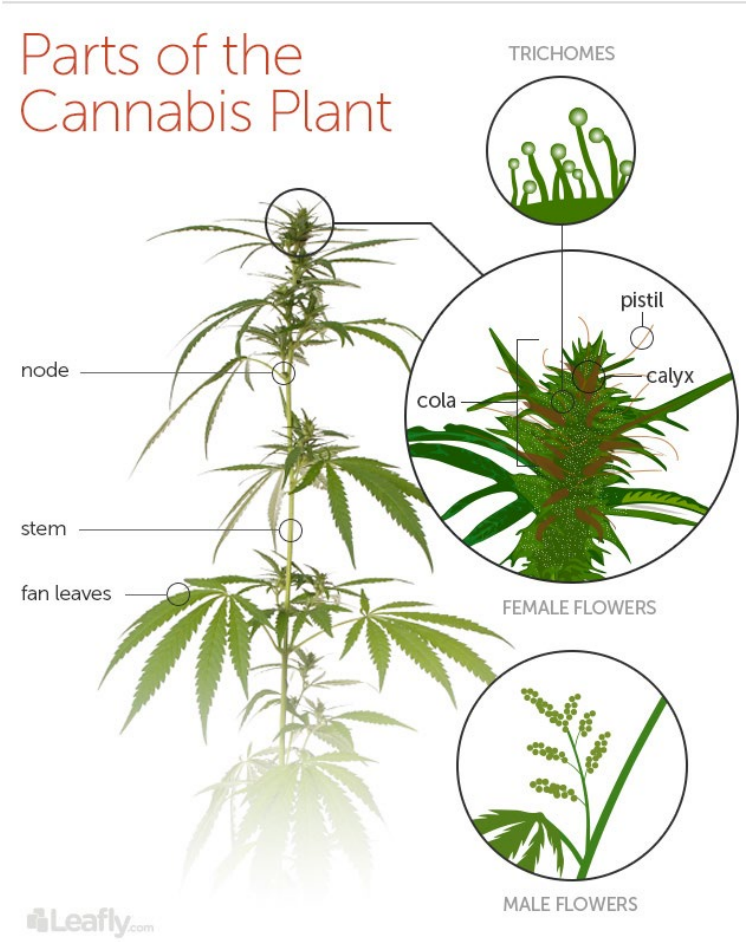
מחקר בקנאביס לשימוש רפואי: חומרים פעילים ומנגנוני פעולה

פרופ' חננית קולטאי, PhD

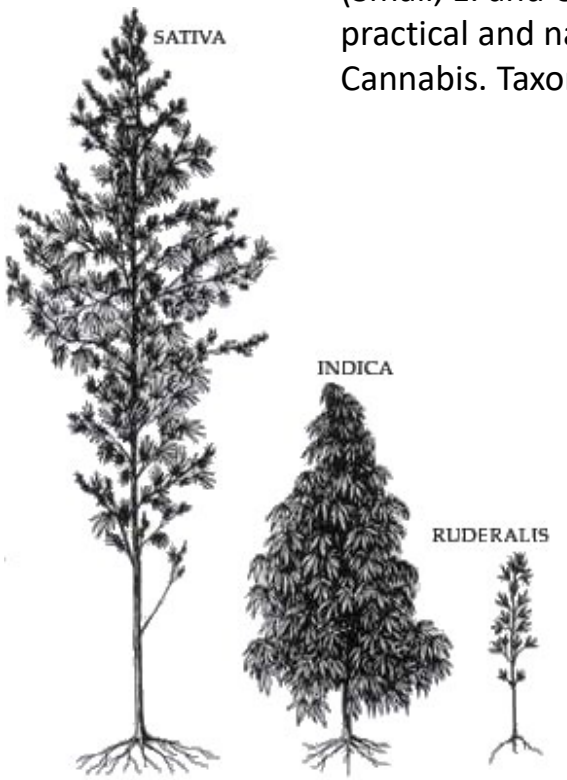
מכון וולקני

KOLTAI LAB

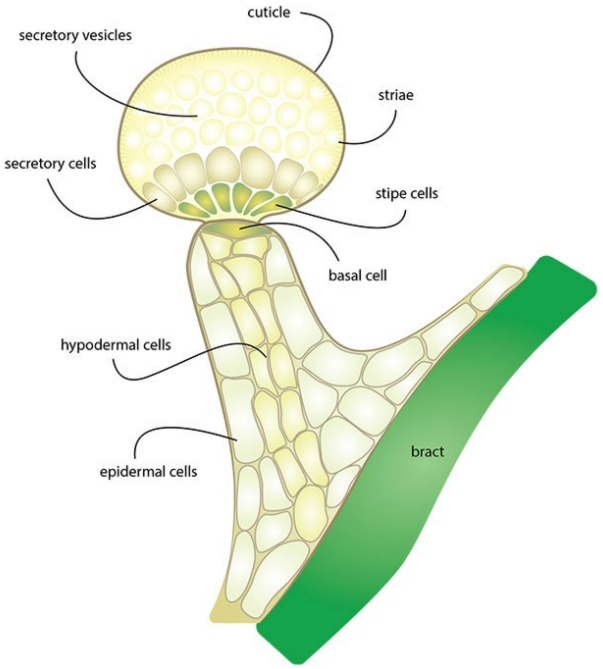
The Cannabis plant: *Cannabis sativa*



- Annual, dioecious, flowering herb
- One *Cannabis sativa* species (Small, E. and Cronquist, A., 1976. A practical and natural taxonomy for Cannabis. Taxon, pp.405-435).

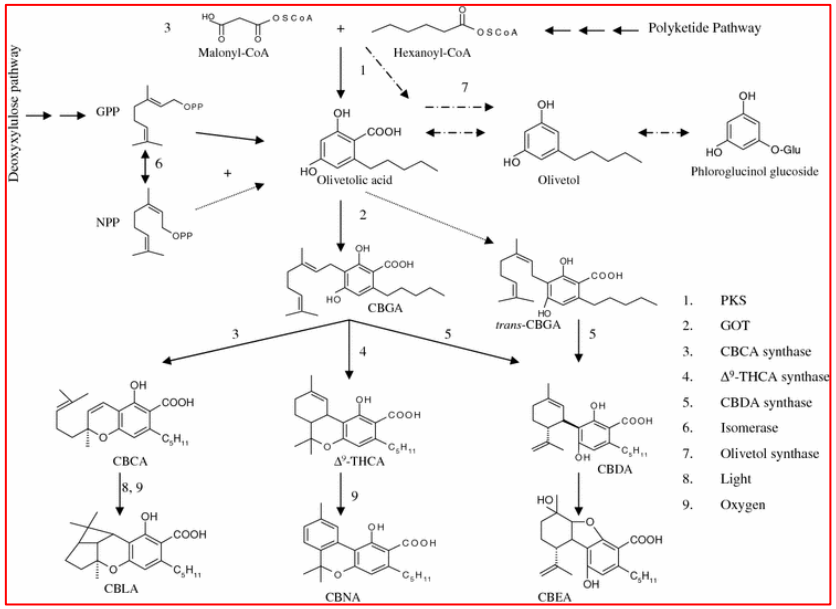


Active compounds- in the female inflorescence

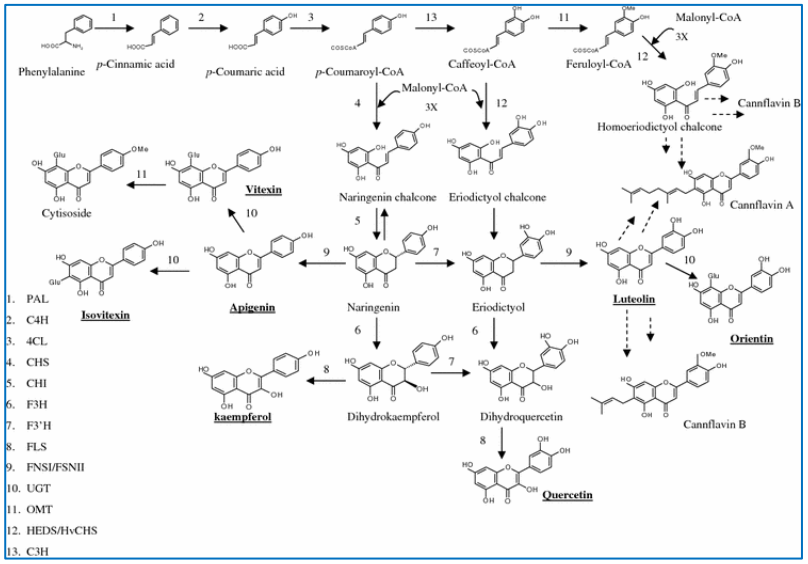


Trichome

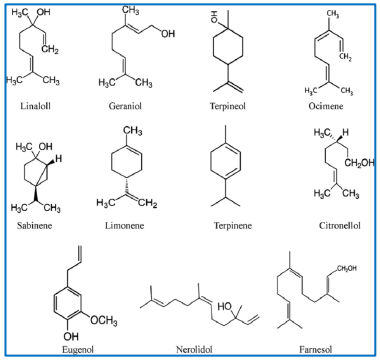
C. sativa produces hundreds of different compounds: phytocannabinoids, flavonoids, phenylpropanoid and terpenoids



phytocannabinoids



flavonoids

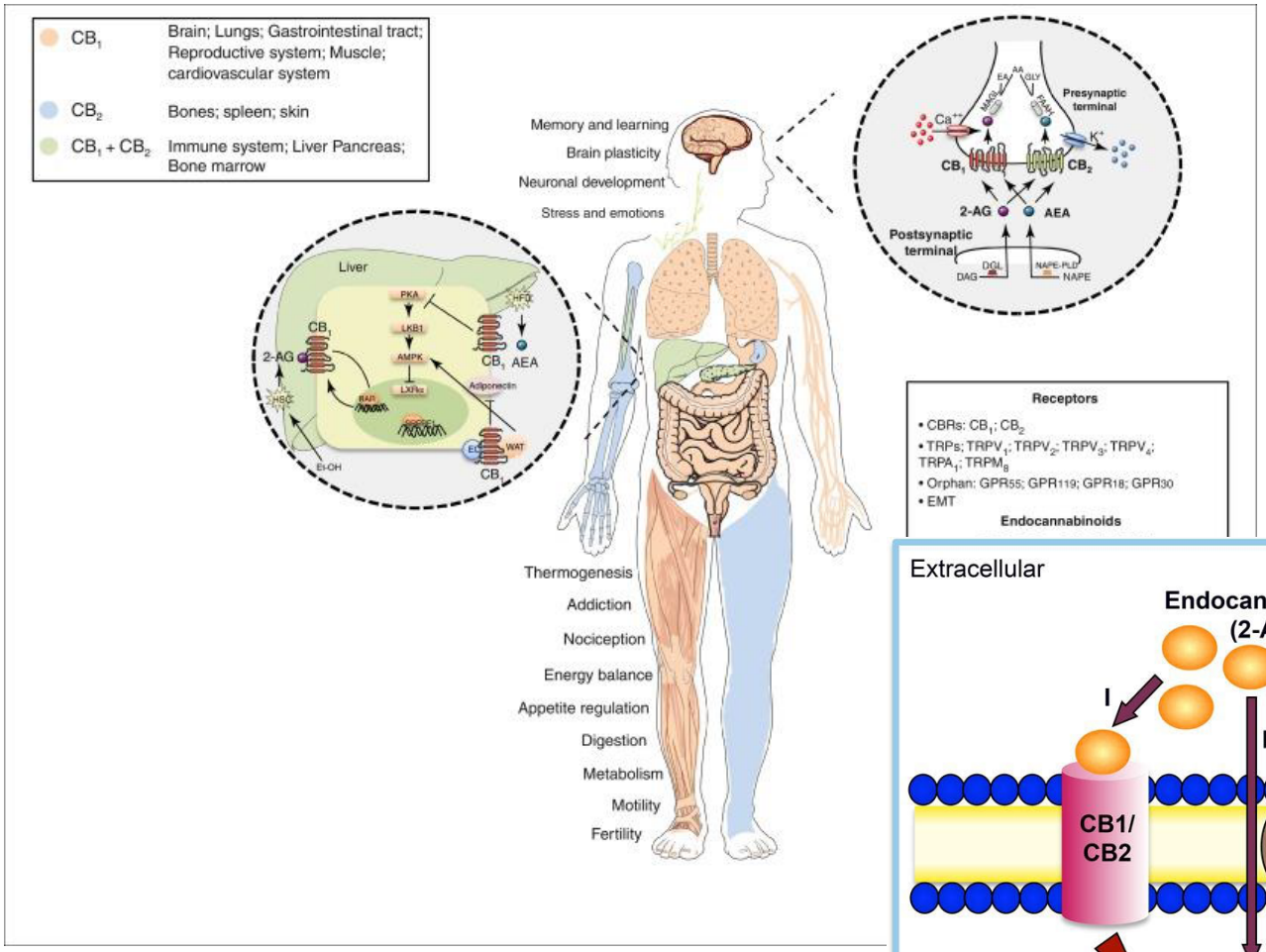


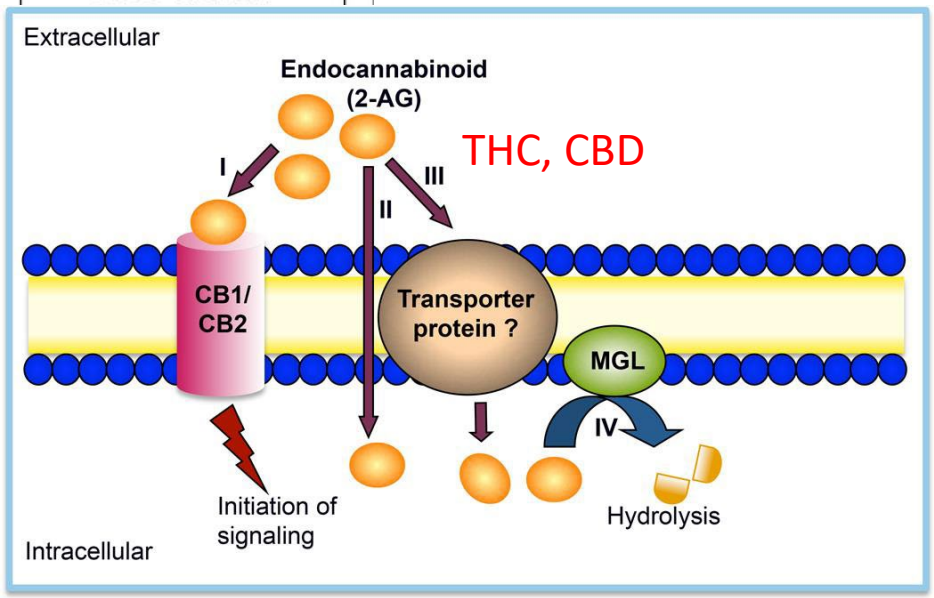
terpenes

Flores-Sanchez, I.J. and Verpoorte, R., 2008. Secondary metabolism in cannabis. *Phytochemistry reviews*, *7*(3), pp.615-639.

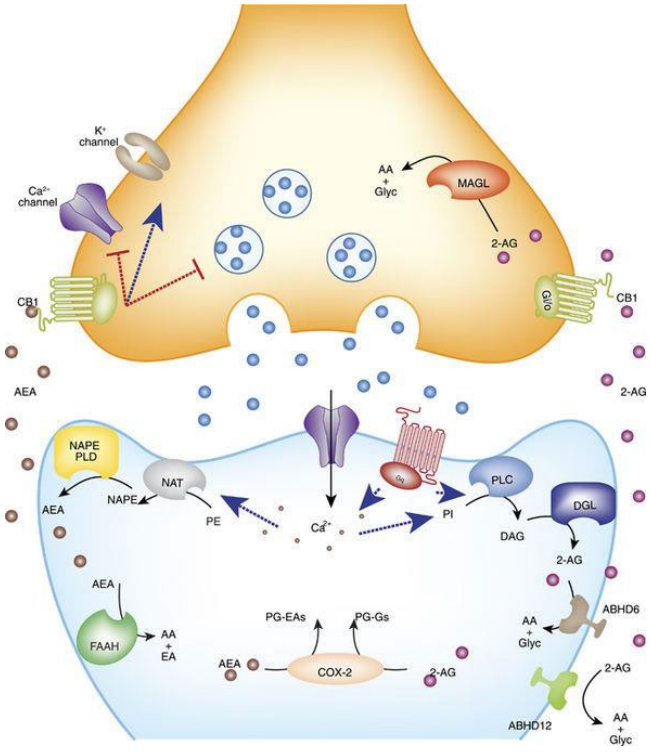
Andre, Christelle M., Jean-Francois Hausman, and Gea Guerriero. "Cannabis sativa: the plant of the thousand and one molecules." *Frontiers in plant science* 7 (2016): 19.

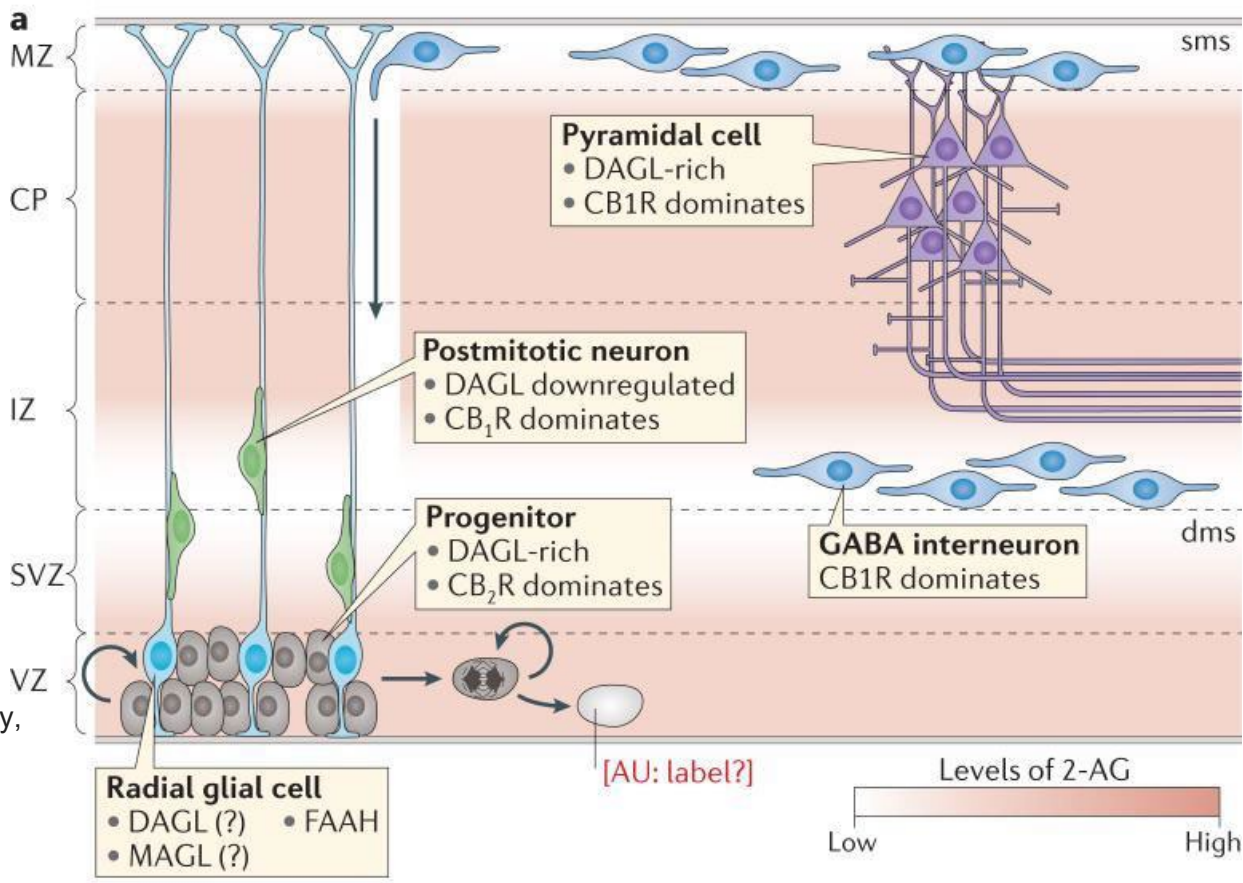
Phytocannabinoids are recognized by the endocannabinoid receptors (CB1, CB2….)





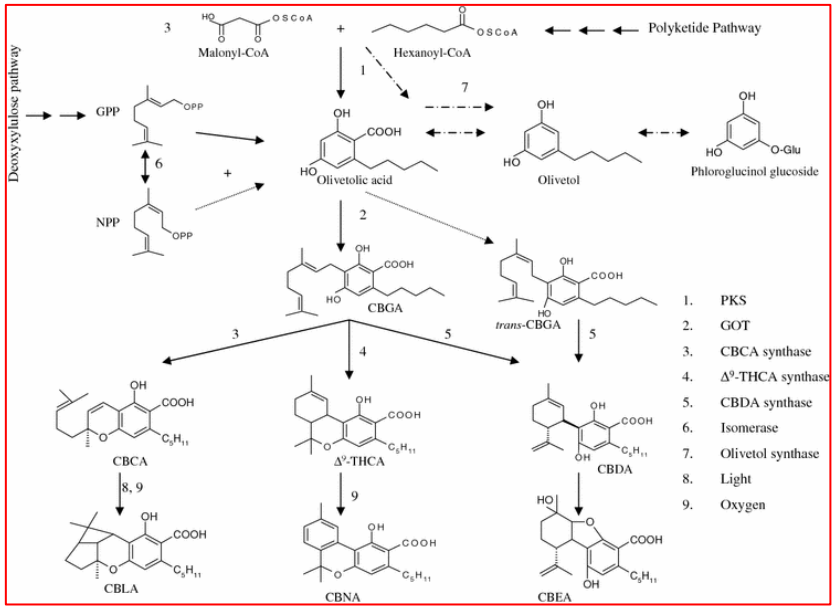
Endocannabinoids receptors in the brain



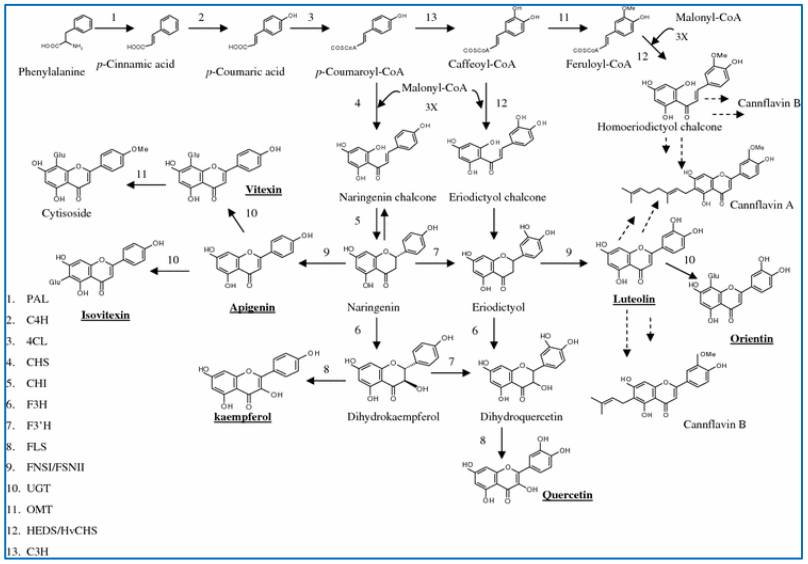


Maccarrone, M., Guzmán, M., Mackie, K., Doherty, P., & Harkany, T. (2014). Programming of neural cells by (endo) cannabinoids: from physiological rules to emerging therapies. *Nature Reviews Neuroscience*, *15*(12), 786-801.

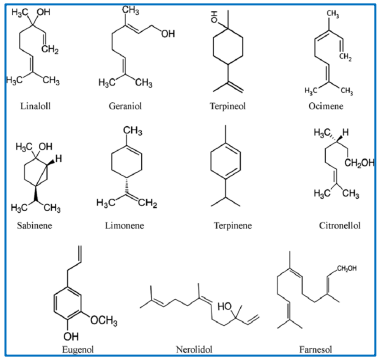
C. sativa produces hundreds of different compounds: phytocannabinoids, flavonoids, phenylpropanoid and terpenoids



phytocannabinoids



flavonoids



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The “entourage” effect

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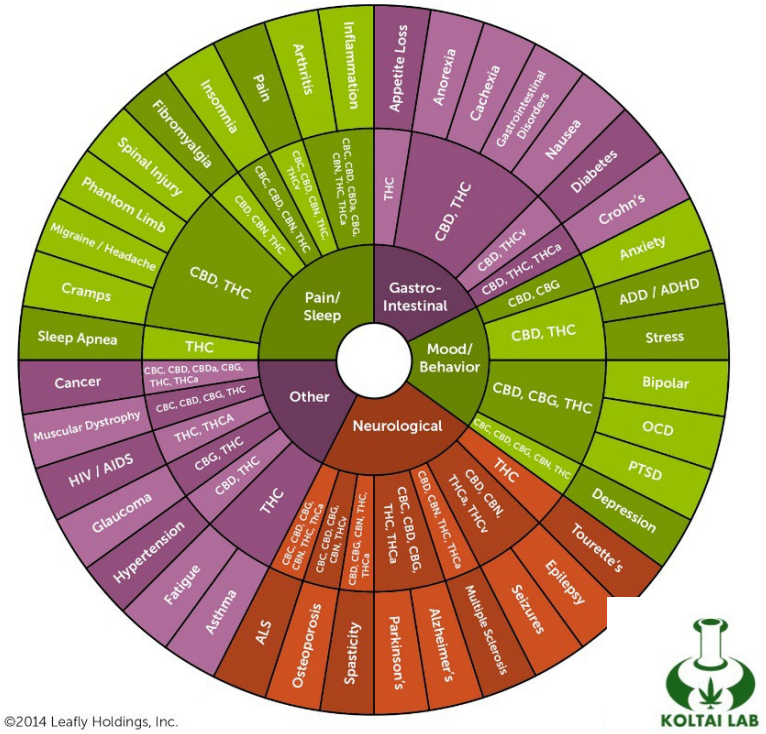
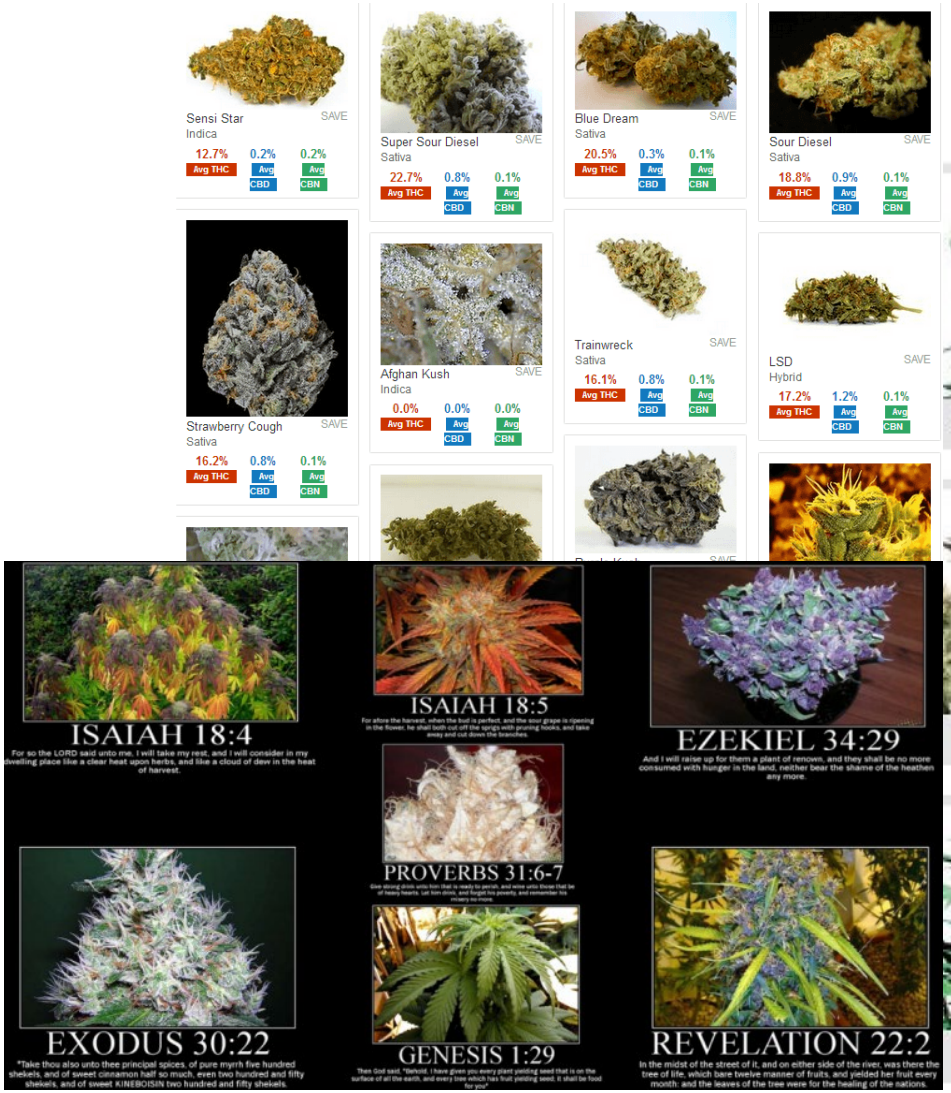


A “buzz” word

Hundreds of different “strains”

Agricultural Research Organization
Volcani Center

Inbred lines…
(sativa vs. indica).



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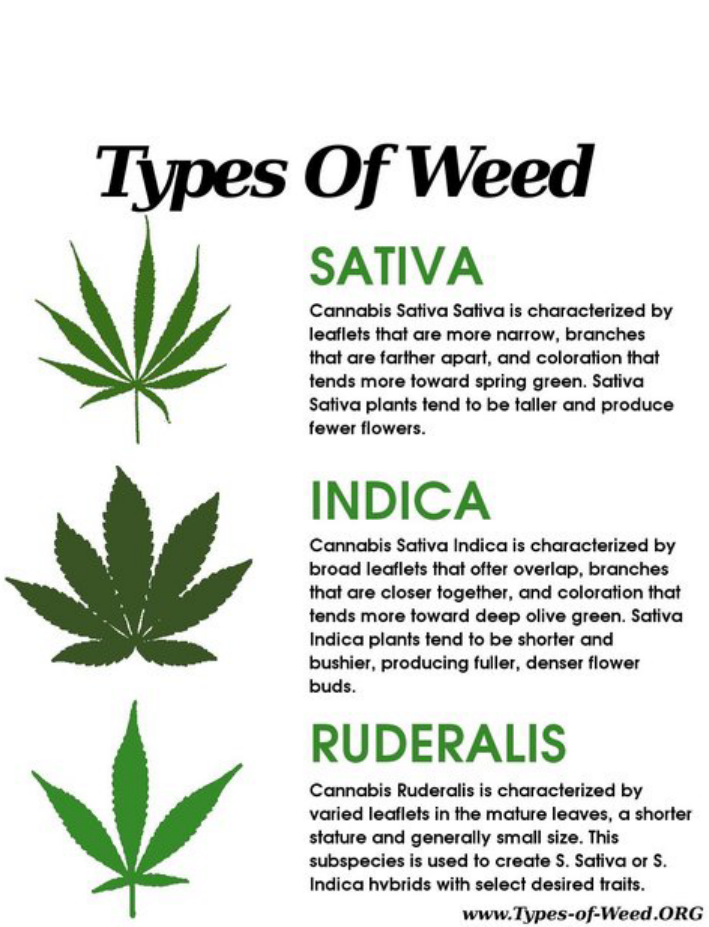
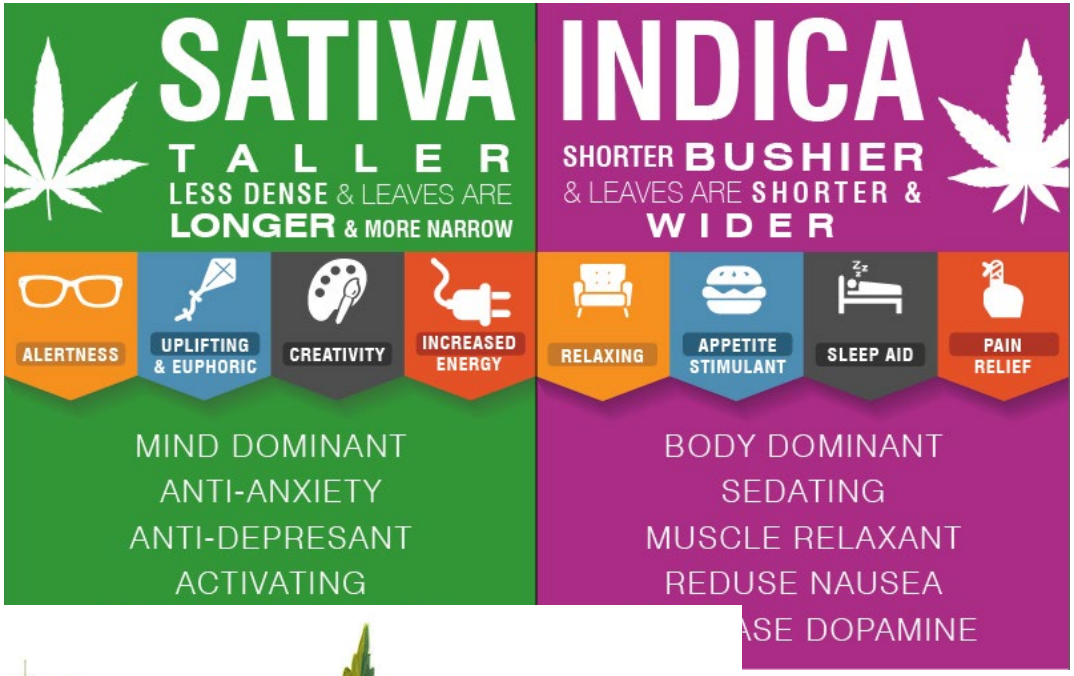
Hundreds of different “strains”

“It is possible that chemically identical or very closely related plant material is being sold under several different names by different producers and there is no clear definition of the concept of a (cannabis) strain” Mudge, E.M., Murch, S.J. and Brown, P.N., 2018. Chemometric Analysis of Cannabinoids: Chemotaxonomy and Domestication Syndrome. Scientific reports, 8(1), p.13090.

Current medical use….





Current medical use….

- Cannabis is used today (medically) mainly as palliative care….
- Can cannabis be used for treatment of medical conditions?

Current medical use….

Primary Condition	Patients, n (%)
	n **= 61,379**
Unspecified Chronic Pain	23,817 (38.8%)
Anxiety	8280 (13.5%)
Post Traumatic Stress Disorder	5143 (8.4%)
Back & Neck Problems	3969 (6.5%)
Arthritis	2395 (3.9%)
Insomnia	2096 (3.4%)
Cancer Related Pain	1641 (2.7%)
Depression	1249 (2.0%)
Migraines	1245 (2.0%)
Muscle Spasms	1038 (1.7%)
ADD/ADHD	1002 (1.6%)
Chronic Nausea	926 (1.5%)
Fibromyalgia	726 (1.2%)
Headaches	707 (1.2%)
Epilepsy	626 (1.0%)
Other	6519 (10.6%)

Table 5 presents a summary of the individuals reporting each primary condition overall and b
Any condition representing less than 1.0% was grouped as an "other". A list of "other" primar
calculated from the sample size for each respective column. *ADD* attention deficit disorder, *A*

Mahabir, V.K., Merchant, J.J., Smith, C. *et al.* Medical cannabis use in the United States: a retrospective database study. *J Cannabis Res* **2,** 32 (2020). https://doi.org/10.1186/s42238-020-00038-w

Current medical use….

- Cannabis is used today (medically) mainly as palliative care….
- Can cannabis be used for treatment of medical conditions?

Alzheimer's disease

- Alzheimer's disease (AD) is the most common type of dementia, accounting for 60 to 80% of cases, and is characterized by the presence in the brain of extracellular deposits of amyloid- (Aβ), a peptide derived from the aberrant processing of the transmembrane amyloid- protein precursor (Aβ PP);
- AD is also associated with neuroinflammation and oxidative stress, two pathological processes that exacerbate neurodegeneration during AD progression.

Aso, E., Andres-Benito, P. and Ferrer, I., 2016. Delineating the efficacy of a cannabis-based medicine at advanced stages of dementia in a murine model. *Journal of Alzheimer's Disease*, *54*(3), pp.903-912.

Evidences that cannabis and the endocannabinoid system improve some aspects of AD

- Selective synthetic agonists of CB1 and CB2 receptors reduce cognitive impairment and brain alterations associated with Aβ production, in at least three different animal models of AD
- THC and CBD application reduced the pathological phenotype in mouse models of AD and tauopathy (deposition of abnormal tau protein) when administered at early stages of the disease

Aso E, Palomer E, Juves S, Maldonado R, Mu ´ noz FJ, Ferrer ˜ I (2012) CB1 agonist ACEA protects neurons and reduces the cognitive impairment of APP/PS1 mice. J Alzheimers Dis 30, 439-459.

Aso E, Juves S, Maldonado R, Ferrer I (2013) CB ´ 2 cannabinoid receptor agonist ameliorates Alzheimer-like phenotype in APP/PS1 mice. J Alzheimers Dis 35, 847-858.

Mart´ın-Moreno AM, Brera B, Spuch C, Carro E, Garc´ıaGarc´ıa L, Delgado M, Pozo MA, Innamorato NG, Cuadrado A, de Ceballos ML (2012) Prolonged oral cannabinoid administration prevents neuroinflammation, lowers -amyloid levels and improves cognitive performance in Tg APP 2576 mice. J Neuroinflammation 9, 8.

Wu J, Bie B, Yang H, Xu JJ, Brown DL, Naguib M (2013) Activation of the CB(2) receptor system reverses amyloidinduced memory deficiency. Neurobiol Aging 34, 791-804.

Aso E, Sanchez-Pla A, Vegas-Lozano E, Maldonado R, ´ Ferrer I (2015) Cannabis-based medicine reduces multiple pathological processes in APP/PS1 mice. J Alzheimers Dis 43, 977-991.

Evidences that cannabis and the endocannabinoid system improve some aspects of AD

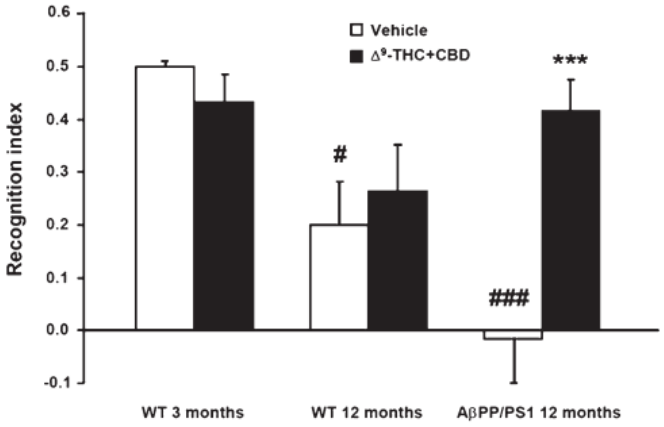


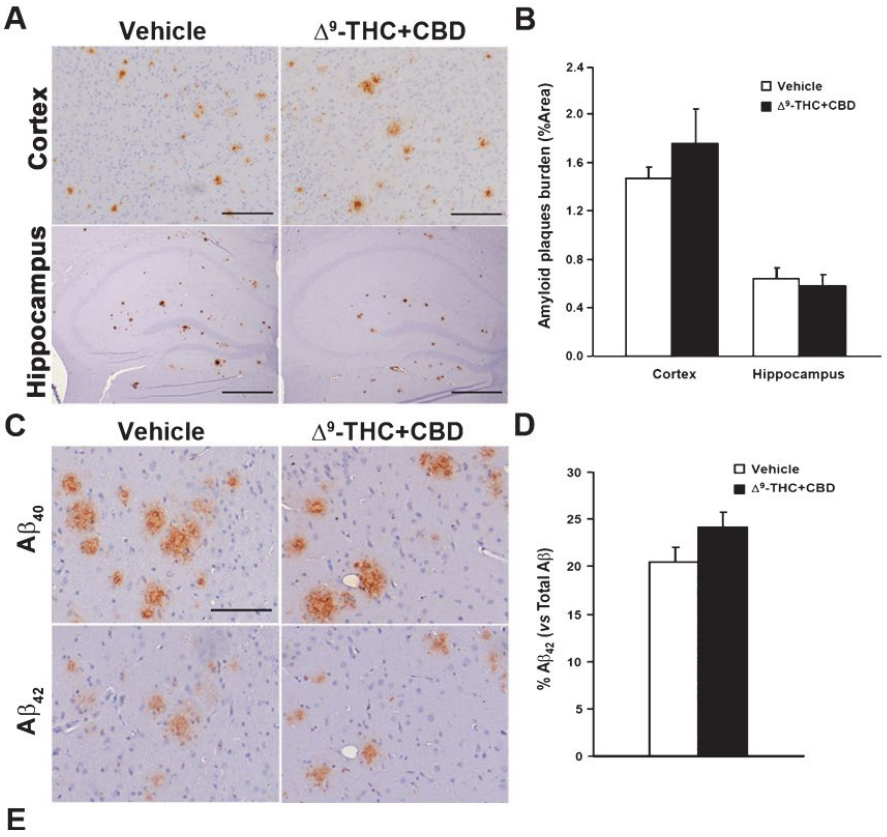
Fig. 1. Memory performance of animals in the two-object recognition test. WT and AβPP/PS1 mice aged 12 months at the beginning of the study exhibit a significant reduction in the recognition index when compared with non-aged control mice (3 months old WT mice). Daily administration of Δ^9-THC + CBD (0.75 mg/kg each botanical extract i.p.) for 5 weeks blunts memory impairment of AβPP/PS1 mice at advanced stages (12 months). However, it is not effective in reducing memory impairment in 12-month-old WT littermates. Data are expressed as the mean values ± SEM; $^{***}p < 0.001$ treatment effect; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ compared to vehicle-treated non-aged control mice.

- THC and CBD administration blunts memory impairment of AβPP/PS1 mice at advanced stages (12 months)
- But they do not affect cognitive impairment associated with healthy aging in wild-type mice.

Exploring the two objects was recorded and an object recognition index (RI) was calculated, as the difference between the time spent exploring the novel object (TN) and the familiar object (TF) divided by the total time spent exploring the two objects

Aso, E., Andres-Benito, P. and Ferrer, I., 2016. Delineating the efficacy of a cannabis-based medicine at advanced stages of dementia in a murine model. *Journal of Alzheimer's Disease*, *54*(3), pp.903-912.

THC and CBD administration do not reduce Amiloyd plaques burden…



Representative images of the Aβ immunoreactivity in somatosensory cortex (top) and hippocampus (bottom) in vehicle- (left) and 9-THC + CBD-treated (right) APP/PS1 mice.

Aso, E., Andres-Benito, P. and Ferrer, I., 2016. Delineating the efficacy of a cannabis-based medicine at advanced stages of dementia in a murine model. *Journal of Alzheimer's Disease*, *54*(3), pp.903-912.

The potential of cannabis treatments

Medical cannabis use provides symptom relief for patients for different medical indications

- However there are above 500 different compounds, so what compounds are the active ones?
- Looking at the effect of THC and/or CBD is not enough.

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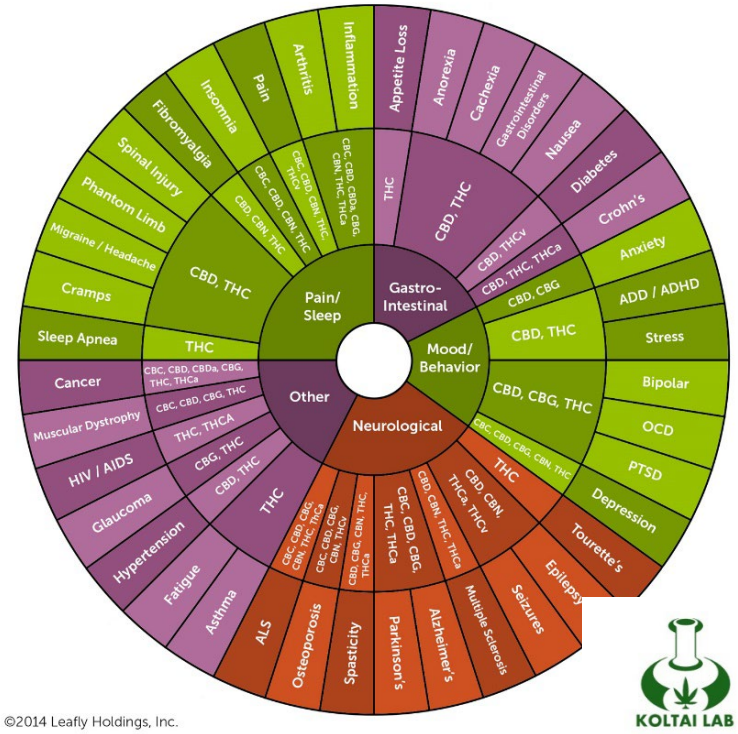
The “entourage” effect

Ben-Shabat S, Fride E, Sheskin T, et al. An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. Eur J Pharmacol. 1998;353:23–31.



A “buzz” word

The potential of cannabis treatments

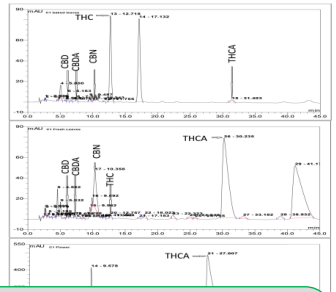
What is the best composition of active compounds for optimized treatment of each medical condition?

- Above 500 different compounds
- “Entourage” effect

Chain of research and development- KOLTAI LAB

To determine the optimal compositions of *Cannabis* compounds for the treatment of different medical indications

We have an authorized farm in Israel for medical cannabis growth for research purposes and contacts with growers in Israel

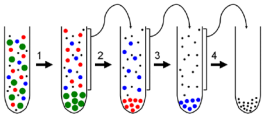


Chemical analysis

Extraction protocols from different cannabis lines

Development of cannabis-based medical products with commercial companies

Separation to fractions



Examination of medical activity in clinical trials

Examination of medical activity in bioassays (*in-vitro, ex-vivo, in printed tissues*)

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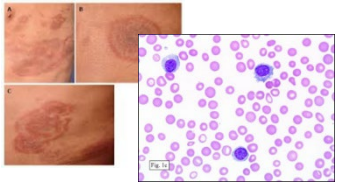
Chain of research and development- KOLTAI LAB

To determine the optimal compositions of *Cannabis* compounds for the treatment of different medical indications

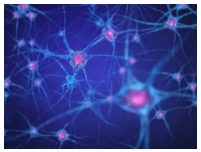
Cannabis for the treatment of Inflammatory Bowel Diseases

Cannabis for the treatment of Cutaneous T-cell Lymphoma



Cannabis for the treatment of bladder cancer

Cannabis for the treatment of nervous system- associated disorders



Cannabis for the treatment of the COVID-19 – related acute inflammation

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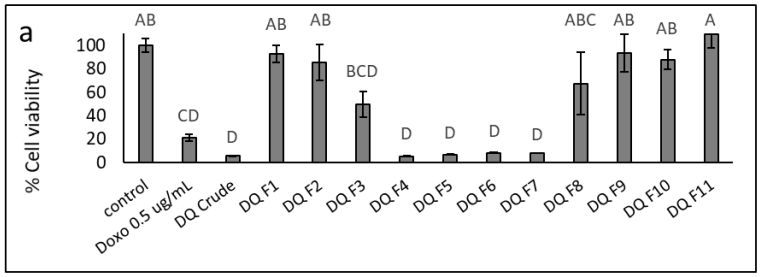
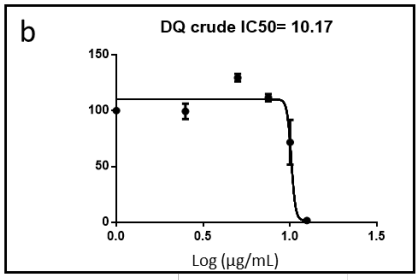
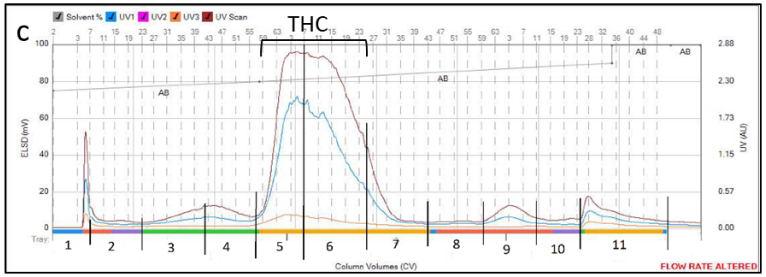
Research towards new cannabis-based drugs: The case of cannabis and glioblastoma (GBM)

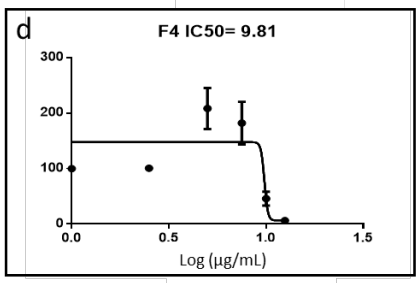
- Glioblastoma multiforme (GBM) is the most frequent, invasive, and lethal subtype of glioma brain tumors.
- Cannabis is commonly used for medical treatment, and individual phytocannabinoids have been shown to trigger GBM cell death.
- However, cannabis contains hundreds of different compounds, and the optimal combinations of molecules with anti-GBM activity are unknown.

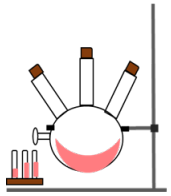
Cannabis and GBM-cell viability

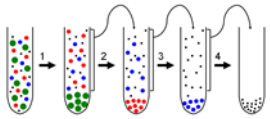


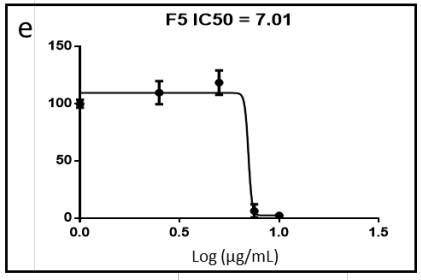


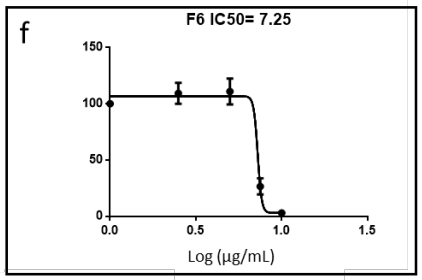


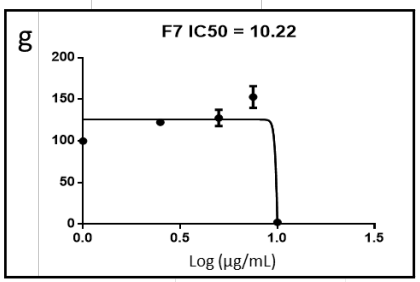












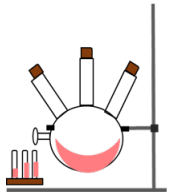
µg/mL

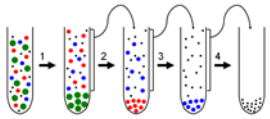
Peeri et al. 2021, Cancers

Active fractions were identified, their activity determined

Cannabis and GBM-active composition

Fraction/ compound	CBD	CBG	CBN	THC	THCV
F4	5.8	80.3	2.8	-	11
F5	-	3.7	4.6	91.7	-





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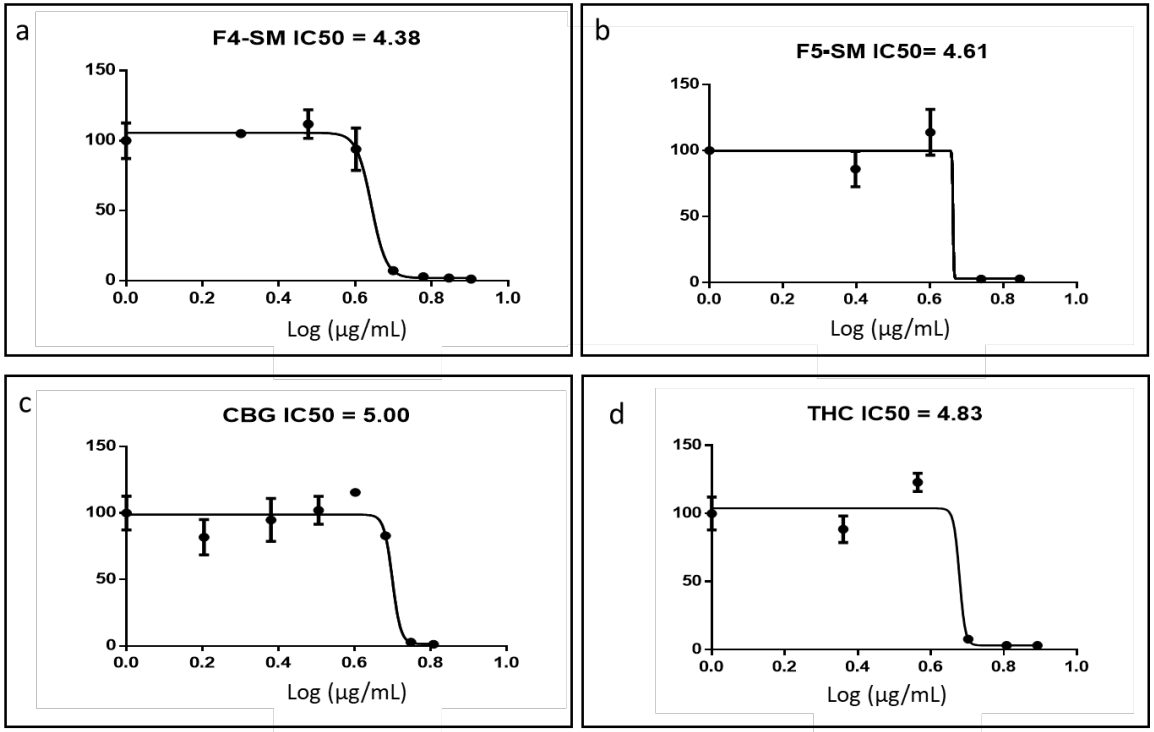
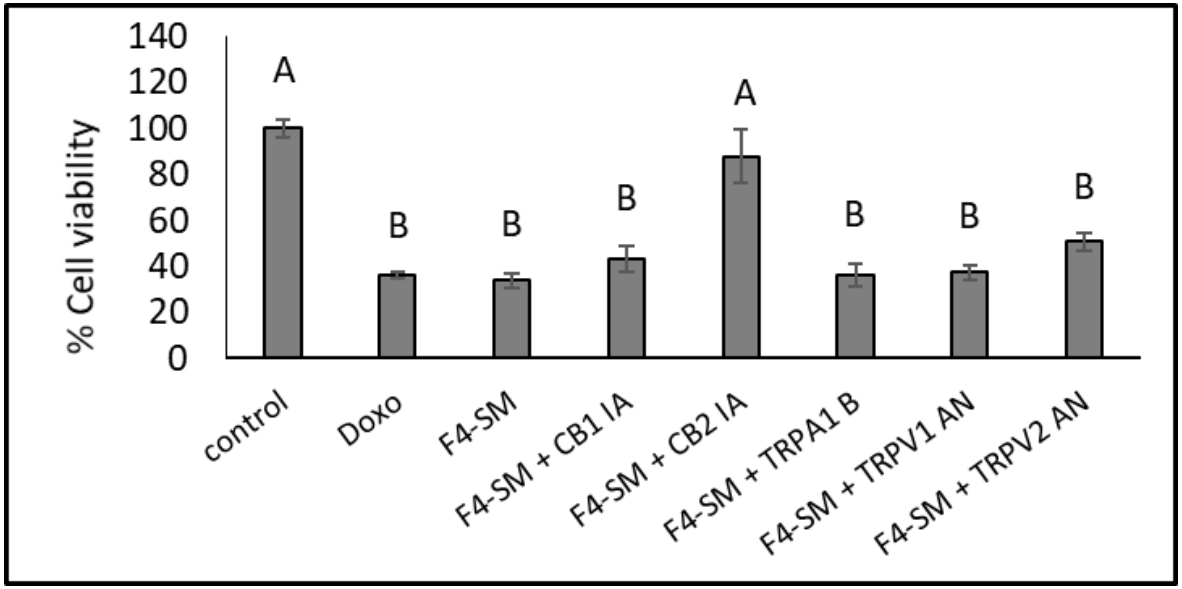


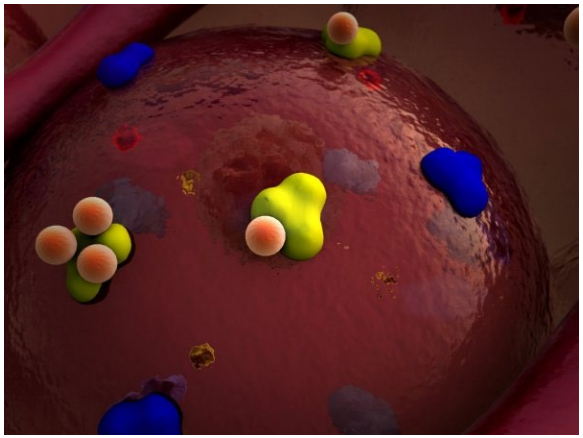
µg/mL

SM (mixture of API) are more active than fractions

Peeri et al. 2021, Cancers

Cannabis and GBM- partial mode of action

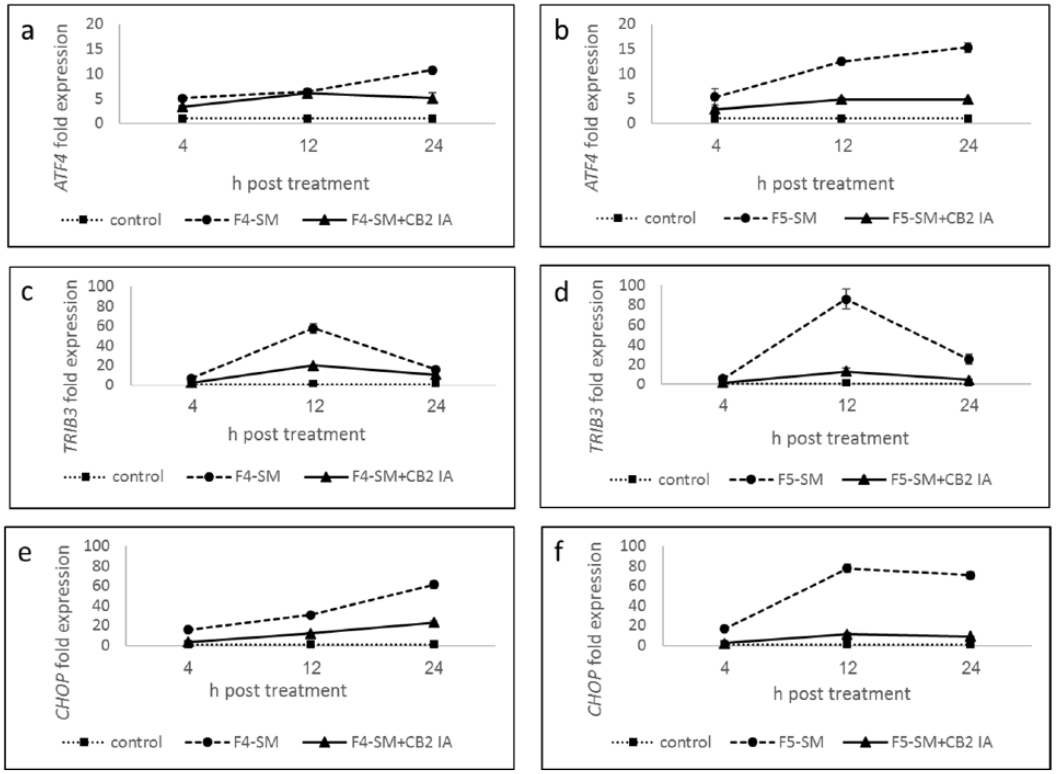


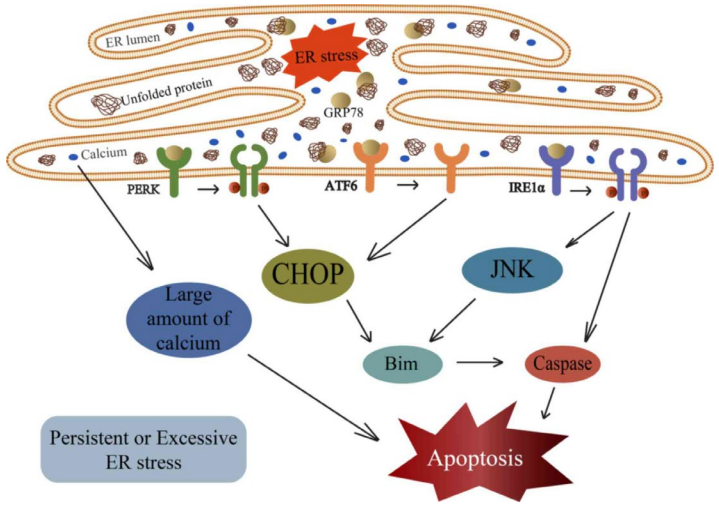


Activity is mediated via CB2 receptor

Peeri et al. 2021, Cancers

Cannabis and GBM- partial mode of action



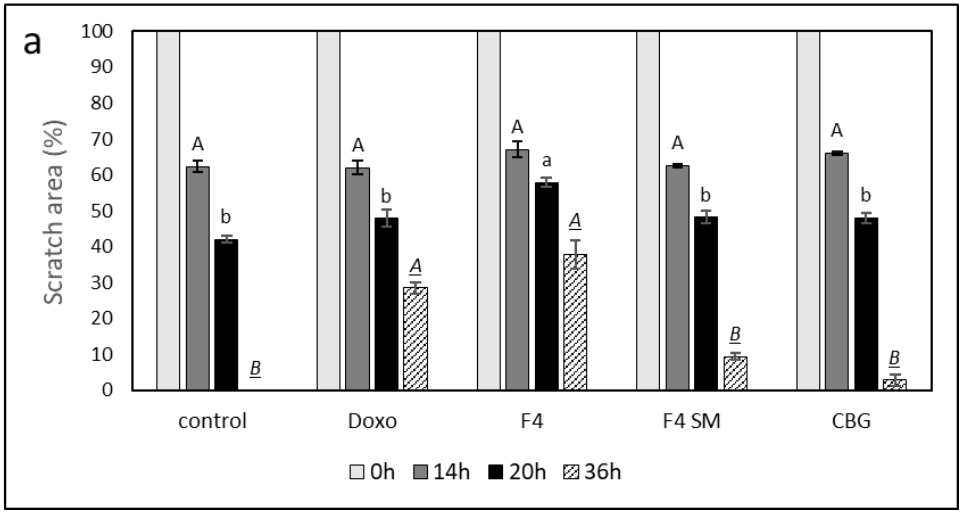


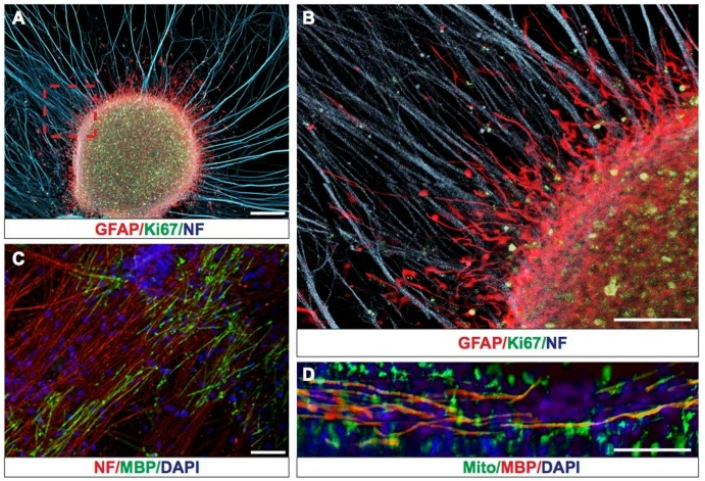
Mao, J., Hu, Y., Ruan, L., Ji, Y., & Lou, Z. (2019). Role of endoplasmic reticulum stress in depression. *Molecular medicine reports*, *20*(6), 4774-4780.

- Cell death is mediated via ER stress
- CBS IA reduced expression of these genes, again suggesting involvement of CB2 in this process

Peeri et al. 2021, Cancers

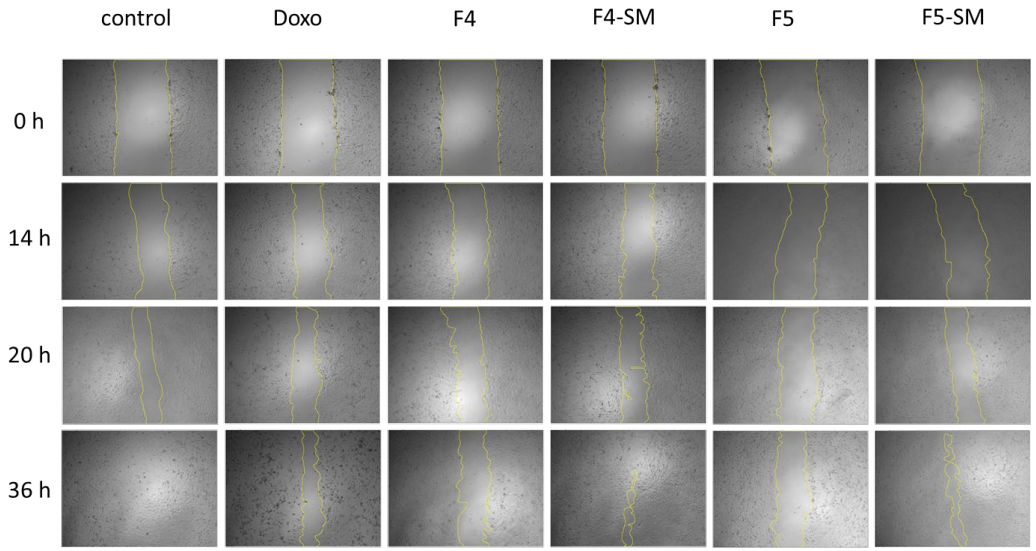
Cannabis and GBM-cell migration





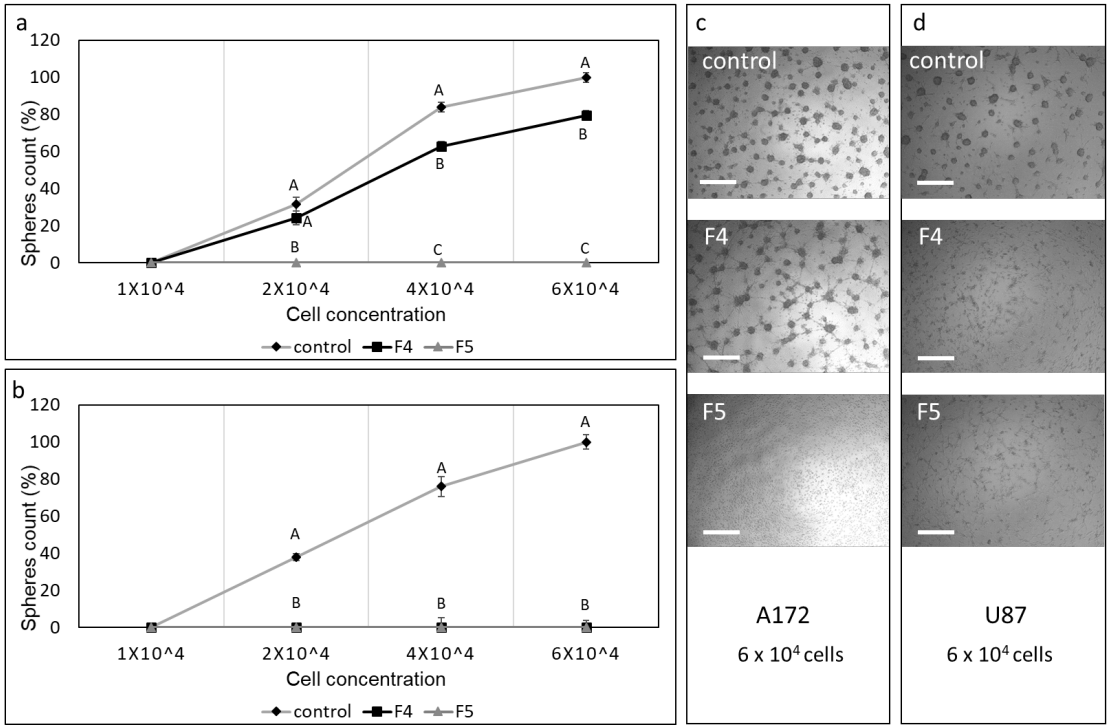
Zepecki et al., Oncogene 38, 1734–1750 (2019)

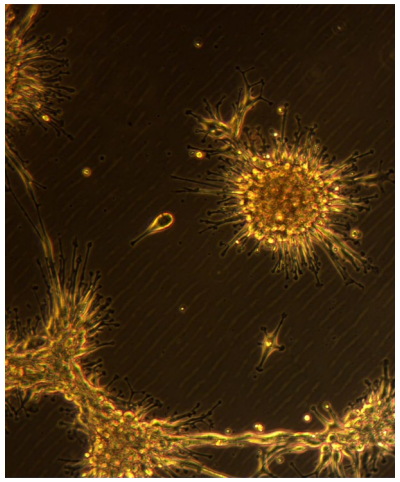
Fractions inhibit cell migration



Peeri et al. 2021,
Cancers

Cannabis and GBM-sphere formation

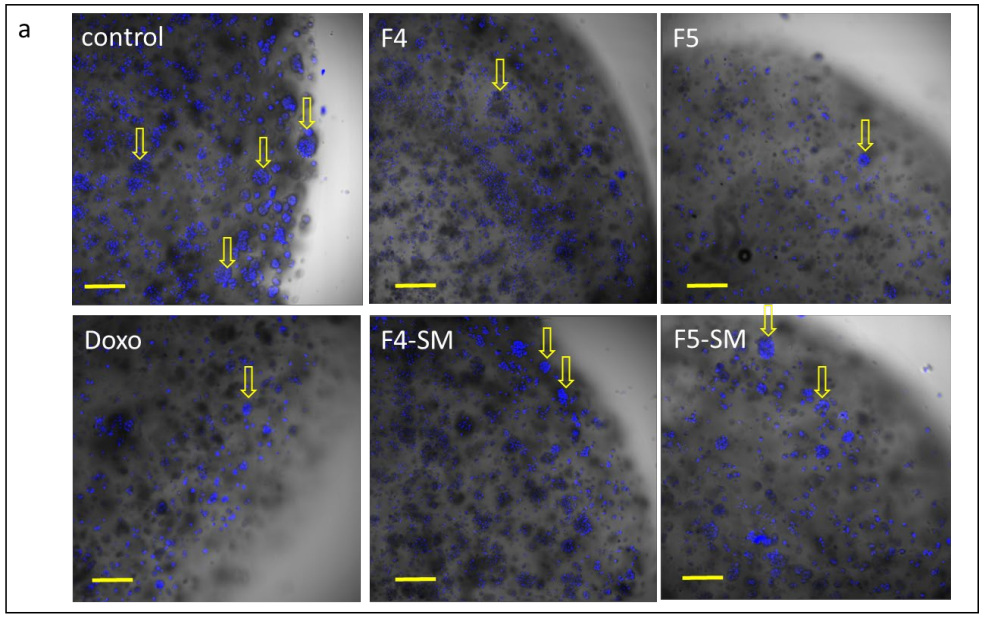


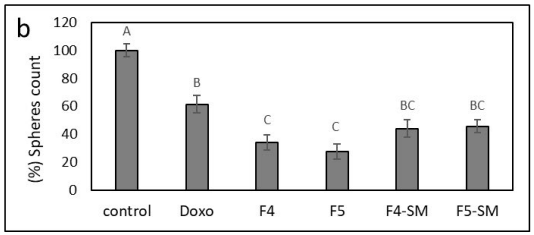


Fractions inhibit colony formation- in 2D model

Peeri et al. 2021, Cancers

Cannabis and GBM-sphere formation

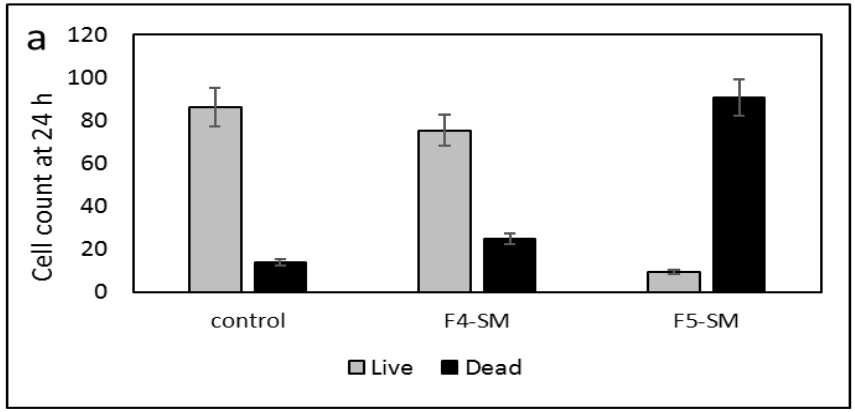


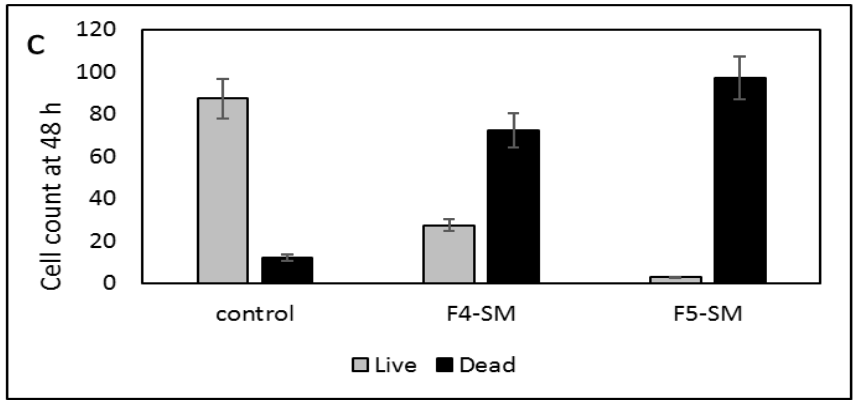


Fractions inhibit colony formation- in 3D model

Peeri et al. 2021, Cancers

Effect on cells isolated from patients' GBM tumor





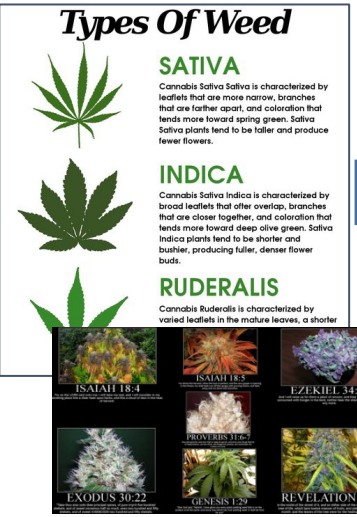
In collaboration with Prof. Chaya Brodie Lab, Bar Ilan University

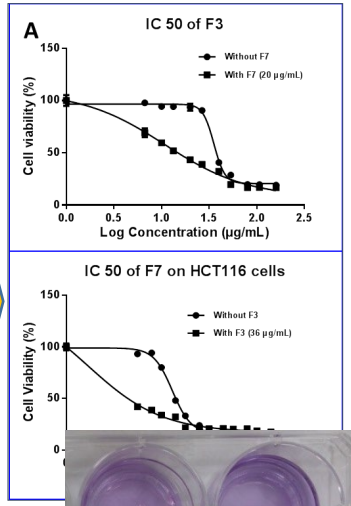
Fractions are cytotoxic to cells isolated from patients' GBM tumor.

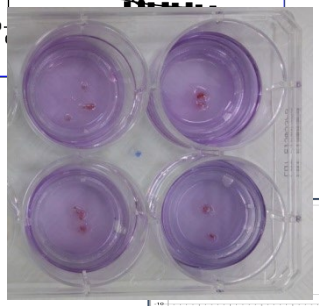
Peeri et al. 2021, Cancers

From “weed” to molecules to drug development-Koltai lab

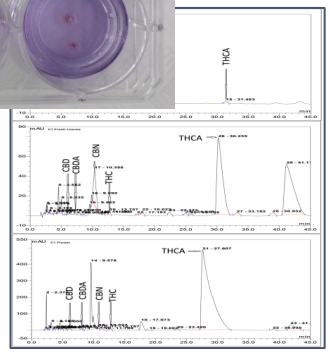
Identification of active compounds and synergies







Bioassays

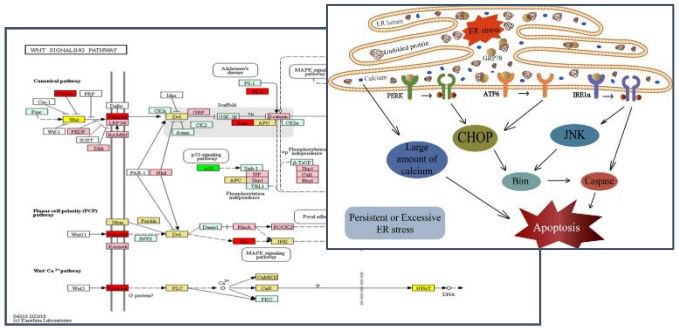


Chemical analyses

Formulation API

CBD	CBG	CBN	THC	THCV
5.8	80.3	2.8	-	11
-	3.7	4.6	91.7	-

Targets and Mode of action



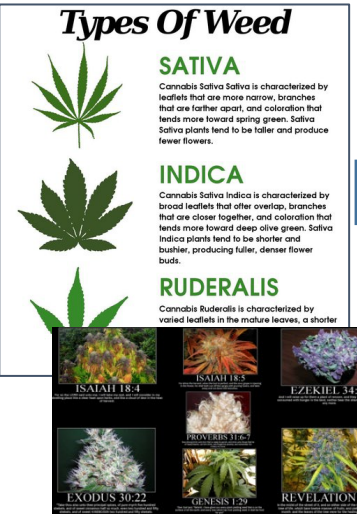
Toxicity; *in vitro* to *in vivo* extrapolations of cell assays; possible drug–drug interactions; development of drug delivery and clinical studies

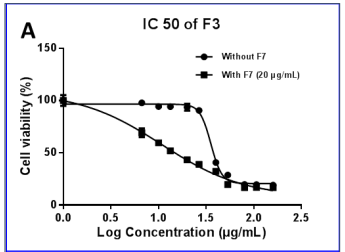
New generation of cannabis-based products

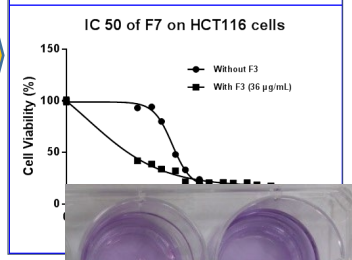
KOLTAI LAB

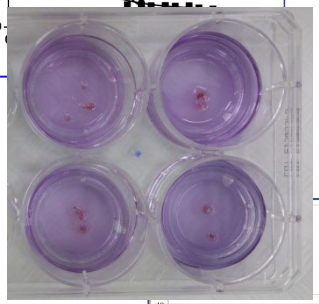
From “weed” to molecules to drug development-Koltai lab

Identification of active compounds and synergies

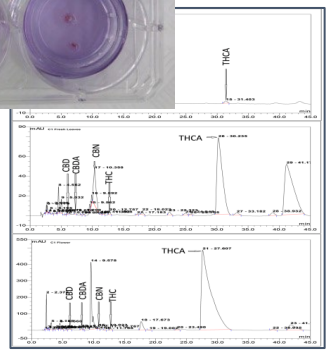








Bioassays



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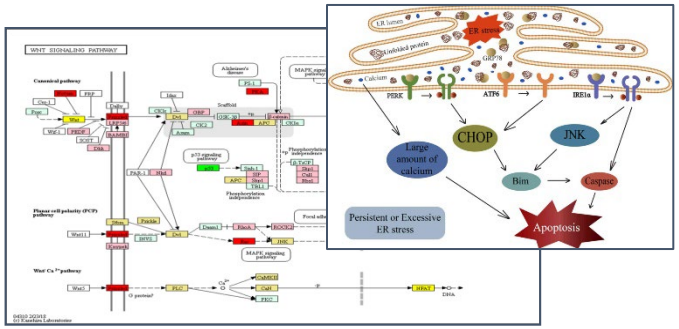

Development of elite chemovars

KOLTAI LAB

Acknowledgments

ARO, Volcani Center, Israel

- Nurit Shalev
- Hadar Peeri
- Dr. Dvory Namdar
- Dr. Ajjampura C Vinayaka
- Dr. Anil SM
- Eddy Belausov
- Dr. Guy Mechrez
- Dr. Karthik Ananth
- Dr. Stalin N. Rajan
- Dr. Adi Faigenboim

Israeli Gene Bank

- Dr. Einav Mayzlish-Gati

Bar Ilan University

- Prof. Chaya Brodie
- Rephael Nizar
- Dr. Gila Kazimirsky
- Irit Shoval

Research is evidence-based and unbiased


Thank you