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TITLE: Anticancer Efficacy of CBD Pure Isolates and Commercially Available Water-Soluble CBD in Colorectal Cancer

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14. ABSTRACT

According to the most recent information from the American Cancer Society (ACS), colorectal cancer (CRC) is the 3rd most common cancer in the United State. Moreover, rates are rising in younger age groups. Amongst Military Veterans, approximately 13,000 cases of CRC have been reported from 2009-2012. Critically, while early-stage cancers respond well to treatment, metastatic colorectal cancer has a 5-year survival of only 14%, emphasizing a need for new therapeutic approaches. CBD, which is a non-psychotropic cannabinoid from the Cannabis sativa plant, has been studied since the 1970s. There is some evidence that it has anti-tumorigenic properties such as slowing of tumor progression, apoptosis induction, and proliferation inhibition although the studies are few. However, CBD like many other drugs, has low water solubility leading to poor bioavailability. Several companies have apparently developed what is being called Water-Soluble CBD, which could theoretically improve bioavailability. This water-soluble CBD would allow for higher concentrations of CBD to be available to a tumor and a dosage regimen that maintained optimal levels for anti-cancer activity to be identified. Given the availability of such agents, the claims being made for their efficacy, as well as the dire need for effective treatments for CRC, this study is designed to determine the efficacy, safety, and validity of water-soluble CBD compounds in colon cancer in vitro and in vivo studies.

15. SUBJECT TERMS
None listed.

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Introduction

Cannabidiol (CBD) has become very popular since the Farm Bill Act of 2018 and while it has been studied since the 1970s, the research has been quite spare. There has been is evidence of CBD being anti-tumorigenic by slowing progression, inducing apoptosis, and having anti-proliferative effects in various cancer cells. However, CBD like many other drugs, has low water solubility that leads to poor bioavailability, with bioavailability based on route of administration reported as: 5% oral, 13% sublingual, 30% inhaled. Several companies have apparently developed what is being called Water-Soluble CBD, and state without much evidence that it has improved bioavailability taken orally. This water-soluble CBD, if proven true, would allow for higher concentrations of CBD to be available to a tumor, and a dosage regimen that maintained optimal levels for anti-cancer activity to be identified. Given the availability of such agents, the claims being made for their efficacy, as well as the dire need for effective treatments for Colorectal Cancer (CRC), this study is designed to determine the efficacy, safety, and validity of water-soluble CBD compounds in colon cancer *in vitro* and *in vivo* studies.

Keywords

Anti-proliferative

Anti-tumorigenic

Bioavailability

Cannabidiol (CBD)

Colorectal Cancer (CRC)

Pharmacokinetic (PK)

Accomplishments

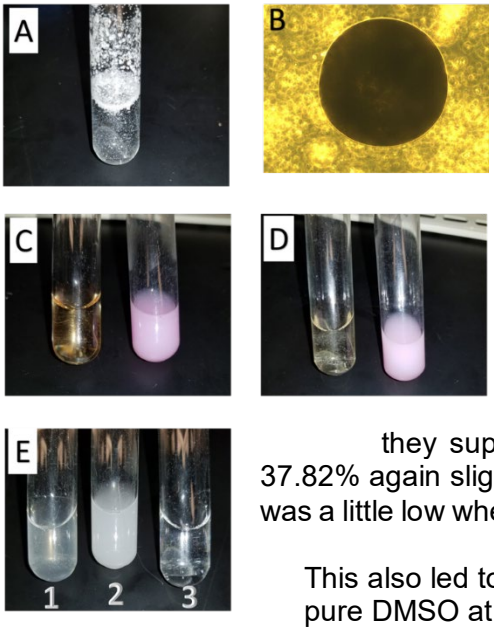
- **What were the major goals of the project?**

SOW Changed Submitted February 22, 2021

- **Major Task 1-** Determine the anti-tumor effects of CBD and water-soluble CBD on colon cancer cells
  - Analyze all CBD compounds using Mass spec. February 28, 2020
  - Evaluate CBD effects on proliferation, cell death, clonogenic survival, migration and invasion in 5 human and 2 mouse colon cancer cell lines.
- **Major Task 2-** Determine the anti-colon cancer mechanism of CBD in the responsive cell lines.
  - Subtask 1 - Evaluate the role of CBD's effect on proliferation of colon cancer cells.
  - Subtask 2 - Analysis of data to identify possible pathways responsible for sensitivity to CBD
- **Major Task 3-** Determine endocannabinoid receptor expression and mutations in sensitive colon cancer cell lines that affect their sensitivity to CBD
  - Subtask 1 - Investigate the presence of endocannabinoid receptors (CB1, CB2, GRP55, and TRPV1) in responsive colon cancer cell lines.
  - Subtask 2 - Compare the mutations found in colon cell lines that possess the most and least resistant to CBD.
- **Major Task 4**- Analyze data and prepare manuscript
  - **Not Finished**

- **What was accomplished under these goals?**
  - SOW Changed Submitted February 22, 2021 to no longer use mice and continue on mechanism of CBD isolate on Colon cancer.
- **Major Task 1**
  - Determine the anti-tumor effects of CBD and water-soluble CBD on colon cancer cells
  - Subtask 1- Analyze all CBD compounds using MS

Isolate is crystalized pure CBD known in the industry as Isolate. MI, TN, and NC are three water-soluble CBD formulations from three different companies and using the only identifier as the state from which they are from.



	Provided COA	Results of LCMS
Isolate	100%	84.76%
MI	11.50%	11.53%
TN	41.82%	8.77%
NC	10.62%	6.99%

Isolate was found lower than the third-party lab test but this happens to most isolated CBD because it absorbs water in the air and if not, completely sealed moisture contaminates the purity. Still comparable to what you would find in a dispensary. On January 7th 2021 I received 10mg pure CBD from Sigma-Aldridge catalog number PHL85705 that was purified to 99.9%. MI was very much on target with what their third-party sent. When I spoke with the company in TN they said that they think there was a mix up and they suppled a new sample. The TN water soluble new sample showed 37.82% again slightly lower but a great deal closer then original sample. NC sample was a little low when it was tested but again over time the moisture could contaminate.

This also led to determining the best way to solubilize CBD Isolate. I found that in pure DMSO at 1M or even 10mM the drug would form spheres in culture. I tested mixing with EtOH but this caused necrosis and finally determined that 1M Isolate in DMSO 4 parts to 6 parts media to 10mM concentration would work and stay in solution. While PBS worked well for the water-soluble CBD formulations.

Figure 1, Solubility of different CBD formulations.
A. CBD Isolate at 1M concentration in PBS.
B. CBD Isolate in DMSO mixed with Cell Culture Media at 100uM under microscope at 10X.
C. CBD Isolate in EtOH at 1M (left) CBD Isolate at 10mM in 4:6 ratio of EtOH and Media.
D. CBD Isolate in DMSO at 1M (left) CBD Isolate at 10mM in 4:6 ratio of DMSO and Media.
E. 1. CBD water-soluble solution from NC at 1mM. 2. CBD water-soluble solution from MI at 1mM. CBD water-soluble solution from TN at 1mM.

- Subtask 2 –
  Evaluate CBD effects on proliferation, cell death, clonogenic survival, migration and invasion. On 5 human colon cancer lines and 2 mouse lines.

- Human Lines

- DLD-1 cell line was first to be tested due to its efficiency in growth and because of data shown in Jeong et at 2019 experiments. They reported using DLD-1 cells in a WST-8 assay, Clonogenic assay, and Annexin V assay all showing an IC50 of 6uM CBD Isolate. I followed the concentrations, cell numbers and length of time used in their experiments to replicate this data. Using 8000 cells per well in 96 well, 24 hr treatment, followed by MTS assay or CyQuant assay. However, after many different attempts with solvents, cells numbers, time of treatments, and different concentrations this was reevaluated and found that the IC50 for Isolate was about 60uM. MTS assay and the more sensitive CyQuant assay, that shows a 40uM IC50 both confirmed this data with replicates of 3.

Migration assays were then performed. This assay looks into both growth of cells treated and the cells ability to migrate toward each other to close a gap. Cells were placed in 6 well plates and were seeded at 100% confluency. Once cells were attached, the plate was scratched down the center with a 1ml pipette tip. And then treated with different formulations of the CBD (Isolate, MI, NC, and TN) or controls (media or media with DMSO), pictures were taken after 24 hrs. The migration assays showed that at 10uM Isolate CBD did not migrate significantly with p value > 0.05. This is the same for the water-soluble compounds MI and NC. However, TN cells showed growth and migration at 10uM with a p value <0.05. When cells were treated with 30uM both the Isolate and MI had reduced migration and growth compares to their respective controls p value <0.05 and NC showed no change, and TN still showed growth and migrations with a p value <0.05. Cells were also treated at 60uM and 90uM and Growth and migration was increasingly inhibited with Isolate, MI and NC but had little to no effect on TN. All these assays were measured 3 times with 4 replications. This data confirmed the above MTS assay showing that the water-soluble CBD from TN had no effect.

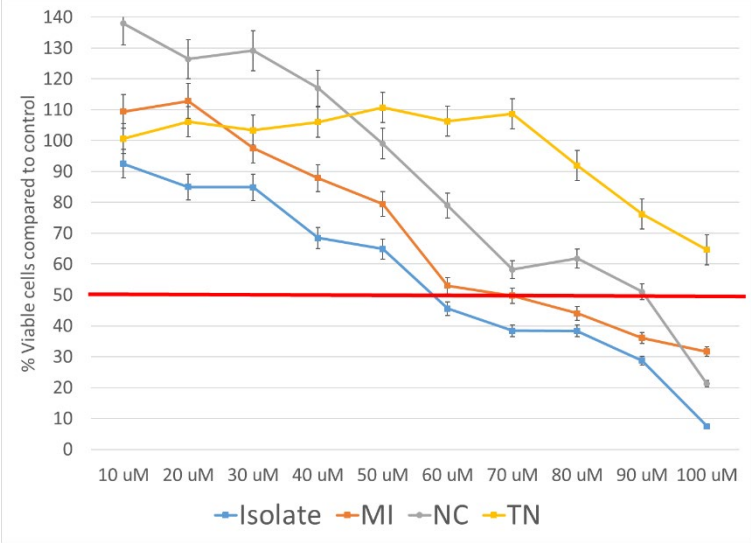


Figure 2. MTS assay of different CBD formulations DLD-1 cells after 24 hr. MI, NC, and TN are three different water-soluble formulations from companies within those states. Graph shows treatments depicting the percent viable cells compared to the control, red line indicates the IC50. Isolated IC50 found to be at 60uM P<0.005. MI IC50 found to be at 70uM P<0.05. NC IC50 found to be at 90 M P<0.005. TN never reached IC50. All treatments replicated 3 times, N=3.

The clonogenic assay was performed by seeded cells at 50% confluency in 6 well plates and the following day treating cells with 30, 60, or 90uM of Isolate, MI, NC, TN, DMSO, or nothing in media for 24 hours. Cells were collected after the 24 hrs being careful not to disregard any dead cells and using trypan blue did a live/dead cell count. DLD-1 cells were seeded at 250 cells per well and given untreated media for 2 weeks then stained with crystal violet and colonies were counted. This data confirmed again that at 30uM, DLD-1 cells showed little response to treatments of 30uM, but by 90uM no colonies survived Isolate, MI, and NC whereas, TN still showed very little response n=3.

EDU assay show the proliferation of cells by labeling the DNA at the start of the experiment and with each doubling of cells the fluoresces intensity degreases. Cells were seeded 8000 cells per will in 96 well and of different CBD formulations were added after cells attached along with EDU for 24 hrs. The Isolate found reduction of proliferation significantly (p value<0.005) at 30uM = 45% reduction, 60uM = 60% reduction, and 90uM = 60% reduction of proliferation compared to the control, as well as MI 90uM = 62% reduction, and NC 90uM = 52% reduction, and a 27% reduction in TN 90uM (p value<0.05) n=3.

- HCT-116 cell line was the second human cell line to be tested due again this was because of the data shown in Jeong et at 2019 experiments. They reported using HCT-116 cells in a WST-8 assay, Clonogenic assay, and Annexin V assay all showing an IC50 of 4-6uM CBD Isolate. Again, using the same number of cells and length of treatment but due to the information gathered from DLD-1 higher concentrations were used to determine the IC50 of HCT-116 which was found to be at 70uM (p value <0.05 n=3). This assay also evaluated the water-soluble formulations and found MI IC50 was at 50uM (p value <0.05 n=3). However, the response for MI seems to plateau at 60uM.

The migration assay of different CBD formulations HCT116 cells after 24 hr. MI, NC, and TN done following same protocol as DLD-1 for 10, 30,60, and 90uM. This showed that both Isolate and MI had a significate reduction of migration in 10uM (p value <0.05 n=3). The 30uM, 60uM, and 90uM also showed

a significant increase on Isolate, MI, NC and even TN (except 90uM). This led to reevaluations of MTS assay and replicates are in progress as well as the CyQuant assay for confirmation.

EDU assay show the proliferation of HCT-116 cells that were seeded 10000 cells per will in 96 well and of different CBD formulations were added after cells attached along with EDU for 24 hrs. The Isolate found reduction of proliferation significantly however to a lesser extent then DLD-1, (p value<0.05) at 30uM = 15% reduction, 60uM = 20% reduction, and 90uM = 35% reduction of proliferation compared to the control, as well as MI 90uM = 28% reduction, and NC 90uM = 35% reduction, and a 20% reduction in TN 90uM (p value<0.05) n=3.

- HT29 cell line was the third human cells line to be tested and in Jeong et at 2019 experiments they only looked at a WST-8 assay. Showing that the IC50 was only slightly lower then 6uM of the DLD-1 cells line. However, they did not give much detail to how many cells were used per well and no other experiments were done in their study. I have begun to look at the MTS assay looking at 10uM to 100uM and found that the IC50 is over this amount. I have been reevaluating this assay and will be continuing to work on this throughout this month and next. Once the IC50 has been determined the Rest of the experimental assays will follow.

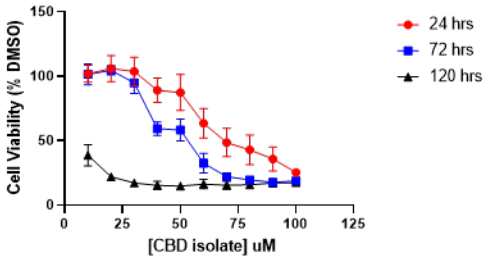
- CT26 are a mice colon cancer cell line and were first experimented with in February along with DLD-1. This was because both DLD-1 and CT26 use the same media and seemed reasonable to do these experiments alongside of each other. When doing the CT26 cells MTS assay the proper solvent method had not been developed yet. Also, when working on the migration assay I noticed that the cells didn't so much as migrate into the area of the scratch but they seem to detach during proliferation and reattached in areas that had available space. This needs to be reconsidered how to best use and analysis this assay. This is being reevaluated in the upcoming month after a conversation how to best address this with my committee.

- MC28- Returning to the literature it was discovered that the cells were serum starved either 24 hours before treatment or just as treatment was introduced. This confounds the results in my opinion and makes the cells more responsive then they would be in the natural microenvironment of the colon tumor. Once more I also believe that if all experiments were conducted in exact same methods that the IC50 being 60uM of the isolate and not 6uM would have little to no consequences of the question that was initially being address: which was if water soluble would have the same effectiveness as isolate CBD on colon cancer? I however was not able to convince my mentor and the other committee members at this time of this question and they pushed me into another direction.

  Since data was not showing acceptable GI50 on CBD Isolate I was instructed to determine the length of the assay and with or without treatments for each cell line. First experiment was done using MTS assay again and treating the cells with Sigma Chemical CBD Isolate for 24 hours, 72 hours, and 120 hours. GI50 was determined for each line using graph pad. The best human line was DLD-1 at 5 days being 7.6uM and the least responsive was Caco-2 5 days at 70.8 uM.

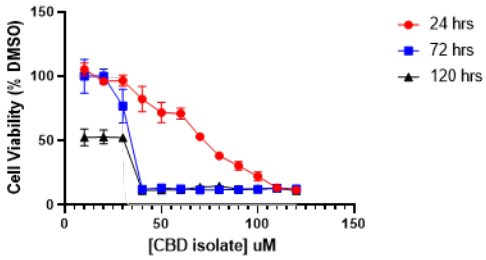
  After which I ran an experiment to study with and without serum but cells only survived out to 48hrs. This showed a significate decease in the GI50 and once returning to the literature found that the previous studies were serum starving cells with CBD treatment. I felt that this was not an appropriate way to determine the efficacy of CBD Isolate verse the water-soluble treatment but my committee disagreed. I found a paper that stated "According to Eastman [3], serum should be kept in cell cultures to avoid both false positive and negative results due to its effects on cell proliferation, stipulating the importance of replicating organic conditions to obtain clinically valid results." Sainz-Cort A, Müller-Sánchez C, Espel E. Anti-proliferative and cytotoxic effect of cannabidiol on human cancer cell lines in presence of serum. BMC Res Notes. 2020;13(1):389. Published 2020 Aug 20

DLD-1 Isolate CBD MTS assay



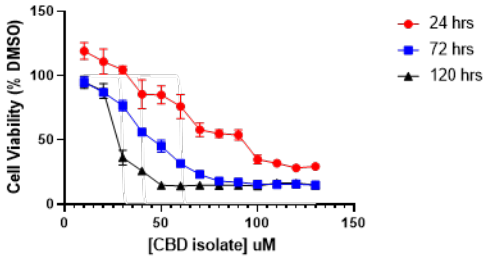
1 Day GI50: 60.24 uM
3 Day GI50: 49.9 uM
5 Day GI50 (extrapolated): 7.6 uM

CT26 Isolate CBD MTS assay



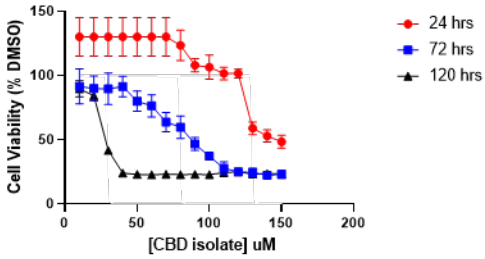
1 Day GI50: 70.05 uM
3 Day GI50: 30.52 uM
5 Day GI50 : 30.04 uM

HCT116 Isolate CBD MTS assay



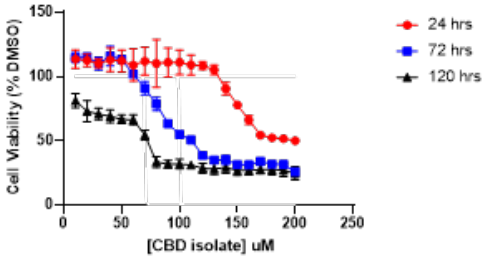
1 Day GI50: 60.50 uM
3 Day GI50: 40.11 uM
5 Day GI50 : 29.75 uM

HT29 Isolate CBD MTS assay



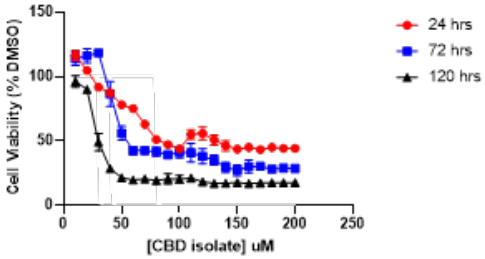
1 Day GI50: 130.2 uM
3 Day GI50: 80.17 uM
5 Day GI50: 29.86 uM

Caco-2 Isolate CBD MTS assay



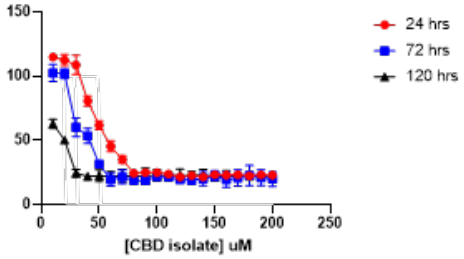
1 Day GI50: Unstable
3 Day GI50: 100.1 uM
5 Day GI50 : 70.8 uM

Lovo Isolate CBD MTS assay



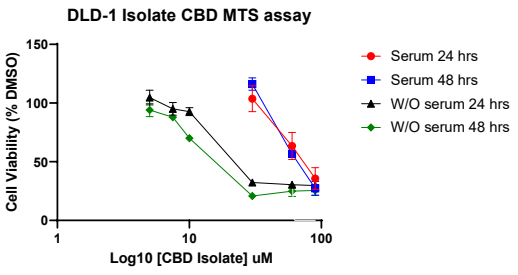
1 Day GI50: 80.02 uM
3 Day GI50: 40.08 uM
5 Day GI50 : 29.98 uM

MC38 Isolate CBD MTS assay



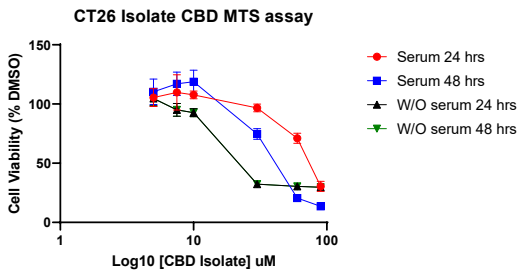
1 Day GI50: 51.21 uM
3 Day GI50: 30.18 uM
5 Day GI50 : 20.00 uM

CBD Isolate GI50 Study on all cell lines with an without serum for 24hrs and 48 hrs.



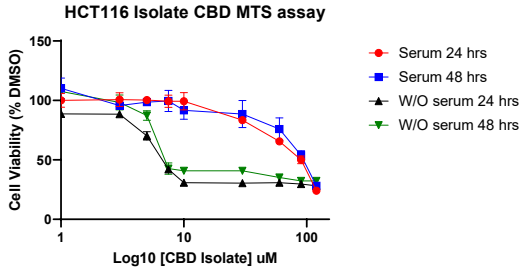
GI50 of CBD Isolate in uM

Serum 24 hrs	Serum 48 hrs	W/O serum 24 hrs	W/O serum 48 hrs
60.24	60.12	11.10	10.37



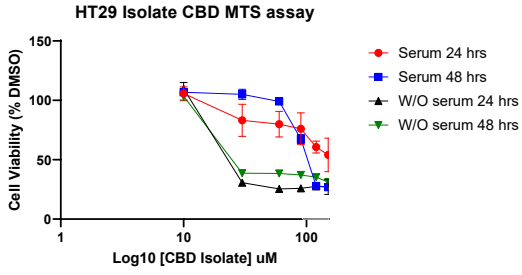
GI50 of CBD Isolate in uM

Serum 24 hrs	Serum 48 hrs	W/O serum 24 hrs	W/O serum 48 hrs
60.39	30.47	11.10	11.10



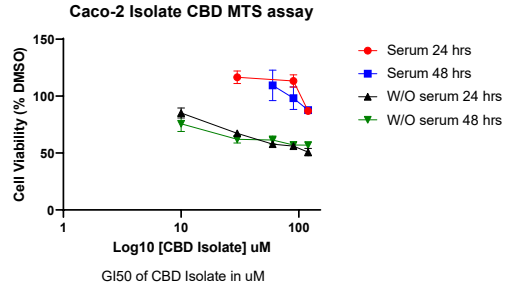
GI50 of CBD Isolate in uM

Serum 24 hrs	Serum 48 hrs	W/O serum 24 hrs	W/O serum 48 hrs
60.28	60.50	5.400	7.376



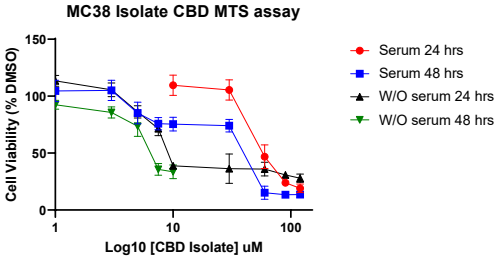
GI50 of CBD Isolate in uM

Serum 24 hrs	Serum 48 hrs	W/O serum 24 hrs	W/O serum 48 hrs
90.50	90.32	29.65	29.80



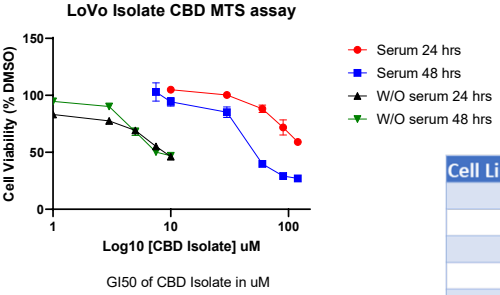
GI50 of CBD Isolate in uM

Serum 24 hrs	Serum 48 hrs	W/O serum 24 hrs	W/O serum 48 hrs
Unstable	91.73	30.31	Unstable



GI50 of CBD Isolate in uM

Serum 24 hrs	Serum 48 hrs	W/O serum 24 hrs	W/O serum 48 hrs
59.94	30.45	7.931	5.477



GI50 of CBD Isolate in uM

Serum 24 hrs	Serum 48 hrs	W/O serum 24 hrs	W/O serum 48 hrs
60.87	37.80	5.384	5.358

Cell Line	24 hrs	72 hrs	120 hrs	W/O Serum 24 hrs	W/O Serum 48 hrs
DLD-1	60.24	49.9	7.6	11.1	10.37
CT26	70.05	30.52	30.04	11.1	11.1
HCT116	60.5	40.11	29.75	5.4	7.376
HT29	130.2	80.17	29.86	29.65	29.8
Caco-2	Unstable	100.1	70.8	30.31	Unstable
MC38	51.21	30.18	20	7.931	5.477
LoVo	80.02	40.08	29.98	5.384	5.358

At this point a new SOW was approved and Major task 3 and 4 were changed to **Major Task 3-** Determine the Mechanism of CBD on colon cancer cells and **Major Task 4-** Determine possible mutations in sensitive colon cancer cell lines that determine outcome.

- **Major Task 2**

- Determine the anti-colon cancer mechanism of CBD in the responsive cell lines.
- Subtask 1 - Evaluate the role of CBD's effect on proliferation of colon cancer cells.
- Subtask 2 - Analysis of data to identify possible pathways responsible for sensitivity to CBD

- **Major Task 3**

- Determine endocannabinoid receptor expression and mutations in sensitive colon cancer cell lines that affect their sensitivity to CBD
- Subtask 1 - Investigate the presence of endocannabinoid receptors (CB1, CB2, GRP55, and TRPV1) in responsive colon cancer cell lines.
- Subtask 2 - Compare the mutations found in colon cell lines that possess the most and least resistant to CBD.
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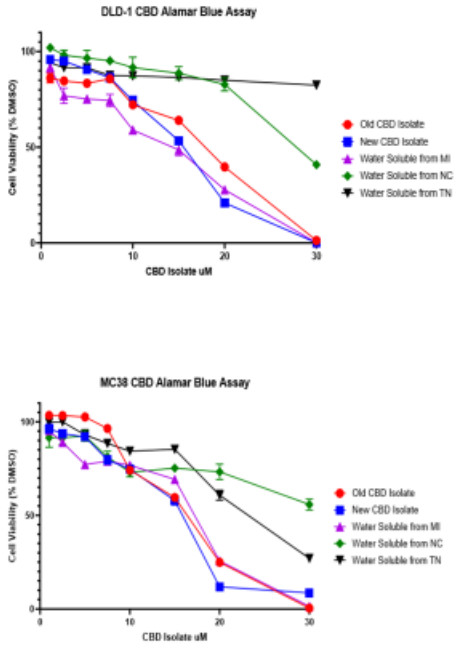
- **Major Task 4**

- Analyze data and prepare manuscript

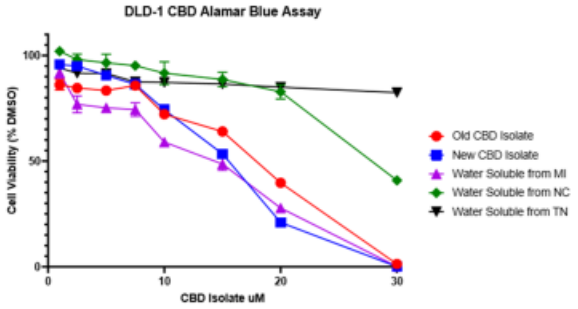
This is the work I had done to justify the SOW change.

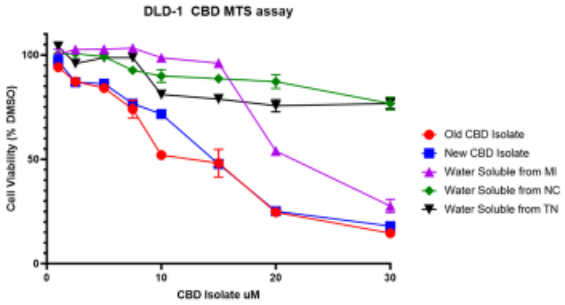


5 Day Isolate CBD, and 3 Water Soluble Treatment Alamar Blue Results

GI50 uM	Old CBD Isolate	New CBD Isolate	Water Soluble from MI	Water Soluble from NC	Water Soluble from TN
DLD-1	16.60	15.03	11.15	56.05	102.0
MC38	18.37	14.85	15.46	40.32	33.64

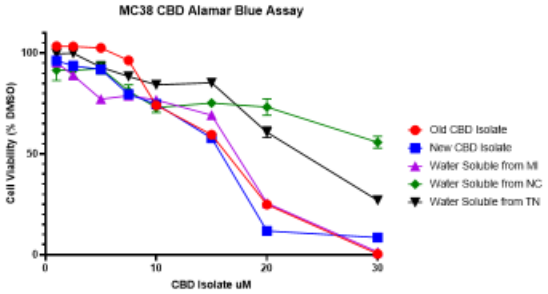
Compare Alamar Blue vs MTS

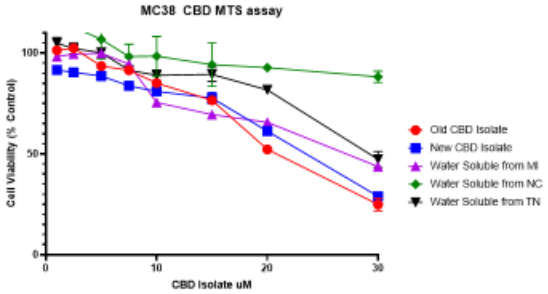




GI50 uM	Old CBD Isolate	New CBD Isolate	Water Soluble from MI	Water Soluble from NC	Water Soluble from TN
Alamar Blue	16.60	15.03	11.15	56.05	102.0
MTS	12.32	14.68	43.43	112.1	76.03

Compare Alamar Blue vs MTS



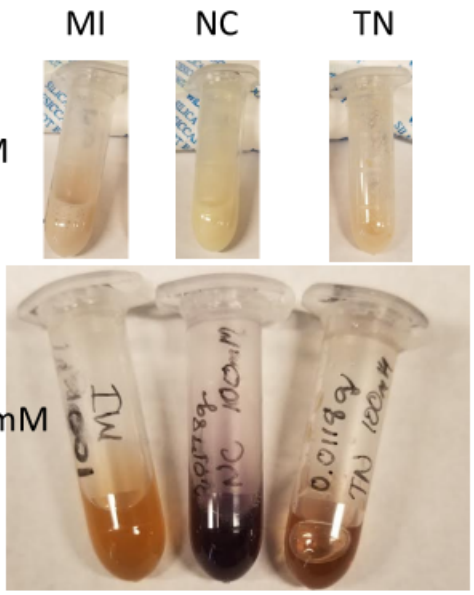


GI50 uM	Old CBD Isolate	New CBD Isolate	Water Soluble from MI	Water Soluble from NC	Water Soluble from TN
Alamar Blue	18.37	14.85	15.46	40.32	33.64
MTS	29.65	29.28	36.49	312.4	60.20

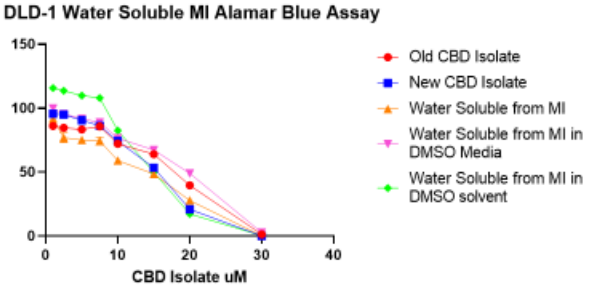
Water Soluble in solvents

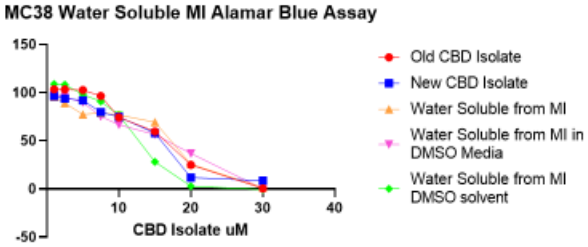
Tested

1. PBS only solvent (normal)
2. PBS Solvent Added 0.4% DMSO to Media
3. DMSO only Solvent Final concentration 0.4%



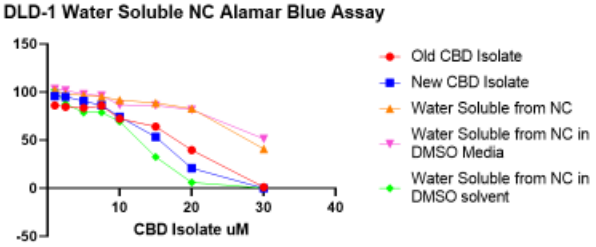
Water Soluble MI

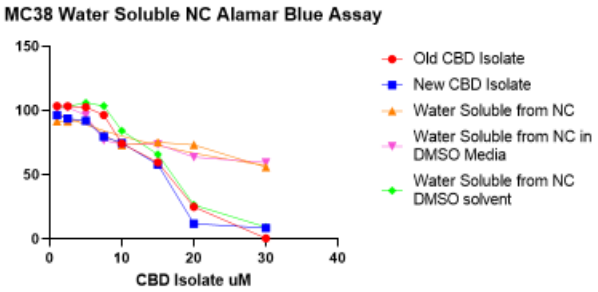




GI50 uM	Old CBD Isolate	New CBD Isolate	PBS	DMSO in Media	DMSO solvent
DLD-1	16.60	15.03	11.15	20.81	19.65
MC38	18.37	14.85	15.46	15.16	13.28

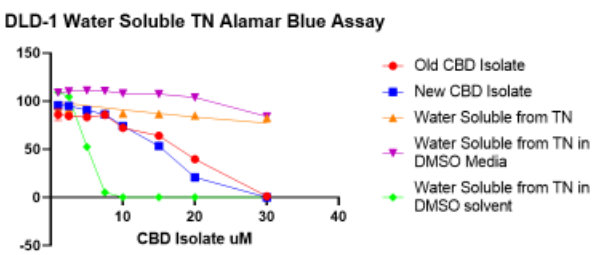
Water Soluble NC

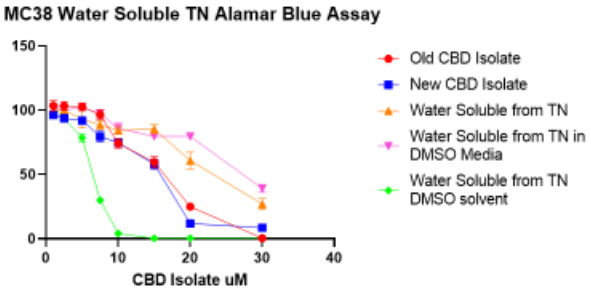




GI50 uM	Old CBD Isolate	New CBD Isolate	PBS	DMSO in Media	DMSO solvent
DLD-1	16.60	15.03	56.05	61.36	10.64
MC38	18.37	14.85	40.32	38.84	22.59

Water Soluble TN





GI50 uM	Old CBD Isolate	New CBD Isolate	PBS	DMSO in Media	DMSO solvent
DLD-1	16.60	15.03	102.0	4745	3.775
MC38	18.37	14.85	33.64	48.32	4.722

GI50

Cell Line	24 hrs	72 hrs	120 hrs	W/O Serum 24 hrs	W/O Serum 48 hrs
DLD-1	60.24	49.9	7.6	11.1	10.37
CT26	70.05	30.52	30.04	11.1	11.1
HCT116	60.5	40.11	29.75	5.4	7.376
HT29	130.2	80.17	29.86	29.65	29.8
Caco-2	Unstable	100.1	70.8	30.31	Unstable
MC38	51.21	30.18	20	7.931	5.477
LoVo	80.02	40.08	29.98	5.384	5.358
LoVo with Alamar Blue	52.48	29.51			

While DLD-1 cells express both CB1 and CB2, Caco-2 cells only express CB1. Depending on the stage of the cancer, endocannabinoids can either inhibit or promote the growth of CRC. Thus, based on the stage of the cancer, both activators and inhibitors of the endocannabinoid system may be useful in combating CRC.

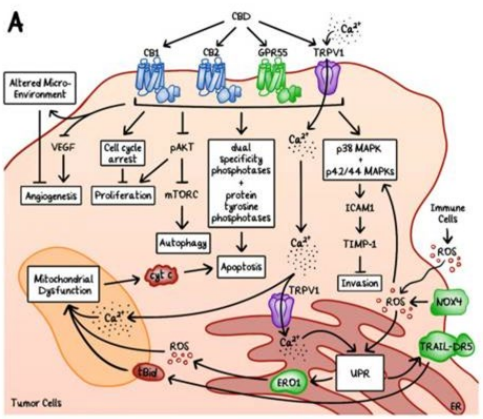
Seltzer et al. Cannabidiol (CBD) as a Promising Anti-Cancer Drug. Cancers (Basel). 2020 Oct 30;12(11):3203.

Mutation

Cell Line	APC	KRAS	ERBB3	PIKCA	TP53	SMAD4	BRAF
DLD-1	mut	mut	mut	mut	mut	wt	wt
CT26	wt	mut	wt	wt	wt	wt	wt
HCT116	wt	mut	mut	mut	wt	wt	wt
HT29	mut	wt	wt	mut	mut	mut	mut
Caco-2	mut	wt	mut	wt	mut	wt	wt
MC38	wt	wt	wt	wt	wt	mut	wt
LoVo	mut	mut	wt	wt	wt	wt	wt

KRAS belong to the small GTPase superfamily. RAS proteins recruit and activate downstream effectors, such as those of the AKT and ERK pathways that in turn affect cell growth, differentiation and survival and may be predictive of a very poor response to some cancer drugs (e.g. those that inhibit EGFR).

PI3K operates as part of the PI3K/AKT/mTOR pathway to mediate cell proliferation, survival, migration and vesicular trafficking.



Seltzer et al. Cannabidiol (CBD) as a Promising Anti-Cancer Drug. Cancers (Basel). 2020 Oct 30;12(11):3203.

Table 1. The effects of CBD on different cancers.

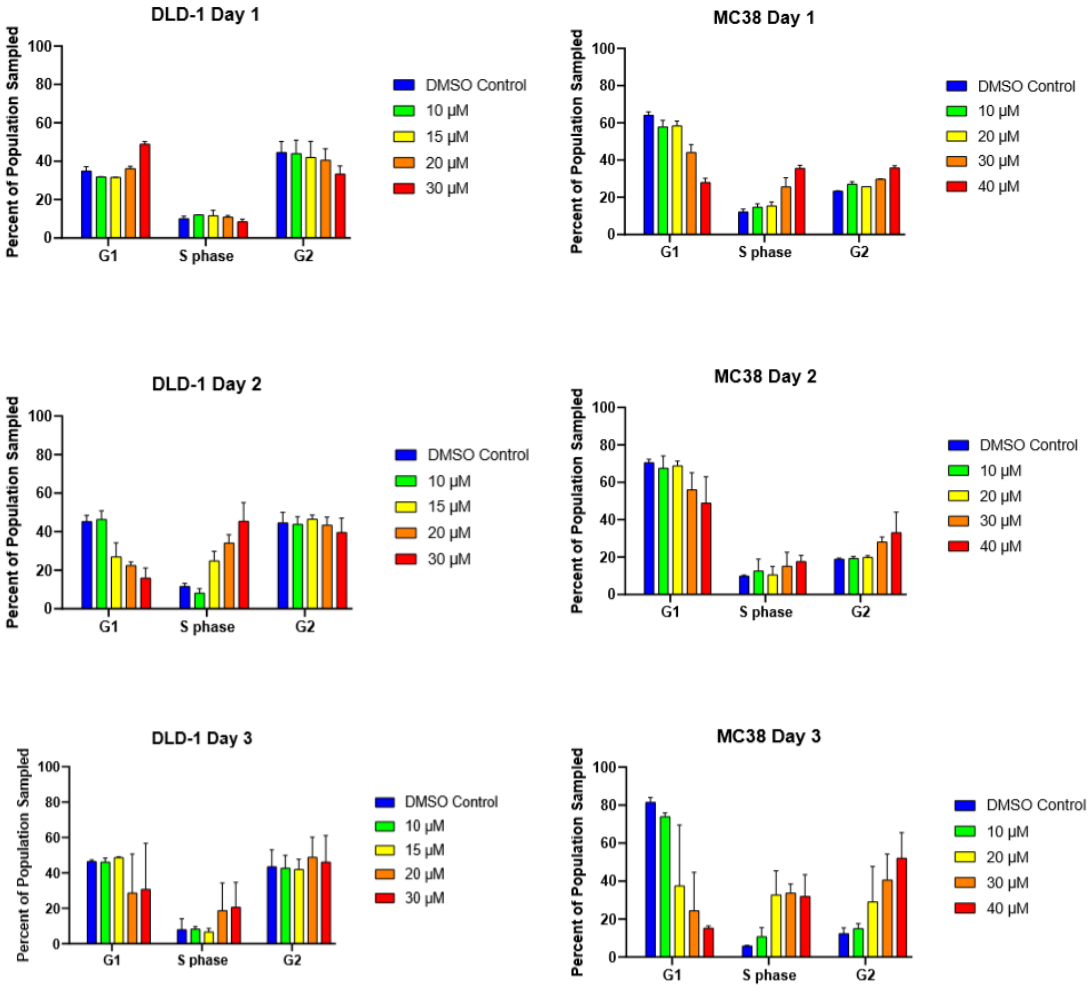
Tumor Type	ROS	ER Stress	Inflammation	Potential Cellular Targets CB_1	CB_2	TRPV1	TRPV2	GPR55	Anti-Tumor Pathway PI3K/AKT/mTOR	MAPK	Autophagy
Glioblastoma	↑	↑	↑	X	X	X	↑		↓	↑	↑
Breast	↑	↑		X	X	X			↓		↑
Lung				↑	↑	↑				↑	
Colon	↑	↑		↑	↑	↑			↓		↑
Leukemia/lymphoma	↑	↑		X	↑	X			↓	↑	
Prostate	↑	↑	↑	X	↑					↑	
Cervical				↑	↑	↑				↑	
Gastric	↑	↑									
Pancreatic								↓		↓	

↑: increase the activity/amount; ↓: decrease the activity/amount; X: not involved.

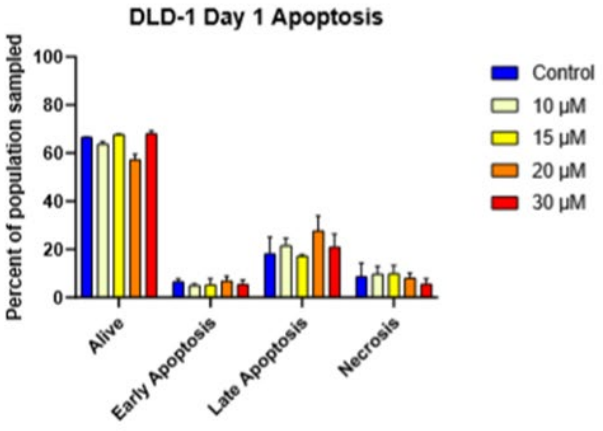
4.1. The Cellular Targets of CBD

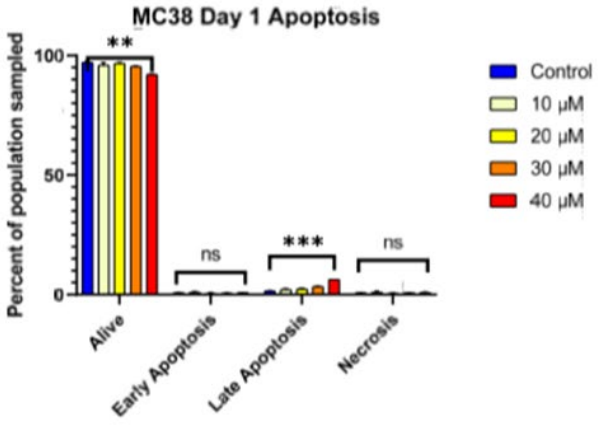
Though the affinity of CBD to CB_1 and CB_2 is considered relatively low, both CB_1 and CB_2 could still be the targets of CBD in certain cancer cells and in infiltrating cells in the tumor microenvironment. Other identified cellular targets of CBD include TRPV1, TRPV2, GPR55, and possibly other GPCRs or non-GPCRs. As summarized in Table 1, these cellular targets can vary depending on cancer types. For example, CBD's effects in glioma are dependent on TRPV2, but not on CB_1, CB_2, and TRPV1 [58,66,67,69,72,106]. On the other hand, CBD's effects in lung, colorectal, prostate, and cervical cancers are largely dependent on some combination of CB_1, CB_2, and TRPV1 [91–93,95,98,113]. The simple presence of these receptors on the surface of cancer cells is not necessarily a good predictor of CBD sensitivity. For example, CB_1, CB_2, and TRPV1 are highly expressed on the cell surface of the thyroid cancer cell line, SkiMol; however, inhibition of these receptors only mildly affected the anti-proliferative effect of CBD in SkiMol [67].

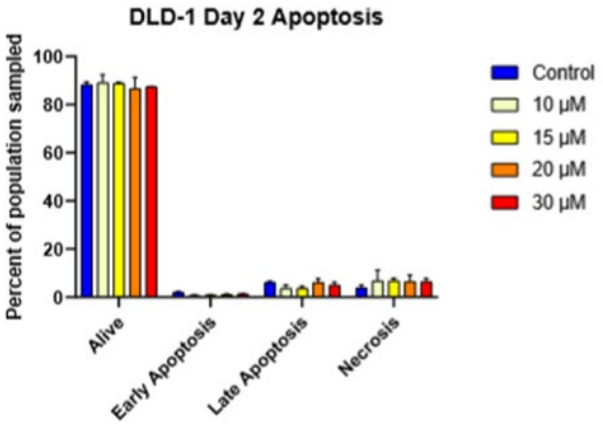
Cell Cycle Data

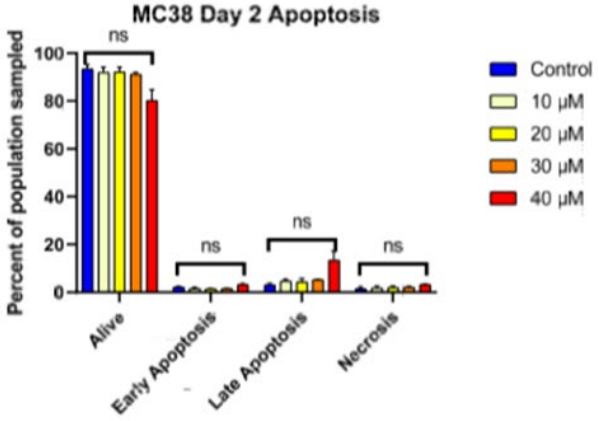


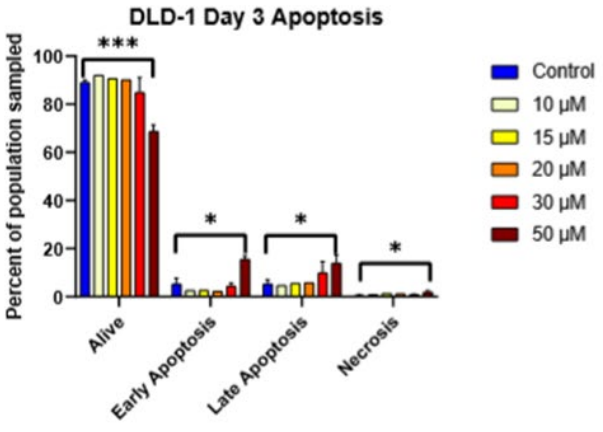
Apoptosis Data

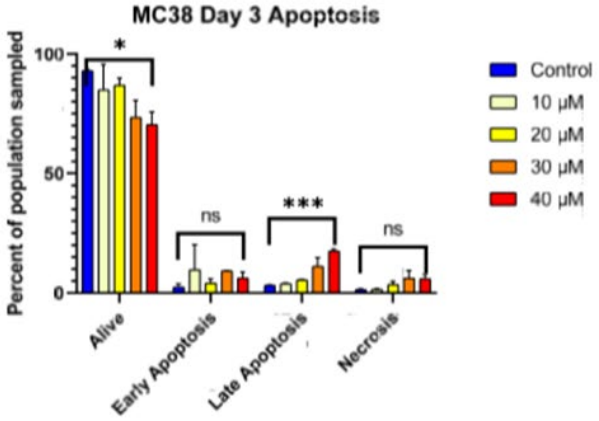


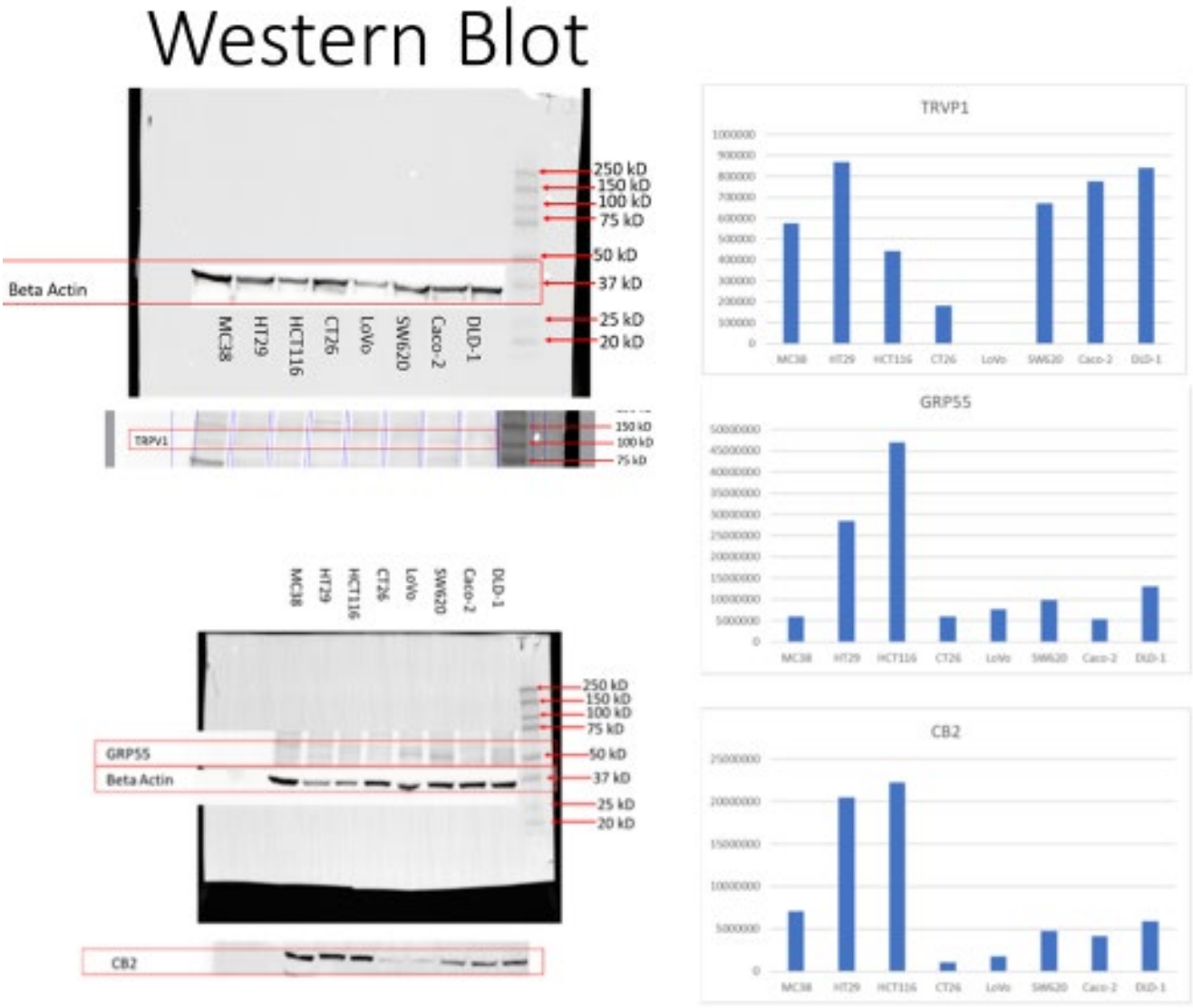












I had not by August found a CB1 antibody that worked and with medical conditions had to stop at this point.

- **What opportunities for training and professional development has the project provided?**
    - Development of mentorship for an undergraduate student for undergraduate research.
    - Internal grant proposal submitted for MTSU Special Project Grant.
    - Guest lecturing “Nutraceuticals & Hemp”, “CRISPR in Agricultural Biotechnology” and “Principles and Application of Flow Cytometry” at the School of Agriculture at MTSU.
    - Mentorship Committee to guide my grant work and professional development.
        - This Committee was appointed in October 2021 and was dissolved by January 2021

The College of Basic and Applied Sciences at MTSU has provided extra resources to support my grant work. My mentorship committee consists of three Biology faculty and one Agriculture faculty. The Committee is available for questions and advice on my research and will schedule regular monthly meetings with me to review my research progress and discuss any research questions.

- **How were the results disseminated to communities of interest?**
    - Poster presentation “Anti-cancer efficacy of CBD Pure Isolates and Commercially Available Water-Soluble CBD in Colorectal Cancer”, MTSU Scholars Week, April 2020.
    - Oral presentation “Hemp and Health”, Pick Tennessee Conference, February 21, 2020.
        - Hosted by Tennessee Department of Agriculture and Tennessee Organic Growers Association (TOGA).
    - Many scheduled conferences were canceled due to Covid-19 in year one, but further virtual conferences and opportunities will be sought during year two.

IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**
    - This project helped me determine the career path I wanted to pursue. I have left academia after recovering from seizures, and have been seizure free since leaving MTSU for 5 months now, to a job in industry that allows me to determine if a product is effective and safe and move on from there. I have never been interested in the why and how something works, as much as I have an interest that if it does in fact work and is it safe. I found my desires are more geared to industry and have recently taken a job with WOW Life Science in Nashville as the Chief Science Officer.
- **What was the impact on other disciplines?**
    - Nothing to Report.
- **What was the impact on technology transfer?**
    - Nothing to Report.
- **What was the impact on society beyond science and technology?**
    - Nothing to Report

CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**
    - Cell lines appear to be responding differently based on my initial experiments. Before proceeding with PK/animal experiments the mechanism of action of CBD on CRC cells needs to be further investigated. The fact that these cell lines appear to be responding differently may indicate that there are potential sub-classes of CRC that are more sensitive to CBD. This has also shown to be different then in the few reported literature articles on these cell lines and CBD, and should be explored further before proceeding with any mice studies. New SOW was submitted and accepted in February 2021.
    - The university has set up a mentorship committee consisting of three Biology faculty and one Agriculture faculty to guide successful completion of my grant work. However, they all resigned by January 2021 and both Jeff Porter the dean of research left by March of 2021 and Dean Bud Fisher left by June of 2021, leaving only Ying Gao who was difficult to work with by this time.
- **Actual or anticipated problems or delays and actions or plans to resolve them**
    - The number one delay that this project has encountered is due to the Covid-19 pandemic. This has kept regulatory bodies from meeting and has been unclear due to my hi risk status due to my MS as to how I was to respond in March and April.

- The second issue has been my health. The pandemic increased my stress levels. I had a surgery in April, hospitalization due to high blood pressure in June, adverse medication interactions, seizures, and tachycardia in July 2020.
    - As the year progressed the seizures increased having 30 between March and June of 2021 and having to relearn how to read and write each time and then finally by August 4th 2021 I have a seizure so bad it lasted 2 days and was unable to read, write, drive or anything else. I had to stop all activities in the lab because this became clearly a laboratory environmental trigger. I have no idea if this was ever resolved at MTSU but I have not stepped back into the building except to drop off my keys.
- As I transferred to MTSU, the orientation, safety training, procurement training, getting familiar with the new lab and facility took about a month.

- **Changes that had a significant impact on expenditures**
    - I had to buy many antibodies and western blot equipment because either the equipment available did not work or no one was will to share.
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
    - No vertebrate animals were used with the change in the SOW.
- **Significant changes in use or care of human subjects**
    - Nothing to Report
- **Significant changes in use or care of vertebrate animals.**
    - Nothing to Report
- **Significant changes in use of biohazards and/or select agents**
    - Nothing to Report

PRODUCTS:

- **Publications, conference papers, and presentations**
    - Nothing to report
- **Website(s) or other Internet site(s)**
    - Nothing to Report
- **Technologies or techniques**
    - Nothing to Report
- **Inventions, patent applications, and/or licenses**
    - Nothing to Report
- **Other Products**

- Nothing to Report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	*Gina Bishara*
Project Role:	*Undergraduate Student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*Ms. Bishara has performed work in the area of cell culture and assisted in cell treatments of colon cancer lines with the CBD formulations*
Funding Support:	NA

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
    - Nothing to Report.
- **What other organizations were involved as partners?**
    - Nothing to Report

SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:**
- **QUAD CHARTS:**

APPENDICES: