Phytocannabinoids as an anti-tumor tool

Preclinical evidence has confirmed that cannabis induces cancer cells to commit suicide. Whole plant extracts are better therapeutic tools than isolated compounds.

By Cristina Sanchez

Cancer is a word that we use to define many different diseases —more than 200 diseases, according to the World Health Organization. They all have something in common and present challenges to oncology.

We need to improve our tools for the early diagnosis of patients. We need new treatments for those patients who have no effective therapy. And for those patients who respond, we need therapies with less toxicity.

We have more than enough evidence to say that we can use cannabis to help address these three issues. Early diagnosis requires a political solution —public investment in health care. Where you live should not determine the treatment the system provides you —or the tools for determining if you have cancer or not.

I won't speak about this today because I am not a politician, I'm a scientist. I'm going to present data supporting the use of cannabis to improve oncologic treatments.

It's important to bear in mind that cancer patients have to deal with many things that are related to the pathology indirectly. The side effects of the antitumor therapies we use include nausea and vomiting, lack of appetite, and pain. There are also psychological effects of the disease that impact in a negative way. When someone has cancer, not only the patients but the families suffer anxiety. We have trouble sleeping, too.

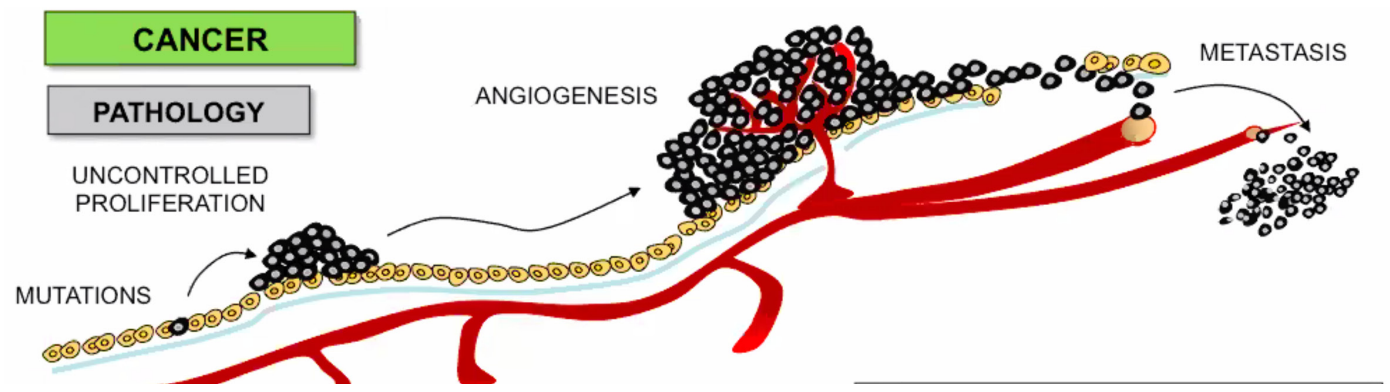
We have known for many years that we can use cannabis as a palliative agent. It has been demonstrated in the preclinical setting and also the clinical setting that we can treat or prevent the nausea and vomiting induced by chemotherapy with cannabis. We can use it as an analgesic to reduce and endure pain, as a tool to stimulate appetite, decrease anxiety, and help improve sleep.

Our research has been focused not on the palliative aspects of cannabis but on its potential as an anti-tumor agent.

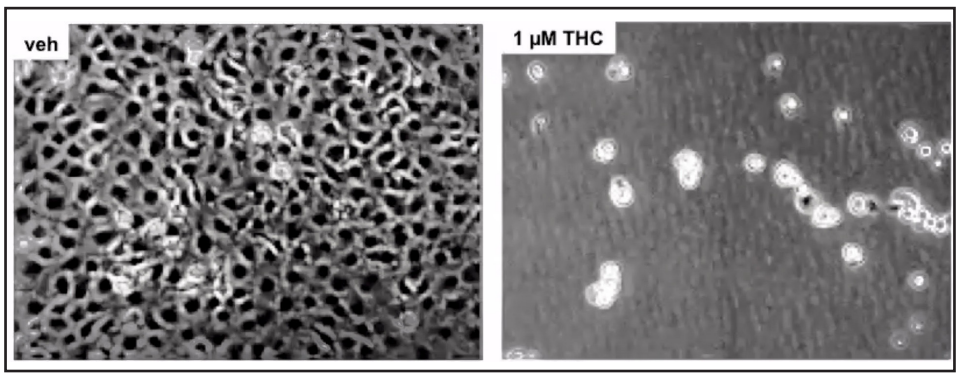
As with any other anti-tumor compounds, with cannabis we have to prove two things; one, that this tool is efficacious, that cannabis is actually an antitumor agent, and second, that this tool is safe.

In the late 1990s we conducted a series of experiments confirming that cannabinoids induce the death of cancer cells. We cultivated human glioblastoma cells and treated them with THC, which caused them to die.

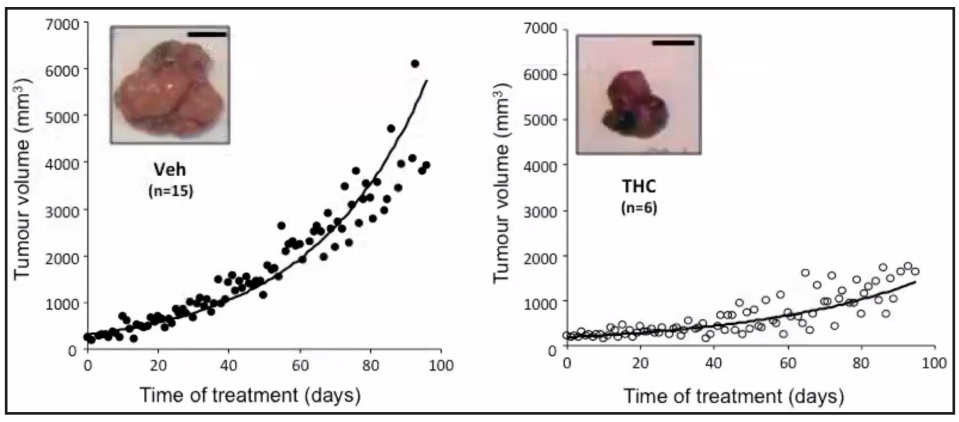
Subsequently we moved on to more physiological models in cancer, and we have seen anti-tumor responses to cannabinoids in glioblastoma (GBM), breast cancer, skin cancer, and many others. In an experiment with MMTV-neu mice, illustrated above, half were treated with THC, and half with vehicle (sesame oil) In mice that did not receive THC treatment, the tumors grew exponentially. In those treated with THC we saw a drastic reduction in tumor growth.



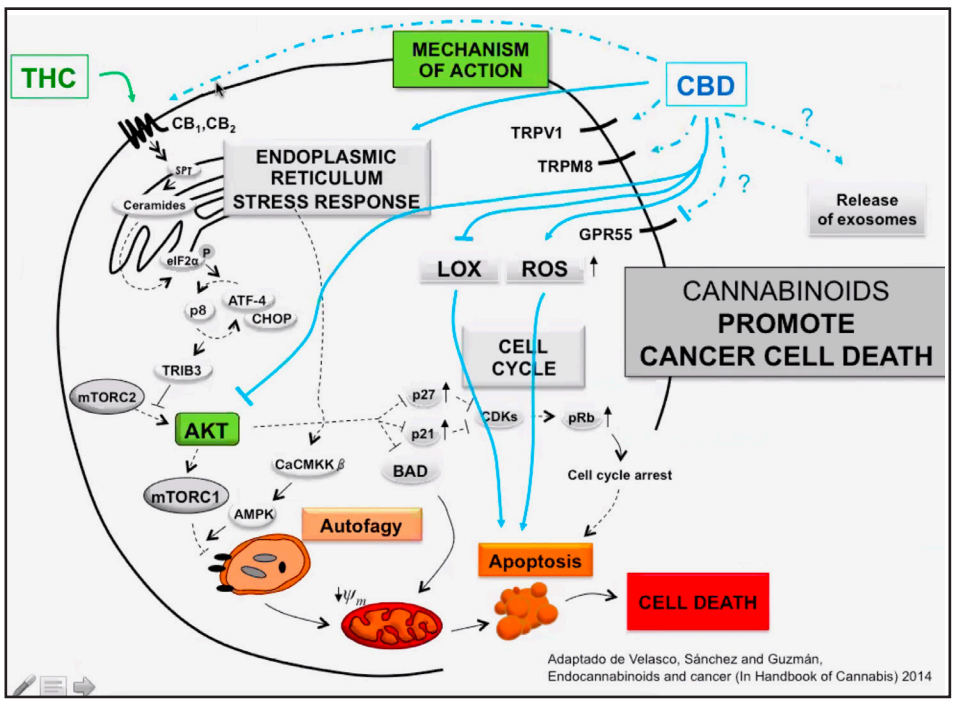
Progression of the disease begins with a mutated cell proliferating. Tumor growth is accompanied by angiogenesis (the formation of new blood vessels) and metastasis (the process by which cancer cells break away from the tumor in which they formed and travel through the blood or lymph system to form new tumors elsewhere in the body). Cannabis blocks proliferation, angiogenesis, and metasisis.



Antitumor effect of THC was documented by Sanchez and colleagues in 1998 using cells from a human glioblastoma line cultured in petri dishes. In the micrograph at left, cancer cells have proliferated. In culture at right, treatment with THC has caused widespread cell death.



THC drastically slowed proliferation in experiment involving MMTV-neu mice with HER2+ breast cancer that were separated into two groups. Graph at left shows exponential tumor growth (vertical scale) over 100 days (horizontal scale) in mice treated with vehicle. Graph at right shows reduction in tumor growth following treatment with THC.



Endoplasmic reticulum stress response is triggered by THC (activating CB receptors), and CBD. CBD also acts on other receptors and targets in the cell to promote cell death.

Biochemist Cristina Sanchez, a professor at Complutense University in Madrid, has been doing research in the cannabis field for 22 years. After obtaining a PhD in the lab of Dr. Manuel Guzman, she did post-doctoral work under Daniele Piomelli at UC Irvine. She resumed work with Guzman in Madrid in 2003. She now leads a small group of researchers working on breast cancer. This article is from a presentation to the Society of Cannabis Clinicians in September, 2018. The whole talk —and Sanchez fielding questions from the SCC doctors— is online at www.cannabisclinicians.org

What is going on inside the cell when we treat them with cannabinoids?

THC induces the death of cancer cells mainly by binding and activating CB1 and CB2 receptors. Activation of these receptors triggers the synthesis of pro-apoptopic molecules, ceramides among them, and leads to a response called the endoplasmic reticulum stress response.

THC, by binding to CB1 and CB2, switches off the regulator of cell survival.

This response occurs when cells detect problems in the folding of their proteins and forms of damage. It usually is the trigger for suicide, so that the cell avoids being a problem for the rest of the organism. The response involves changes in the levels and activities of a number of proteins. All these changes converge in the inactivation of a protein called AKT, which is the master regulator of cell survival.

So THC, by binding to CB1 and CB2, switches off the regulator of cell survival. This triggers a process called autophagy, which means "self-digestion" and leads to apoptosis, which is programmed cell death. That is the general mechanism of action of THC on glioblastomas, breast tumors, and many others.

With CBD the main mechanism of action seems to be the generation of reactive oxygen species, which trigger apoptosis. CBD also activates the endoplasmic reticulum stress response and inactivates AKT. CBD inhibits lipoxygenase (LOX) activity, which also leads to apoptosis. It binds other receptors, including TRPV1 and TRPM8.

Recently, a couple of papers were published suggesting CBD anti-tumor action might be mediated by the blockade of GPR55, a proposed cannabinoid receptor. However, numerous experiments done in our lab did not show that CBD antitumoral action was produced by CBD inactivating the GPR55 receptor. More research is needed to determine whether GPR55 is involved in CBD anti-tumoral action.

Another paper that just came out suggests that CBD anti-tumor action is produced by the release of exosomes —small vesicles released by cells that cancer cells can also release. It's been proposed that exosomes carry molecules that can control the expression of genes that help tumors be more aggressive. But we don't know if the release of exosomes is the cause of the anti-tumor effect, or just the result of anti-tumor action.

Not only can cannabinoids block and control cancer cell proliferation, they can also block angiogenesis, the formation of new blood vessels to support the tumor. Pharmaceutical companies are working hard to develop anti-angiogenesis tools.

To study angiogenesis we generated glioblastomas in the backs of immune deficient mice. We treated half of the mice with vehicle and half with THC. As shown in the

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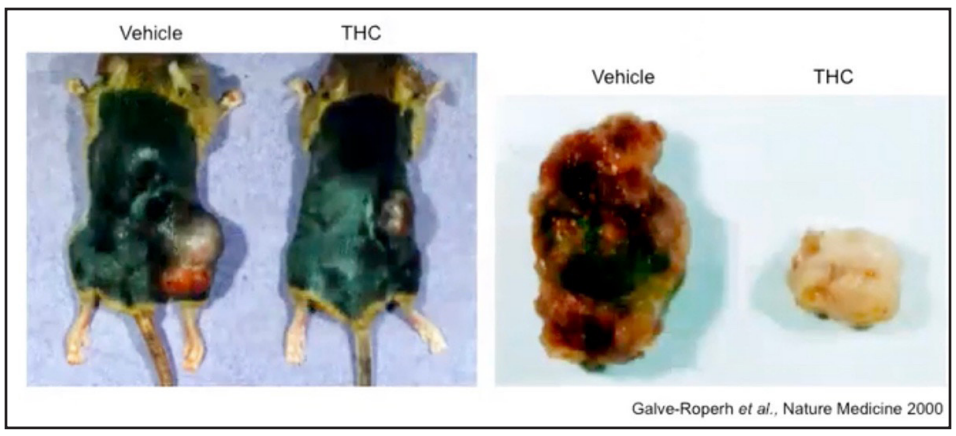
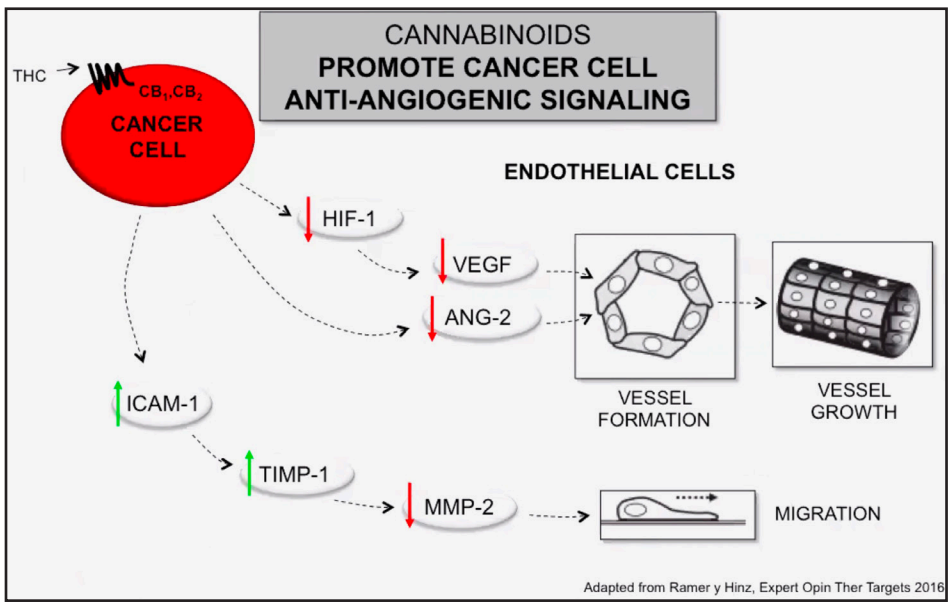
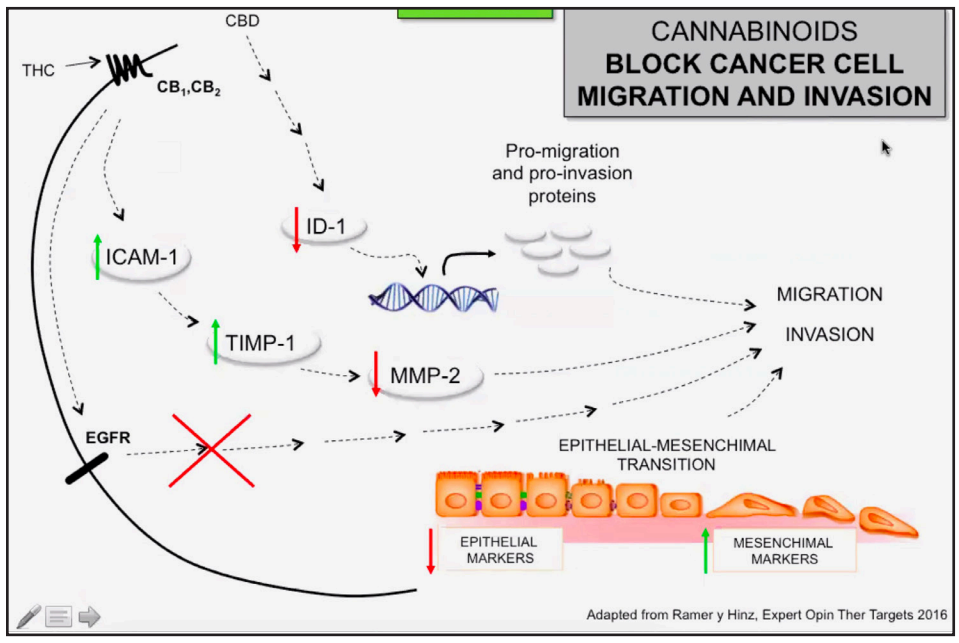


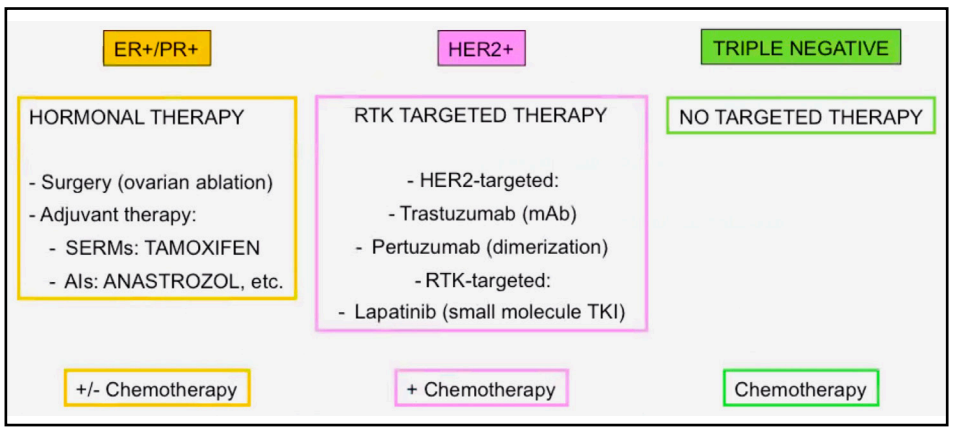
ANTI-ANGIOGENESIS EFFECT OF THC was observed in mice genetically engineered to develop glioblastomas on their backs. Photo at left shows reduced tumor on mouse treated with THC. In photo at right, untreated tumor is larger and red color shows it to be well supplied with blood. Tumor from mouse treated with THC is white, indicating that new blood vessel formation was blocked.



THC BLOCKS ANGIOGENESIS BY ACTIVATING CB1 AND CB2 RECEPTORS IN CANCER CELLS. Modulated as a result are HIF-1, the main regulator of angiogenesis, and VEGF, the main growth factor regulating vessel formation and vessel growth. Proteins involved in endothelial cell migration are also modulated and cease to function.



Cannabinoids, especially THC, modulate proteins involved in cancer cell migration and block the epithelial to mesenchymal transition. CBD down-regulates the ID-1 gene, which controls expression of pro-migration proteins.



BREAST CANCER TYPES AND TREATMENTS.

illustration above, the tumors in the THC-treated group were small and unable to supply themselves with blood.

What was the mechanism of action? Were the cannabinoids targetting cancer cells or the endothelial cells of the blood vessels?

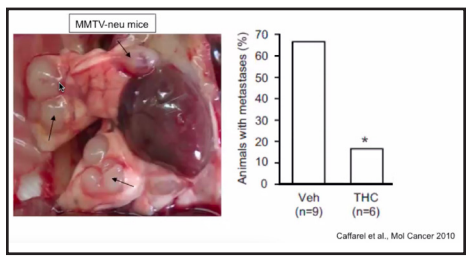
Research indicates that THC blocks angiogenesis by activating CB1 and CB2 receptors in cancer cells. Among the proteins modulated are HIF-1, the main regulator of angiogenesis, and VEGF, the main growth factor regulating vessel formation and vessel growth. The result of these changes is that vessel formation and vessel growth is impaired.

In addition, cannabinoids block the migration of endothelial cells by inhibiting a family of proteins that break down the extracellular matrix that surrounds the cancer cells.

We also have proof that cannabinoids block the metastasis processes that occur in the later stages of the disease. In an experiment with MMTV-neu mice that developed breast tumors, 70% metastasized in a control group that received no treatment. In the animals that were treated with THC, this number went down to 20%. *(See illustration at bottom of page.)*

Again, we know the mechanism of action. THC acts by binding and activating CB1 and 2 receptors, and modulates the family of MMPs, and also the activity of other growth factor receptors like EGFR, which trigger migration and invasion. So if cannabinoids block these proteins, they are blocking these processes.

THC also blocks what we call the "Epithelial-Mesenchymal Transition." In our tumors, most of the cells have low-epithelial phenotypes, and show epithelial markers, but when they have to migrate and invade other tissues to generate metastasis, they have to start moving, and to do that, they have to change their shape, their phenotype. They have to acquire mesenchymal markers instead of epithelial markers, so they start to produce mesenchymal phenotypes. They change shape and start moving. Cannabinoids, especially THC, can block these transitions.



Metastasizing tumors (in photo) occurred in almost 70% of MMTV-neu mice treated with vehicle (bar at left in graph) and fewer than 20% of mice treated with THC.

Cannabinoids block the capability of cancer cells to migrate and invade surrounding tissues.

In the case of CBD, it seems the main mechanism of anti-metastatic migration is the down-regulation of the ID1 gene, which controls many of the expressions of pro-migration and pro-invasion proteins. By decreasing the levels of these proteins we hamper the invasion capabilities of the tumor.

Maximum efficacy

We asked ourselves: is it more efficacious to use pure cannabinoids or whole-plant preparations? The cannabis plant has more than 140 cannabinoids, and some of them have potential therapeutic properties. They have been shown to have antiproliferative properties in cancer cells, some have anti-inflammatory properties and antioxidants, and so on. Most of them have not been studied yet.

Same thing with a family of very interesting compounds that this plant produces: the terpenes. There are more than 100 terpenes in the plant. Some of them also have therapeutic properties, and the majority of them have not been studied yet.

When we work with the cannabis plant, we have to keep in mind what Drs. Mechoulam and Russo call the entourage effect. In pharmacological terms, we call that synergism. We have one and one, and the result is more than two.

In 2017 we started a project to compare the anti-tumor efficacy of pure THC provided by a German company, THC Pharm, with THC-rich extract provided by Aunt Zelda's, a nonprofit mutual benefit corporation founded by Mara Gordon.

We used models of breast cancer. When oncologists have to choose the proper treatment for each patient, they classify breast cancer in three different subtypes. The first is called "hormone-sensitive," because these cancer cells express estrogen receptors, or progesterone receptors.

The problem with these cancer cells is that they have very active estrogenic signaling, so the goal of the treatment in these patients is to shut down the estrogenic signaling. You can achieve that by removing the endogenous source of estrogen via surgery, or you can use drugs —a pharmacological approach of blocking the estrogen receptor itself with a selective estrogen receptor modulator, such as tamoxifen, or with compounds that block the synthesis of estrogens with aromatase, the main enzyme controlling the synthesis of estrogen. Anastrozole is one example of this kind of drug. Depending on the prognosis, these patients may also receive chemotherapy.

A second type of breast cancer subtype is characterized by the overexpression of the oncogene HER2+ (Human Epidermal Growth Factor Receptor 2). Compounds that block this specific receptor. include Trastuzumab, which is directed against the extracellular domain of HER2+. Another, Pertuzumab, blocks the dimerization process of HER2+, which is crucial for activation.

Another way to target these receptors is to block their enzymatic activity with tyrosin kinase inhibitors such as Lapatinib. HER2+ breast cancer is usually treated also with chemotherapy.

A third type of breast cancer is called triple negative. The name comes from the

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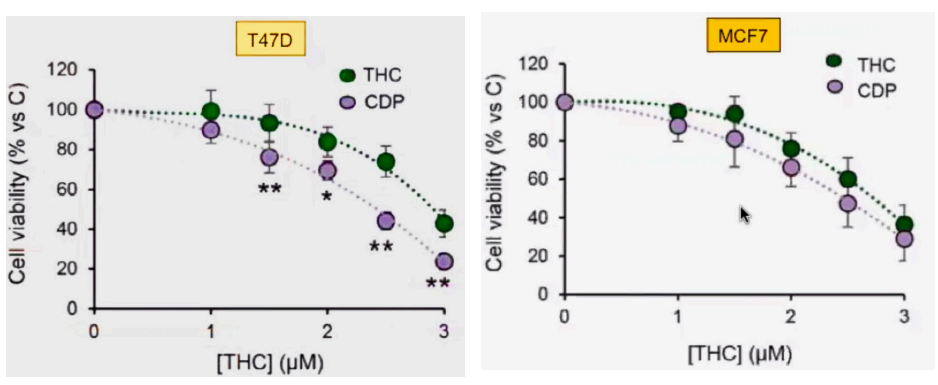
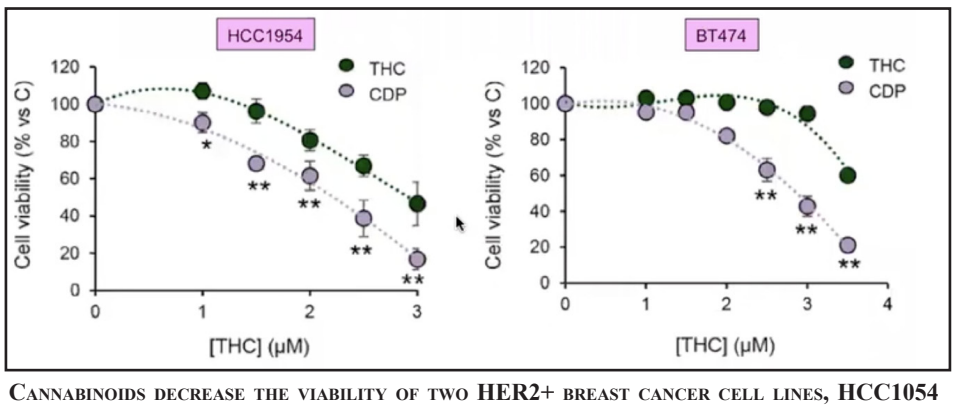


CANNABINOIDS DECREASE THE VIABILITY OF TWO HORMONE-SENSITIVE BREAST CANCER CELL LINES (vertical scale in both graphs) more potently as dosage increases (horizontal scale). Extract (purple dots) decreased viability more potently than THC (green). Experiments summarized in graphs used T47D cells (left) and MCF7 cells (right).



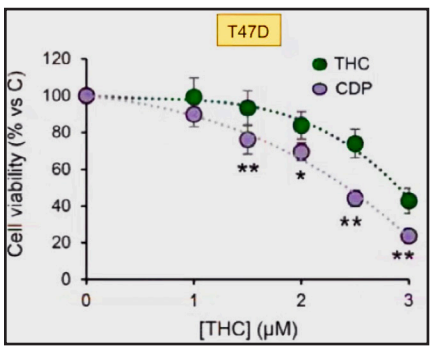
CANNABINOIDS DECREASE THE VIABILITY OF TWO HER2+ BREAST CANCER CELL LINES, HCC1054 (left) and BT474 (right).

Sanchez *from previous page*

fact that it doesn't express either estrogen receptors or progesterone receptors, or HER2+. Unfortunately, this is the most aggressive phenotype, and we don't have any targeted therapy because we don't have any molecules or markers that we can specifically target. The only therapy that these patients can receive now is standard chemotherapy, which indiscriminately targets all cells in the body that are undergoing proliferation.

We started by comparing and analyzing the anti-tumor effect of pure THC versus a whole plant preparation in the cell model of hormone-sensitive breast cancer, using a cell line called T47D. Both the THC and the extract decreased the viability of the cells. The effect of the extract was more potent.

We next ran the same experiments in another hormone-sensitive cell line (MCF7). The differences were not statistically significant: both pure THC and the extract decreased the viability of the cell line, and again the extracts seem to be more potent than the pure compound.



High-THC Extract (purple dots) and pure THC (green) both decreased cell viability in a model of hormone-sensitive breast cancer. Extract was more potent.

When we replicated the experiment with an HER2+ cell line, we saw the same pattern. Both pure THC and the extract decreased cancer cell viability, and the extract was more potent than the pure compound.

We completed this series of experiments in models of triple negative breast cancer. And in this case again, we used two different cell lines in culture, and we saw the same thing. Both cultures decreased the viability of the cancer cells, and the extract was more potent than the pure compound.

So we concluded that all breast cancer subtypes are sensitive to cannabinoid antiproliferation action. It's important to point out this conclusion, because there are comments out there that hormone sensitive-tumors may not respond to cannabinoids. We see that they do respond, and in the same way the other types of breast cancer do.

All breast cancer subtypes are sensitive to cannabinoid antiproliferation action.

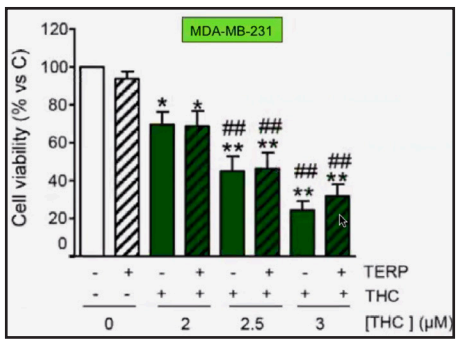
We also concluded that the THC extract was more potent than pure THC in killing cancer cells.

Next we tried to see if combining pure THC —provided by a German Company, THC Pharm— with some terpenes added would recreate the effect from the plant extract. We made a cocktail with the five terpenes most abundant in the extract from Aunt Zelda's —Beta-Caryophyllene, Linalool, Alpha-Humulene, Beta-Pinene and Nerolidol 1— and we conducted experiments on cell viability.

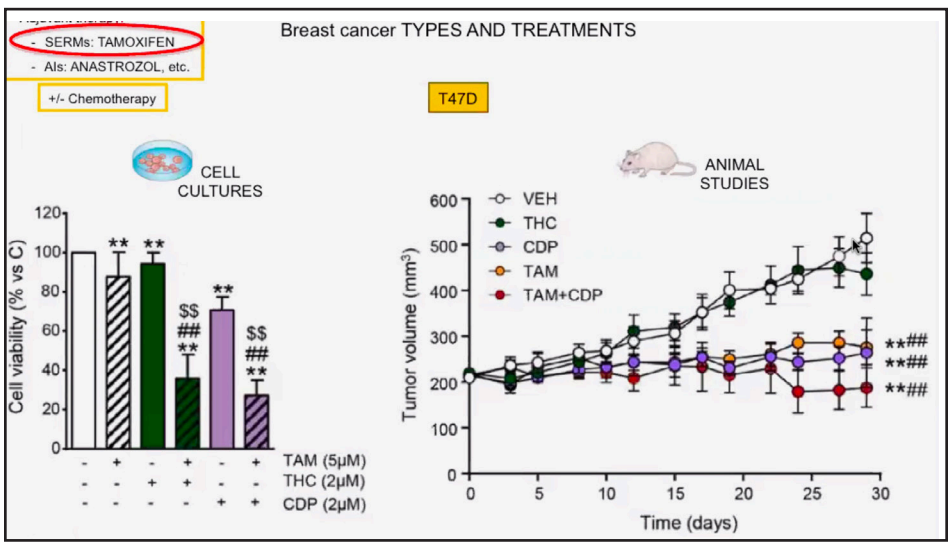
We started with the hormone-sensitive subtype and found that when we combine the terpene cocktail with the THC, we do not see any statistically significant difference. Adding terpenes —at least these five terpenes— doesn't improve the killing effect of pure THC.

With HER2+ and triple-negative breast cancer cells we saw exactly the same thing. The combination of THC with these terpenes does not recreate the potency of the extract.

For the next step, we decided to test cannabinoids in combination with the standard cancer treatments in the three subtypes of breast cancer. We started with hormone sensitive tumors, and chose Tamoxifen as the drug of study.



Combination of THC and the five terpenes most abundant in whole-plant extract did not equal the effectiveness of the extract in killing hormone-sensitive cancer cells. Terpenes with no THC (second bar from left) killed no cells. Vertical scale shows cell viability, horizontal scale shows dose administered to cell culture. Similar results were obtained using HER2+ and triple negative cancer cell lines. Adding a terpene cocktail to THC does not improve its ability to kill breast cancer cells to a statistically significant degree.



Tamoxifen plus cannabinoids as a tool to block hormone sensitive tumors was tested in vitro (on cell cultures, results in bar graph) and in vivo (animal studies, graphed at right). In bar graph, vertical scale measures cell viability. Small doses of Tamoxifen and THC by themselves (second and third bars from left) were minimally effective. Adding THC to Tamoxifen greatly improved potency (fourth bar). Extract (fifth bar) was more potent than THC. Extract plus Tamoxifen most potent of all. Graph at right shows extract (purple) to be as potent as Tamoxifen (yellow). Superior potency of extract plus Tamoxifen (red) was not statistically signifcant.

In vitro studies showed that submaximal doses of tamoxifen produced a very small decrease in cell viability, and so did submaximal doses of THC. But when we combined tamoxifen with THC, we could see a synergistic response. This is an example of one plus one is more than two. We see an unexpected decrease in cell viability.

There was also a decrease in cell viability when we combined tamoxifen with the whole plant extract.

When we tried this in vivo, we found that the extract we were using was more potent than pure THC and as potent as tamoxifen, which is the drug that most patients are using nowadays. Disappointingly, when combining tamoxifen and the extract, we didn't observe the synergistic response that we observed in vitro.

We did the same studies in HER2+ breast cancer cells using Lapatinib as the drug combined with cannabinoids. And we observed the same thing: The extract is as potent as the current treatment that these patients receive. But again we observed no statistically significant improvement of efficacy when the extract and Lapatinib were combined.

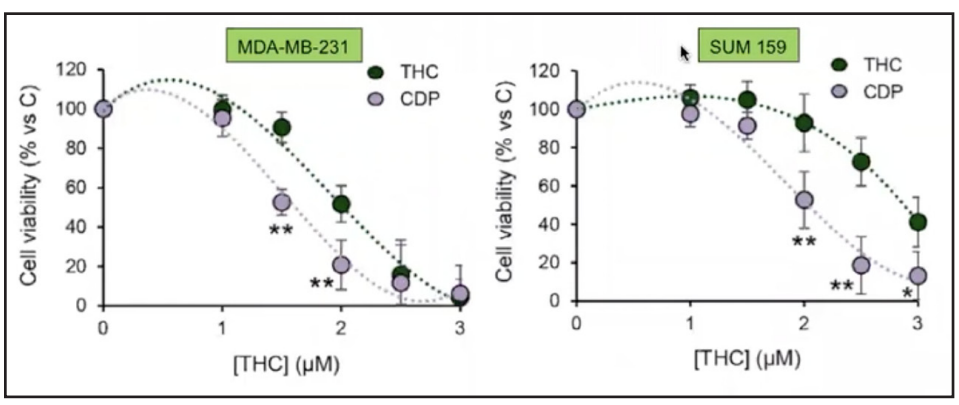
In triple negative breast tumors we used cisplatin as the chemotherapy, and we observed no synergy in vitro. THC added to cisplatin produces the same effect as cisplatin alone. Adding the extract in combination with cisplatin is more effective.

When we move to the animal setting we observe that cisplatin seems to be slightly more potent than the extract. When we combine the extract with the cisplatin, we see a slightly higher antitumor response, but it's not statistically significant either.

The take-home message is that the extract is as potent as standard therapies, and that the extract is more potent than the pure THC compound in vivo, which is important. Often you see in vivo experiments do not reproduce the in vitro results, but in this case the extract is more potent in both cell cultures and test animals.

A second conclusion is that the THC extract was as potent as the standard therapies tested in this study. Also, the combination of cannabinoids with the tested anticancer therapies tested do not improve the potency of the individual treatments.

We would like to repeat these experiments with lower doses of the treatments. The dose of cisplatin we used was so high and produced such a strong effect, that it left very little room for improvement from the extract. So we would like to repeat this experiment with a dose of cisplatin that would produce less of an effect.



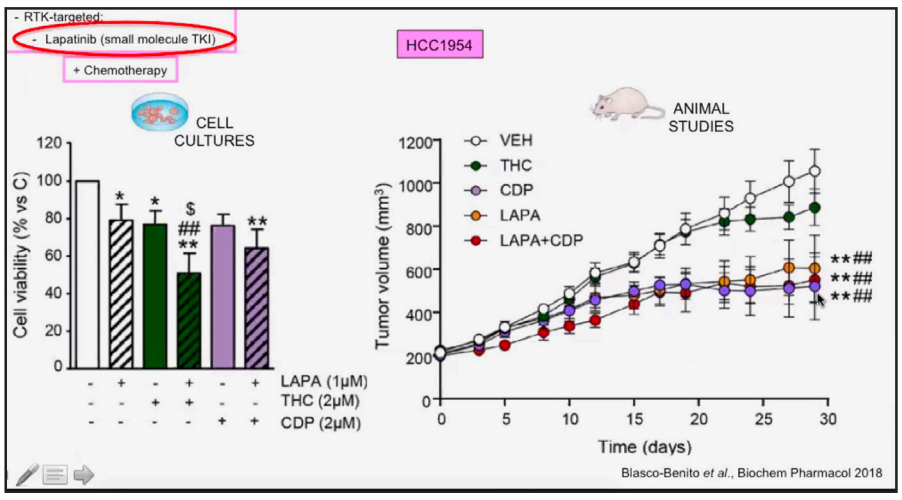
Cannabinoids decrease the viability of two triple-negative cancer cell lines ("remarkably," said Sanchez), MDA-MB-231 (left) and SUM 159 (right).

CANNABINOID	Concentration (mg/g)
THCA	3.449
THC	551.308
THCV	ND
CBD	ND
CBDA	ND
CBG	3.667
CBN	ND
CBC	ND

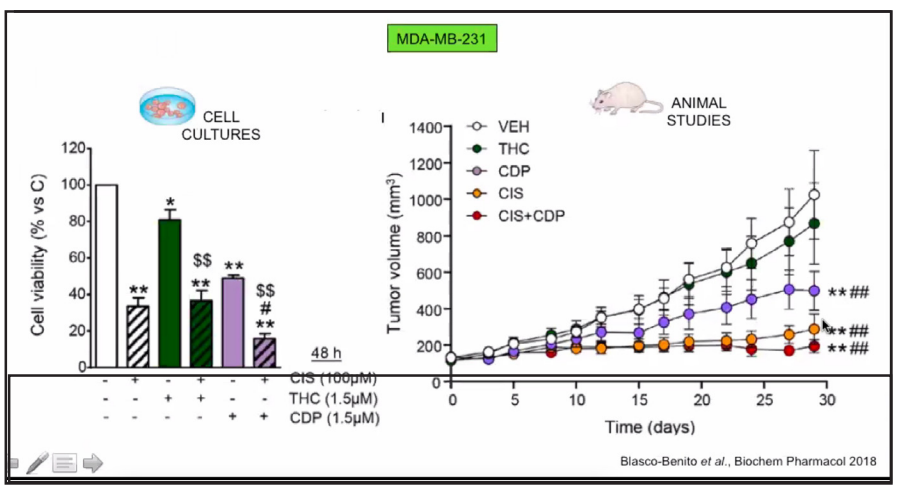
ND = Not detected.
BDL = Below detectable limit.

High-THC cannabis extract used in the study was provided by Mara Gordon of California-based Aunt Zelda's (which also funded the study). The pure THC to which the extract was compared came from THC Pharm, a German company.

TERPENE	Concentration (mg/g)
α-Bisabolol	0.177
Camphene	BDL
3-Carene	BDL
β-Caryophyllene	1.948
Caryophyllene Oxide	0.032
p-Cymene	0.178
Geraniol	ND
Guaiol	ND
α-Humulene	0.557
Isopulegol	0.023
D-Limonene	ND
Linalool	0.620
β-Myrcene	0.025
Nerolidol 1	0.357
Nerolidol 2	0.081
Ocimene	0.049
α-Pinene	0.015
β-Pinene	0.317
α-Terpinene	0.013
γ-Terpinene	0.013
Terpinolene	0.017



Lapatinib plus cannabinoids as a tool to block HER2+ tumors



Cisplatin plus cannabinoids as a tool to block Triple negative tumors

Graphs from Blasco-Benito et al., Biochem Pharmacol 2018