>> Good morning everyone and welcome to Day Three in the session on [unclear] and Prevention. And on behalf of my NCI Co-chair Dr. Ron Johnson, we welcome Dr. Sean McAllister as Co-chair for this session. He is currently at the Pacific Medical Center Research Institute and Affiliate of Sutter Health. And Dr. McAllister’s group has an active research program focusing on the anti-tumor activity of both synthetic and plant-based cannabinoid compounds, with the goal of developing therapeutic interventions for aggressive cancers. His findings have supported multiple clinical trial concepts for targeting brain cancer. Additionally, Dr. McAllister has investigated the potential of cannabinoids to inhibit neuropathic pain and neurodegenerative disease and contributed to development of cannabinoid-based weight loss drugs. So with that I'm going to give the controls over to Sean McAllister and thank you and everyone enjoy the session. And we’ll see you at Q&A. >> Thank you very much. Good morning everybody. Let’s get this session started. Our first speaker is Dr. Mary Abood from Temple University. And she’s going to be giving us an overview of cannabinoid signaling and biology. >> Hello, everybody. I’d like to thank the organizers for inviting me to give this general talk this morning, especially Drs. Ross, Johnson and McAllister. So am going to talk to you about cannabis signaling and biology. Just a very general overview. I have no conflicts to disclose. Next slide, please. So this is adapted from, years ago, I’d like to start with talking about sort of the sociological and scientific uses of cannabis. So the first record of cannabis was an archeological record of hemp cord in 8000bc. Medical use in China was 2700bc. Religious use in India also, bc. And then we got the Arab world about 1,000ad. And then there’s a gap in the timeline until the 19th century when the western world really started to follow cannabis bioactivity. So for many years cannabis was part of patent drugs. They call it patent drugs. And they were used in tinctures, etc. And available to the general public. The first part of when cannabis started to be regulated was in the United States was during the Marijuana Tax Act in 1937. But it wasn’t really made a controlled substance until 1970 when it was made a controlled substance under the Controlled Substance Act. So this is still the case. And as you know in the 90s several states started to legalize medical use. The first state was California and Arizona though practically speaking that really didn’t happen for many years. In 1999 Dronabinol which is synthetic THC was rescheduled to schedule three, which is a lower schedule drug. And you’ve heard about Dronabinol from previous speakers, we’re going to talk about it more today as well. In 2005 the first cannabis-based medicine was approved from treatment of multiple sclerosis. It’s called Sativex or Roibixamals [phonetic]. And in 2006 a cannabinoid receptor antagonist was initially approved for the treatment of obesity. But subsequently withdrawn in 2009 due to the psychiatric side effects. From the scientific standpoint Delta 9 THC was isolated by Rafael Machulum [phonetic] in 1964. And for many years thereafter there was really not much known about exactly how THC worked at a molecular level. In 1984 Lyn Haler’s [phonetic] lab clearly showed that cannabinoids could inhibit the adenylyl cyclase and subsequently showed receptor binding with synthetic ligand. And this really started the whole scientific field was able to really advance quite rapidly thereafter. So the first CB1 receptor was cloned in 1990 and the receptor was mapped in the brain – you’ll see a picture of that in a minute – and a couple of years later the first endogenous ligand was isolated, again in Rafael Machulum’s lab. That’s called Anandamide for the Sanskrit [unclear]. The second cannabinoid receptor was cloned in 1993. And Rimonabant, which I just spoke about from a drug point of view, was discovered in 1994. In 1995 the second analogous cannabinoid was isolated. That’s [unclear] glycerol. And also we saw that we could precipitate withdrawal with cannabis. This is the first example of, one of the main animals and an example of having withdrawal signs. So in the late 90s, receptor knockout mice were created and the role of [unclear] system in retrograde signaling in the neuro system was established. And then in 2000s we find that there is a, there is some other candidate cannabinoid receptors – two which I’ll briefly mention today, GPR55 and GPR18. Next slide, please. So this is a summary of the effects of cannabis really attributed for the most part in this slide to the principle psycho active ingredient, Delta9 THC, coupled in humans. So we know they have the anti-emetic effects, there’s appetite stimulation, there’s analgesia, lowered intraocular pressure, reduced spasticity, euphoria, drowsiness, alterations in cognition and memory and immunomodulation. Next slide, please. So as I went over on the time line three have been two receptors that clearly have been identified to date. The CB1 and CB2 cannabinoid receptors. They’re members of the G-protein coupled family of receptors. And so in the CNS, activation of CB1 results in inhibition of neurotransmitters release. This has been shown due to hyperpolarization of membrane potential, mostly in pre-synaptic neurons, but there’s evidence for that in post-synaptic neurons as well. The CB2 cannabinoid receptor is primarily associated with immune cell function, although you’ll hear of other functions as well. And as I mentioned GPR55 and GPR18 may be cannabinoid receptors. So those are the receptor part of the endo cannabinoid system or the endogenous cannabinoid system. Next slide, please. So here is a snake pot, a schematic of the CB1 and CB2 receptors. And the point of this is to show that they have the molecular signature of G-protein coupled receptors of Class A G-protein coupled receptors. The CB1 receptor’s a little bigger. It has a long and terminal domain that is important for its function. Both receptors inhibit cycla MAP [phonetic] and forskolin-stimulates and adenylyl cyclase. Both receptors are involved in activation of MAP kinase signaling pathways. And both receptors can increase intracellular calcium. The CD1 receptor is unique in that it can inhibit calcium channels and stimulate inwardly rectifying potassium channels. And the other thing I want to point out is that there is overall about 44 percent identify between the two receptors. This rises to 68 percent in the transmembrane domains, which is the area where the ligands are thought to bind. Next slide, please. So as far as the ligands, there are two primary endogenous ligands that have been identified. Anandamide and 2AG. The pharmacological properties of Anandamide and 2AG are quite similar to those of THC. And if you block the degradation of both Anandamide and 2AG this actually can mimic the effects of THC. These ligands are synthesized from phospholipid precursors and in the nervous system release has been demonstrated by in vivo dialysis and liquid chromatography mass spec. It’s also been shown that these ligands are removed via re-uptake by specific carriers, although the identification of these carriers is still controversial. And they are degraded by fatty acid amide hydroslase and monoacylglycerol lipses. Additionally, there are many other endocannabinoids. I’ve listed a few of them here. Palmitoylethanolamide has gotten some attention lately. 2-arachidonyl glyceryl ether and arachidonyl-glycine or NAGly, which Sean will mention later. Next slide, please. So I wanted to show the structures of the endocannabinoid and the synthetic cannabinoids. And so Anandamide and 2AG are [unclear] acid derivatives. That’s the similar structure that you see with the two structures on the top. And THC is shown. And you can see that the auto ligands, especially CP55940 is quite similar in structure to THC. WIN 55212 is a different chemical structure. And SR141716A or Romana Band [phonetic], that’s CB1 receptor antagonist is a different structure as well. And there are many synthetic cannabinoids that are now available for study in animals. Alright. Next slide, please. So we’re going to walk through this signaling slide. And I think you’ll pick up some other signaling as well. But this is the sort of classical signaling. So on the left hand side where it says CB1, CB2, the G-protein signaling is via inhibition of adenylyl cyclase and decreased in cyclic MAP [phonetic]. Can you click, please? And there’s also evidence for increases in cyclic MAP in certain conditions. Next, please. For that synthetic antagonist win that I mention there’s a second pathway that’s been shown. Click, please. Which is via Gq and phosolate ligade C [phonetic], dia glycerol [phonetic] and intracellular calcium. Click, please. Dibata Gama [phonetic] subunit of the G-protein also plays a role. And [unclear] signaling, activation of DI3 kinase. Click, please. Again activates a different protein kinase C. You can see that all of these will converge upon MAP kinase or ERK signaling. Click, please. In addition, PI3 kinase can activate AKT and lead to activation of beta katinium [phonetic]. And I’ve given control of neuronal cell survival as an example of one of these pathways that can be activate by cannabinoid receptors. Next, please. So the receptors, the CB1 receptor is the most highly expressed G-protein coupled receptor in the nervous system. It’s widespread expression. As I mentioned it’s pre-synaptic in the central nervous system. Although there is evidence for limited post-synaptic localization that’s less well characterized. In general, the distribution parallels the known pharmacology of THC. Other areas that are important in the brain are nucleus solitary tract, hypothalamus, and motor systems. Outside the CNS we have expression throughout the, albeit low or levels, throughout the body. The sympathetic nervous system and the immune system for CB1 as well in a number of cancer cell lines and other peripheral sites like the heart, lung and adrenal. All these sites that are listed below. Next, please. So this is an old slide from one of the early mapping studies that is meant to illustrate the high level of expression of CB1. In this case in a rat brain. And superimposed on the brain areas are kind of their main function. So cerebella expression where CB1 is very high. It’s involved in balance and coordination. The hippocampus in learning and memory. The [unclear] in motor control and the nucleus [unclear] in addiction. Next, please. So in addition to expression in tissues, there’s subcellular localization of cannabinoid receptors as well that may play important roles. So that the receptors generally thought of as being plasma membrane receptor, but it’s also present on the endoplasmic reticulum. There’s perinuclear association and there’s association with other organelles; lysosomal receptors and mitochondrial CB1 receptors that have been associated with regulation of energy metabolism. Next, please. So the CB2 receptor is highly expressed in cells from the immune system, particularly bone marrow, thymus, spleen and tonsils, lymphocytes, all sorts of CB2 receptors and cells derived from the immune system. And one of the interesting things that’s been observed is that the level of expression of the CB2 receptor increases during activation and differentiation in the cells. In addition to immune cells the CB2 receptor’s been found in the uterus, in the lung, and in bone cells, ostoclasts, osteoblasts and osteocycles. It’s also expressed in microglia and there’s evidence for limited expression in the central nervous system, specifically in brainstem neurons. Next, please. So this slide is a schematic to show the enzymes that are involved in synthesis and degradation of the endo cannabinoid ligands. So as I mentioned both arachidonoylglycerol 2AG or 2 arachidonoylglycerol and Anandamide are synthesized from phospholipid precursors in cellular membranes. So for 2AG there are two lipises [phonetic], the thag laypase [phonetic], alpha libada [phonetic] and for Anandamide there are several pathways that have been elucidated. As illustrated by the three arrows, so there’s a fossil lipis pathway. There’s NAPE PLD pathway. And then there’s a APHD4 fossil diastrase [phonetic] pathway. So both Anandamide and 2AG act on CB1 and CB2 receptors and both are degraded. And that’s how they’re gotten rid of primarily. So the 2AG is degraded by MAG Lipis or these ABDH6 and 12 enzymes into arachidonoylglycerol and the Anandamide is degraded by fatty acid amahydralace [phonetic]. And again [unclear]. Okay. Next, please. So as you may know there’s a wide range of therapeutic potential for cannabinoids for the cannabinoid system. And so nausea and anorexia, click, please, are the primary, are actually the only right how therapeutic indications of cannabis in the United States. Marinol or Janabonol [phonetic], which is the oral form of Delta9 THC. Naboline or Casamet, which is a synthetic analog. These are approved for patients undergoing chemotherapy and were approved for AIDS wasting syndrome. Next, please. For pain and spasticity, click, please, we have Sativex or Nabiximal [phonetic], that are approved in many countries, not yet here in the United States. Next, please. So obesity, I mentioned that Robodovat [phonetic] was indicated for that, but was withdrawn due to psychiatric side effects. Next, please. And most recently CBD has been approved for epilepsy, specific forms of epilepsy, severe epilepsy in children. And, of course, we think that there’s therapeutic potential in antitumor agents as well as in addiction. Next, please. So I wanted to bring this slide up to show that in addition to THC the cannabis plant has over 50 cannabinoids. These are some of the more prevalent ones. CBDH I’ve already mentioned. And they have been shown to have a variety of effects as well. Anti-cancer, analgesic, anorexic. So the cannabinoids and the cannabis plant have numerous effects as well. Next, please. So I just wanted to show CBD versus THC. You can see that they’re structurally quite related. But they’re very different in terms of their effects. So the story I think that many people are aware of, but if you’re not, was that Charlotte’s Web high CBD cannabis was found to be useful in Dravet’s syndrome. And this led to the study of many companies to form epidiolex. And GW was able to get CBD approved by the FDA for Dravet’s and Lennox-Gastaut’s syndrome which is another intractable form of childhood epilepsy. Next, please. So I also wanted to mention that the synthetic agonists can be a problem. So there are many compounds that were used for, initially for scientific purposes but have gone onto the abused market. And they were sold as spice or incense and I’ve got a picture of it on the bottom right hand corner. They’re basically selective potent agonists of CB1 that were developed as pharmacological tools and are used, were temporarily not illegal, so they were used and they were able to be bought in gas stations and things like that and can be still obtained over the internet. There are reports of their affects that are similar to cannabis but they produce intense withdrawal. And they’re being banned, but they’re still a problem. So next slide, please. So just to point out that while cannabis has a number of physical effects, it’s shown in blue, the synthetic cannabinoid seem to have amplified effects as well as different effects, including anxiety and agitation, confusion and hallucinations, paranoia and psychosis, headache, seizures, very increased heart rate and blood pressure, myocardial infarctions, vomiting, diaphoresis and there have been reports of death with synthetic cannabinoids. Okay. Next, please. So I just wanted to bring up the evidence for additional cannabinoid receptors. So as I mentioned earlier there have been receptor knockout mice in several laboratories. And the persistence of biochemical, electrophysiological and behavioral responses in these knockout animals suggest that there might be additional sites at which are cannabinoid receptor subtypes. So Anandamide, for instance, produces the full range of behavioral effects, antinociception, catalepsy and impaired locomotor activity in CB1 knockout mice and there’s been a non-CB1, non-CB2 site that was described in microglial cells. This receptor seems to respond to both endocannabinoids and 2AG as well as synthetic cannabinoid.   
Next, please. So this receptor GPR55 is an orphan. You can tell it’s an orphan because it doesn’t have a name associated with it. So they’re indicated as GPRs. So it was isolated in 1999, had high level of expression in human striatum. It’s like CB1 and CB2 it’s a Class A GPCR and has some identity, albeit very low with CB1 and CB2. Next, please. So this receptor’s been studied in my lab and several others and the emerging evidence indicates that it may be involved in nociception. Interestingly it’s proposed endogenous ligand, lysophosphatidylinsitol which is not an endo cannabinoid. But several cannabinoid ligands do interconnect with GPR55. And despite limited homology, there are putative3 binding sites that have been identified by molecular modeling and we and others have identified selective GPR55 agonists and antagonists, which are allowing pharmacologically to characterize this receptor. Next, please. So why am I bringing it up. Well, it has potential physiological relevance in people, as well as being a potential endo cannabinoid receptor including an association with cancer cell proliferation and pro invasiveness. So it is an interesting receptor to study. Next, please. So the other receptor that is a putative cannabinoid receptor is GPR18. It’s another Class A GPCR and again it has some limited identity with CB1 and CB2. And interestingly some identity with the Delta Opioid receptor. Next, please. And it turns out to be a receptor for an endo cannabinoid derivative, which I mentioned earlier, and N-arachidonylglycine. NAGly is an anandamide metabolite. And GPR18 is still a candidate cannabinoid receptor. It’s been difficult to study for various reasons. But we think that part of the reason is that there are several signal transduction pathways that might be causing biased agonism. And that’s made it difficult to study. Next, please. So, for instance, just to show you that it can respond to cannabinoid ligands, if we, this is from a paper that a few years old now that we looked at GPR18-expressing cells. And what you can see in Panel A is that upon application of THC there’s a rise in intracellular calcium. And in Panel C, I think it’s easiest to look at, you can see that a number of synthetic and natural cannabinoid ligands activate GPR18, including abnormal cannabinoid and THC. Next, please. So to summarize kind of what I tried to present here today is that cannabinoid receptors were identified as GPCRs responsible for the effects of THC. Subsequently, an endogenous cannabinoid system or the endocannabinoid system consisting of lipid ligands and GPCRs has been elucidated. I think, I tried to establish that CB1 and CB2 are established cannabinoid receptors and that GPR55 and GPR18 are candidate cannabinoid receptors. And I showed you the structures yet again. Next, please. Finally, I’d like to thank the folks in my lab who’ve helped with the studies that I described as well as Eugen Brailoiu at Temple who did the calcium imaging in that one single data slide I showed you today. And thank you for your attention. >> Thank you very much Mary. Our next talk is by Dr. Manuel Guzman, University of Madrid. And his talk is focused on mechanisms of THC anti-tumor activity. >> Hello, everyone. My name is Manuel Guzman. And I'm going to summarize what we know now with this, about the mechanism of THC anti-tumor activity. First, the slide with my potential conflict of interest is July 2020. I’ve been a member of the Advisory Board of [unclear] Therapeutics from which I receive personal stipend as well as grants for research. Currently I'm a member of the Advisory Board of Canna Foundation from which I receive personal stipend. And I’ve co-authored six patents for GW Pharma, one with Plytoplant [phonetic] Research and one with [unclear]. The first report in the anti-tumor activity of THC and other cannabinoids dates back to 45 years ago when Munson and co-workers published this paper entitled Antineoplastic Activity of Cannabinoids in the Journal of the National Cancer Institute. This report had a strong impact at the time not only scientifically but also on the general mass media. And this is an example from the Washington Post saying that Cancer Curb is Studied, the active chemical agent of marijuana curbs the growth of three kinds of cancer in mice as discovered by the team of Medical College of Virginia. ‘What we think is that this work will open up a vast new area of research’ an MCV spokesman said. ‘Years of work may stem from it.’ Well, unfortunately the prophecy of the MCV spokesman didn’t fulfil. And we’ll have to wait 35 years that is going to 2000 for renaissance of the studies of cannabinoid tumor in fact to a cure. So I will try to summarize in this talk what we have learned during this last 20 years based on the main pillars on which these studies have been mostly [unclear]. First, using THC as a cannabinoid receptor agonist. Second using mice as species model for modeling cancer. And most of these studies have been conducted in brain tumors of glio origin. That is glioblastoma. In 2000 our group found out that THC was capable of inducing an anti-tumor activity in mouse and rat models of glioma [phonetic]. With this slide you can see mice harboring glioma cells and there is [unclear], there is supercantaneous [phonetic] tumors. Mice were treated either with [unclear], [unclear] mice with [unclear] cannabinoid THC and with the synthetic cannabinoid WIN 552122. And as you can see tumors from mice treated with cannabinoids either the natural cannabinoid or the synthetic cannabinoid had a much smaller size than control tumors. On the right you can see a rat harboring a glioblastoma on its right brain hemisphere in actual progression up and in [unclear] projection. Down on the left column before THC treatment and on the right column after one week of THC treatment. As you can see as pointed by the arrow THC was capable of inducting a stroke regression of the tumor and we didn’t observe any relapse of the tumor in the following months in which we monitored the animal. After those findings our group and many other groups all around the world have been trying to define the mechanisms of this anti-tumor action elicited by THC. We know nowadays that most, if not all cancer cells express on their surface amount of CB1 and RC2 receptors. And by activating these receptors THC and another cannabinoid receptor antagonists can trigger different anti-tumor processes. For example, cannabinoids can inhibit the process of angiogenesis that is tumor vascularization. And they do so by activating cannabinoid receptors and inhibiting the production and the signaling on the main molecular factor, the main cytokine which is involved in angiogenesis that is in tumors. That is vascular [unclear] growth factor. Cannabinoids as you have seen can also activate cannabinoid receptor on the surface of cancer cells to inhibit the migration, the invasion of cancer cells and therefore the colonization of other organs and there is metastases. And they do so by blocking the activity of different enzymes. For example, meta [unclear] 2 that is involved in breaking [unclear] and therefore allowing cancer cells to migrate to other sites in the tissue and to colonize other organs. THC and other cannabinoids by activating their receptors can also block the process of tumor cell proliferation. Cannabinoid can block some of the essential transitions in the cell cycle. For example, the G2M transition by inhibiting CDK1. Cannabis via cannabinoid receptors can also enhance the differentiation pattern of malignant, very little differentiated cancer cells. Therefore, allowing a more post [unclear] and more differentiated and less malignant phenotype. And they do so by inhibiting the expression and activity of different [unclear] receptors in [unclear] growth factor. However, the most widely reported action of THC and other CB1 and CB2 [unclear] agonists in cancer cells is in an induction of cancer cells deaths by the process of [unclear] death called apoptosis. And it does so essentially by inhibiting the activity of a key protein kinase for cancer cell survival that is AKT. This cartoon summarizes what we know now what there is about the mechanism of THC pro-apoptotic activity. THC and other cannabinoid receptor agonists can bind to and activate CB1 and all CB2 receptors on the surface of cancers. One of the main hallway events that occurred in THC treated cancer cells is the accumulation of a particular lipid called seramite [phonetic] in the membranes of a particular cell organal called the endoplastoparticular [phonetic], ER. This accumulation of seramite [unclear] restores the function of this orgonal and therefore induces the so-called ER [unclear] wrist bones. This response elicits the activation of different proteins started with the transcription factor P8 and ended up with the activation of a pseudo [phonetic] kinase called TRIB3. And TRIB3 can bind to and inhibit AKT. When AKT is inhibited within cancer cells some of its substrates can also be blocked of course, entered in this mTORC1 complex. And when it is inhibited the process of autophagy or cell self [unclear] can be activated. The activation of autophagy we know can damage mitochondria [phonetic], you know, the main orgonals involved in the production of [unclear] of ADPs for the cells. And so by decreasing the potential of the mitochondrial inner membrane autophagy can damage mitochondria, reducing the production of ADP and inducing the release of different factors that can activate some protase in the spaces that are involved in the process of [unclear]. So as you can see this is a very complex mechanism, but we can define it as a kind of a cul de sac or death corridor for cannabinoid treated cancer cells. So that when THC activates cannabinoid receptors on the surface of these cells by means of this progress, this sequential events cannabinoids can end up with inducing apoptosis in cancer cells. This process of apoptosis as induced by THC was discovered in glioblastoma cells, but we know nowadays that it is very widely expressed among different models of cancer. At least this core mechanism of THC induced activation of CB receptors on the surface of cancer cells, the induction of [unclear] stress, the [unclear] of the AKT signaling pathway and the activation of autophagy and [unclear] it can serve in models of a skin carcinoma, on a skin melanoma, breast cancer, on [unclear] cancer, a [unclear] carcinoma, [unclear], etc. This THC induced apoptosis is not only very [unclear], but it’s also pre-selective for cancer cells. And it does not occur in non-cancer cells. Essentially as we have seen in glioma glioblastoma cells THC by activating cannabinoid receptors can enhance seramite accumulation therefore blocking AKT and inducing apoptosis. However in primary glio cells of the mouse brain, cannabinoid, by activating cannabinoid receptors cannot decrease but enhance AKT activity and not produce cell death but cell survival. Moreover in these cells seramite is capable of killing cells and cannabinoids in primary glio cells can block seramite induced apoptosis. So in other words, in glioma cells THC uses seramite to kill the cells, whereas in primary glio cells it’s capable of preventing seramite induced apoptosis. So this jing jang [phonetic] action of cannabinoid activity that is selected for cancer cells and does not occur in non-cancer cells seems to be very interesting to allow, say clean chemotherapies in experimental models and maybe in patients, unlike general toxic chemotherapies that are usually applied to cancer patients. How good or bad is THC compared with established chemotherapies and also how California THC synergize or combine with other chemotherapies? Following with the example of glioblastoma, the standard treatment of glioblastoma, the benchmark treatment of glioblastoma is temozolomide. Temozolomide is a DNA alculating [phonetic] in DNA damaging agent that can induce DNA damage response and therefore kill the glioblastoma cells. It is known that temozolomide does not only trigger this DNA damaging action, but can also trigger autophagy the same as THC and other cannabinoid agonists can do. Therefore contributing to apoptosis. So how can THC combine with temozolomide in this mechanism of glioblastoma cell death? This is a very original, a very first experiment that we conducted 17 years ago on this issue. We induced glioblastomas in mice by using this cell line and we measured tumor [unclear] along this, of treatment. As you can see, we treated mice exponential growth of the glioblastomas, THC as the only agent that had some affect. But decreasing tumor size as the [unclear] unique agent did. However, here the most striking data came when combining THC and temozolomide. As you can see by using together THC and temozolomide we had a very strong supra additive, a very strong [unclear] action so that we can even induce a net regression of the tumors by combining THC and temozolomide. Moreover, THC does not only synergize with the temozolomide. But also as noted by Scott and co-workers with the other benchmark treatment for glioblastoma that is [unclear] therapy. So maybe THC by combining so potently with temozolomide and [unclear] therapy could be a promising agent for glioblastoma patients indicated. So to summarize what we know nowadays from a legal perspective, I think I’ve provided data, summary of data sustaining there is substantial evidence that THC and other cannabinoid receptor agonists are efficacious and safe compounds to inhibit the growth of a great array of cancer cells including glioblastoma cells in small laboratory animals, as mice and rats. There are also case reports and case series reports suggesting that maybe THC containing cannabis preparations and other cannabinoid preparations have exerted semantic tumor activity in some particular cancer patients. This is likely and this would be of course desirable. However, the currently available anecdotal evidence is in my opinion pretty weak nowadays and we have to reinforce it. From a clinical trial perspective there are two clinical studies, which more clinical studies have already been conducted on recurrent glioblastoma patients. One of them with nine patients and the second of them with 21 patients. And these two clinical studies have reported some I would say encouraging, optimistic signs of THC anti-tumor activity. This issue will be elaborated by my colleague Giaimo Valesco [phonetic] in another talk. However, these pilot clinical studies, although encouraging should be reinforced by more robust clinical studies to determine whether THC could be used as an anti-tumor drug not only in glioblastoma but also in other types of cancer. So as I have summarized in this talk we know some things on how THC induces anti-tumor responses in a clinical model. But, of course, we have to learn more in the future. For example, we have to increase our think on the [unclear] of THC action. But only if THC action that we know pretty well, but especially on the action of other cannabinoids, for instance, CBD and also of cannabinoid medicine [phonetic] that is at the end what most of our patients are taking nowadays. It would be of course ideal to identify molecular biomarkers that can predict the response of a particular tumor [unclear]. In other words trying to get to know in advance whether a tumor is expected to respond or not to respond to cannabinoids. It’s also nice to reinforce our knowledge on the [unclear] for clinical, combination of therapies so that we can they’re to jump into the clinics with higher chances of success. I’ve mentioned that [unclear], for instance, in the glioblastoma models in mice can synergize with the temozolomide. They can also [unclear] with [unclear] therapy. So we have to do that work also in other types of tumors. And nowadays there are some signs of evidence that indeed can have a good synergize with other chemotherapies in other clinical models of cancer. For example, with dymoxophine [phonetic] in ER positive breast cancer models with [unclear] in [unclear] models and with [unclear] in pancreatic myo carcinoma models. We know that cancers expressed cannabinoid receptors, they express signs that synthesize and are great in looking at evidence, cancer cells product endo cannabinoids. But at the end we don’t know still what is the actual role of the endo cannabinoid system in tumor generation and in my opinion another interesting line of future research. And, of course, last, but not least, we should conduct robust control clinical standards with cannabinoids to get to know whether cannabinoids could be anti-tumor drugs in cancer patients. Well, that’s it. Thank you so much for your attention. >> Thank you very much, Manuel. That brings us next to my talk, which we’re going to focus now on cannabidiol. Again, cannabidiol doesn’t interact efficiently with CB1 and CB2 receptors, in contrast to THC. Next slide, please. So I have no conflict of interest disclosed for this presentation. Next slide. I’d like to start out with the cannabidiol therapeutic funnel. I think we’re all very familiar with the palliative effects of THC in terms of treatment of cancer patients. But on the right hand side we’d also like to talk about the pre-clinical evidence that shows direct inhibition of cancer progression, both with THC and CBD. The ability of both these compounds to inhibit chemotherapy-induced neuropathy. And also both of these compounds desensitize cancer to specific first-line therapies. And again it’s just really this combination which personally excites me with the research in the sense of having a drug that improves palliative care, can inhibit cancer progression and we hope through well run clinical trials will lead to prolonged survival in patients. Next slide. So I like to start out when I originally got interested in this project was actually after reading a paper by Christina Sanchez who’s in Manuel Guzman’s lab, where they were looking in culture at targeting brain cancer cells with THC. And it was really a surprising finding to me. So from reading that paper my specialty at that time really was structure activity relationship of cannabinoids. So I had a host of cannabinoid like, cannabinoid type compounds, many different classes and I screened them across two types of cancer cell lines, brain cancer and breast cancer. Here SF126, UT51 and UD7 are brain cancer cell lines. And the MDA cell lines are triple negative breast cancers. And what we found is that actually when we compared all these cannabinoids, the cannabidiol was actually more potent than the other cannabinoid agonists we tested. And again cannabidiol does not interaction efficiently. CB2 receptors, again moderately more potent. But of significant interest. Next slide. The reason being. Next. Is again the THC produces this high that we all know and when you think in terms of long term clinically [unclear] compliance that can actually be an issue. Next. Whereas CBD does not produce this high. So again we were definitely very interested in following this in terms of looking at its direct anti-tumor activity. Next slide, please. So I want to first present some of our first really significant findings in this space. And all this work was done in collaboration with Pierre-Yves Deprez, who’s a cancer biologist and specializing in breast cancer, but most of this work has been in collaboration with ER [phonetic]. So if we look at the way to the left, we look at this protein called Id-1. I’ll talk a bit more in the next slide about this. But we can consider Id-1 as protein, a master regulator of metastatic progression. That’s where the tumor actually goes from its initial site and spreads throughout the body, metastatic progression. But we looked at CBD, at its ability to down regulate this protein because of some visual effects that we had seen on the cells and culture and lo and behold CBD is quite effective at down regulating this protein. In fact, if we then go to the center when we compared CBD against other cannabinoid agonists it was significantly more potent at targeting this protein and we went on to show that this was due to targeting the promoter region of this particular protein. Next slide. So again Id-1. There’s hundreds of articles on Id-1. It’s called a basic helix protein. It’s actually a transcriptional regulator in the sense that it binds transcription factors and stops them from functioning, which ultimately in the case of Id-1 being a master regulator leads to many changes downstream that gives the cancer cells a selective advantage to travel throughout the body and metastasize. And I’ve listed a few major articles talking about this particular protein in the cancer space. Next slide. So with that in mind we found a model, a triple negative breast cancer model in mice, called 4T1 cells that’s driven, its metastatic progression is driven by the expression of Id-1. So we tested CBD in this line and indeed it did produce a down regulation of Id-1. So we took these cells and inoculated them in the tail end of a mouse and over time what happens is metastatic pho sites form. There’s some pictures there of the acone [phonetic] CBD. And you can look at the white nodules. Those are the metastatic tumors forming in the lung and we found by treatment with a CBD that we produced this dose dependent inhibition of metastatic progression. So next, please. Well, as a pharmacologist what we like to do is take natural compounds and see if we can improve upon their activity. So with any form of collaboration, with Anu Mahadevan working on X [phonetic] and we went through a library of compounds that had structural similarities to CBD and we discovered this compound called 01663. And we compared this against CBD and found that indeed this analog was more potent, and as I’ll show you in more efficacious, is that targeting Id-1 in metastatic progression. So if we go to the bottom two curves. These are called survival curves. So as you look out to the left, especially the red line, this means that that cohort of mice is surviving longer under treatment. And what we found is that where there is more potent and efficacious analog that if you treat these mice in comparison to CBD they actually, it extends the survival greater compared to CBD. And these are actually models of, you’re looking at the graphs at the bottom. These are models of advanced metastatic progression. So these actually are really in the case, you’re starting to treat these animals after the tumors [unclear]. Next slide. We’ve also gone onto look at the control of targeting Id-1 with CBD brain cancer as well. In this case glioblastoma one of the most aggressive kinds of brain cancer. And here again we can see that if we look right at the middle panel, this is basically what we call an orthotropic model. And this is where the cells have actually been injected into the brain of the mouse and they form a tumor. If you look up at this vehicle you can see the purple area, that’s tumor, and if you look below, you can see the CBD treated and you can see a much less tumor burden in the brain of the mouse. If we go all the way over to the right, this is just staining of that tumor. So you actually remove and you actually stain the tumor itself after treatment. And again what we found, which wouldn’t be surprising in vivo is the CBD is affected by targeting this chain Id-1. Ultimately leading to reduction in tumor burden in a tumor driven by the expression of this gene. Next slide. So with that in mind, the infected in breast cancer CBD affected in brain cancer. We then screened across multiple types of cancers that are driven by the expression of Id-1 in terms of their aggressiveness. And what we found if you look at all these: we have breast, prostrate, salivary gland, head and neck and glioblastoma again. In brain cancer what you find is that CBD is effective at targeting Id-1 expression. Ultimately, the suggestion being that this compound could be affective in cancers that are driven by Id-1 in terms of their aggressiveness. Next slide, please. So Id-1 is just one of the many downstream targets that are modulated by CBD. In regard to its anti-tumor activity there have been many studies with CBD now. On the left hand side I have a long list of targets that have been implicated. And these control proliferation, invasion, metastasis, angiogenesis and apoptosis, showing that this molecule can target multiple pathways that are involved in cancer progression. And this also includes targeting immune function as well. And if you’re interested there’s this really nice recent review article by Dr. Heinz and Reimer [phonetic]. If you’re interested in this type of thing, I suggest you read that article. Next slide, please. So now that’s really showing you significant amount of pre-clinical evidence. Let’s talk a little bit about clinical evidence. So on the left hand side, you know, often if you’re in the space of looking pre-clinically at the anti-tumor activity of cannabinoid you get emails from patients who like to tell you their success story and sometimes you’ll get some who will also tell you when new drugs actually didn’t work. So we all know there’s a lot of self-ministration going on with cannabinoids at this time. So on the left hand side it’s just a scan from a patient who had initially received first line therapy. This is a patient with triple negative breast cancer. Over time unfortunately, you see all those black dots, that’s where the metastasis reoccurs. So you can see there’s metastatic progression all over the entire body unfortunately. So in the second round of treatments the patient added on a cannabis-based extract containing CBD and THC as well as first line therapy. And you can see after many months that this was really a dramatic effect. So you can see why these anecdotal [unclear] studies in patients have really driven people to be interested in these compounds. On the right hand side showing you three clinical trials that have been run in the cancer space. So really the first by GW Pharmaceutical, probably one of the most well run albeit unfortunately the details of that trial are not out. So it’s really hard to critically analyze that. But hopefully we’re going to hear that in Dr. Glascow’s talk coming up. And the other two studies are really case studies, again showing a benefit. But again these are really case studies and as Dr. Gisium [phonetic] was saying earlier we really are in need of well-run clinical trials in this space. Next slide. So stepping back to CBD showing now a list of downstream target that have been shown to basically control cancer progression. So we have a really active interest in what’s the initial site that CBD interacts with? It does not interact efficiently with CB1 and CB2 receptors. So what’s this initial site that it interacts with? Well, one of the most unifying themes across labs has been this ability of CBD to initially produce a production of reactive oxygen species in cancer. And this, again too much ROS in the context of a cancer cell is deleterious and it will result in cell death. And so basically if you look at A, this just is a comparison of production of ROS in two triple negative breast cancer lines, 231 and 421, in comparison to a normal breast cell line where you do not produce this enhancement of [unclear] at similar concentrations. This effect occurs rapidly. We can detect it even at 24 hours. It’s concentration dependent, that’s C. And this effects, if you look at D can be blocked by Vitamin E or [unclear], this is an antagonist to reactive oxygen species as well. Some partial blockade by calcium antagonist bapta [phonetic]. And then finally in F, I just go onto show that this, again this analog that we discussed earlier was more effective decreasing more or less in regard to CBD. And that will make sense later here in some of later slides. Next slide, please. So I want to return to Dr. Guzman’s work and this will make sense in slides like this. Again they’ve done some really elegant work and defined the CB1, CB2 mediated anti-tumor activity, the pathways involved in anti-tumor activity. I have three things circled here: Trip2, AKT and then ultimately autopaghy mediated cell death, which Dr. Guzman talked about. And I want you just to keep those in mind as we talk about the next slides. Next slide, please. So as I showed you earlier, we looked at cross cell lines targeting Id-1. So what we decided to do is look across all these cell lines and used a micro analysis. And that’s really just looking at gene expression, you look at thousands of genes, and the ability of what your drug does, your anti-tumor agent, what it does to change the expression of these genes. And, of course, these genes are controlling proteins that of course ultimately are helping the cancer to progress. And so when we did this comparison against vehicle 1 CBD across cancers we found some important proteins that were really modulated across all the cancers. And if you look at the bottom what might not be surprising is one of the top genes there was Id-1, which again I talked about earlier. But then if you go to the top what we found is initially you get this release of this stress factor called GDF15. Now that’s actually a common stress factor. It’s not just cancer-specific. It can happen in all cells when they’re targeted by drugs, stress. What was particularly interesting though was this over expression of TRIB3. And that again is an upstream marker of downstream autophagy, it’s cell death. So here now we see the shared property between THC, CB1, CB2 receptor agonists, CBD. So two cannabis plant compounds targeting the same pathway involved in inhibiting cancer progression. Additionally, we found the modulation of this specific transport, the transport of it was involved in modulating basically dealing with reactive oxygen species, which again makes sense with the data I showed you on [unclear]. Next. So we led on really to verify again that GDF15 is up regulated. And what we found is that actually one of the other critical factors, FOXM1 which is involved in cancer [unclear] is also down regulated by CBD. We went on to look at whether GDF15 controlled either Id-1 or FOXM1 through these transcription factors that were down regulated in this analysis. Next, please. And what we found in that is that GDF15 could partially control FOXM1, but still we didn’t have a pathway that really up streamed of Id-1 to explain how CBD is modulating protein. So here, again the slide before was really an analysis across cancers. Here we focused on patient derived brain cancer in tumors. Again, glioblastoma. So these are tumors taken directly from patients after surgery, brought to our lab. And again we performed this micro array analysis, specific in this case to primary brain cancer. So basically to review what we found again is probably not surprisingly was this down regulation. But again if we look all the way at the top we see this release of this stress factor, GDF15, and importantly again TRIB3. Again part of this autophagy mediated pathway showing this now overlap between the effects of THC and CBD. And again we found this up regulation of this transport that deals with reactive oxygen species. Next slide, please. So we went onto then take these patient derived tumors, create orthotropic models of mice and treat the mice again with DBD and indeed if we then looked at other pathways, one implicated again autophagy mediated cell death is AKT. We saw down regulation of AKT, which would be expected if you were targeting that pathway. We saw an up regulation of this protein called NFR2, which is evolved again in reactive oxygen species. And finally again this channel, this channel I talked about earlier called XCT, which deals with [unclear] as well, which is up regulated. The data making sense of this initial production of ROS. Next, please. And just on the right side is just to really show you, these are the primary tumors we’re talking about in culture. You can see vehicle, it gets smaller when treated with CBD. But when you use this ROS antagonist you can reverse these effects. And I just showed you a suite of signal transduction where the down regulation, the presence of CBD treatments is basically blocked using these ROS [unclear]. Next slide. So we’re not the only group that has published on CBD producing effects in autophagy mediated pathway, there’s also this study by Dr. Presaud’s [phonetic] group, which shows that again CBD is effective at targeting this pathway as well, even triple negative breast cancer. Next slide. So I just want to review a bit of the data and talk about what I consider some of the important knowledge gaps. If we go up to the top again we have this production of ROS that appears conserved with CBD across cannabis. I think one of the most important questions is, what is the initial interaction site for CBD cancer cells that leads to this production of ROS? And how does ROS translate into specific alterations in gene transcription? For instance, is modulation of ID proteins, up regulation of GDF15 and down regulation of FOXM1? And also importantly there’s clearly, if you look to the left of the bottom of the slide, there’s clearly this overlap between anti-tumor activity with THC and CB1 and CB2 receptor agonists and CBD which does not interact efficiently with the receptors. Next. So one of the issues with CBD is it’s not that potent in terms of its ability to inhibit cancer cell by ability and cancer progression. So basically when it’s been tested across multiple studies it’s been shown to have interaction with multiple types of initial sites. And we’re just listing a few here. So that’s really one of the issues with CBD is its lack of potency in these analogs. Next slide. So what we spent some time over the last few years doing is really trying to develop analogs that are more potent at targeting this initial interaction site. And I talked earlier about this analog 01663 that we created to be more potent than CBD at targeting Id-1. So what we did is we spent more time creating more analogs. And the theory here is that we would create analogs that were highly active at targeting these specific downstream events and ultimately that would allow us to come up with a compound that would potentially be targeting this initial interaction site. And through many rounds of synthesis we came up with drugs that were similar between 50 bold more potent than CBD. And so on the right hand side of this basically slide, what we show is that we compare CBD and the initial what I call first generation analog against – one moment, please. I have a little bit of a time out on my computer. But basically what we’re showing is that these compounds are more, much more potent at targeting Id-1 and downstream events that happen when you target Id-1, such as invasion. Next slide. So we then went onto look at this markers, Id-1, FOXM1 and GDF15 with these analogs. And again the compounds were much more effective and much more potent, suggesting that we do have molecules that are more active at these sites. Next slide. So with that being said, I wanted to present some things that I think are important knowledge gaps and areas where we target future research. I talked a lot about this initial interaction site. Obviously I think that’s quite important. We want to look at specific events leading to alterations in the protein expression of these shared pathways that I showed you: Id-1, GDF15 and FOXM1. And then further more I think again there’s always this question of whether THC, CBD or a combination of THC and CBD would be more effective as a treatment ultimately for targeting cancer. We’re interested, I think some interesting studies would of course look in more detail at cannabinoids and their ability to enhance first line agents that are used across cancers. Some very important research, which is lacking, is really a detailed analysis of the modulation of the microenvironment and immune response in the presence of cannabinoids and it’s going to be very important. And most ultimately is really well-developed clinical trials across cancer, which I hope will include a biomarker potentially that will really pick specific patient populations, which you expect would show more responses to the drug. Next slide. And I just want to thank all of my collaborators and all the staff that collaborated, the whole team in producing all of this data. Thank you very much. Dr. Joseph Califano is from UC San Diego. And he’s going to talk about HBV and cannabinoids in that space. Thank you. >> [unclear] but let me double check. He might have had to drop off. I know it looked like he was in clinic when he first joined. So hold on one moment before he gets started. >> Yeah, you guys, it just took a while for you guys to unmute me. Can you hear me okay? >> Yes, we can hear you. >> Great. Super. Thank you so much. I appreciate the opportunity to present here with such extraordinary investigators. And thank you very much for your time. Just to do a little bit of a mayo culpa. Unfortunately I have a day job as a surgeon and due to Covid I'm going to have to step out and go ahead and take care of patients at this conclusion of this, so my sincere apologies. So go ahead you can present the first slide. So we’re going to talk a little bit about HPV or oropharynx cancer. In particular, cannabinoid in interaction with this is kind of an interesting topic. And I’ll tell you a little bit historically about HPV and oropharynx cancer, because I think understanding the biology and the epidemiology will be helpful to understand why this may be an interesting subject of investigation. You can go ahead and do the next slide. So I don’t have any conflicts of interest to dispose of. Next slide. So head and neck cancer is mostly squamous cell carcinoma. You can click it one more time. Really when we talk about what we think about traditionally with head and neck cancer causes is smoking and alcohol exposure related and usually long term exposure to smoking. And I put this slide up just as a reminder that this is still a very dominant feature of this particular disease. Click it one more time. But increasingly really human papilloma virus in the throat is really becoming a more dominant part of the head and neck squamous cell carcinoma landscape. There are some other causes. But really HPV is fairly extraordinary. And when you talk about this most folks in the United States and in western countries really get exposed to high risk HPV sometime in their teens and 20s. And when we look at this extraordinary burden of HPV cancer we’re experiencing in the current day, it has to do with exposure that happened to people 30 or 40 years ago that are maturing and being expressed as an incidence of human papilloma virus [unclear]. Next slide. So we’ll talk about some basics about the disease that you can understand. It’s mostly explained as a carcinoma, usually men, ages 40 and over. Usually presents actually with regional [unclear] because it can be asymptotic and doesn’t usually become symptomatic in the throat until it gets to be of a certain size and often painless. Next slide. HPV, mediated head and neck cancer is a little bit different. It happens at a younger age. Most people smoking related cancers happen in their 50s and 60s, but HPV mediated 50s and even 40s and even some patients in their 30s. It is primarily in the oropharynx, in the lingual and palatine tonsils. The palatine tonsils are the ones you always think about when you’re a kid and you went to the doctor and somebody said you had to have your tonsils out. But it turns out there is conflict tissue and a ring around the oropharynx which includes the basaloid tongue, the very back of the tongue that we can’t see when we look in somebody’s mouth – we have to see using specialized equipment – and also in the nasopharynx. And interestingly enough we are seeing that HPV mediated head and neck cancer is actually occurring in the nasopharynx, hypopharynx and the nasal cavity with some significant regulatory in addition to the oropharynx. The oropharynx would be the major site. People tend to be nonsmokers and nondrinkers, tend to be male, and it has a very particular histology associated with it. Next slide. This is really a classic study that really nails down the fact that HPV is an independent risk for oropharynx cancer. The Scandinavian population based study that at the time was reported in 2001 really looked at essentially a million subjects and did a next in case control study. Really quite nicely done. You can see the odds ratio for the presence of high risk HPV antibodies prior to development of oropharynx cancer is extraordinarily high. And the mean enrollment was 9.4 years prior to development of cancer. And this is the presence of antibodies. And we don’t think that this means there’s a latency period of ten years. We think it’s probably more on the order of 20 to 30 years. But certainly for this study this is what it was able to demonstrate that a really key definition is showing that the presence of the virus or response to the virus predates development of cancer. Next slide. In 2009 was a landmark year in the United States where human papilloma virus associated cancers were found to be established to be tongue in the oropharynx. The oropharynx actually exceeded cervical cancers in the United States in 2009. So if this doesn’t convince you that this is a bit of an epidemic, you can go to the next slide. And this shows you the projected increase in oropharynx cancers, which is currently estimated to probably be 30,000 people annually by 2030. One thing you’ll notice is that there is an increase not only just in White men, but also Hispanic men as well. And that also there’s increase in women. So this epidemic tends to be relegated to males, but increasingly we are actually seeing it in women as well. And this actually shows no signs of decreasing. So why is this happening? Next slide. Well, as it turns out there is really some nice epidemiologic data that show that when we look at risk behaviors and development of head and neck cancer that sexual activity is linked independently to the development of HPV and oropharynx cancer. And particularly oral sex partners is linked to HPV and oropharynx cancer. And obviously the thought is that it’s spread through either oral-vaginal sex is probably one of the primary modes of transmission. That being said, other indicators sexual activity area also associated with the development of the cancer. So the transmission of the virus is probably aided by other mechanisms as well. Here you see kind of a variety of different measures for sexual activity that are associated with HPV or oropharynx cancer. Next slide. So this is kind of a key slide here. Investigators in 2008 reported in the JNCI that prolonged daily marijuana use is independently associated with HPV and head and neck cancer. And this is a bit unexpected and I just want to go through kind of go through the slide in a little bit more detail. The title here you see the association and dose responses for HPV and oropharynx cancer. In the bottom row you see the dose responses and associations for HPV negative oropharynx cancer. And you see going from left to right, tobacco, alcohol, poor dentition, oral sex and marijuana. And just so you know the marijuana use here that’s associated if you look up there it’s actually equal to 15 joint years. And that is daily use or daily use of marijuana for 15 years, which is really a fairly high and consistent exposure to marijuana. And the interesting thing is that when this was looked at the adjusted OR ratio for daily marijuana use was independent from tobacco, alcohol and sexual exposures as well as HPV exposure. So, you know, there were a lot of questions then this came out. People actually kind of said, well, you know, is this incompletely assessing the relationship between marijuana use and sexual exposure? Are we maybe haven’t quite separated out those two founding variables? But the dose responses pretty interesting. And then the minimal amount of use, talking to the audience that knows far better than I that daily marijuana use is not necessarily associated with joint sharing and the concern for oral transmissions perhaps a little bit different in the population that uses it on a daily fashion. So I think this is what kind of prompted me to kind of scratch my head and say this is kind of a curious association. You don’t really see this association in any of our head and neck cancers and it’s kind of an odd and unique thing. So we decided to investigate it in a little bit more detail. Next slide. I'm going to move on, because I know this has already been presented and keep on going to the next slide. And this just tells you about the San Diego. The only reason why I was interested in this is that the San Diego is in California and, of course, we have had legalized cannabinoid products for really many years here. And it’s really endemic to the culture. So we have extraordinarily high use. So that’s really been kind of been apropos and in interesting question, particularly in our population where there’s such a high endemic use of not just combustible marijuana projects, but also just ingested cannabinoids. Next slide. And we’re going to keep on going to the slide after this. I do want to point out that when you look at the downstream targets of cannabinoids both as anti-cancer agents and it’s progrowth agents that the audience is looking at this and saying that we know this very well. But when I was introduced to this field what really struck me was that I'm looking at a lot of the pathways and the molecular networks that we really think of in head and neck cancer as being central networks in both HPV and non-HPV needed head and neck cancers. So the first question that really comes to mind in the head and neck cancer context is that both the GPC pathways associated with cannabinoid ligand stimulation in addition tend to be really exactly those pathways like P38 junk [unclear] that we know are really dominating control mechanisms for this particular tumor type. So certain it’s an interesting question to ask what the effects of these are in this particular context. Next slide. So we published a paper on Clinical Cancer Research and you’ll see a lot of data from that plus a little bit new data from hereon in. But the first thing is we just decided to go ahead and see what happened when we took cannabinoids on here in cell cultures of HPV oropharynx cancer cells and particularly wanted to make sure that we chose appropriate physiologic concentrations that were analogous to those that one would find in patients who had had recreational marijuana use and also we’re always suspicious of new associations, so we made sure we did it in a lot of different cell lines and then looked at both the simulation inhibition. And you can see the effects here are not optically strong, but they’re definitely consistent. If you look at the cartoon down in the lower right hand part of the slide you’ll see we have specific CNO1, CNO2 agonists as well as a mixed THC agonist. And then CNR1, CNR2 inhibitors as well. And then across and agreeing with our particular cell lines with red is stimulation, non-response in black and inhibition, blue. And generally you see that we’ve slanted towards, surprise, that you tend to see stimulation for CB1 and CB2 agonists, but inhibition for antagonists, while they’re not universal. And this is intriguing to kind of move on and try to do some next steps. Next slide. And you can advance the slide, please. Great. Thank you so much. So we’re not having obviously since we’re looking at some of the downstream effects we want to go ahead and look at apoptosis. And this is just one slide then in a particular cell line, which is an oropharynx cancer cell line showing significant inhibition of apoptosis when we treated it with THC. And just again the concentration we’re using are more physiologic. If you really jacked it up to non-physiologic concentrations you’ll tend to see how it effects. But in physiologic concentration you see an additionally apoptosis. Next slide. And I see a summary slide here looking at both apoptosis and migration. The apoptosis on the left and migration on the right, where you can see a variety of CNR agonists on the left, CNR1, 2 on the left and the antagonists on the right for Apoptosis. And you can see there really are significant effects in this particular tumor type. Similarly when we looked at migration which includes trans as well as assays, the CNR agonists tended to cause migration whereas the antagonists inhibited. And this was probably done in a variety of our cell lines as well. So at this point in time we had thought, okay, so we show some phenotypical set, the sedialogic [phonetic] concentration of cannabinoids and cannabinoid agonists and antagonists in this particular system. Can we at least take a look at our canonical [phonetic] downstream pathways and see if we’ve got an appropriate and reasonable signal that may indicate this is the case and makes some sense. So if you can go to the next slide. So here a couple of experiments. So for those of you who are western block, there’s nice western block there showing that in one of our cell lines, a sponsored one, that when you go ahead and treat with THC as well as CRN1, CRN2 agonists, you do see an increase in MAP kinase activity, particularly if FOXOP38 MAP kinase. This is a classical MAP kinase downstream, here activation as well as you see some increase in fossil heat protein 27 also downstream. But as well just for those of you who don’t hang out in cancer circles this is a very well known, well characterized downstream sector of ligand xenotype [phonetic] in this particular tumor type. And then on the left hand side you’ll see that when we actually use [unclear] a specific P380 MAP kinase inhibitor we’re actually able to aggregate some of the proliferate effects, the growth effects of THC in our CNR1, CNR2 agonists in these inherent cell cultures. So indicating, but not completely but significant portions, so indicating that we know that in this particular system we are working partly through the P38 MAP kinase though not exclusively through the P38 MAP kinase pathway to get these things, specific effects. Next slide. We did do this in the xenograft model. So we have a xenograft model here, which you can see over here on the left and also an intact immunocompetent model. So on the left is the xenograft and on the right is the immunocompetent model. We used both agonists in the xenograft and the immunocompetents used THC. And you can see actually kind of strikingly, you saw a little bit greater effect in our immunocompetent model as well. And, in fact, actually took the tumors that actually had thickened significantly responded and actually assayed them for possible P38 activity and actually slowed increased [unclear] relations with P38 MAP kinase as well, indicating that this was associated with an increase in MAP kinase activity. We began to look at other models, including other HPV models, but I don’t have that data here. Next slide. So this is kind of an interesting question. As a surgeon who works in this particular tumor type one of the distinct advantages I have is that we tend to be very adept at getting tissue of various types associated with our disease state that we study. So for this particular patient population we actually have a very highly characterized cohort about a little less than 40 patients where this patients had HPV. At our center’s campus we collected their tumors, their plasma, their serum, their salivated rinses and did extensive genomic analysis including a whole genome expression arrays, DNA methylation and also this whole genome sequencing and the like. And so our question was, gosh, you know, we found these genotypic effects in both the [unclear] cell, other kind of apoptosis and invasion assays as well as in xenografts and other mouse models. What can we find in a patient population? So to explain a little bit about this experiment we took these patients, everything classified by whole genome RNAC and we quite luckily had archival serum associated with these patients that had been drawn from their blood just prior to therapy work during their biopsy. And when I say prior to therapy it’s usually in the operating room. So we actually have this interesting cohort of both patients with well characterized genomic-ally with also pre-treatment sera. We assayed the sera for a presence of cannabinoids and those are indicated in the top little diagram you see there with the black circles. And then actually used two different techniques, [unclear] genomic pathway activation. The one below I’ll introduce first. That’s standard gene set enrichment analysis. So we went ahead and looked at various gene sets. And quite interestingly enough we did find activation of MAP kinase and [unclear] growth factor receptor pathways in a particular state. And in that state we saw significant association with the black circles/vertical bars, which each represents a tumor. Those are the ones that had cannabinoid exposure. And if you look at that, A is just more graphical. Inside of that where you see that four out of those five, those patients that actually had active cannabinoids in their blood at the time that the tumor was sampled. It did have activation of downstream pathways, including these classic MAP kinase pathways. So I thought this was particularly interesting to us that you can actually see that in patients themselves one may actually at the time of exposure to a cannabinoid. This was undoubtedly all THC, of course. They do have activation pathways in their tumors. Next slide. So we’ve done more work as you noticed there’s some real questions about whether you’d have some interactions in terms of microenvironment immune response. We have been able to look in the specific mouse head and neck models where we’re looking at the effects of THC on antigen specific T cell activation. And you do see that physiologic concentrations you get some moderate inhibition of T cell activation. So it’s possible that some of these effects are not exclusively tumor mediated. Next slide. And then I do have some collaborators we’ve reached out to. These are data from a colleague of mine Sylvia Gookin [phonetic] as well as Dr. Inaway [phonetic], looking at specific GPCR and G protein coupling. And the interesting thing is is that we don’t really, the prior speakers have so eloquently demonstrated the way in which these particular GPCR ligands interact with the GPCRs and their coupled G protein is really so obscure. This demonstrates that a nicked CB1, CB2 antagonist actually has some G alpha 12 and G alpha 13 activity, which previously we’re demonstrating we’re trying to investigate this as potentially other reasons and other downstream effects of these molecules. Next slide. And finally just a little tease to share, we’ve done a little bit of work in CBD, really not much. But I thought for this audience it would be interesting when we looked in these specific head and neck HPV media or oropharynx cancer cell, we have noticed significant burke [phonetic] activation as well as some proliferate effects for appropriate physiologic concentrations of CBD. It is early data and we haven’t published it yet, but these particular data presented are very robust. So it will be interesting to see whether this holds true in kind of more complex systems. Next slide. And finally we do have, as you know, all of our patients and a lot of patients I see use cannabinoids for symptom management and it can be quite effective. And obviously the thing that happens with this is we kind of get a little concerned when our patients are using particular cannabinoids in this particular tumor type. And this is some proactive data where we actually looked at cytotoxic effects of radiation and showed that we do get some inhibition of cytotoxicity when we go ahead. So there’s some ammunition of apoptosis when we have radiation exposure in cell line systems. Next slide. So just to summarize. There is this epidemiologic association that is kind of interesting. We tried to look at kind of how this worked out in a variety of cell line and mouse models. And, in fact, were able to actually demonstrate some association of P38 MAP kinase upregulation, patients who had direct measurement, a presence of cannabinoids and HPV mediated oropharynx cancer. So it’s an interesting story and I have to say that it’s kind of been a fascinating example of potentially some interesting effects that could be context dependent. I should say that parenthetically we looked at this in non-HPV mediated cancers and do not see any of these effects. So it appears from what we’ve seen that this is pretty much limited to HPV the oropharynx cancer. But given the epidemic of it, it does warrant certainly some more investigation and some concern about how we kind of treat this particular tumor type. So I think I’ll stop there. And one more slide please if you don’t mind. I just want to thank particular folks in my lab: Chao Liu, Koji Ebisumoto, John Pang and Takuya Nakagawa who were just extraordinary in doing a lot of this work and all the folks funded and particularly my collaborators involved. Thank you all for your kindness and listening. >> Thank you very much. I again want to thank all the presenters for the informative talks and of course for the committee organizing this session. I think that brings us to questions, which we want to start with Dr. Califano, since I know you have to potentially get back to the clinic. Okay. So I'm going to try and pull up the some of the questions here. >> I have a question actually for Joe before he leaves. >> Sure. >> If he’s still available. >> I am. I'm here. >> Okay. I missed, effects in HPV positive cervical cancer, so I'm wondering if the effects are more general to the virus effected tumors? >> Yes. Yeah, it’s a great question. And, first of all there aren’t great data physiologically. It’s a quirk of the fact that the epidemiology group and HPV in oropharynx cancer became very strong because people kind of couldn’t figure out what was going on here. Right? The epidemiology of cervix cancers are really pretty straightforward, mainly straightforward. So, you know, this is kind of a quirk that prompted us to do that. So we have not looked in cervical cancer cells. So I do not know … I actually don’t know of any epidemiologic data that even asks the question. So it’s provocative. It would be interesting to find out, but I don't think we have any data. I don't know have any data that I know of in particular. But I do think you make a really good question and that is: is there an interaction between the HPV genome gene products and these pathways and how the interact with cannabinoid ligand induced pathway interactions, because I think that’s the real interesting question. The other interesting question is could this be an effect where cannabinoids facilitate HPV infection? The data, and don’t specifically know that for sure, but the data that maybe speaks against that is that we only see this association in the long term cannabinoid exposure. Right? It’s only like the grave in 15 joint year population, which as we all know there’s probably a lot larger population than that that has cannabinoid exposure and ages and a sexual debut where they get first exposure to the virus because it’s is the first exposure to the virus during sexual debut in an epithelial service that is thought to be the most highly likely site for virus persistence and potentially transformation 20, 30 years down the road. >> Just one other question I had was: in your model systems have you knocked down either CB1 or CB2 and see if that nullifies the effects by your agonists? >> Yes. We just had an update. I didn’t present it here, just for … but, yes, we’ve done SI and SH knockdowns which did demonstrate that, yes. >> I'm sorry, is it working through those receptors or what’s going on? >> Yes. So you can actually blunt, if you knock down you’ll blunt it. >> Okay. >> Not completely though. And I don't know if we actually put those data in a paper. But even in the absence of exogenous ligand if you knock down receptors you will see growth inhibition. >> Okay. Thank you. >> So I have one other question for Dr. Califano, if I may before you are leaving us. And I'm trying to find. There was some interest in the concentration and maybe the low concentration might be the culprit of the increased cancer proliferation of head and neck tumors. And also … Go ahead. >> I actually think so. So, you know, we specifically really wanted to make sure we used, you know, we spent a lot of time actually combing the literature to look at ketamine [phonetic] plasma concentration in recreational users, because, you know, the hypothesis is that this chronic recreational exposure somehow predisposes the transformation. Right? So, you know, you had to be really careful about that. You know, if you’re right it begs the question. Right? Dosage and context are kind of everything. So the question always is does this mean that you do, you know, super physiologic concentrations, will you see inhibitions? We haven’t noticed that much, you know, maybe a little bit. But we’ll use really high concentrations and didn’t see particularly dramatic inhibition in any way. In some slides we still saw the same growth effects. Is that helpful? >> Yes, yes. Thank you. Related to the context, are there other tumors not related to HPV that you think might be stimulated by THC? >> I have absolutely no idea. But I will tell you that epidemiologically when we kind of said we were interested in it, the only tumor type that I’ve seen in a literature and we’d love to hear this from the other panelists is testing cancers that have some epidemiologic association with cannabinoid exposure. But it just doesn’t exist, you know. And people have looked. And in fact it’s really kind of interesting. If you look for non-HPV median cancers, which tend to be [unclear] there’s actually an inverse effect. So there is some slight attenuation of risk for daily marijuana smokers and oral cavity cancers at increased risk in the risk for oropharynx cancer. So even in the same epithelia, you know, tobacco exposed. And it’s slight, it’s not great data, but it’s very clear that for oral cavity cancer you’re not seeing the same effect. >> Thank you. >> So there’s another question that showed up in the chat. And it’s, ‘could we expect an interaction between THC use in HPV vaccine efficacy? >> I don't know. I have no data. I mean, that really has to do with what’s the ability of the vaccine to provide, you know, a cell mediated or antibody mediated immune effect. And I don't think we have great data on that. There’s a lot of data out there on the use of THC as immune inhibiting agents, particularly in diseases like multiple myeloma and other things like that. So the hypothesis would be reasonable based on existing data. But I can’t comment. I don't think we have data. >> Okay. Thank you. >> Thank you, Dr. Califano. And thanks for your excellent talk. >> I appreciate it. And thanks to the speakers. I apologize for having to leave. >> Take care. So maybe we have some additional questions for the other speakers. We do. Mary, Dr. Abood, there’s a lot of interest in the endo cannabinoid system, even from this meeting. And some interest in its importance as a regulatory system. And I was just wondering if you could comment on the importance of it and how it’s related or interacts with other regulatory systems, just some clarification about, you know, there was some talk about it, it being the larger regulatory system of the human body. >> That’s a big question. >> That’s a big one. [Laughs] >> So I mean there’s just two things. One thing that I didn’t really emphasize in my talk is that the endo cannabinoid ligands are produced on demand. And so they’re very important for regulation, but they’re not like sort of always floating around. So there are some hormonal. And I think some of the other speakers could speak to this too. There are some hormonal effects of anandamide. But mostly they’re produced when needed. There is, and this is not my work, but there is a literature on endo cannabinoid deficiency syndromes. Ethan Russo has published on this. And so there’s a whole literature on that that I didn’t really touch upon. But, yeah, I think the endo cannabinoid system’s very important. And it interacts with many other neurotransmitter and hormonal systems in our bodies. So we’re really at the … there’s still lots to be learned is all I have to say. Sorry. >> That’s great. That’s great. Thank you. I was also wondering in regards to tumor biology, what, maybe another tough question, what would you say is something that we need to find out about for this system with regard to tumor biology and cancer? What’s important next step in research in this system? >> I mean, I focus mostly on the receptors, so I think that, you know, as everybody pointed out in this session there’s a lot of signaling systems that are bound to contraindicated in certain cancers and perhaps useful in others. So I think we just need to know more about the mechanisms of action. Anybody else can chime in here. [Laughs] >> Thanks, Mary. >> So I have a question regarding, in several talks we see cannabinoid effects on cell migration, either to inhibit or provoke migratory behavior. And I'm just wondering in the normal epithelial cells is there evidence that the cannabinoid system modulating motility? >> Say that one more time. I lost you at the end. I lost the end part that you said. >> The question would be cannabinoid effects on cell motility and normal epithelial cells, what sort of, what’s known about that? Since we see it in cancer, in tumor cells that cannabinoid’s altering activity both promoting and inhibiting, whether there is an analogous sort of role in just normal cell motility in epithelial cells? >> Hmm. Yeah, that’s interesting. I'm not aware of a lot of work in that space. I mean, there’s quite a bit of work, you know, in terms of the endo cannabinoid CB1, CB2 receptor agonists and CBD in controlling immune cell migration throughout tissues, and not a lot about the normal endo-thio [phonetic] cells. I mean, really I think what’s shown that in many cases there’s not a lot of expression in some normal cells. But in sort of a pathological, in a condition of where, you know, maybe there’s inflammation or tissue damage that’s when you get this re-expression. But Manuel. >> Yeah, there are some indications that cannabinoids may have a dual role on cell migration depending on whether the cell is [unclear] normal cell. [unclear] are well known to increase migration. For instance, macrophages of the [unclear] cells at least at low signal input there is endo cannabinoids and not for very prolonged times of exposure. And there are also some indications for [unclear], cannabinoids may have a [unclear] action on cell migration being inhibitory at high concentrations and pro migratory at low concentrations. That is an interesting topic because we never get that whenever we speak on cancer the cancer is a very complex organ. I would say organ. And it can take very different types of cells. So whenever we talk, for instance, about cannabinoid receptors in cancer cells, which is also as Mary pointed out, a very complex issue because cannabinoid receptors are expressed at different amounts in different types of cancers and different amounts of different degrees of malignancy in the same cancer. And cannabinoid receptors may have different roles in different stages of cancer, depending on whether CB1 and/or CB2 are activated. And in this case regarding migration as you pointed out is also a very tricky question and something that we should learn about. In general, we have found, we and others, have found that cannabinoids inhibit cancer cell migration. But it is also true that in some instances, [unclear] cancer cell migration. So, yeah, it’s complicated. And something we should define for the future, I guess. >> Dr. Guzman, another question on another system perhaps. Can cannabinoids interrupt other [unclear] that would also have a more rapid cell proliferation, such as wound healing or during pregnancy? For example, would the inhibition of [unclear] also occur by THC? >> That is also an interesting question. And it happens so many times in the cancer field in general when cannabinoids [unclear] in particular that will keep us bus for many years. [unclear] As many of you know, some biphasic actions of cannabinoids or at least actions [unclear]. It’s very, very important to say that only the cannabinoids can increase or decrease whatever parameter we are talking about. But in which precise conditions we are doing those experiments. For instance, in the case of anthogenesis [phonetic], there are a large number of reports that cannabinoids can inhibit cancer in anthogenesis and other pathological forms of anthogenesis, for example, some retronopotosis [phonetic] [unclear]. But cannabinoid can also enhance, in this case enhance or decrease normal anthogenesis on the different [unclear] in different experimental settings. So in summary we usually see decreased anthogenesis in pathological conditions. But either decreased or increased anthogenesis in normal situations, depending on the dose of the ligand at the time of [unclear], etc. So that is something also that has to be explored more in detail and I'm sure again that there are factors that depend on the cell type with which proteins are cannabinoid receptors interacting in that precise set and what is the precise cannabinoid ligand we have used for and at which concentration for how long. It is not the same, too many [unclear]. So, a complex issue, but with a very short question I would say usually cannabinoids decrease, pathological anthogenesis and they can increase or decrease normal anthogenesis depending on the content. >> Thank you. There are some specific questions, Dr. Guzman. Are you aware of any epidemiologic studies proposing to investigate whether self-reported cannabis use improves or worsens outcome? >> There are, besides focusing on the internet or something like that I don’t give them scientific value. But, yeah, there are case reports and some case series that have been reported. That have been published in the scientific literature. The largest of this series is 119 patient series that was published in anti-cancer research in 2018 and by a British group that followed [unclear] in London and patients along four years. Patients that were taking CBD, either CBD oils or synthetic pharmaceutical grade CBD. And the authors claim that there were some positive actions of CBD in treatment and depending on, there were different types of cancers. The parameters, the end points they used were different in some cases. They were [unclear] tumor cells, in some cases [unclear] responses, etc. I mean, we all know that the [unclear] value of an observational study is very limited. It's not a randomized control trial. But it’s [unclear] that goes in the same direction as the two only clinical studies that we’ve conducted so far in cancer, one with nine patients, another one with 21 patients. They were elaborated on later on in the conversation by Giaimo Glasgow [phonetic] in [unclear]. And those two small trials also point to potential cannabinoid anti-tumor activity, or at least to an overall survival of the patients. So I mean we are in a situation in which we have a very strong critical evidence of working in [unclear] tumor and actions. But unfortunately we are very, very far from the clinics yet. So far as I said two clinical studies we conducted. One clinical study, as far as we know is being conducted in Australia, also with glioblastoma patients. One clinical study is close to start here in Spain. And that’s it. And at the same time millions of cancer patients are taking cannabinoids all over the world. There is a wealth of data there that are getting lost and it’s really, really important for the field that health professionals take action in this respect and follow close trying to get to know not whether cannabinoids are knocking the tumor out and drugs overall that is very naïve, but as to whether certain cannabinoid combinations together with some certain first line treatments that are being used for many patients all over the world in different types of cancers in different stages of cancer there could be some say niches in which cannabinoids would be helpful. >> I have a question for I guess Sean and Manuel. In both your talks you mentioned a need of a biomarker, a treatment response for tumors. What’s known about such biomarkers currently? And what would be ideal candidates? For instance would it be a gene expression signature or presence among metabolite? I'm just wondering what your thoughts were. >> I can start that. I think, this goes back to well-run clinical trials, I'm hoping that in some of the clinical trials that are going on that they’re able to collect patient tumors not from the treatment but probably before treatment from these patients, because most of the patients before they go on treatment will have past blocks of their tumor that they have archived. What would be really interesting is to take a look at the genetic signature between responders and non-responders in well controlled clinical trials, which hasn’t, I'm not aware of that being done yet. But, you know, it’s a difficult task, but I think that might help us. And the reason I feel it’s so important is because, you know, obviously they’re running these clinical trials, but as we all know this is a big push I know for NIH too, is really to have biomarkers associated with the trial, because you really do risks of putting a false/negative if you treat across all comers. And so that is a concern. So I'm really hoping that someone is doing this collection that we can get a hold of this material and do those experiments. >> Has there been work done with cell lines just to get an initial sort of survey of potential markers? >> Yes. Dr. Guzman can talk about that. >> I mean, yeah, going back to the clinics of course it would be ideal to have biomarkers that predict another response. But we have to put reality in context. I mean, the World Health Organization recognizes about 150 different types of cancers from an anatomical point of view and maybe hundreds if not thousands from a molecular point of view. And for most of them, for the big bulk of them, there are no biomarkers. Of course, we have PSA, we have ER for positive breast cancer, we have R2 for positive breast cancer. But there are very few types of cancers, in spite of a big effort that has been made by the scientific community that can, very few cancers we do have reliable, predictors. So I mean, of course, as I said it would be ideal to have predictors for cannabinoid receptors, but we are very far from that there being many, many different types of cancer and in general many different types of diseases. And yet we made an effort, Giaimo Valesco did in our lab, published a paper nine years ago, in which we compared glioblastoma cells coming from patients with different sensitivities in vitro to cannabinoid induced apoptosis. We made a series of cells in which we did array experiments, global transcriptomics. And we did the same in cells that were resistant to cannabinoids and [unclear] action. So we compared general gene expression profile on cells that were resistant and sensitive and we found out that there was a growth factor called midkind [phonetic] that can be a predictor for cannabinoid response in glioblastoma cells. So that when glioblastoma spreads very high midkind levels that can first resist cannabinoid induced anti-tumor activity and the other way around. That of course we did those experiments in cell cultures and then we went to mice and corroborated that notion. So, yeah, I mean it’s feasible to do pre-clinically. But it’s much more complicated doing the clinics. And we don’t know whether this pre-clinical information we got for midkind glioblastoma is really applicable to humans. It would be ideal to know, but maybe we will know in the coming years. But yet at the end it’s only one type of cancer out of 150. So come on. [Laughs] We have to work. >> Thank you. There is a question in the chat for Dr. McAllister in regards to what your thoughts were, you mentioned about the CBD interaction site in terms of the Ross [phonetic] production and if you had any particular candidates in mind and what that target might be. And then also how that’s connecting to the Ross production. >> Right Yes. I would love to say that I have a primary candidate right now. But we don’t currently. That list I showed is sort of potential interaction sites. What’s been difficult is in the assays we have been using, even ROS assay it’s still really not early enough in the chain of events to really do detailed antagonist studies. So we’ve been working towards actually collaboration with Dr. Abood to really look at, you know, use calcium imaging in some assays that look at initial effects of these compounds to release out which sites might be more primarily linked to this production of reactive oxygen species. >> And there is actually a follow up. Is there any evidence for endo cannabinoid being able to drive these ROS processes in terms of the endogenous pathway as opposed to the CBD? >> Yeah, I'm trying to think off the top of my head if any of the endo cannabinoids have shown this. Yes. Yes. There is evidence of preclinicals that they also product similar effects. >> So I have a question maybe each of you could potentially address in that our title of the session is biology and cancer prevention and we haven’t really, you know, talked so much about prevention. But I'm wondering what your thoughts are and what evidence there might be on the influence of cannabinoids on modulation of cancer risk markers, early indicators of cancer risk or any studies for which intermediate endpoints have been modulated, say in probably preclinical models? >> Yeah. I can start. I mean, prevention is an extremely complex issue as you all well know. Yesterday we heard about, for instance, a chemotherapy-induced neuropathic pain prevention. But, of course, that is a super clinical space [phonetic] in which we can define when we start the pain and when we start the pharmacological intervention. And that’s very, very difficult in the real life in real patients and in real life humans. And talking about patients, but people who still are not suffering from cancer when the cancer starts how we can deal with that. I mean, that is extremely complicated. But there is a report mid-90s by the NIH in which they treated mice and rats wit high amounts of THC for all their lives, the lives of the animals, more than two years, trying to find in principle that cannabinoids were portomogenic [phonetic] in compounds and to the surprise of everyone, cannabinoids were shown to be anti-tumoral. Those animals produced less tumors, less spontaneous tumors. Mice and rats are different from humans, and that is one of the problems, one of the many limitations of these animals to be models of cancer, because we humans mostly develop carcinomas whereas mice and rats [unclear] tumors such as lymphomas. So most of the tumors that were detected in these animals were lymphomas. But anyway, besides this [unclear], it was shown that high amounts of THC given periodically to these mice along with two generations, an appearance of less tumors, less tumor [unclear] in both mouse and rat models. I mean, there is a single experiment but it was a very painstaking experiment. It took two years of the life of the animals. And maybe it can point to the notion that maybe in some instances, at least biologically in species, and for some tumors cannabinoids could be anti-tumoral components when prophylactically given. But I mean in humans we have no idea and I think those studies would be great if we conduct them. But going back to real life I think it’s almost impossible to get to know. But I believe that in patients that have been diagnosed with cancer a very interesting niche for therapy would be neoadjuvants. So start to give cannabinoids not first line, not in direct phase of the tumor, but before, in the short period of time, before the standard of care of core chemotherapy, radiotherapy is given. Those trials I think they would be easier maybe than other more complex trials and it would involve a short period of time and maybe for having proof of concept as to whether cannabinoids may be added to [unclear] would be really ideal for trying to get into this issue clinically, because as I said we have many, many different lines of evidence, but clinically speaking unfortunately we’re basically at lost in the clinics. >> Thank you. Any other comments about cancer prevention or best ways to study with cannabinoids, thoughts there? >> Yeah, it reminds me of, you know, he study, it was Dr. Tashkin [phonetic], I think who did this really large study, looking to see whether, basically for a smoker, who also smoked marijuana, if you would see this. The expectation at which a hypothesis made sense, because marijuana does have a high amount of tar and, you know, benzoparines [phonetic]. So that hypothesis did make sense. So they looked at this patient population and actually what they found is almost the opposite result. They weren’t, again they weren’t testing for the specific hypothesis, but what they found really is the data suggested that those marijuana users actually would a lower incidence of cancer in comparison to not smoking. Almost suggesting a protective effect, which you know it does make sense. Again, this is a complicated study, but it does make sense with the preclinical data. You know, cannabinoids have actually anti-oxidant properties. Again we’re showing you all the [unclear] for anti-tumor activity, so it made sense in terms of what was being studied at that time. So if we’re talking about maybe protective effects for patients that may have cancer, you know, over time. That’s one of the better studies I think. >> Thank you. Yeah, it’s a complicated area in context and dose and all these factors and understanding the endo cannabinoid system is going to be important. And I think the results from a couple studies that I think both Manuel and Sean had shown about the ROS activity may play some role there as well, as you mention. >> So during the session we’ve heard, you know, studies showing both anti and pro tumor agenic [phonetic] effects from cannabinoids. And I just want to ask the panel in terms of understanding the cancer biology are there particular areas that we need to know now that are most important for understanding how cannabinoids effect tumor biology? And I think broadly other aspects of cancer biology or perhaps particular needs in cancer models to better understand these both pro and anti-tumor effects? >> [unclear] >> I'm sorry? >> I will start. I mean, that is an extremely complex issue. I would say that the clinical evidence supports very strongly that pharmacological activation of cannabinoid receptors has anti-tumor activity. And that is supported by many different experimental models both with culture cells, both with mice, with rats, not only in xenografts, atopic xenografts, but also in orthotropic xenografts, also in genetic models of cancer, etc. So I would say that the general conclusions of the studies is that in one sentence, but there are situations in which cannabinoids seem to be pro-tumor agenic. For instance, there are some studies also by Tashkin and others showing that when animals are injected with cancer cells that do not express cannabinoid receptors and therefore they are not prone to cannabinoid induced apoptosis, for instance, and if we give those animals large amounts of cannabinoids, of THC, for instance, during prolonged periods of time, what appears is that the effect of cannabinoids on the animals’ anti-tumor response overcomes the capability of cannabinoids to [unclear] or other anti-tumor effects directly on the cancer cells because those cancer cells do not express cannabinoid receptors. So at the end I believe if a balance of very complex factors which overall are in the balance they are diverted to anti-tumor responses as long as the conditions of the assays are appropriately and especially as to whether cannabinoid receptors are expressed on the surface of cancer cells. I would like to say that most of the cancers studied so far and most of the cancer cells lines studied so far express relatively high amounts of cannabinoid receptors from their surface. They express essentially CB2 receptors many times, which is also a very interesting question going back to Mary when she was talking about the endogenous role of the cannabinoid receptor, because why, for instance, in many cancers from a stage one to a stage four there is an induction of CB2 receptors? What is the meaning of that? We don’t know really. But at least [unclear] they are targets for different pharmacological activation of the receptor with the cannabinoids. It’s also difficult to, and very tricky, what is the similarities and differences between pharmacological cannabinoid receptor activation and endogenous cannabinoid receptor activation with an [unclear], because many times the outcome is different regarding, for instance, [unclear] cell migration or also cell survival. So maybe there are also some indications that at least in some stage of tumor initiation, and there is also there is clinical work, cannabinoid receptors could be [unclear] in each of these stages of tumor generation whereas at the end of a tumor formation, if we activate those receptors pharmacologically with THC, for instance, we have to be tumor responses. So there is again this kind of jing jang action of cannabinoid system in tumors that makes this complicated. But overall I would like to [unclear] in spite of these complexities the bout of the clinical evidence supports that by activating pharmacologically cannabinoid receptors we have anti-tumor outcomes. >> Yes. Well said Manuel. I would agree with those major points. I think, you know, again I'm back up on my soapbox, we would love to understand a little bit more about this difference, the shared pathways and the differences between THC and CBD. It’s pretty fascinating how, you know, one CB1 and CB2 receptor agonist, the other undefined sites or multiple sites. But they have these common overlapping pathways. I’d love to learn more, see us learn more about that. And I think really, again there’s many types of cancers and many types of therapies for those cancers. I think we could definitely increase our understanding of how cannabinoids work in these different spaces in terms of their ability to enhance first line therapies and obviously with the HPV work that we saw today, understanding which cancers we should really be careful in terms of potentially using cannabinoid therapeutics. >> Do you think studies, should there be more studies on let’s say the endogenous signaling pathway, manipulating that and looking at outcomes in regards to cancer biology? Or focus more on the purified components or even perhaps looking at the effects of more complex mixtures or the cannabis, you know, extract itself? Are there particular aspects that haven’t been looked at as well that we need more information for currently? >> My answer to that would be, yes. We need more information in both of those spaces. I will say the more I follow the literature with the cannabis mixtures it still seems to always circle back to the, you know, the primarily THC and CBD as being the primary active components in those mixtures. So at least the papers I have read. I don't know if there’s some that I’ve missed that maybe Manuel would like to talk about. >> I do [unclear] and it would be ideal to have, the more information, the better, of course. But complex mixtures are very complex of course, pharmacologically speaking. So, I mean, it would be really nice to know, to get to know, which part, what’s the interactions, co-dynamic [phonetic] interactions of the different cannabinoids or complex cannabinoid mixtures. And, of course, if we talk about all animals or about patients, it would be ideal to get to know also the pharmacological interactions. Yes, there are some indications in the literature. For instance, there is a work in mice conducted by Christina Sanchez in our lab. She found that complex crude cannabinoid preparations that were enriching THC were more potent than THC alone in inducing anti-tumor responses in three different models of breast cancer, triple negative breast cancer and ER positive, PR positive breast cancer and R2 positive breast cancer. However, pointing in the opposite direction in that case here as we mentioned before that one conducted in UK with 190 patients there were some suggestions by the authors, of course it wasn’t a [unclear] study and there were no doses and compounds were not very much control, that P or CBD exerted higher [unclear] than CBD oils. And therefore complex mixtures. It’s something we still do not know, but at least I would say the [unclear] work and our work and the work by others THC and CBD seem to work by different mechanisms to induce anti-tumor responses at least preclinically. So I suggest that if we combine the THC mechanism of action with the CBD mechanism of action we could have a stronger anti-tumor responses as [unclear] some status on this. So, yeah. And that may be different for every type of cancer and maybe for every type of patient. So if we pull very far the problem, maybe even patient, individual patient wise. So, yes. But in general I believe that combining in this case, because [unclear] is really at least until say 100 milligrams. I think it wouldn’t make any harm to activity to them, to the least to anti-tumor therapies based on THC and the other way around. If CBD has a good tolerability we could add for a patient maybe 20, 30 milligrams a day of THC. And in theory I think we would have a stronger effect when we combine both mechanisms of action. And the clinical literature also supports that I would say. >> Okay. Thank you. Well, I think our time is drawing near. So I will just like to thank the session speakers for wonderful presentations and a very stimulating discussion. And Karen do you have any final words? >> Yeah, thank you all for your presentations and this discussion. And I think, you know, some additional questions will likely be answered perhaps in the next session about preclinical treatment. You know one to think about may be for Friday is also barriers in addition to, you know, the models that we’re using, but what other barriers are there for studying in preclinical models in cannabinoid, in endo cannabinoid systems. So I think we’re finished. Thank you all. **(END OF FILE)**