacokinetic type of effect where we might have reached steady state, but it's unclear at this point. Next slide, please. So to get a little bit more into the physiology of pain, we next removed the primary afference, the DRGs, from the mice that were treated with paclitaxel or vehicle as well as with KT109 or vehicle. So that was all done in vivo. The cells were then plated in cell culture. And 24 hours later, we harvested the DRGs and we recorded from them. Excuse me, 24 hours later, we recorded from the DRGs. And it's been shown that paclitaxel will cause a hyperexcitability of these primary afferent neurons. So our question was, will this DAG lipase beta, will it inhibit this hyperexcitability caused by paclitaxel. And the next slide shows our results. And first, as you can see, on the left, this shows the number of action potentials from the DRGs that were harvested from vehicle-treated animal versus paclitaxel-treated animals. And as you can see, there is about twice the number of action potentials following paclitaxel treatment from vehicle. The rheobase shows that less stimulation was needed to get these action potentials with paclitaxel. So over on the right, that shows that the threshold for the action potentials were reduced with paclitaxel. So what happens with KT109? In the next slide, we'll see. And KT109 reduces the action potentials down to the level of the control animal or at least the controlled DRGs. Next slide, please. And we get the same effects with the rheobase as well as it returns the thresholds back to control levels. So it makes the thresholds higher for the action potentials of the DRG. Next slide, please. So in treating paclitaxel-induced neuropathy or any type of neuropathy, it's important to know whether or not that these potential treatments will alter cancer cell growth or will they alter the antitumorigenic or antiproliferative effects of paclitaxel. So I'll just show you in the remaining minutes some very quick data in which we use A549 human non-small cell lunger cancer cells. And we tested both JZL184 and KT109 in the cells both with and without paclitaxel. So just to see what it does by itself as well as does it interfere or augment the antiproliferative effects of paclitaxel. And the next slide shows the effect of the MAG lipase inhibitor JZL184. And over on the left, this shows cell proliferation. And our control cells or the cells that were treated with JZL184 shows rather strong proliferation of the tumorous cells. And paclitaxel completely blocks this tumor growth whether or not JZL184 was in the bath. And on the right, this shows apoptosis. And again, paclitaxel significantly increases that apoptosis. And JZL184 has no effects on apoptosis alone or it doesn't interfere with the paclitaxel's proapoptotic effect. Next slide, please. So this also shows KT109 now in the same lung cancer cells. And we get the same pattern of effects. KT109 has no effects by itself, either on the left in terms of proliferation of the tumor cells. And it does not interfere and it does not augment paclitaxel's effects on proliferation. And again, the same pattern of effects on the right of apoptosis. KT109 does not interfere with the apoptotic effects of paclitaxel and does nothing by itself. Next slide, please. So just to really kind of wrap things up, these enzymes, MAG lipase inhibitors and DAG lipase beta inhibitors do reverse paclitaxel-induced allodynia. We believe this is through very distinct mechanisms of action where MAG lipase inhibitors require CB1 and CB2 receptors. But the DAG lipase beta is obviously independent of these activation of these cannabinoid receptors. We also believe that the DAG lipase beta inhibitors are showing some sort of macrophage neuronal mechanism of action. And again, because DAG lipase beta is not expressed on the neurons, particularly in the DRGs, so it must be interacting somehow upon the macrophages that are releasing mediators that may lower the threshold of the nociceptors. With repeated administration with MAG lipase inhibitors, low dose MAG lipase inhibitors retain their antinociceptive effects where tolerance happens with repeated high dose MAG lipase inhibition. And that's accompanied, not shown in this study, though, but in other studies, that's accompanied with CB1 downregulation and desensitization. And the DAG lipase beta inhibitors given repeatedly gives us prolonged antinociceptive effects. And neither, at least, the JZL184 and the KT109 had any effects on these human lung cancer cells. And they did not interfere or augment the effects of paclitaxel. And finally, I would like to give you the take home message here. So we've got biosynthetic and degradative 2-AG enzyme inhibitors. They each reverse paclitaxel-induced neuropathic pain through distinct and possibly overlapping mechanisms of 2-AG regulation. And finally, in the last slide, I would like to thank the NIH for support for this research as well as acknowledge the team here at VCU as well as our collaborators throughout the United States and throughout the world. So thank you very much. And questions will be taken after these presentations.

>> Good morning, everyone. My name is Daniele Piomelli. And I would like to first of all thank the organizers for having me today. I'll start with some disclosures. Please, next slide. So the funding of the work in my lab has been throughout the years from NIH, mostly NIDA and NINDS. But I'd like also to disclose that I serve as an advisor and I own equity in a company called Excel Pharma. And I'm an advisor also for two biopharmaceutical companies, Therapix in Tel Aviv and Aelis in Bordeaux, France. Next slide, please. So I'm sure that many of the viewers today are the familiar with the World Health Organization three-step analgesic ladder you can see there on the left of the screen. It gives guidelines for analgesic treatment, as you know, and states that for low levels of pain, non-opioid therapy is recommended whereas with increasing pain, mild to moderate, eventually to the severe pain, then opioids of increasing strength need to be applied along within non-opioid therapy. Next slide, please. The WHO analgesic ladder highlights something that we all know, that opiates and opioids are still the mainstay treatment for cancer pain. And one would imagine that they being so widespread in use the indication would be supported by a large amount of clinical evidence. However, as you can see in the slide, a recent meta-analysis published by Cochrane Reviews in 2017 states that the qualitative evidence for efficacy of opioids in cancer pain is still disappointingly low. In addition to efficacy being problematic, side effects are also an issue, dose limiting in many cases, at least in 10, 20 percent of the patients. And this, of course, in a cancer situation can be extremely problematic. Now, folks have been self-medicating with cannabis. Folks who have suffered from various forms of cancer have been self-medicating with cannabis for a long time. And cannabis has emerged, as we know, because we're here today to talk about it, as a potential option and alternative to the opioids. I'd like to go over a comparison between these two drug classes, opioids and cannabinoids. Next slide, please. Between these two drugs classes and compare and contrast them because I think there is a lesson to be learned there. Next slide, please. So the two classes of compounds have what I like to call an almost parallel scientific history. So to the left, you see the history of opium has been known for many, many, many years, although its use as an analgesic was questioned in antiquity. But the discovery of its main principal, active principal, morphine dates to 1804. The structure was described, of course, many years later. Eventually, the key discovery that brought the opioids to the center of attention was the discovery and existence of an opioid receptor in the body, actually a family of opioid receptors in the 1970s. And this led, eventually, to the discovery of the endogenous opioid system first in 1975 and then the endorphins and endomorphines. Next slide, please. Cannabis has, as I said, an almost parallel scientific history. It's parallel all the way to the discovery of an endocannabinoid system, basically with a delay of approximately 140 years. The active principal of cannabis, which also has been used since antiquity, although not throughout entire known world. But has been used as an analgesic. And the discovery of THC dates between 1944 and 1964. In 1964, its full structure was elucidated by Raphael Mechoulam. And what you see here is the structure of the THC. And that led to the discovery of the cannabinoid receptors, CB1 and CB2. Dr. Lichtman before me has described them and their actions. Eventually leading also to the identification of an endogenous system, that of the signaling molecules that activates those receptors. Those are the endocannabinoids. And the endocannabinoids activate those CB1 and CB2 receptors throughout the body. Next slide, please. Now, these two very similar histories at one point diverge, however. The trajectories diverge. Since about 1854 until 1942, they were both on the United States Pharmacopeia and also in pharmacopeias throughout the world. And they were used for many things, including the treatment of pain. But next slide, please, in the late 1930s, a divergence occurs. On one hand, folks kept using, the medical community kept using the opiates and we know now that they're one of the most popular drug classes that are available to the physician. On the other hand, cannabis was literally criminalized, was delegalized, by the Marijuana Tax Act in 1937, which was deemed unconstitutional and was then replaced in 1970 by the Controlled Substance Act, or CSA, under the Nixon administration. So I think we need to ask ourselves, next slide, we need to ask ourselves why these two drug classes, which had so similar developments, had such different fates. And one thing I like to clarify right away is that toxicity and addictive potential were not at all a factor in the decision to go over to opiates and not pursue in any way the cannabis and cannabinoids. And we know that for sure because in the 1930s, the toxicity, the addictive potential and toxicity of the opiates were extremely well known. In fact, they've been known for at least 300 years. And also it was clear that cannabis was not at all comparable in terms of toxicity to the opiates. The reason, I believe, it's a historical reason. It's not a scientific reason and has to do with racist politics. It's not the topic of my talk today, but it's one topic that I think we all as scientists and society should have -- it's high time that we reckon with. Be what it may, the legal constraints created by the delegalization of cannabis and the social stigma that followed from that delegalization really retarded, slowed down, substantially research on cannabis in general and as an analgesic in particular. Next slide, please. Now, it slowed it down, but it didn't completely bring it to a full stop. And actually, in 2016, the National Academy of Sciences, Engineering, and Medicine convened a committee to study what was known about cannabis in terms of both therapeutic potential and risks. And the committee was able to develop a 470 almost pages long report indicating there was, of course, a lot of information available about cannabis. They also drew 15 conclusions and 4 recommendations. Next slide, please. And one of those conclusions, conclusion 4.1, is that there is substantial, and I highlight here, evidence that cannabis is an effective treatment for pain in adults. Now, the use of the adjective substantial is actually quite thought out. It is not a random word decision. Substantial had a very specific meaning within the context of this report and was distinguished from conclusive, conclusive being the evidence, for example, that cannabis is effective in the treatment of nausea. Substantial still requires work. Next slide, please. And so the question that we need to ask ourselves as scientists, as a society, is is it worthwhile to move the needle of evidence, to do the work that is needed to move needle of evidence from substantial to conclusive knowing that this work will be actually fairly substantial. And I'd like to propose here that this is indeed worthwhile and is worthwhile for three reasons. The first one is that the clinical evidence available is, as I said, quite substantial already. So we are off to a good start. The second reason and the one on which I will focus the rest of my talk, this being a preclinical session, is on the fact that there is a strong biological possibility for endocannabinoid and cannabinoid regulation of pain. I'll spend some time on that. And finally, I'd like to say a few things about the health risks posed by cannabinoids, which are at this point, I think, the consensus of the scientific community is pretty clear, lower than those posed by the opioids. Next slide. So the available, as I said, I won't go into detail. This is a preclinical panel today. And also Dr. Abrams has talked about it yesterday and others will later on today. But I'd like just to say that since 2016, that's when the National Academy committee stopped looking at the literature, from 2016 on, there had been a few clinical trials on cannabis in cancer pain and other forms of pain. And these have shown -- many of them, some of them, rather, have been placebo controlled randomized clinical trials using often a compound called nabiximols, which is a sublingual spray containing 50 percent of THC and 50 percent of the nonintoxicating cannabinoid cannabidiol or CBD. And by and large, these trials have not been extremely successful in that most primary endpoints have not been met. However, what folks have found fairly consistently is that the use of nabiximols improved general quality of life and some effectiveness also appears to have achieved for folks less than 65 years old. Next slide, please. So I'd like to move on now to something, the real topic of my presentation today, the focus of my presentation today, which is really the biological possibility for utilization of cannabis and endocannabinoid-based drugs in the treatment of cancer pain. Just as a reminder, pain, including cancer pain, starts most often at the periphery at the body. There is in the case of cancer pain the release of pro-analgesic factors or the pressure of the cancer on an organ. And that leads to the activation of peripheral sensory fibers that have their cell bodies in the dorsal root ganglia, which is the highlighted, magnified at the center of the slide here. And from the dorsal root ganglia, goes into the dorsal horn of the spinal cord. And from there, it travels. It crosses over and travels up to the brain. Next slide, please. And each and every relay station in this pathway, there are cannabinoid receptors. This is in the dorsal root ganglia, in the dorsal horn of the spinal cord, in the thalamus, in the cortex. At each and every individual relay station, we have the cannabinoid receptors potentially poised to control the pain process. But do they actually do that? Next slide. Next slide, please. So if you go and look at the literature, and a few weeks ago, I actually did that. The PubMed search shows that there have been almost 3,400 papers on cannabinoid and pain published from 1972 to 2020. So we do have a lot of taxpayer money that has gone into this. And we do have, I think, a lot of good data that it's time for us to tackle carefully. I'd like to just bring up one study, an archeological study, really an old study from my lab, showing one point I'd like to make today. Is that the control of pain by endocannabinoid and cannabinoid receptors does not just occur in the central nervous system as one would be tempted to think based on what we know about cannabis but actually starts in the periphery of the body. It starts in the terminals that have their cell bodies in the dorsal root ganglia. And this is work, archeological work, dated to the 1990s where in collaboration with a group in Italy we showed that administration of very small doses of anandamide in the paw of an animal caused very profound analgesia whereas administration of doses, of similar doses, either intraperitoneally or intravenously had no such effect. Next slide. The receptor is indeed present in the periphery of the body. Next slide. This was shown by a variety of investigators. The paper you've seen a glimpse before shows the localization of a receptor on a sensory terminal. But others, including Andrea Hohmann, have shown, for example, that cannabinoid receptors are transported to peripheral nerve terminals. Rohini Kuner has shown that genetic ablation of cannabinoid CB1 receptors in the dorsal root ganglia selectively stops the analgesic effects of THC and enhances pain sensitivity. So two different things. Stops analgesic effect of THC, indicating that peripheral receptors are important to the analgesic action of cannabis but also enhances pain sensitivity, indicating the cannabinoid receptors are important in the normal aiding of pain stimuli. Next slide, please. And in fact, there is a theory now suggesting that endocannabinoids act together with other lipid mediators as peripheral gatekeepers for pain processes before the pain signal reaches the central nervous system. So what you see here is a schematic showing in the center the two cell types present in the dorsal root ganglia that are involved in early steps of pain initiation. Through the top is the C nociceptor, which is a small diameter. And it has unmyelinated fibers. You see the C receptors are pseudo unipolar, so they have two axonal branches that go one to the periphery and the other one to the central nervous system. And they're surrounding by satellite glia cells as well as by endoneuria macrophages, which are also involved very much in pain sensing. The cell type on the bottom is the delta nociceptor, which has a broader, larger diameter and is thinly myelinated. So it will be faster in conduction. So the pain stimulus, of course, starts at the nerve ending toward the periphery and proceeds toward the central nervous system. But it is regulated by a variety of cell types. And you see some of them, not all of them, possibly most of them, listed here in this schematics. And this include cells that are resident to the skin or to the mucosa of the body like mast cells and macrophages and to some extent neutrophils. But also cells that infiltrate during, for example, damage, such as the damage caused by a chemotherapeutic agent or the damage caused by the cancer growing mass itself. It includes the monocytes and neutrophils that come, of course, from blood vessels. So all these cell types release mediators that could either stimulate pain, they're pro-algesic, or inhibit pain. So typically, cancer cells release a whole host of cytokines, which tend to be pro-algesic in their action. But there are also analgesic mediators that are produced by resident cells. And among the analgesic mediators are the endocannabinoids and particularly the endocannabinoid anandamide. Next slide, please. In addition to working in the periphery of the body, the endocannabinoid system also works in the central nervous system. And this has been now known for quite a while. One example that I like to highlight here is the phenomenon of stress-induced analgesia, which is something we all have experienced, any one of us who has ever played any sports or engaged in any physical activity. During the physical activity if you get hurt, you often don't feel pain. The pain emerges later on. And that is part of the -- the reason for that is because the stress, the presence of a stress hormone in your system induced by the sporting activity or whatever causes an analgesic response. And the opioid system has a very important role in this. It's been known for many years that opioids mediate a substantial component of stress-induced analgesia. In some models, approximately 50 percent of it. So now dating back to now 15 years, Andrea Hohmann working with my lab, we discovered that the known opioid component of stress-induced analgesia is mediated by endocannabinoids, by both anandamide and 2-AG working in concert actually. They divide labor in certain ways. But that finding and many others from many other labs shows that there is a central component to endocannabinoid mediated analgesia, not just the peripheral component I underscored before. Next slide, please. One important point that I would like to make with this slide is also that cannabinoids and opioids often serve nonoverlapping functions that are converging. So in the case of stress-induced analgesia, the opioid component is separate from the cannabinoid component, but together they make up the bulk of the stress-induced analgesia. So they are nonoverlapping, but they're converging. They both are analgesic, but they engage different cellular circuits. That is clearly shown also by localization of these receptors that is overlapping but not completely overlapping. Next slide, please. So when you have two biological systems that are nonoverlapping but converging in their function, one thing that happens, particularly when we're talking about pain control, is that they might engage in greater than additive or synergistic interactions. And this is something really important because if you can give a drug that they took together, two drugs that are synergistic in their mode of action, you can lower the dose of each drug and you can also achieve greater efficacy for a lower dose. Next slide. And so this has been actually studied quite extensively in the animal literature and a little bit also in the human literature. And what you see here is a meta-analysis by Bernard Le Foll published a few years ago about the opioid-sparing effects of cannabinoids. So the ability of cannabinoids to engage in a synergistic interaction with the opioids such that the effect of the opioids is magnified. And so from their systematic review of the animal data, they concluded that, and I'm going to read, the median effective dose ED50 of morphine administered in combination with THC was 3.6 times lower than ED50 of morphine alone. Now, for those of you in the audience who are not card-carrying pharmacologists, next slide, let me explain this with a graphic. So basically, you can see to the right that if you want to achieve a certain effect, that effect with morphine, a certain degree of analgesia, and that degree of analgesia is achieved with 3.6 approximately doses of morphine alone, the addition of a dose of THC will lower the dose of morphine from 3.6 to 1. So that is a very substantial effect because it will lower the side effects of morphine. It will lower the constipation. It will lower the risk to develop addiction. It will lower the risk to cause respiratory depression. So those are potentially very favorable outcomes and desirable outcomes. Next slide. So we still don't have a very clear view of if this animal work will translate into human treatments. There is one paper that I'd like to point out published by the group of Meg Haney with Ziva Cooper out of Columbia University showing that the same synergism could happen also in people. The synergism was demonstrated in an experimental human study, so under very controlled conditions. It needs to be replicated in a more real-world context. But it's encouraging. Next slide. So you heard already about the endocannabinoid. I'd like to spend some time over this with you because the endocannabinoids themselves, the existence of the system and the existence of the role, the play in the regulation of pain signals is further evidence that the cannabis and cannabinoids are potentially useful for the treatment of chronic pain and particularly of cancer pain and need to be evaluated as such. So there are two main endocannabinoids. You heard Dr. Lichtman about them. Anandamide and 2-arachidonoylglycerol or 2-AG. I'm not going to delve into the chemistry today, but they are both derived from phospholipid precursory membranes. And they both activate CB1 and CB2 receptors with anandamide being a little bit less good at activating CB2 than 2-AG is. 2-AG also is a full agonist, if we can use that term loosely, whereas anandamide has lower efficacy at CB1 receptors. What I like to point out today is also not just the fact that they are able to activate these receptors but also the fact that they are cleaved by enzymes that terminate their actions. And they're two different sets of enzyme. Again, Dr. Lichtman has introduced them. Fatty acid amide hydrolase is the enzyme that cleaves anandamide into arachidonic acid and ethanolamine whereas monoacylglycerol lipase, MAGL, is the enzyme that cleaves 2-AG into arachidonic acid and glycerol. Next slide.

>> Daniele, we are running out of time, so you have about a minute to wrap up your presentation.

>> Yes. Next slide. So this offers multiple therapeutic targets. We have two cannabinoid receptors and multiple deactivating systems. Next slide. Next slide. I'd like to just say this to conclude, that one important point that makes us also hopeful that inhibitors could be useful for the treatment of pain and cancer pain is that we have individual persons who carry hypofunctional FAAH mutations. And these individuals experience less or no pain. In one particular case, you see here the photograph of this lovely lady from Scotland. Her name is Jo Cameron. And she has microdeletion in a pseudogene, a FAAH pseudogene that essentially removes the FAAH activity completely. And she experienced little or no pain. Next slide. So I'm going to wrap up here. I had a few slides on dependence and addiction, but let me quickly go to the next one. So what do we know about cannabis and dependence? We know that the frequent use of cannabis can result in dependence and loss of control over use. And we know that sudden cessation of heavy and prolonged cannabis use can cause with withdrawal. And this goes under the rubric of cannabis use disorder. Next slide. However, even though cannabis use disorder is not a joke, it's really a serious thing and needs to be taken seriously, it is not as dangerous as opioid withdrawal. And also, even though cannabis can cause acute and chronic toxicity, there is very little or no evidence of fatalities. Next slide. Next slide. To sum up, I'd like to posit the cannabinoid-based drugs play key roles in pain regulation. The endocannabinoid system has such roles. There is a place, in my opinion, for cannabis and cannabis-based medications in cancer pain management. This place has to be demonstrated based on experimental and evidence. And we also need to understand better the potential benefits and risks of opioid-cannabinoid interactions. And last slide, just a thank slide to all the people who have collaborated in this work. And thank you for listening.

>> So today, I'm going to talk about something that I believe we haven't covered yet in the conference, and that is cannabidiol or CBD and the potential role that CBD may play in the treatment of pain associated with cancer. Next slide. So what is CBD? Interestingly, CBD was actually identified structurally one year prior to delta-9-THC. So both THC, CBD, and some other cannabinoids were isolated by Roger Adams in the 1940s. And Raphael Mechoulam, pictured here with a nice chalkboard drawing of cannabidiol, identified the stereochemistry of CBD one year before he identified the stereochemistry of THC. And he also conducted very important pharmacological studies which determined that the cannabinoid that is responsible for the so-called psychoactive effects of cannabis were mediated through THC. So really from that point moving forward, THC received more attention in the research field than its so-called non-psychoactive counterpart CBD. However, that doesn't mean that CBD research didn't continue from the 1960s moving forward. It did, including in the 1980s when Dr. Mechoulam investigated in humans the pharmacological property of CBD, which is anti-seizure properties. And other studies as well moving forward looking at other potential pharmacological effects of CBD. So while it was shown to be pharmacologically active, as I mentioned, one distinction between THC and CBD was a lack of so-called psychoactive effects. Another difference that was identified early on between THC and CBD is that CBD did not show sort of overt analgesia to physiological pain such as thermal pain. So in the preclinical world, for example, testing mice or rats on a hot plate for thermal sensitivity. While it was shown that THC could decrease thermal sensitivity, it was shown that CBD couldn't. So although other pharmacological properties of CBD such as its antiseizure effects continue to be investigated, the potential for CBD to serve as a therapeutic for the treatment of pain really fell by the wayside. And so as I mentioned, the effects that we were aware of for CBD were antiseizure effects first identified in humans in the 1980s. And then this really moved into the popular world and public awareness in the 2000s with Charlotte Figi who really became famous through Sanjay Gupta's coverage of her story on CNN. And so when she was about five years old, her family famously treated her with a cannabis extract from a cannabis constituent called hippie's disappointment because it was very low in concentrations of THC but relatively high in concentrations of CBD. So this again sort of repopularized an increased interest in research again into CBD. And I became introduced to CBD at a international cannabinoid research symposium conference where I sat in on a presentation by Dr. Sean McAllister who was presenting on the antitumor effects of CBD, which he has found in both in vitro tumor studies as well as using in vivo mouse models of various cancer types. And that was around 2010, 2011 where Dr. McAllister was reporting on these findings, and I became fascinated with learning more about CBD and some of its potential pharmacological effects. So if you fast forward 10 to 15 years from there and now we all know CBD has really exploded in the public, its availability now with the passage of the 2018 Farm Bill and associated Hemp Act. Also with the FDA approval of the first phytocannabinoid-based FDA approved drug Epidiolex for the treatment of Lennox-Gastaut and Dravet syndrome. So we now have an FDA approved CBD formulation. We have over-the-counter sales of hemp-derived CBD formulations. And you can find anything from CBD containing shampoos to edibles to CBD treatments for your pets who may suffer from things like anxiety. So the main reasons that people name anecdotally for using CBD are for decreases in anxiety, help with sleep, as well as treatment of pain. And so as I mentioned, there is a little bit of a disconnect between the historical animal data showing that CBD is not a frank analgesic as we may think of analgesics, being able to block physiological or nociceptive pain, to people reporting that they use CBD for painful conditions. And in contrast to there being what Daniele pointed out as substantial evidence for the use of cannabis as a therapeutic for pain conditions, we really are missing clinical evidence for CBD alone as a treatment for various pain conditions. And this is really because the clinical studies in this realm, if we didn't have enough, which we don't have enough cannabis and THC clinical studies for the treatment of pain, we are really far behind in the availability of clinical data to look upon for CBD alone. So I've already mentioned sort of a wide range of different therapeutic targets for CBD. Next slide, please. This is something that is really fascinating with CBD and probably similar to THC as well. So those of us who study CBD and present on CBD often use this graphic that was published by Dr. Mechoulam in 2009 in a Trends in Pharmacological Sciences publication. And so this is a pie chart showing phytocannabinoids outside of THC, some of their potential pharmacological effects and some of the proposed mechanisms of action underlying these potential pharmacological effects. And you can see half of this pie chart is dedicated to CBD's potential pharmacological effects and the potential mechanisms of action. So there are many things to point out here. One of them, again, being the wide list of potential pharmacological effects anywhere from antipsychotic, antiepileptic, anti-ischemic, antidiabetic, antibacterial. And you can see here also analgesic. And again, the majority of the reason why these different indications make this list are either based on in vitro evidence or in vivo preclinical evidence. And there still remain only a small handful of clinical studies to support these pharmacological acclaims outside, of course, of the antiepileptic effects that have now been determined through Epidiolex's clinical trials. The other thing that's very important about this graphic is the list of the potential mechanisms of action. And the first thing that I want to point out here is that almost none of these potential pharmacological effects are linked with the potential mechanism of action of cannabinoid receptor activation. And so although many of the pharmacological effects of CBD are similar to those of THC, CBD does not bind with appreciable affinity to either CB1 or CB2 receptors. There are some reports in vitro of either direct or indirect interactions between CBD and cannabinoid receptors. But the preponderance of evidence from preclinical in vitro or in vivo studies suggests that there are other mechanisms of action to support CBD's wide range of pharmacological effects that don't involve interaction with the canonical cannabinoid receptors. And these potential mechanisms of action are really fascinating and support, I think, the possibility that CBD could be involved in therapeutic effects of a wide range of indications. Many of these are either neuroprotective mechanisms, direct ways to protect neurons from stress, as well as ways in which to suppress neuroimmune activation and decreasing inflammation. Next slide, please. And so to tie in some of these mechanisms of action into pain and the research that I want to talk to you about for the remainder of my presentation, as I mentioned, after sitting through Dr. McAllister's presentation on CBD, I went back to the laboratory and wanted to learn more about what is CBD and what do we know about some of its potential effects. And there was the epilepsy literature and the cancer literature. But quite surprising to me, there was almost no pain literature with CBD at the time except to say that it didn't seem to be able to block nociceptive pain. But there were two preclinical studies suggesting that CBD may be anti-inflammatory in some animal models of pain. And so in learning more about the potential mechanisms of action of CBD that I've listed here, I was intrigued that they seemed to match up very nicely to potential mechanisms of action of CIPN, which Dr. Lichtman has already discussed in the first presentation of this session. And sadly, the reason why I at this time was thinking about CIPN is that one of my lab mates, Dr. Gladys Corley, had been recently diagnosed with stage four breast cancer. And she was undergoing Taxol chemotherapy and was suffering very significantly from CIPN. And I hadn't known about CIPN prior to this. And so sort of these two different things happened at the same time for me, learning about these mechanisms of action of CBD, the mechanisms of etiology of CIPN. And I was very intrigued that there seemed to be a potential for CBD for the treatment of CIPN. Next slide, please. So as Dr. Lichtman has mentioned, the rodent model of CIPN is a very well-established animal model. We can inject mice or rats with different dosing regimens of different classes of chemotherapeutic agents. Most of the work that we conduct in the laboratory uses the chemo agent paclitaxel at this dosing regimen shown here. And you get reliable both mechanical as well as thermal sensitivity that we can measure in the animals. One of the things that I decided to do when I first brought this model up in the laboratory was to look at the potential for CBD to prevent chemotherapy-induced neuropathic pain. In most cases of neuropathic pain, we don't have the ability to think about a prophylactic treatment strategy, but we do with CIPN because for patients who have yet been diagnosed or started a chemo dosing regimen, we can predict the onset of CIPN. So the studies that I started with were pretreating mice with CBD prior to each exposure of paclitaxel. And what we found is that if we pretreated mice with CBD prior to each paclitaxel injection, the animals never went on to develop what is shown here, mechanical sensitivity. So in this graph on the Y-axis, I have the mechanical threshold needed to elicit a withdrawal response from mice. And along the X-axis, this is their mechanical threshold at baseline. And in the black squares, you can see how that threshold is significantly decreased after paclitaxel treatment. In the white circles, you can see that mice that have been pretreated with CBD never went on to develop the mechanical sensitivity. And so in contrast, if you test one of the gold standard analgesics, morphine, in the prevention paradigm, obviously or not surprisingly a pretreatment with an opioid doesn't go on to protect against the development of CIPN. Now, you can also do the studies that Dr. Lichtman presented, which were reversal studies. Right? You can treat mice or rats with a chemotherapeutic agent and wait for the mechanical sensitivity to develop and then treat the rodents with a particular intervention to see if it can reverse CIPN. And importantly, and we haven't done a lot of this work with CBD, but the work that we have done so far with CBD, we haven't seen very robust reversal of an established CIPN. So I point that out because I think it's important when we are reading the preclinical literature to pay attention to people's methodology, whether or not they're looking at prevention or reversal, and looking forward to the initiation of clinical trials to think about these two distinct aspects of CIPN that may respond differentially to potential therapeutics. And I think both of these need to be looked at in clinical studies with cannabinoid. Next slide, please. And so we went on to very fully characterize the effects of CBD at preventing paclitaxel-induced mechanical sensitivity. And one of the first things that I wanted to look at was whether or not CBD would interact with other phytocannabinoids in this model. And this was because of some really elegant writing by Ethan Russo that I again had been reading up on the time talking about the so-called entourage effects of phytocannabinoids, that specific combinations of phytocannabinoids may interact uniquely, either to decrease the adverse effects of one phytocannabinoid or potentially to increase the therapeutic effects of a phytocannabinoid. And Dr. Piomelli mentioned nabiximols, this combination of THC and CBD that has been studied clinically as well as preclinically. And one of the rationales for developing this combination of THC and CBD was the potential for the presence of CBD to mitigate some of the adverse effects of THC. And not really with the thought that CBD might magnify some of the therapeutic effectiveness, although I think that many of us have come to believe that that may also be a possibility. And so I was interested in learning more about the phytocannabinoid effects in these models and in investigating if there was any scientific basis to this notion of the entourage effect. And at this time at Temple University, I was really honored to be in the same department as a very well-renowned pharmacologist, Dr. Ronald Tallarida, who was a pioneer in looking at drug synergy and being able to study using animal models how and to what extent two different drugs would interact with one another. And so I met with him, and we planned this experiment to look at potential entourage effects of CBD and THC in this model. And so this is a very full dose response curve of CBD in the preventive assay. You can see here that CBD in our hands and in many other hands has a very complex dose response relationship. We actually had sort of two efficacy peaks here, somewhat of an N-shape dose response curve. And we tested a very wide range of THC doses as well in the preventative model. And we saw sort of a similar pattern of these two different peaks. And so basically, what we did with consultation with Dr. Tallarida was to look at the ascending limb of these dose response curves for CBD and THC. Next slide, please. And determine what the ED50 was for CBD in this assay and what the ED50 was for THC in this assay. And then what we did is we modeled testing combinations based on Sativex, looking at a relatively one-to-one ratio of CBD and THC. And so you could predict based on the known ED50s of either drug alone that an ED25 of CBD combined with an ED25 of THC should experimentally give you the ED50 of the drug combination if these two drugs were working additively. If these two drugs were working synergistically, the ED25 combination of this drug would produce more than an ED50 effect. If these two drugs in combination were working sub-additively, you would see the opposite. Next slide, please. And so when we tested a wide range of these combinations on a one-to-one ratio, first of all, we still got this interesting shaped dose response curve, somewhat of an M-shaped dose response curve. And we found that much lower dose combinations than the ED25s produced an ED50 in the combination. And so you can see here the combination dose to produce the ED50 was 0.146. So we're looking at roughly 0.08 mgs per kg CBD and 0.08 mgs per kg THC in combination producing the ED50. And you would predict that these combinations really shouldn't have any effect in this assay. And so we demonstrated for the first time experimentally that CBD and THC may work very profoundly synergistically in this prevention model of neuropathic pain. Next slide, please. And so we have a lot of different experiments planned in our laboratory to explore the mechanisms of action of CBD and THC in these models, mechanisms of action of synergy, as well as to take this research beyond CBD and THC and look at other (inaud.). What I'm really the most (inaud.) taking place within the next five to ten years. I'm very excited (inaud.) researchers such as Dr. Meg Haney and Dr. Marisa Weiss (inaud.) clinical trials (inaud.) preclinical literature on pharmacological and neuroprotective and anti-inflammatory effects of beta-caryophyllene as well as recent reports that beta-caryophyllene may act as an agonist at cannabinoid CB2 receptors. And so we've started comparing the effects of CBD and BCP in some of our animal models, including our CIPN model, and looking at their combinations. So again, here is a concentration or a dose response curve for CBD in the Taxol prevention model. Here is a dose response curve for CBD. And we've started looking at their combinations. And what we are seeing so far, these data are not complete, what we're seeing so far is that we would combine CBD and BCP in this model. We're getting hints at synergy, but nothing as extreme as really this tenfold shift in potency that we saw when we combined CBD and THC. And so we're going to continue this combination work with CBD and BCP as well as other phytocannabinoids and terpenes as well. Next slide, please. But one of the more interesting aspects of this work with CBD and BCP is that while we're not seeing very robust synergistic interactions behaviorally, we do believe that we're seeing some potential synergistic interactions on a neuroinflammation level. So what we do in these CIPN mice is when they're done with their studies we harvest their spinal cords and do immunohistochemistry around the L5 region. And specifically, we look at microglial iba-1 staining. You can see that paclitaxel increases iba-1 staining, showing an increase in microglia activation in mice that were treated with paclitaxel. Interestingly, in this model and other models that we've been looking at, we don't see, even at doses of CBD and BCP that show robust behavioral effects, we don't show decreased microglial activation with these agents alone. However, when we look at the mice that received BCP and CBD in combination, we do see a profound reduction in microglial iba-1 staining in the dorsal horn of the spinal cord. And again, this is an early quantification of these data. And although these are preliminary data, I feel confident in showing them to you because we've actually seen the identical finding in our mouse model of stroke. So we have tested CBD and BCP alone and in combination in a mouse model of permanent ischemia, and we see the exact same pattern with microglial activation. That CBD is effective in reducing infarct size. BCP is as well. But neither of them are effective in the stroke model of reducing microglial activation. However, when we look at the combination, we see this reduction in microglial activation. So this suggests that a potential synergistic mechanism of action of the phytocannabinoids and terpenes may be on the neuroimmune level due to these different cannabis constituents having different mechanisms of action of decreasing inflammation and neuroimmune activation. Next slide, please.

>> And Sara, I just want to let you know we'll need to start wrapping up your presentation.

>> Right. Thank you. And this is just my last data slide. I just will quickly mention that another avenue that we've taken with our CBD research for neuropathic pain is looking at synthetic analogues of CBD. And there are several reasons why we're interested in synthetic analogues. One, to potentially increase the bioavailability of CBD to potentially reduce some of the potential adverse effects of CBD such as some drug-drug interactions and hepatic toxicity. But a more sort of purely scientific reason that I'm interested in studying analogues of CBD is as I mentioned there are so many potential mechanisms of action of CBD that it can be a very daunting task to try to tease apart what the important mechanisms of action are for CBD for different therapeutic endpoints. And I think it's very interesting to think of the designing of synthetic analogues of CBD that may be more selective to more specific mechanisms of action and to look at what types of pharmacological effects they produce to start to tease apart some of the different important mechanisms of action of CBD for these different therapeutic targets, including neuropathic pain and the prevention of CIPN. And if you're interested in this work, we have some of this work already published in Journal of Molecular Neuroscience and another publication soon to be coming out in British Journal of Pharmacology. Next slide. And next slide after that. And these are all of the people that I need to thank for inspiring me to do this research and for being wonderful collaborators in this space. And thank you very much for your time.

>> I'd like to begin by thanking the conference organizers for inviting me to this very timely and important meeting. The authors have no conflict of interest. In order to study nausea and vomiting in the laboratory, we use two animal models. The first is a Suncus murinus or house musk shrew. And these animals wretch and vomit in response to the administration of a toxin such as lithium chloride. We use this species to evaluate whether a drug reduces vomiting. The other measure that we use in our laboratory is conditioned gaping. Although rats can't vomit, they do show conditioned gaping in response to the administration of a flavor that is paired with an illness inducing agent like lithium chloride. And they also show conditioned gaping responses upon re-exposure to a context that has been previously paired with illness produced by a drug like lithium. We use this measure over that of conditioned taste avoidance because it is more selective. Taste avoidance is produced by almost all drugs paired with the flavor, even rewarding drugs. Unlike conditioned taste avoidance, conditioned gaping is produced only by emetic drugs, not by rewarding drugs. Also, unlike conditioned taste avoidance, conditioned gaping is attenuated by anti-emetic drugs whereas conditioned taste avoidance is not. Topographically, the condition gape in rats requires similar musculature as the shew wretch before the shrew vomits. So conditioned gaping is a standard measure that we use in our lab to evaluate whether a drug reduces or produces nausea. To test if a compound reduces acute nausea during conditioning, rats are pretreated with the compound before we make the taste illness pairing. So during the conditioning treatment, they're given the pretreatment or vehicle prior to saccharine paired with lithium or saline. Then a test. When we re-expose the animals to saccharine, if the rats show less conditioned gaping than vehicle controls, the pretreatment compound is thought to reduce nausea, presumably because it lessened the impact of the nausea produced by lithium during conditioning. So in this figure, you see animals pretreated with vehicle prior to lithium during conditioning show gaping but when compound X is given, let's say a cannabinoid, is given prior to lithium, you see there's less gaping at test in a drug-free test. This suppression of gaping is not interference with learning per se because the pretreatment typically does not interfere with the establishment of conditioned taste avoidance when rats are subsequently given saccharine to drink. We measure the amount of saccharine that they consume, and you can see the animals given vehicle prior to lithium show suppressed consumption. But animals given the compound X prior to lithium also show suppressed consumption. So this treatment does not affect taste avoidance. It only affects conditioned gaping, this nausea-induced behavior. To assess anticipatory nausea, which is a model of the nausea that chemotherapy patients would experience upon returning to the treatment environment, rats undergo four conditioning trials where a distinctive context, in this case a black plexiglass box, is paired with illness-inducing lithium chloride. After four conditioning trials, rats are placed back in the box, this time in the absence of lithium, just as a chemotherapy patient was walking back into the clinic, and the rats show conditioned gaping to the contextual cues. So to assess if a compound reduces the expression of anticipatory nausea, before returning to the context at test, rats are pretreated with the compound. If the rats show less conditioned gaping than vehicle pretreated controls, then the compound is thought to have anti-nausea properties. So we've looked at the effect of cannabinoids on these preclinical models of nausea and vomiting. And the cannabinoids that we focused on are THC, CBD, CBD acid, and CBD acid methyl ester, a more stable version of CBD acid. Anecdotal reports from patients indicating that smoking relieved their chemotherapy-induced nausea and vomiting prompted oncologists to begin looking at the antinausea and anti-vomiting effects of cannabis. Certainly THC, the only intoxicating component in cannabis, has been shown to be effective in reducing nausea and vomiting in cancer patients undergoing chemotherapy. And results from lab using shrews, the species that is capable of vomiting, has shown a dose dependent reduction in lithium chloride-induced vomiting by THC. So here we see the number of vomiting episodes on the Y-axis and the various doses of THC or controls injected with vehicle on the X-axis. We see that the doses ranging from 3 to 20 milligrams per kilogram IP reduced vomiting in the shrews relative to the vehicle controls. Then we tested the ability of THC to affect acute nausea in rats. That's the model where we pair a novel sweet saccharine with nausea-inducing lithium chloride. And again, we see a dose dependent effect of THC. Rats pretreated with 1 and 10 milligrams per kilogram IP showed significantly less gaping than vehicle controls, indicating that THC indeed reduces acute nausea. THC also reduced anticipatory nausea pairing contextual cues with nausea inducing lithium chloride. As we see, we see a dose dependent reduction in anticipatory nausea. The downside of THC, though, is at high doses it is sedating. Because it's intoxicating, it may not be the best treatment for some patients, although other patients may find this aspect of the treatment beneficial. As most of you probably know, the cannabis plant does not just contain THC. It contains a number of other cannabinoids which are not intoxicating. One such compound is cannabidiol. Interestingly, CBD does not bind to the typical endocannabinoid receptors, but a number of CBD's other behavioral effects such as reducing anxiety or inflammation have been shown to be 5-HT1A receptor mediated. And there is evidence that CBD displaces 8-OH-DPAT, the classic 5-HT1A receptor agonist from the 5-HT1A receptor at micromolar concentrations, indicating that CBD does exert its action at this 5-HT1A receptor. So we then asked whether CBD reduces toxin-induced vomiting in shrews and acute and anticipatory nausea in rats. So looking at the effect of CBD in acute vomiting in the shrews, we see that relative to the vehicle pretreated shrews, CBD effectively reduces nicotine-, lithium-, and cisplatin-induced vomiting at a dose of 5 milligrams per kilogram SC. To determine the mechanism of action for CBD's effects, we administered the 5-HT1A antagonist WAY 100 135 prior to CBD to see if CBD's effect on vomiting was 5-HT1A receptor mediated. And we see that we are able to block the CBD-induced suppression of vomiting by administering the 5-HT1A receptor antagonist, indicating that CBD's effects on vomiting are 5-HT1A mediated, acting through serotonin. We also wanted to look at the effect of CBD in our rat model of acute nausea using both male and female rats. As you can see, both male and female rats treated with 5 or 20 milligrams per kilogram of CBD gaped less at tests than vehicle controls. There was no sex effect in the suppression of gaping, in the effectiveness of CBD on the suppression of gaping. And similarly, when we administered either of the two classic 5-HT1A receptor antagonists, we were able to block CBD's suppressive effects on acute nausea, again indicating a 5-HT1A receptor mediated effect for CBD on acute nausea. So really to understand how CBD was working at these 5-HT1A receptors, we needed to go into the brain. These 5-HT1A autoreceptors are located on the soma and the dendrites of serotonergic neurons. And they are discretely localized within the raphe nuclei of the brain stem, predominantly in the dorsal raphe nucleus. When administered systemically, 5-HT1A agonists inhibit serotonergic cell firing to the DRN. And similarly, when directly applied to the DRN, we see decreases in serotonin levels in terminal regions. So this was the target for us to deliver CBD directly to the brain to determine if indeed CBD is acting as a 5-HT1A agonist on these somatodendritic autoreceptors to reduce the release of serotonin and have an antinausea effect. So when we administered the CBD systemically, we replicated the suppression of acute nausea produced by 5 milligrams per kilogram of CBD systemically. And when we administered the 5-HT1A receptor antagonist, WAY 100 635, to the DRN, we blocked CBD's effect. And when the antagonist was administered outside of the DRN because of misplaced cannula, the red triangles here on the right figure, we did not see CBD's effect being blocked, indicating that the effect of CBD was mediated by 5-HT1A receptors, agonism of the 5-HT1A receptors selectively in the DRN. And conversely, when we delivered CBD directly to the DRN, we saw that the CBD-induced suppression was blocked by systemic administration of the 5-HT1A antagonist. So our working hypothesis then is that CBD is acting on these 5-HT1A receptors in the DRN to ultimately reduce the release of nausea-inducing serotonin to forebrain regions. I don't have time to give detailed information about this slide, but we have subsequently shown in a publication in eNeuro in 2018 that the forebrain region whereby CBD prevents serotonin release is the interoceptive insular cortex. So as you can see, animals that were treated with lithium and pretreated with vehicle show elevated serotonin in the interoceptive insular cortex shortly after lithium administration and for the first 20 minutes. However, CBD prevents that elevation in serotonin in this region. Unfortunately, CBD has a limited window of efficacy in treating acute nausea as only lower doses are effective with higher doses being ineffective. This is a typical biphasic effect of cannabinoids in CBD and other models. We've previously shown that this high dose, 40 milligrams per kilogram, actually potentiates lithium-induced vomiting in shrews. So CBD may not be the ideal therapeutic agent. Interestingly, though, unlike THC, CBD has no effect on locomotion at doses ranging from 1 to 10 milligrams per kilogram. So GW Pharmaceuticals had some data suggesting that CBD acid may be more potent behaviorally than CBD. So we decided to look at its effectiveness in these models. This is the acidic precursor to CBD that is present in the fresh cannabis plant. So upon heating or just even normal drying of the plant, CBDA is decarboxylated to CBD. At the time we started the work with CBDA, there was hardly anything in the literature about this compound, so it was quite exciting to us to be working with this. So we began looking at the effect of CBDA in the acute nausea model, starting with a dose of 5 milligrams per kilogram IP because that was our effective dose of CBD. And we found that it didn't work at all. We got a bit discouraged, and we went to even higher doses still with no effect. But based on data provided by GW Pharmaceuticals, we decided to see what would happen if we went to lower doses. And as we went to these lower doses, CBDA became effective in reducing acute nausea even at 1 micrograms per kilogram or 0.001 milligram per kilogram. CBDA still reduced acute nausea, was highly effective, indicating that it is more than 1,000 times more potent than CBD. Remember, the effective dose of CBD was around 5 milligrams per kilogram. Certainly CBDA has a wider therapeutic window than CBD. So finally getting the dose sorted out, we went on to look at the mechanism of action of the antinausea effects of CBDA. We went right for 5-HT1A receptors like CBD. And as you can see, the WAY compound 5-HT1A receptor antagonist blocked the suppressive effective of CBDA on acute nausea, indicating that, like CBD, it also is 5-HT1A receptor mediated. Then we determined if a synergy might occur by combining sub-threshold doses of ondansetron, a typical antiemetic drug, and with CBD acid. And as you can see, the combination of these two ineffective doses of each of those compounds, when combined, completely prevented nausea-induced conditioned gaping. Then we looked at the ability of CBDA to reduce contextually elicited anticipatory nausea in rats. That is the model of anticipatory nausea in chemotherapy patients. And we likewise found that very low doses of CBDA were highly effective in suppressing anticipatory nausea in this rodent model. And again, we were able to block CBDA's effect with the 5-HT1A receptor antagonist, indicating that CBDA's effect on anticipatory nausea is also 5-HT1A mediated. And importantly, like CBD, CBDA does not impair locomotor activity at effective doses. Finally, we tested the ability of CBD acid to reduce vomiting in shrews, toxin-induced vomiting. And we looked at lithium and we looked at cisplatin as the two toxins. Each of these compounds produce vomiting in vehicle treated animals. But CBDA at 0.1 and 0.5 milligrams per kilogram reduced lithium-induced vomiting and at 0.5 milligrams per kilogram reduced cisplatin-induced vomiting in the shrews. One problem using CBD acid, however, is that it is relatively unstable. It's quite easily decarboxylated to CBD. It may even be partially decarboxylated at room temperature. So with this in mind, Raphael Mechoulam, our collaborator in Jerusalem, synthesized CBDA methyl ester, which is more resistant to conversion to CBD. Actually, Mechoulam is the discoverer of CBD acid many years ago. So we tested this CBDA methyl ester or HU580, to see its effectiveness in reducing nausea and vomiting. And this in collaboration with Raphael Mechoulam and Roger Pertwee at University of Aberdeen. Rats were pretreated with vehicle or one of three very low doses of CBDA or CBDA methyl ester HU580 ranging from 1 to 0.1 micrograms per kilogram before being conditioned with saccharine paired with lithium. They were then tested 72 hours later. As you can see, HU580 but not CBDA reduced lithium-induced gaping at a dose as low as 0.1 micrograms per kilogram or 0.001 milligrams per kilogram IP. At a dose of 1 microgram per kilogram IP, both reduced conditioned gaping. So as you can see, CBDA methyl ester or HU580 is not only more stable but also may be a bit more potent than CBDA in reducing nausea. The final two bars represent two additional groups that were pretreated with a 5-HT1A antagonist prior to vehicle or 0.1 micrograms per kilogram of HU580. As with CBDA, 5-HT1A antagonist prevented the antinausea effect of HU580 or CBDA methyl ester. So this is 5-HT1A mediated. As with acute nausea, HU580 was also more effective than CBDA in reducing anticipatory nausea, even at a dose as low as 0.01 micrograms per kilogram, amazingly low. And HU580 also had no effect on locomotor activity. So to summarize, each of these cannabinoids reduced vomiting, reduced acute nausea, and reduced anticipatory nausea in this model. But CBDA methyl ester may be the most potent of each of these and appears to be more stable than CBDA. So finally, we wanted to take this work to a preclinical translational level. And this work, this has been published recently in Psychopharmacology. We wanted to determine whether chronic administration of these compounds would modify their ability to reduce nausea and also whether repeated treatments with these compounds might reduce their effectiveness in reducing nausea. This slide presides the mean number of gapes seen at test among rats given repeated, that is seven times, exposure to CBD, the top section, CBDA, the middle section, and HU580, the bottom section at an effective acute dose of each compound prior to treatment for lithium-induced nausea in a single conditioning trial. As is apparent in each section, rats given repeated pretreatment displayed similar suppression of gaping as rats given acute pretreatment for each of these compounds. Tolerance did not develop to the potential of these compounds to reduce nausea. As well, in the final two bars of each of these figures, pretreatment with a 5-HT1 antagonist WAY 100 635 prevented the antinausea potential of each compound even following repeated treatment. Then we tested to see if the pretreatment would maintain efficacy across repeated treatment sessions, that is conditioning trials. And as you can see, all pretreatments maintained their efficacy across four conditioning trials, suggesting that they would maintain efficacy across repeated treatments in clinical trials. Then we evaluated if repeated CBD, seven daily pretreatments, would modify its potential to reduce lithium-induced vomiting in shrews and there were no significant differences between acute and repeated CBD in the reduction of lithium-induced vomiting. Both reduced vomiting relative to vehicle treated controls. We have not tested chronic administration of CBDA or HU580 on lithium-induced vomiting in the shrews. Finally, I'd like to thank all of the students and collaborators who have been involved in this work as well as the people who funded the work. And I thank the conference organizers for the opportunity to share it with you.

>> As Dr. Fu hopefully gets the audio fixed, I'm going to begin just by going through some of the questions that came up with that last presentation. And again, I want to thank my fellow presenters for a very outstanding session. So Dr. Parker, one question that came up was THC tested at these very low doses like the CBD and the CBDA?

>> THC was tested certainly in shrews at low doses for vomiting, at doses below 1 milligram per kilogram, and it was ineffective. And other people have shown that too. So these very low doses of THC are ineffective against vomiting. We haven't done quite as broad of a dose response curve with the nausea model, but we have compared a low dose of THCA and a low dose of THC. And THCA is much more potent than THC. So I didn't present the THCA data, but THCA at a dose of 0.5 milligrams per kilogram is effective. I'm sorry. 0.05 milligrams per kilogram is effective, but not THC at that low of dose. But THC at a dose of 0.5 milligrams per kilogram is effective in reducing nausea. So in various studies, we've tested lower doses, and we don't see this dramatic effect that we see with CBDA. And THCA is about ten times more potent than THC in these models, but we haven't really explored this low dose range with THCA. But I do think it's time to consider some clinical trials with this, especially the HU580 compound, which is stable and like a drug can be administered at a stable dose. It's stable over 21 days. Mechoulam left it on a shelf at 4 degrees centigrade. This is the way it's been tested in the paper. At 4 degrees centigrade, CBDA did show some decarboxylation over 21 days, but CBDA methyl ester didn't. That's the reason I chose to present on this particular topic because I feel quite excited. I think it has real clinical potential for nausea and vomiting.

>> And Linda, that came out of the chat function too. One of the members of the audience asked what else needs to be done to further validate your studies. Are there other models? Why isn't CBD or CBDA being assessed in patients?

>> I agree. I think it's time. But I don't know. I don't do clinical trials, so unfortunately my lab kind of ends at the preclinical level. The clinical trials, I'm hoping to get the information out there so somebody who does clinical trials might want to take this on. I'm happy to work with anybody who is interested in it.

>> Hopefully this whole forum and this symposium will stimulate that.

>> Yeah.

>> Moving up to Dr. Ward, one question that came up in the chat is have you looked at the time course of the inflammatory biomarkers after your chemo treatment? Does it change over time?

>> We haven't gotten a chance to do that yet. Can you hear me?

>> Yes.

>> No. So far, we've only looked at the day 14 time point with the immunohistochemistry. But I think that that's an excellent idea to look. There's going to be different neuroimmune changes at different time points. So I think it will be really important to look 24 to 48 hours after either the first or the fourth injection and then also to look for other -- but so far, we've only been focusing on that day 14 time point when we're measuring the peak level of allodynia in the mice.

>> So question. This is Yali. Hello?

>> Hi, Yali. We hear you.

>> Yeah. Okay. Sorry. Let me address some questions for Dr. Lichtman yourself, my co-chair. Very exciting data on DAG lipase. One question is are there any clinical trials of these enzyme inhibitors that are planned?

>> So right now, MAG lipase inhibitors, at least there's one that has made it into phase two clinical trials. And that was for a different endpoint. That was for Tourette syndrome. And those trials ended because of lack of efficacy. So there aren't clinical trials presently with these drugs. Again, that's something that could be advanced with any of the drugs that have already passed through phase one. And I didn't really even get into FAAH inhibitors, which Dr. Piomelli has one that is advanced in clinical trials for other indications. Any of these could be evaluated.

>> That's my question. Are they orally available or they are all by injection?

>> Well, the ones that have been in clinical trials are orally available. And I think Dr. Piomelli could jump in on this. Your B597, that's orally available, right? So yes.

>> Okay. Another question. Did you publish data that suggests that nabiximols are associated with a reduced use of opioids in cancer pain?

>> So the clinical trials that you're referring to, the patients were specifically instructed to maintain their current level of opioids. So we weren't able to assess any so-called opioid sparing effects in those studies.

>> Okay. Thank you. Now, the other question addressed to the second speaker, can cannabis be considered less addictive than opioids, Dr. Piomelli?

>> Could you repeat the question?

>> The cannabis be considered less addictive than opioids. So be better for pain management.

>> The question is whether cannabis is less addictive than opioids? Well, that depends what opioid you're talking about, right?

>> Yeah.

>> Talking about carfentanil, fentanyl, morphine or are we talking about codeine. These are different opioids and they have different addictive potential. But by and large cannabinoids have less of an addictive potential than opioids do. I think that the overall consensus of the literature of the investigators is such. (inaud.) do know and I think that is also very well established, that cannabinoids can cause dependence and they do cause dependence. What I do think still needs to be understood correctly is to what extent they do. If we look at, for example, nicotine or alcohol or psychostimulants and we compare cannabis to those drugs, what is the rate at which cannabinoids promote dependence compared to those drugs. It is a very important type of analysis which at least to the best of my knowledge has not been fully conducted. And I think it's really important to do it now.

>> I see. Thank you. So another question for I think it's Sara, Dr. Ward, is what is the time course of inflammatory response in your CIPN model?

>> Yeah. Dr. Lichtman asked me that right before you jumped on.

>> Sorry. Sorry. I didn't hear that. I was fiddling with the audio.

>> No problem.

>> Yeah. There are a bunch of questions for Dr. Parker. You said why wasn't THC studied at low levels like the methyl ester?

>> I'm sorry. Pardon?

>> Why wasn't THC studied at low concentrations like the CBD methyl ester. Is that biphasic also?

>> Oh, are they biphasic?

>> Is THC biphasic too?

>> We've gone to higher concentrations. So we started at 5 milligrams per kilogram, which is kind of where our best dose for CBD. And we went higher. And we just went to a few higher concentrations. They weren't effective. I think we went to 10, and it didn't work. So we started going lower. So it's biphasic. The higher doses, it's more effective at 1 microgram per kilogram than it is at 1 milligram per kilogram.

>> Okay.

>> Strangely enough, but it is. And we couldn't believe it. This experiment was how low can we go. We called it that. We kept taking it lower and lower and lower until it went away. And it went away at -- 0.05 micrograms per kilogram, it got weaker. And then 0.005 it went away. So it's just incredibly -- but it is biphasic. But we didn't test extremely high doses to see if it would come back again. Yeah. So we don't know what happens at very high doses of this.

>> Okay. So Dr. Lichtman, one question is what is the mechanism of KT109 in reducing neural hyperexcitability? The DAGL inhibitor.

>> Yeah. (inaud.) there's been (inaud.).

>> Dr. Aron Lichtman, it sounds like your connection is kind of breaking up, in and out, and being a little spotty. One thing I would recommend if that continues is to maybe just turn off your webcam as that might save on some bandwidth if you're on the East Coast getting hit by some storms.

>> Is it better now? Dr. Lichtman?

>> Okay. Can you hear me better now? Sorry about that.

>> Yes. Yes.

>> Okay. So I'll repeat the answer to that question.

>> Yes. Please.

>> Very rapidly here. So we think that DAG lipase beta inhibitors are working in the periphery because KT109 does not cross the blood-brain barrier. It's most likely on macrophages and it could be as a result of a consequence of reducing arachidonic acid within the macrophages and the downstream mediators. Likewise upstream, it could also be due to an increase of diacylglycerols, which are then going to signal at protein kinase C and can potentially have anti-inflammatory effects as well that way. So it could be a combination of upstream and downstream influences or alone. So we're trying to separate out those.

>> Okay. Thank you. Let me see if there are other questions. So the general question to all speakers are what are the gap areas and your thoughts on future recommendations and directions. We can start with Dr. Lichtman and go down through the speaker list.

>> Okay. Well, I think a lot of the gaps, and I'm going to speak more broadly, with the phytocannabinoids is we really need to have controlled studies. Given the wide availability of THC, CBD, and different cannabis extracts and we really need to know more about dosing and effectiveness. And these controlled studies are I think of paramount importance. Likewise, the other work that's focusing on synthetic types of agents as well as the enzyme inhibitors are of very important interest as well. But I think the lower hanging fruit are the phytocannabinoids and the terpenoids that we heard about from Dr. Ward. Dr. Piomelli?

>> I completely agree with you, Aron. I think what we need is to get final answers to whether cannabis is effective in pain and particularly in cancer pain. There is tantalizing evidence out there. Evidence needs to (inaud.) clinical trials. I do believe that this is an investment worth making at this point in time (inaud.). But I also do believe that we are going to be extremely -- we're not going to see something momentous. This is my own personal bias. I don't think cannabis, per se, will completely change the fate of analgesic management in cancer pain. But I think it can be a useful add-on. Going forward, I have more hope that, I think maybe I'm biased as well, but I have greater hope on the endocannabinoid system offering new sources, new targets for therapy. It must be, however, (inaud.). I like to highlight it must be tailored for the particular type of pain condition that we are looking at. So cancer pain is different from neuropathic. Neuropathic pain is different from CINP. I think this is a very important point that we need to appreciate how heterogeneous pain is as a pathological construct. And we need to design models as well as clinical trials that capture this heterogeneity. I'd like to say just one last thing. While talking about diversity and heterogenetic, I think it's also important to consider that we need to make sure that all the animal work that we do, there is resemblance to the real world. And by that, I mean using hopefully multiple species of animals. Using both sexes. And also using animals of different ages. We will not be able to capture the diversity of the human condition. But at least we'll make one effort toward (unint.) validity, which is something that often our studies lack.

>> Daniele, that also brings up another interesting point that the preclinical studies that are looking at opioid sparing effects of cannabinoids usually use drug naive animals. But that's not really the case in the clinic. Most of these patients have had opioids for some amount of time. What would be your recommendations to better model the clinic situation?

>> I think this is something that a group of experts should sit down and talk about. My own feeling is that if we use multiple species and the two sexes and animals of different age, we have a better sense of reality. Now, having said, we also need models that are valid. Not just models that produce pain but models that have constant validity, phase validity, fully also predictive value. The model you use, Aron, is a great model because it's a model that has clearly phase validity and constant validity. It would be really important to see if it does also have predictive value in terms of what is (inaud.) in the patient and vice versa. But the important point that we as scientists I think need to internalize, and that's a difficult thing to do, but internalizing diversity because the world outside us is very diverse and we often try to simplify things in the lab so that we have robust data. That's (unint.). But we can't pretend that the diversity does not exist in our models. And again, this is the topic that I think would be very good to discuss at the NIH level, having folks from different (inaud.) society contribute to the conversation.

>> Excellent. Excellent point. We need particularly relevant models and multidisciplinary teams like clinical oncologists working together with neurobiologists and psychopharmacologists such as Dr. Parker and her team. Okay. Dr. Ward and Dr. Parker, your thoughts.

>> Yeah. I would just reemphasize the point about dose that Dr. Lichtman brought up. I think for CBD, you could see at least from my data and from Dr. Parker's data, and this is true for THC as well, the very unique dose response relationships that we see. And Dr. Abrams and others have reported this in humans as well. It's not an artifact of the animal studies. There are ultra-low dose effects, low dose effects, high dose effects. And sometimes, they can be in opposite directions. And so we have to have a better understanding of how the doses we test in animals relate to the doses and concentrations that people are using. With CBD, people using over-the-counter CBD use 10 to 30 milligrams a day and report effects anecdotally. And then you look at the Epidiolex clinical trial, and you're looking at 800 milligrams a day. And we don't know if really low doses are effective and really high doses are effective and somewhere in between. We have hints from in vitro, in vivo, and human studies that there are going to be sweet spots in different dose ranges. And then there are going to be areas where you don't see effectiveness. And so it's really scary to me when I do get an opportunity to talk to clinicians who want to run clinical studies with CBD and they ask me, well, what do you think for a dose. That's really scary for me to think that we can try to select a small dose range in human studies, and we may completely miss the mark and miss something that could be very effective if we were to have started much lower or much higher. So I think we need to put a lot more work into understanding the dose response relationship of CBD and plasma levels and CNS tissue levels.

>> I can't hear you, but maybe you're asking me. Okay. I agree. The dose response data with CBD is all over the map in terms of different indications, even in the animal literature. I have a student that's starting to put together a massive review looking at this effect because I know our doses tend to be much lower when we deal with nausea than people who are dealing with addiction who have much higher doses. At these high doses in our vomiting model, we get enhanced vomiting. It's actually bad to go to high doses of CBD. It would be devastating to patients because it would make them sicker. So if the animal model translates, that is. I don't want to overstate it. But if the shrew model translates to humans, then high doses are dangerous. So I think that we really do need clinical trials. We need human clinical trials where these drugs that are relatively safe, we know that CBD and CBD acid are. Certainly in these animal models, they don't produce locomotor effects. They don't produce other kinds of effects. They're antianxiety. Animals that are stressed, highly stressed, CBD acid at the same low doses reduce anxiety. And also CBD acid, other people have reported that CBD acid and this methyl ester also reduce pain, thermal pain, sciatic nerve pain, at these low doses. At higher doses, it effects anabolic effects like obesity. Yossi Tam has been doing work on obesity. And HU580 is effective at milligram doses. So different doses do different things. And we have to make sure that if we go to higher doses for one effect that we're not producing, like enhanced vomiting in the case of the shrews, for other effects. So I think that's something that needs to be sorted out. But that being said, it's relatively safe. And I think it's time for at least clinical trials with CBD and CBD acid because there's nothing. Actually Raphi Mechoulam and I and Erin Rock, my postdoc, have put together a book for MIT Press that they're looking at right now (inaud.) CBD effects. And there's nothing. There's hardly any clinical trial. It's almost all anecdotal. And I think it's time to look at this clinically.

>> Thank you. We are told to wrap up. I want to thank all four speakers for your presentation and very stimulating discussions. Dr. Lichtman, do you have some final word?

>> (inaud.) looking at both sexes and multiple species is going to be very important from a translational sense, especially as we look at the endocannabinoid system as opposed to phytocannabinoids. So I thank my fellow speakers and I thank you very much too.