Predklinične raziskave kanabinoidov kot osnova personaliziranega zdravljenja rakavih bolnikov

Tamara Lah Turnšek

Odd za genetsko toksikologijo in biologijo raka

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ZGODOVINA & PREDSTAVITEV MEDICINSKE UPORABE KONOPLJE

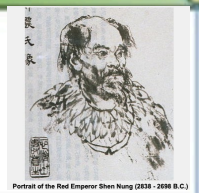
Konopljo (*Cannabis*, lat.) je pračlovek uporabljal že pred 25 000 leti, zapisi od 5000 B.C., a njeno mnogostransko delovanje kot zdravilo za ljudi in živali, še ni dokončno pojasnjeno.

~12,000 BCE — 2700 BCE — 1800 BCE — 200 — 1200 — 1800 — 1900 — 1990

Cultivation of hemp for rope and fiber in ancient China

Emperor Shen Nung explores cannabis and other herbal remedies for human disease in ancient China

Rdeči cesar Shen Nung (2838-2698 BC)

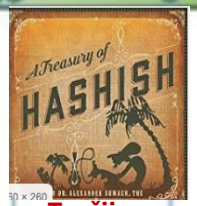


Indijci in druge vzhodne kulture

Therapeutic use of cannabis in ancient India

Sumerci in Asirijci 3-2000 BC.
Egipčani 200 BC

Use of cannabis as medicine throughout Middle East, Far East and North Africa



Turčija
200- AD

Use of cannabis for seizures by Islamic physicians

980-1037 A.D AVICENNA
"Canon of Medicine,"
Perzija

Angleška farmakopeja

1843

William O'Shaughnessy returns from India and promotes cannabis for treatment of numerous conditions including seizures

Bromides introduced for treatment of seizures

Sir William Gowers publishes *Epilepsy and other chronic convulsive disorders*; describes use of cannabis for epilepsy

Phenobarbital introduced

League of Nations prohibition of cannabis

Synthesis of CBD and Δ9-THC

Early human trials of CBD for epilepsy

1857

1881

1937 - 1960

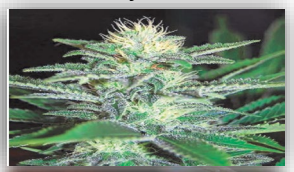
1965

Mechoulam and Carlini – 9 bolnikov ..+ XY danes!

Konoplja (Cannabis, *lat.*).

Drugi deli rastline- vlakna: tekstil, vrvi..

Cvet- ženske rastline smola jo ščiti



Trihomi-Mešički Konoplja veže kovine in druge toksine *Pozor! Ekoremediacija-čisti prtst*

Raphael Mechoulam

Dostopnost do 400 vrst konoplje.

Nagrade

EMET Prize of Art, Sciencenand Culture, EMET Prize of Exact Science

IMCA - Israeli Medical Cannabis Agency

סוכנות ישראלית ממשלתית לקנביס רפואי

Izrael vodi v raziskavah: Osem firm, 4/8 ima GMP produkcija

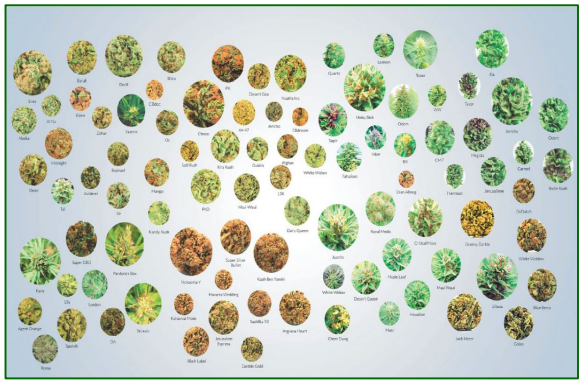
Kvaliteta in odmerki zdravljenja so strogo kontrolirarni, kar omogoča zdravljenje, slednje, in napredek

Renesansa medicinske konoplje

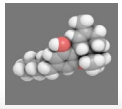
Cannabis Indica **THC** > 0.2 mg/kg

& mixed

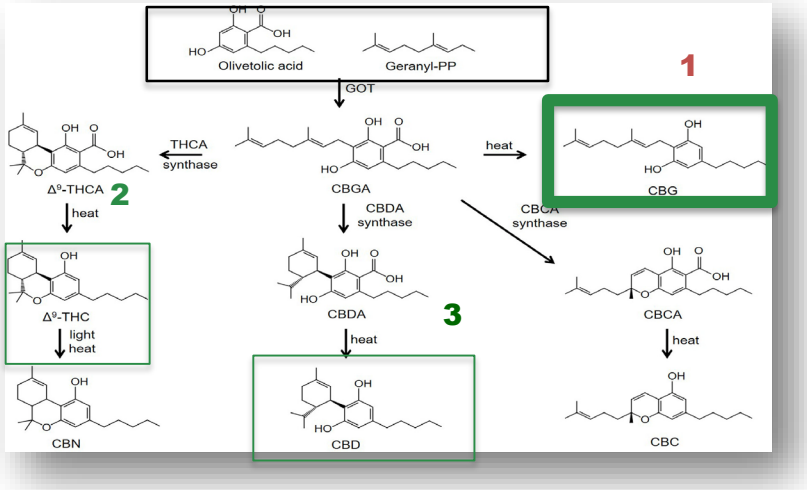
Cannabis Sativa **THC** < 0.2 mg/kg



Utopia ! *,Moby Dick, White Widow, Desert Queen Dairy Quene,Eden,…….*



Biosinteza kanabinoidov v konoplji



Walsh et a, (2021). Front. Pharmacol. 12:777804. doi: 10.3389/fphar.2021.777804

Raznovrstnost naravnih izvlečkov različnih križancev konoplje

The "breeds" contain different cannabinoids - 124 strains can be clustered into 5 groups

Clusters are rich in CBG, CBD, CBDA, Δ9-THC, Δ9-THCA

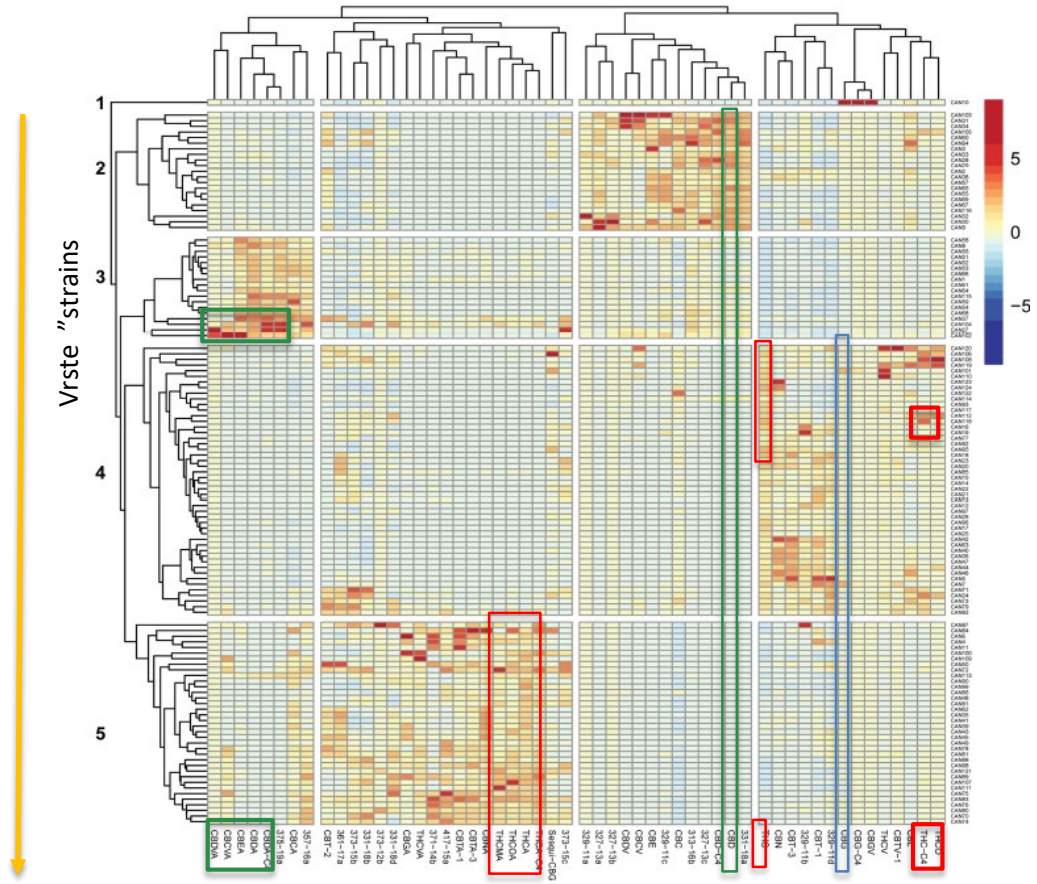


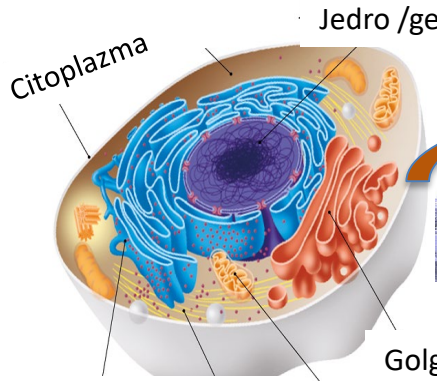
Figure 1: Heat map of unsupervised hierarchical clustering of the cannabinoid profile of 124 *Cannabis* extracts.

Baram & Meiri, Oncotarget 2019

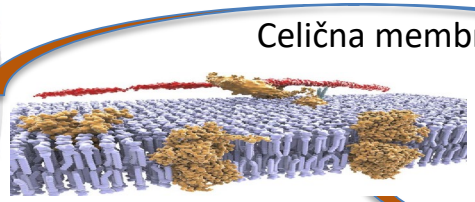
Cannabinoids: Mechanism of acting on human cell

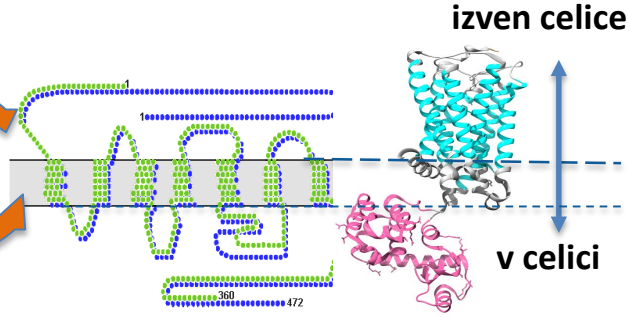
1. Communication

2. Transmembrane receptors



Celično ogrodje in druge organele

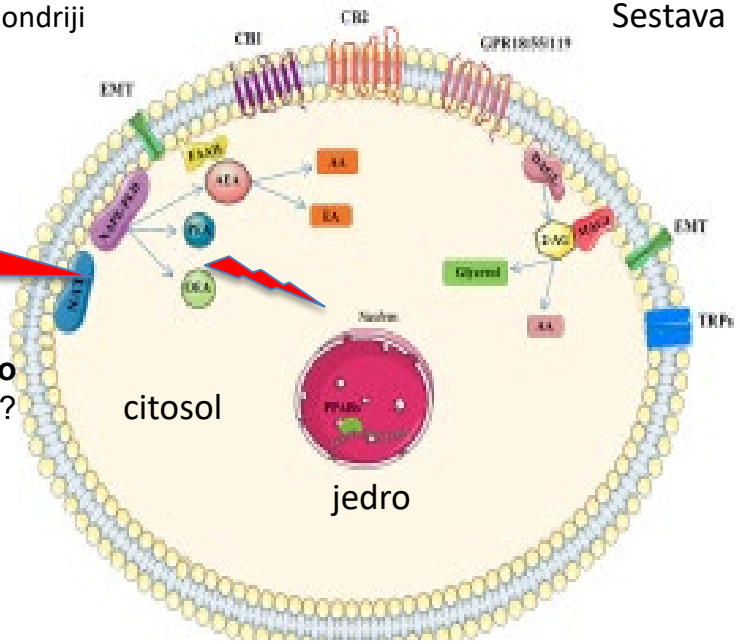




Sestava CBR1 ali CBR2 (aminokisline!)

Signaliziranje v celico in v celici z endo kanabinoidi?

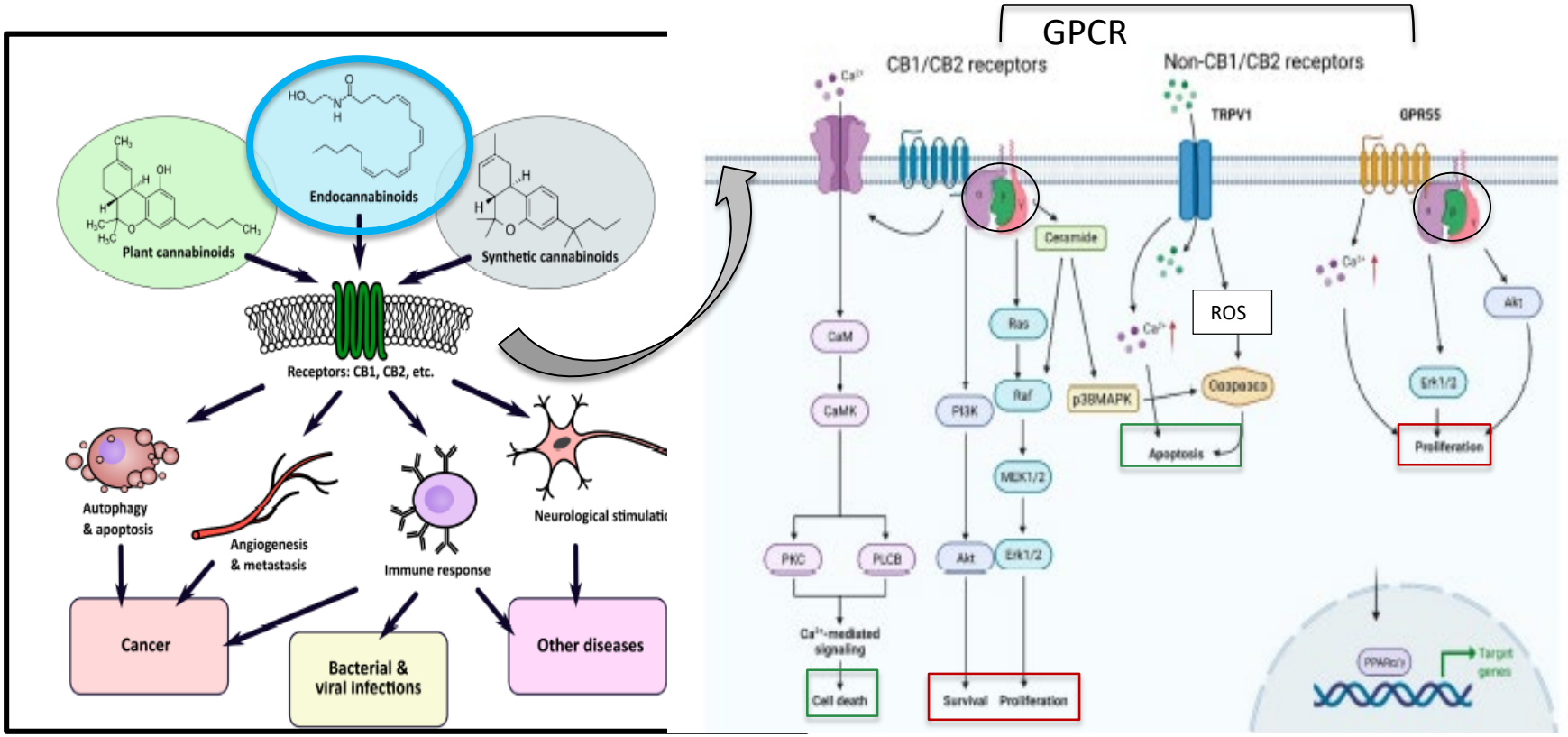
Kaj sporočajo kanabinoidi in zakaj?



3. Intelligent cell response by -
-gene activation and
-protein synthesis
= signalling

Cannabinoids signaling mechanisms in normal and diseased cells

goes *via* specific and non-selective receptors & complex molecular pathways

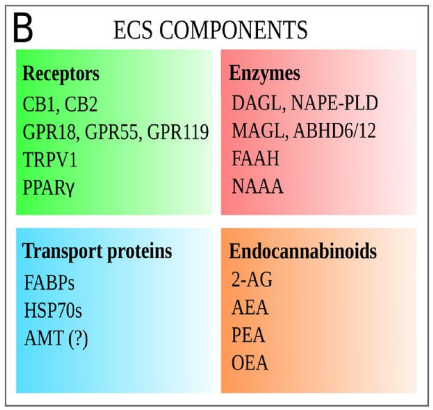


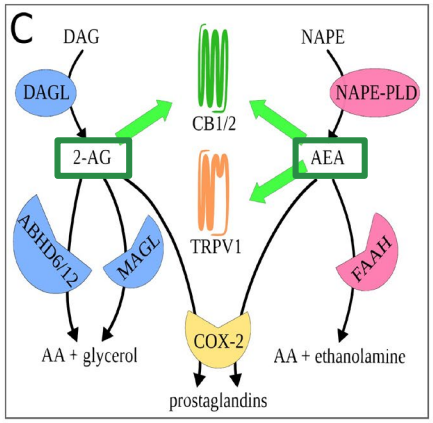
CB1, CB2 & GPR55 = G-protein coupled receptors, **TRPV** – Transient Ion Receptor Protein -Vaniloid

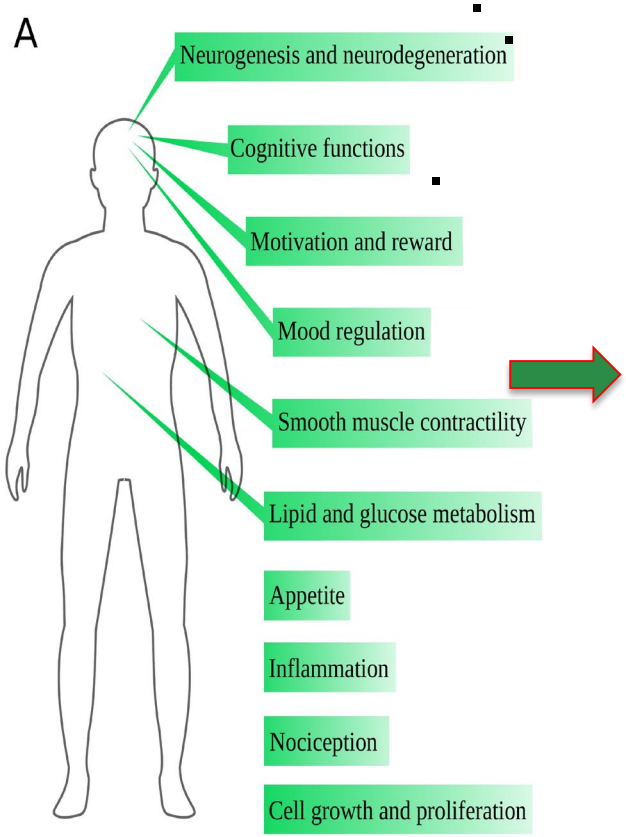
Sledzinski, P Int. J. Mol. Sci. 2021, 22, 263. https://doi.org/10.3390/ijms22010263

Lee, X.C. Cancers 2021, 13, 1211. https://doi.org/10.3390/cancers13061211

Endokanabinoidni sistem (ECS)







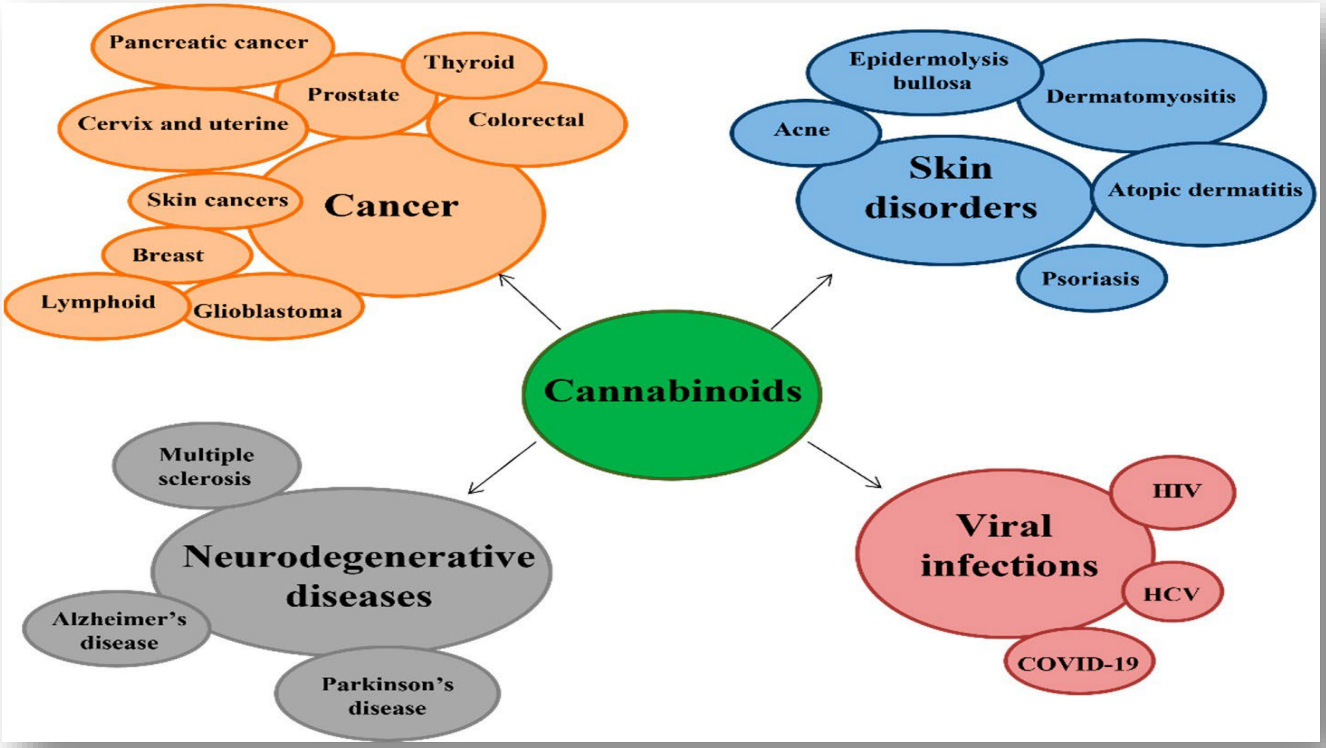
- Obvladovanje stresa, depresije in tesnobe, spomina, zavedanja
- Bolečina (fibromegalija)
- Nevrološke bolezni (Parkinson, Multipla skleroza, epilepsija)
- Črevesne bolezi, (Crohn), bruhanje in apetit
- Imunski sistem, pomirja vnetja in podpira odpornost.
- Kardivaskularno delovanje
- Hormonska neravnovesja
- **Deluje proti razvoju in napredovanju raka –celična rast**

4. Komponente
(1) endokanabinoidi
(2) encimi biosineteze in razgradnje,
(3) prenašalni-proteini in
(4) receptorski proteini

Smo odkrili šele z rastlinskimi kanabinoidi!
Ima vlogo zaščite pred pred prevelikim vzburjenjem celic - organizma ((n.rp. nevrona - regulira sinapse) zaradi bolezenskih povzročiteljev in ohranja homeostazo!

Saisiulewicz (IJMS , 2020)

Terapevtski pomen kanabinoidov



Dobro preskušeno - klinična uporaba

Nezadostno preskušeno –priporočeno?

High quality evidence

- Pain (neuropathic pain): Δ^9-THC
- Multiple sclerosis (spasticity): Δ^9-THC
- Epilepsy: CBD
- Cancer (palliative treatments): Δ^9-THC
- Weight loss (AIDS): Δ^9-THC

CANNABINOIDS

Moderate-low quality evidence

- Parkinson´s disease
- Alzheimer´s disease
- Huntington's disease
- Addictions
- Glaucoma
- Post-traumatic stress syndrome
- Tourette syndrome: Δ^9-THC
- Anxiety: CBD
- Cancer: Δ^9-THC, CBD

UPORABA MEDICINSKE KONOPLJE V RAKU

Incidenca in prevalenca raka narašča !!

Tudi znanje? !

ICR The Institute of Cancer Research

g

American Institute for Cancer Research®

CANADIAN BREAST CANCER FOUNDATION™

FONDAT
CANAD DU
CANCER SE

CANCER ID

LUNG CANCER RESEARCH FOUNDATION
Living. Breathing. Science.

Kure it™
cancer research

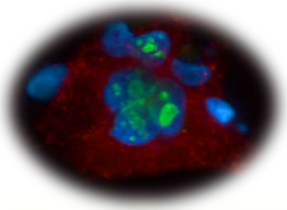
AUSTRALIAN CANCER RESEARCH FOUNDATION

World Cancer Research Fund

MCRC
Driving Cancer Team Science

CANCER RESEARCH UK

Kaj se dogaja v celici tekom karcinogeneze ?

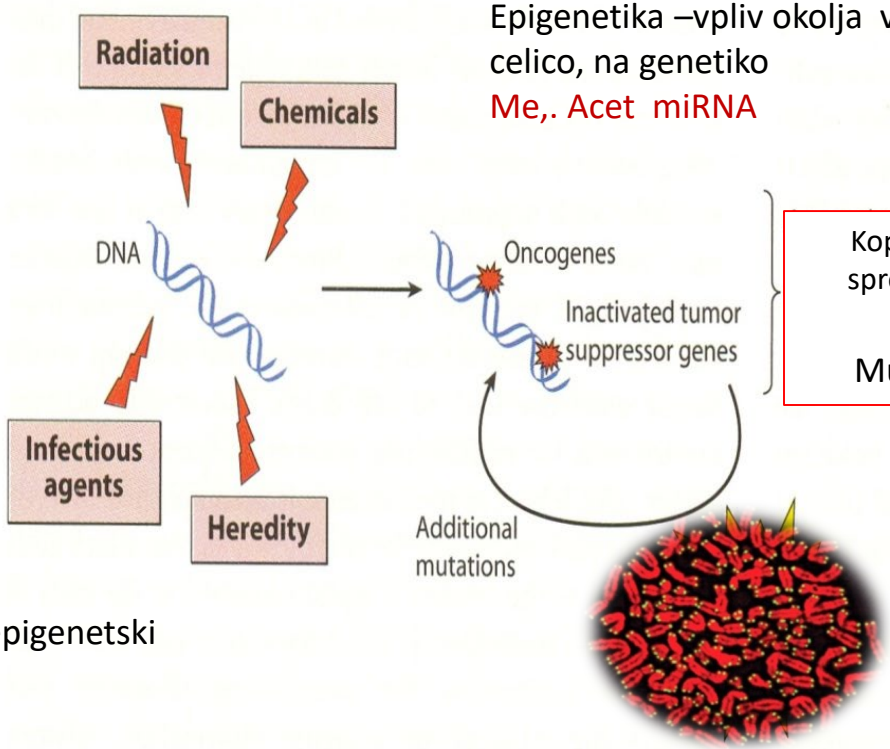


Rakava matična celica

Zunanji dejavniki

Sistemski

Epigenetika –vpliv okolja v /na celico, na genetiko
Me,. Acet miRNA



Kopičenje sprememb = Mutacij

HALLMARKS OF CANCER

1. Self-sufficiency in growth signals
2. Insensitivity to antigrowth signals
3. Evasion of apoptosis
4. Limitless replicative potential
5. Sustained angiogenesis
6. Tissue invasion and metastasis

Mikroorganizmi:
Virusi, bakterije

Dedovani geni in epigenetski učinki

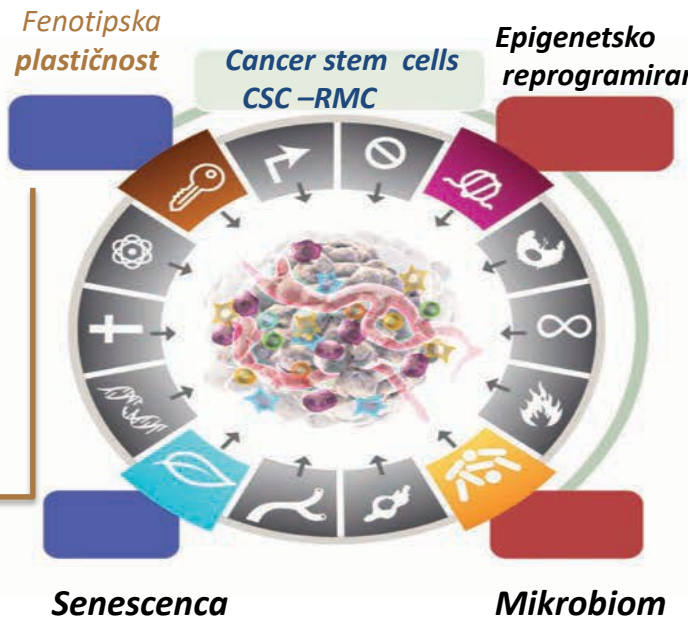
Spremembe,kromosoma genetska nestabilnost

Notranji dejavniki

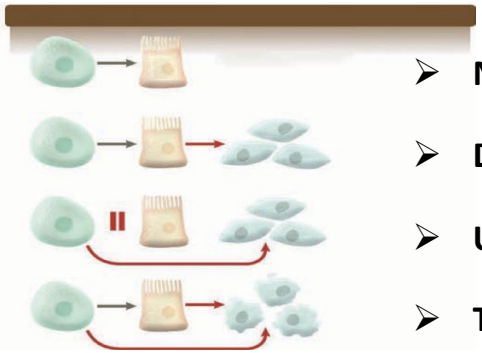
Tumorsko mikrookolje

Hannahan in Weinberg, Cell, 2000

Značilnosti rakavih celic od 7 (leta 2000), 11 (leta 2011) do 14 (leta 2022) in rakavih matičnih celic



Nastanek **rakavih matičnih celic CSC**

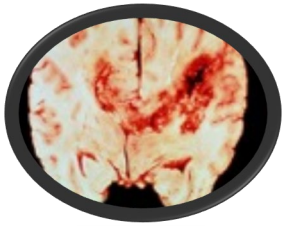


- **Normalna diferenciacija**
- **Dediferenciacija**
- **Ustavljene diferenciacija**
- **Trans-diferenciacija**

*Hanahan in Weinberg , Cancer Discovery, **2022***

MOŽGANSKI TUMORJI –REDEK, ZELO AGRESIVEN RAK

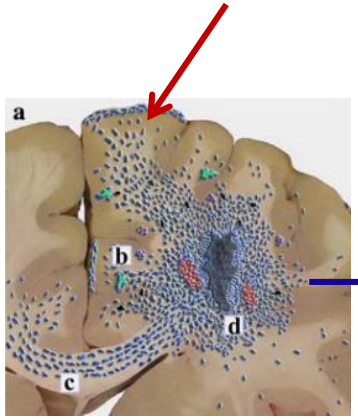
Možganski tumor glioblastom – GBM
Dodatna – kombinirana terapija cilja GBM matične celice ?

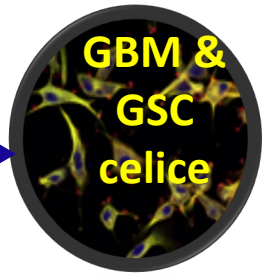


- Ožiljenje *vs* nekroza
- Rast
- Invazija celic v možganovino

Incidenca 7-10 /100 000 prebivalcev

Prognoza cca 14-16 mesecev preživetja





Kirurgija

↓

Obsevanje

↓

Kemoterapija

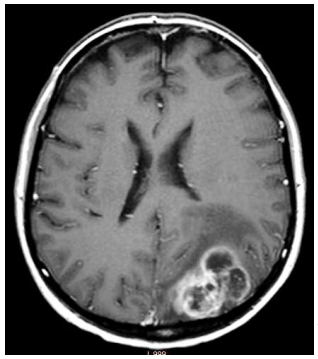
↓

Podaljša preživetje za 2-4 mesece ☹ !

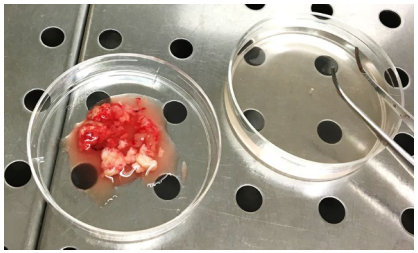
Personaliziran pristop

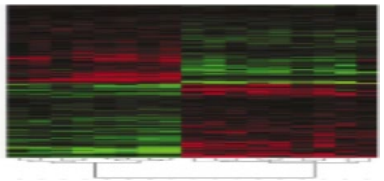
Ali lahko napovemo, kateri bolnik bo dovzeten za sočasni učinek standardnega zdravljenja in dodatnega s kanabinoidi – s katerimi in v kakšnem razmerju?

GBM BOLNIKI



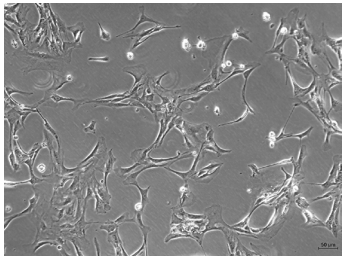
Tumor



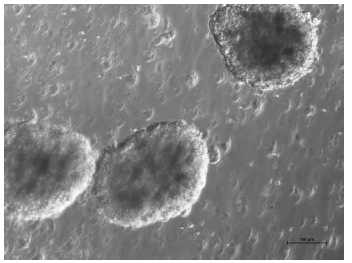


Frozen tissues RNA/DNA/protein

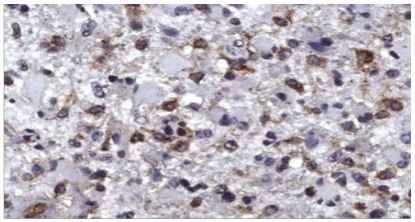
Primary cultures GB cells



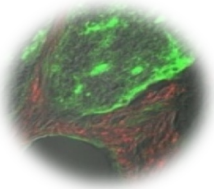
GB stem cells (GSC) spheroids



Tissue slices



GSC organoid -GBM GSC +TME

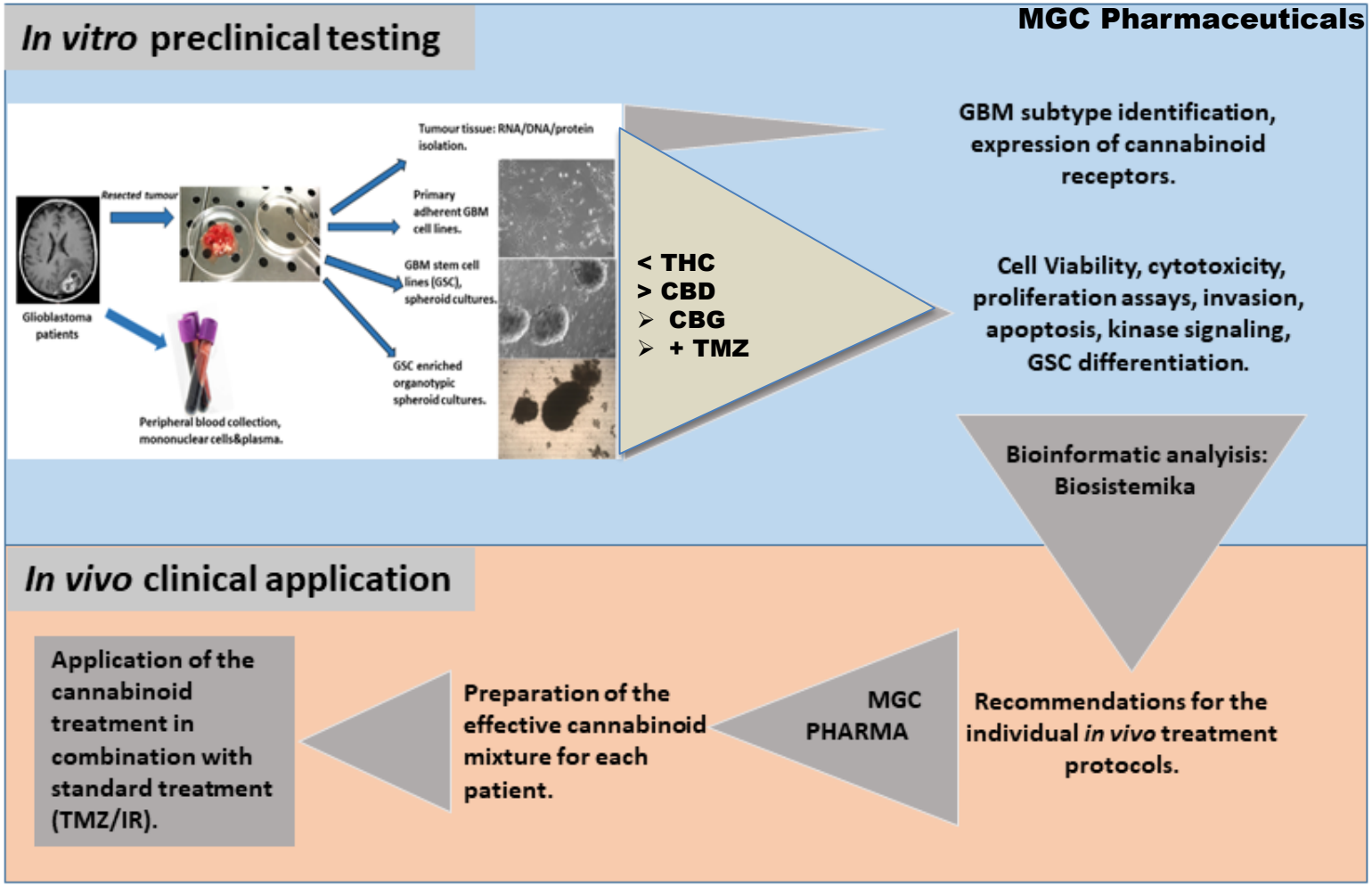


Nevrokirurgija UKC-Ljubljana
Institut za patologijo MF-UL
Onkološki institut

Shema priprave tumorskih vzorcev za analize tkiv in celic: primarne kulture za testiranje in raziskave - personaliziran pristop analiz !

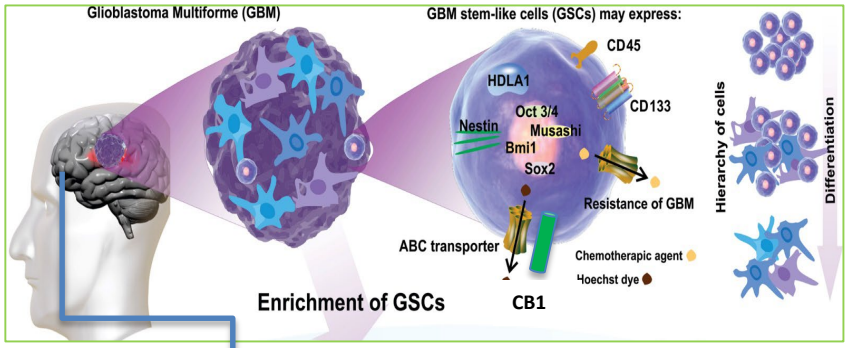
Platforma

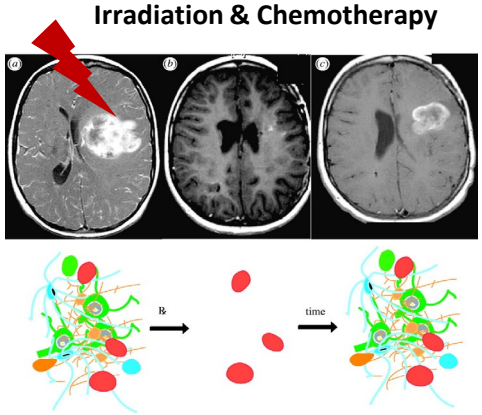
za *in vitro* predklinično testiranje odgovora na tretiranje učinka kanabinoidnov in receptorjev v bolnikovih celicah, in vitro napoved učinka na GBM bolnikih?



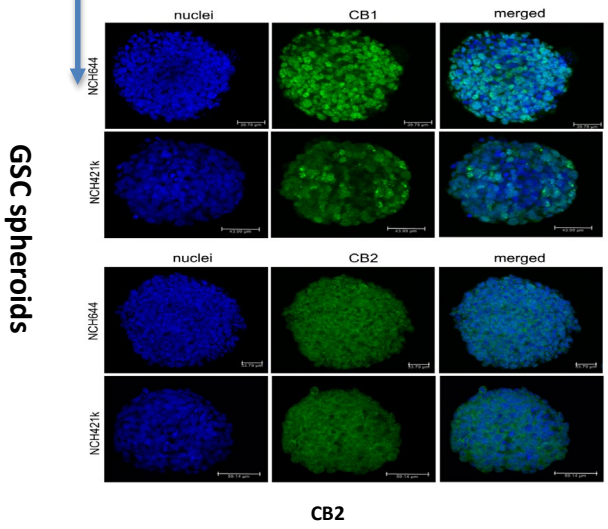
Glioblastoma stem cells

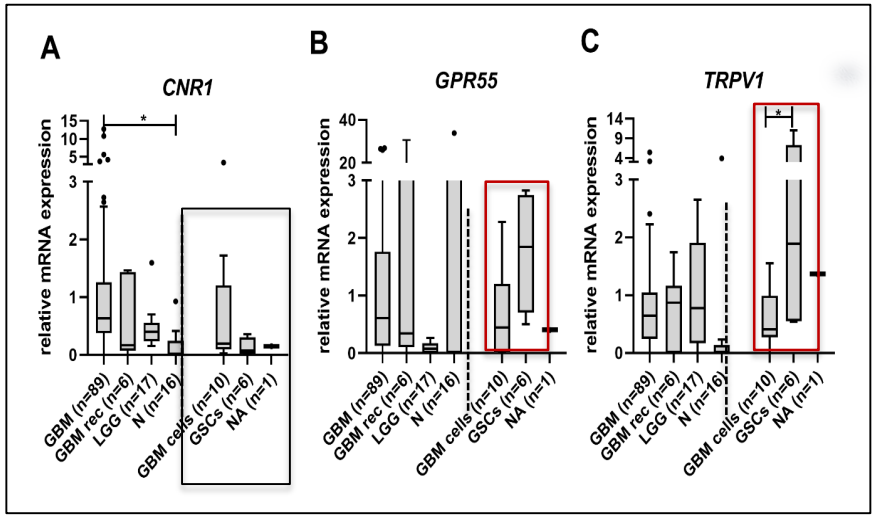
are highly therapy - resistant and express receptors CB1 & > CB2 and much higher GRP55 & TRPV1





Aggresive tumour regrowth from remaining stem cells

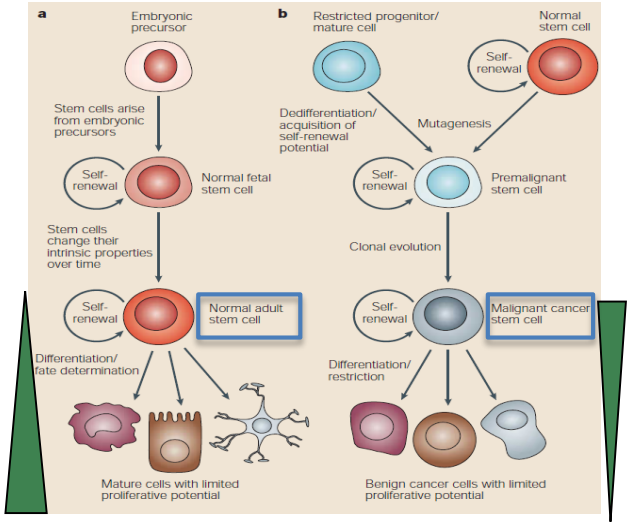


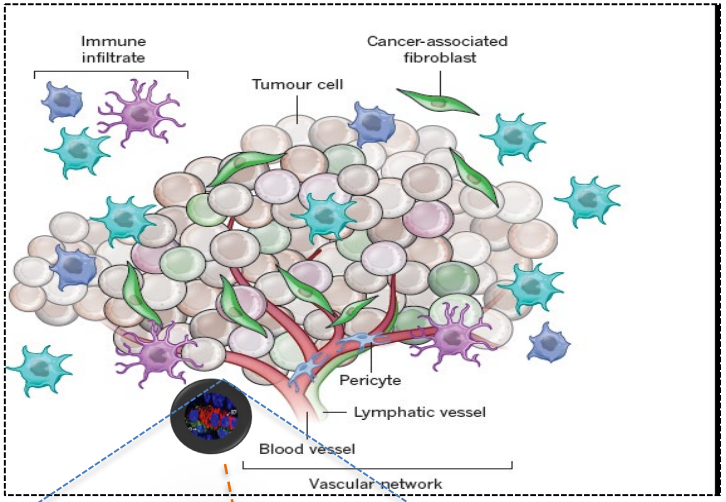


Lah, T.T.; et al. Cannabigerol Is a Potential Therapeutic Agent in a Novel Combined Therapy for Glioblastoma. Cells **2021**, *10, 340.https://doi.org/10.3390/ cells10020340*

Lah, T.T.; et al. The Cytotoxic Effects of Cannabidiol and Cannabigerol on Glioblastoma Stem Cells May Mostly Involve GPR55 and TRPV1 Signalling. Cancers **2022**, *14, 5918.https://doi.org/10.3390/ cancers14235918*

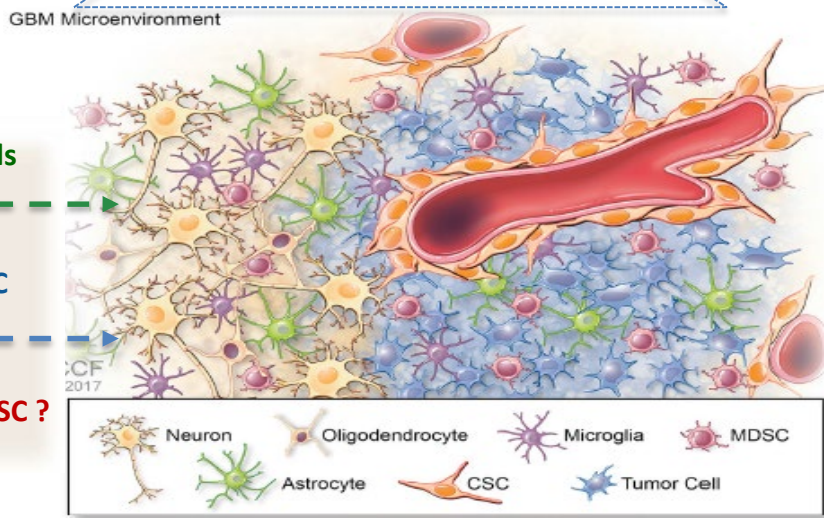
Tumor microenvironment (TME) is NOT affected by applied effective dosed of CBD or CBG





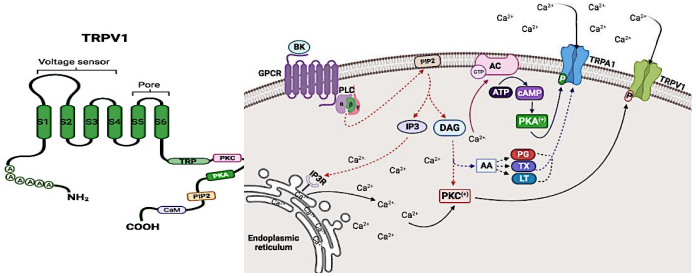
GENETIC STABILITY: **LOW in NEURAL STEM/ PROGENITOR CELLS (NPC)**
but HIGH IN GSC - resistant to theray
TRPV1 RECEPTORS ROLE?

Cancer Stem Cell niche



Pardal et al., Nature Rev Cancer 12/2003

CBD is known “neuroprotective agent*
There is no danger to brain in childhood and in adults –shown also in clinical applications
There is danger consumption in pregnancy

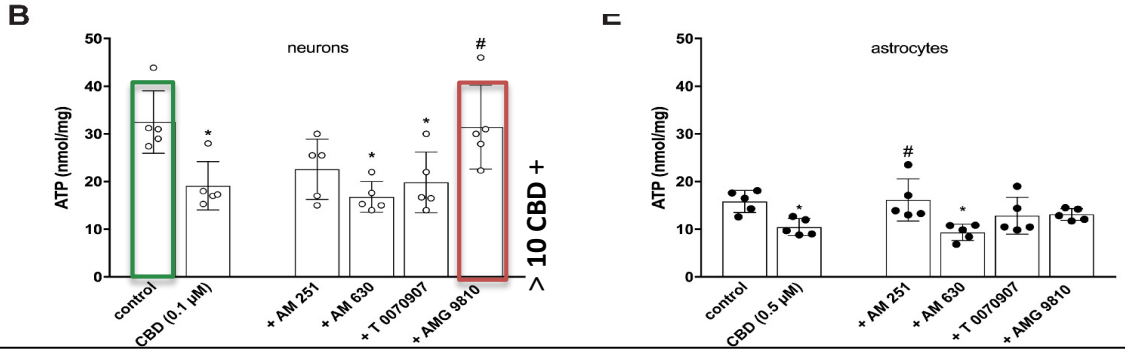


TRV1 is **highly expressed NSC and NPC** during postnatal development, but decreased and is **no/low** in neurons in brain *

Cannabinoid activation of TRPV1 **displays significant dependence on internal and external calcium l**evels and does not appear to induce the highly permeant channels**

Neural progenitor/stem cells
Fetal rat NPC > sensitive to neurons

Astrocytes more resistant



CBD 0.1- 0.5 μM caused cca 10 % cells early apoptosis that not increasing with time and concentration and would be inhibited by in vitro synthetic inhibitor.

CBD is TRPV1 inverse agonist, blocking and desensitising THC would also inhibiting apoptosis at > 10 μM ***

***Jurič, D.M.; Brvar, M.* Cytotoxic Effects of Cannabidiol on Neonatal Rat Cortical Neurons and Astrocytes: Potential Danger to Brain Development. *Toxins* 2022, *14*, 720. https://doi.org/10.3390/toxins1410072**

*** Costas-Insua, C.; Guzmán**, M. Endocannabinoid signaling in glioma. Glia **2022**, 71, 127–138.

****Duitama, M., Co, J.** J. S. **(2021). Biomolecule** TRP Channels as Molecular Targets to Relieve Cancer Pain. https://doi.org/10.3390/biom12010001

***** Stock, K.,** The capsaicin receptor TRPV1 as a novel modulator of neural precursor cell proliferation**. *Stem Cells*, *32*(12), 3183–3195. https://doi.org/10.1002/STEM.1805

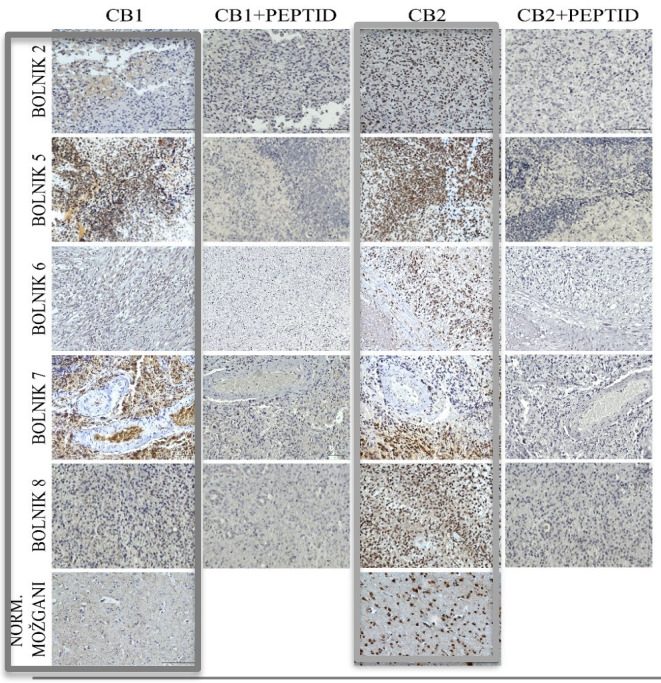
*****Starkus,** J.,, & Turner, H. (2019). Diverse TRPV1 responses to cannabinoids. **Channels, 13(1)**, 172–191. https://doi.org/10.1080/19336950.2019.1619436

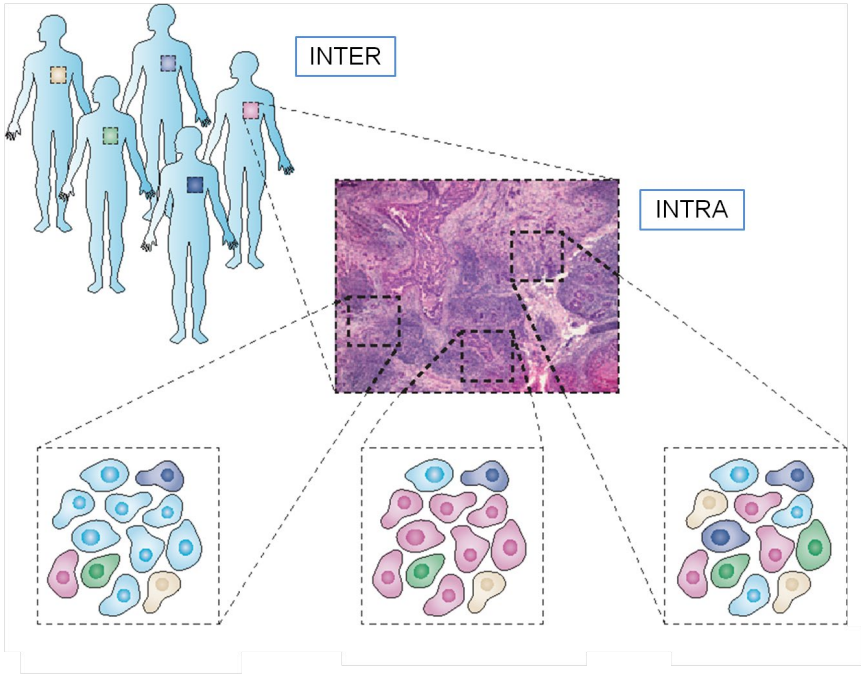
9/2022***

Izražanje receptorjev CB1 in CB2 v tkivih GBM je med bolniki različno - heterogenost tumorjev: personalizirano zdravljenje!

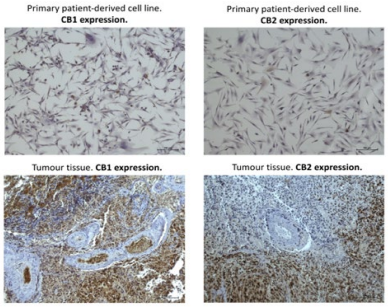
CB 1 CB2

Bolnik Kontrola Bolnik Kontrola





	CB1			CB2		
Vzorec GB	Intenziteta barvanja	Pozitivne celice	Lokalizacija	Intenziteta barvanja	Pozitivne celice	Lokalizacija
Bolnik 2	1	1	C	1	3	J
Bolnik 5	2	1	C	2	3	J
Bolnik 6	0	0	-	2	3	J, C
Bolnik 7	1,5	3	J, C	2	3	J, C
Bolnik 8	1	1	J, C	2	1	J, C
Norm. možgani	2	1	C	3	2	J, C

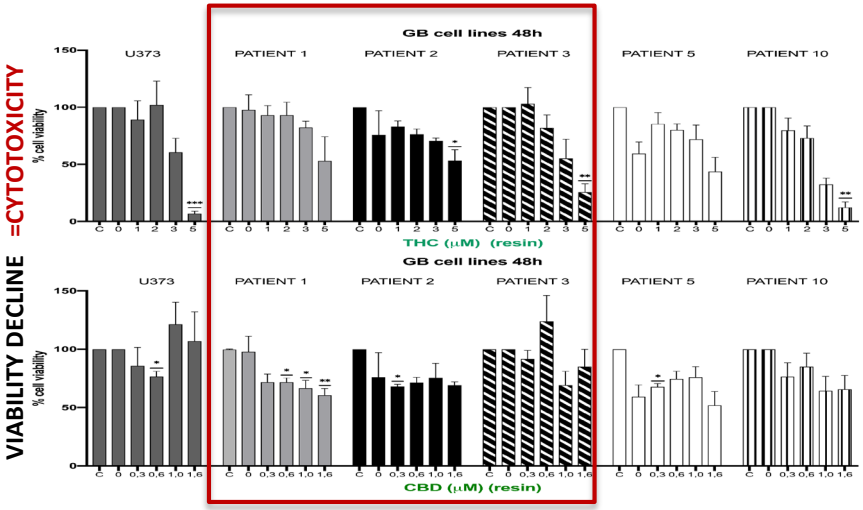


Variability in inhibition of viability = cytotoxicity response of patients-derived GBM cells upon THC & CBD:

Individualize the effective dose!

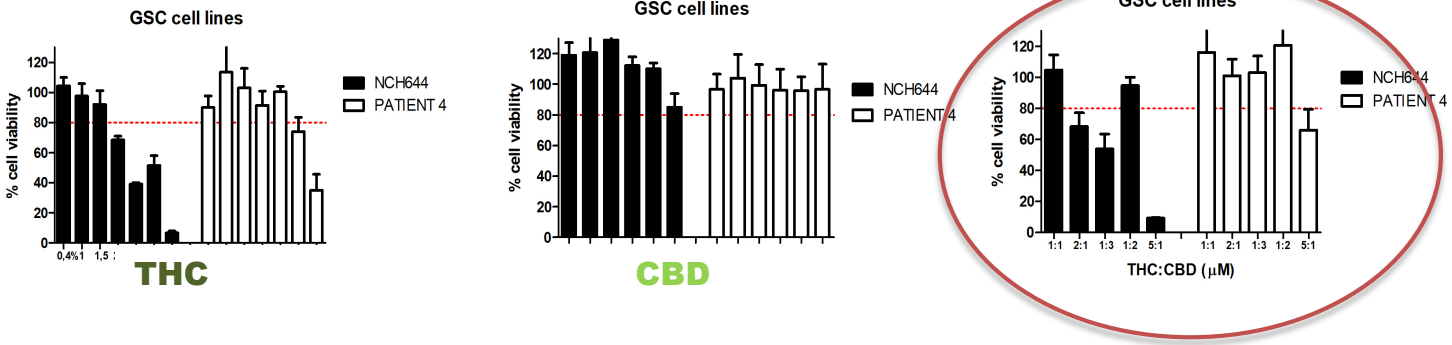
THC
GBM cells

CBD
GBM cells



Synergistic/Additive cytotoxicity on GBM and GSC cells

THC +CBD
GSC cells

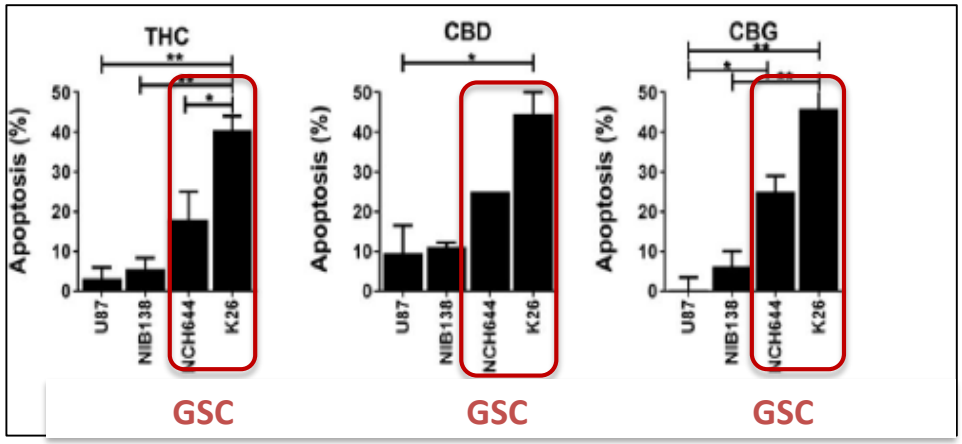


Article

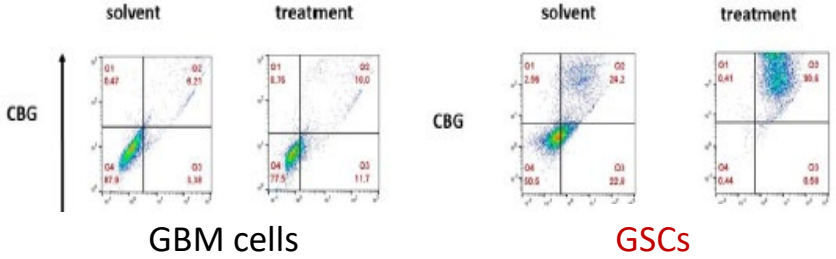
Cannabigerol Is a Potential Therapeutic Agent in a Novel Combined Therapy for Glioblastoma

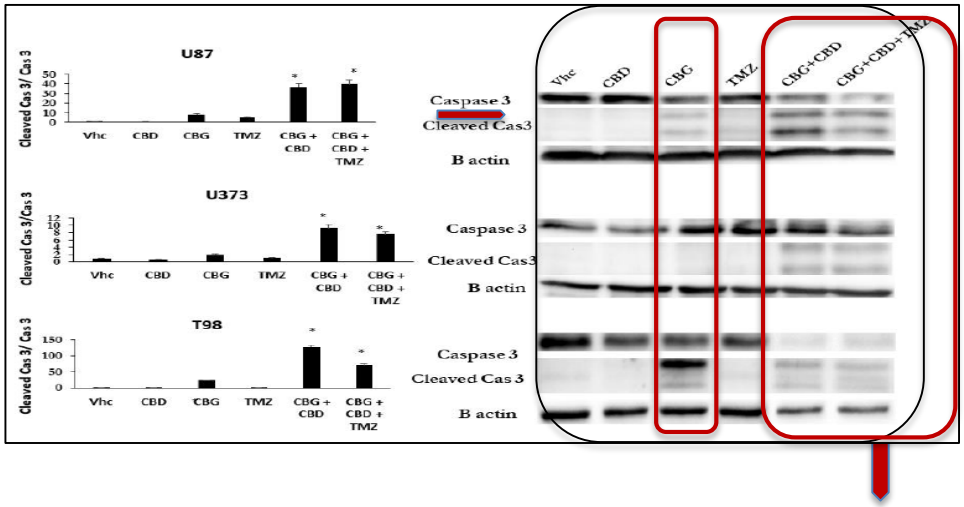
Tamara T. Lah [1,2,3,*], Metka Novak [1], Milagros A. Pena Almidon [4], Oliviero Marinelli [4], Barbara Žvar Baškovič [1], Bernarda Majc [1,3], Mateja Mlinar [1], Roman Bošnjak [5], Barbara Breznik [1], Roby Zomer [6] and Massimo Nabissi [4]

CBG induces apoptosis in GBM and GSC - overcoming therapy resistance!



- Besides **cell cycle arrest, apoptosis** is the second reason for observed cytotoxicity, both, in **differentiated and even more so in GBM stem cells**

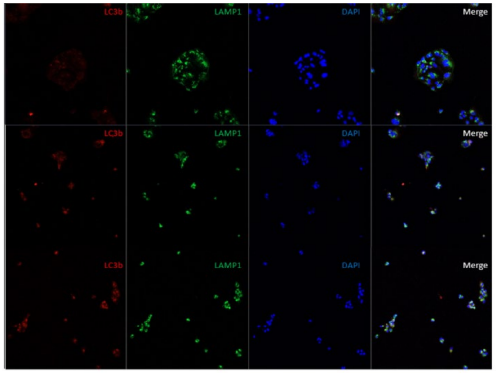




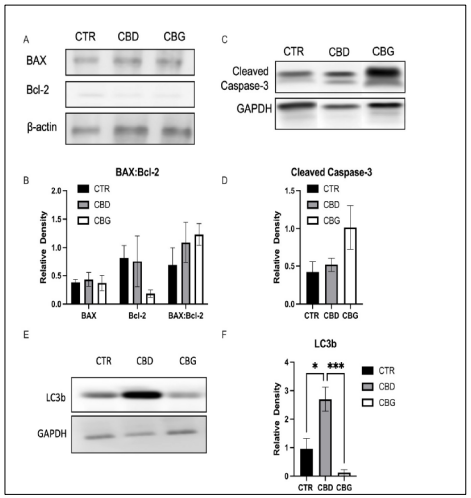
- **Intrinsic apoptosis mechanism goes via activation = enzymatic cleavage of the executive caspase 3**
- This is **only triggered by CGG & accelerated by CBD** in combination!
- **But not by THC, which is affecting apoptosis via autophagy**

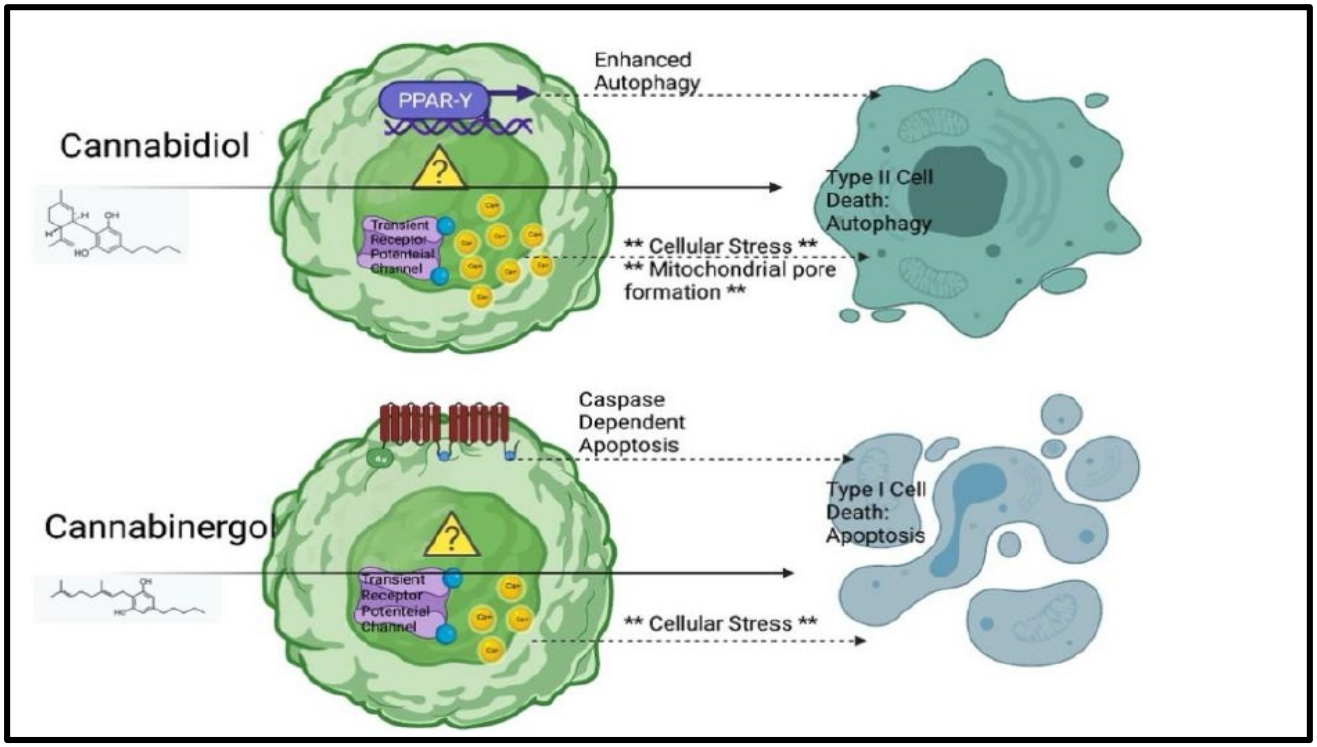
Cannabidiol and Cannabigerol inhibit Cholangiocarcinoma growth *in vitro via* divergent cell death mechanism (autophagy & apoptosis)

Autophagy (LC3)



Apoptosis /Caspase 3



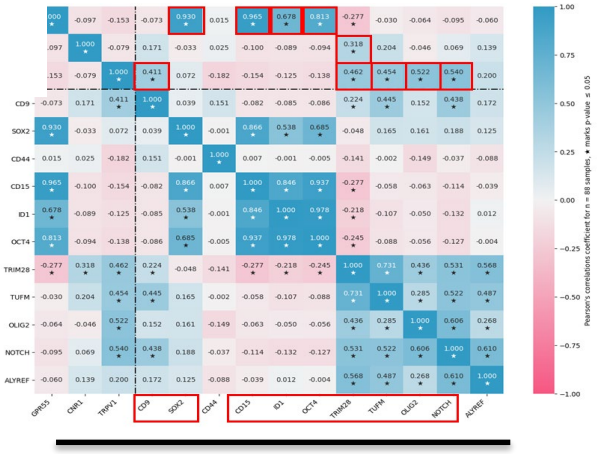


Viereckl, M.J. et al,. Biomolecules ***2022****, 12, 854. https://doi.org/10.3390/biom12060854*

Significant correlation of receptors GPR55 and TRPV1 with stemness (left) and EMT markers (right) suggests:

that inhibition of GPR55/TRPV1 would lead to GSC differentiation to less resistant GBM cells against standard treatment

GPR55
CNR1
TRPV1



GBM Stemness genes

GPR55
CNR1
TRPV

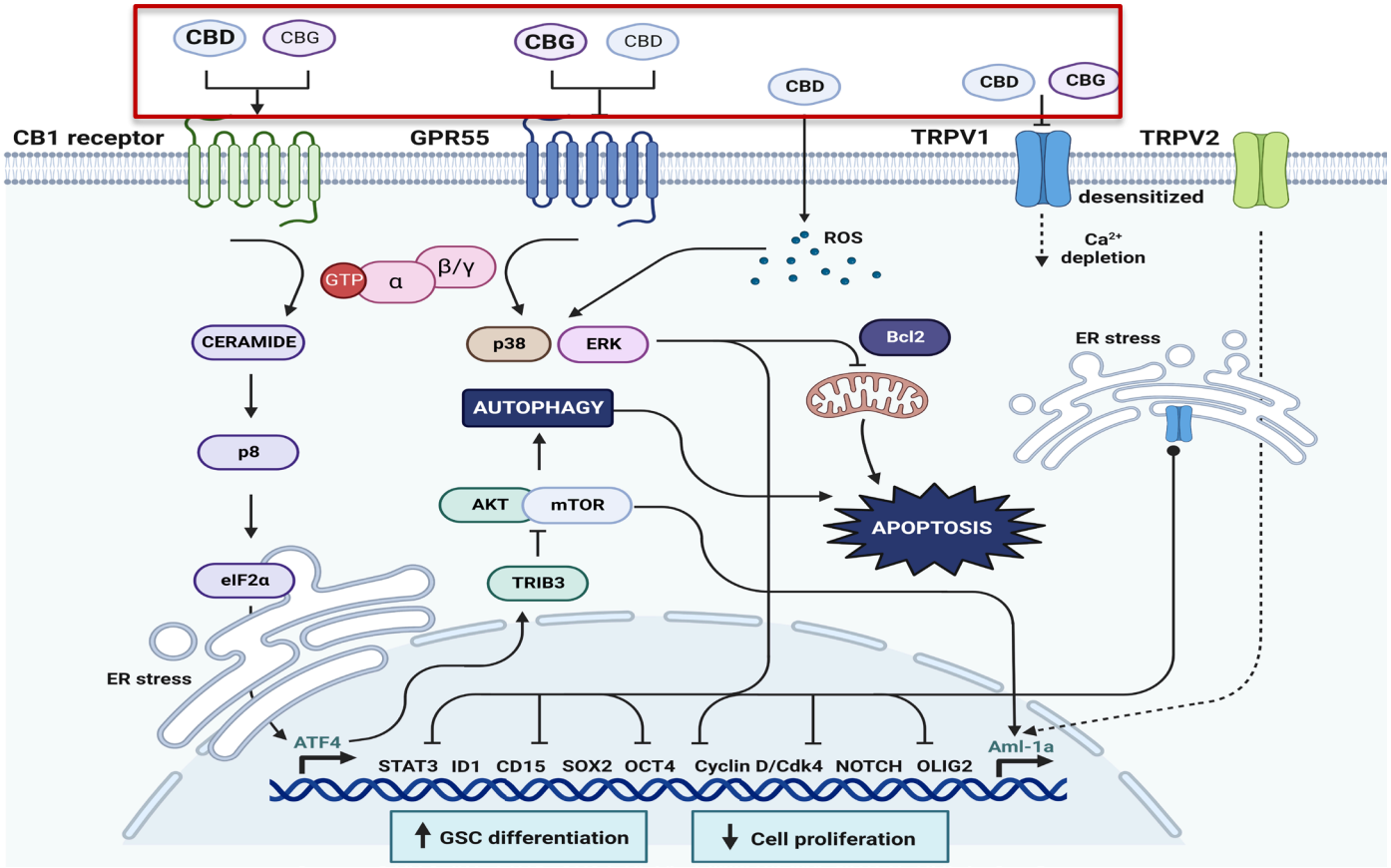
Epithelial – Mesenchymal Transition genes

TRPV1 correlation (r) with ***NOTCH*** and ***OLIG2*** and putative GSC genes ***CD9, TRIM28*** and **TUFM** and with EMT related genes, **like** ***CDH1,NF-Kβ, STAT3, CHI3L1-***

GPR55 correlated with **SOX2, OCT4, CD15 , ID1** and **S100A4 and KCNF** genes

*Correlation of cannabinoid receptors expression with GSC markers and EMT markers in in cohort of 86 GBM patients. Correlations coefficient (r) with cannabinoid receptors are marked in red (-)and blue (+) frames, sgnificance by **

Signaling mechanisms, leading to differentiated GSC upon GPR55 and TRPV1 blocking. This is followed by induced apoptosis of GB cells. which take another route to apoptosis via CB1



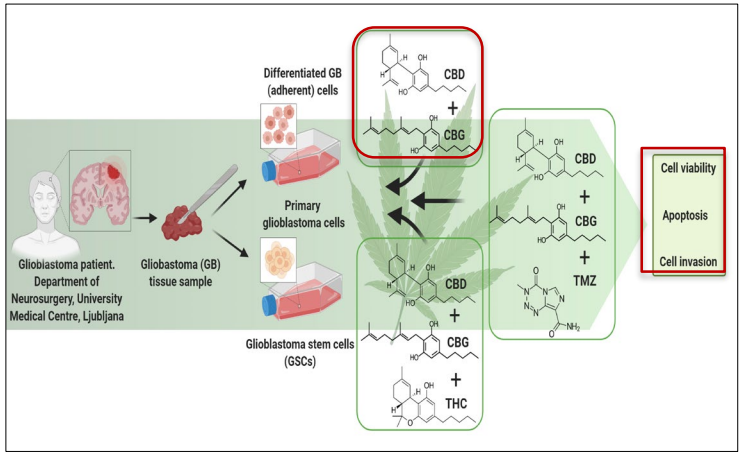
Treatment by CBD:CBG mixture induces two parallel mechanisms in GSCs and differentiated GBM cells.

First, due higher GPR55 and TRPV1 expression in GSCs , the two cannabinoids preferentially **inhibit GPR55** and **desensitize TRPV1 on GSC cells, thereby triggering their differentiation- downregulating stemness genes.**

Secondly, in parallel, CBD (CBG) are **activating CB1 and inhibit differentiated GBM cells** proliferation and induce autophagy as well as apoptosis by the ER stress signalling pathways, **linked to eIF2a, ATF 4-TRIB3 AKT/mTor=** common intermediate signalling molecules

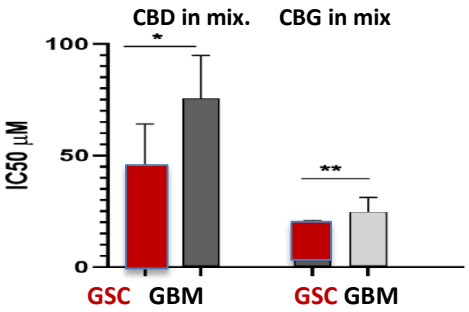
Summary

NOVEL CBG & CBD effects



CBD & CBG combination is more effective to GSCs than GBM cells!
CBD& CBG induce GSC differentiation, inhibiting their resistance.
CBG efficiently replaces THC cytotoxicity to:

- ✓ Arrest cell cycle G(
- ✓ **Proliferation**
- ✓ GBM apoptosis
- ✓ Inhibits invasion



Higher GSC cytotoxicity at CBD:CBG combination ➡

Cannabinoid receptors

CB1,CB2, *GPR55, TRPV1* expression
In GBM cohort of 86 patients
&
Prognosis:
no effect (*GPR55?*)
Subtypes:
no correlation

Stemness and invasion markers:
high correlation: *GPR55 & TRPV1*
SOX2,OCT4, CD15 , ID1
NOTCH, OLIG2, CD9,TRIM28,TUF, STAT3

Gene Expressions *GPR55, TRPV1*
2x GSC > GBM cells

Signaling mechanisms:
Blocking GPR55 and TRPV1 signaling: downregulating stemness genes ?!
GSC differentiation ➡ apoptosis ➡

POVZETEK

CBG predstavlja novo, še neraziskano možnost adjuvantnega zdravljenja bolnikov z GBM, tako kot pri nekaterih drugih oblikah raka –

Sam CBG in dodan CBD najučinkoviteje odstrani matične celice GBM, sicer odporne na terapijo, najprej z diferenciacijo celic po vezavi na receptorje GPR55 in/ali TRPV1

Temu sledi **sprožena. Apoptoza diferenciranih celic , verjetno po** usklajenem signaliziranju tudi s specifičnim CB1 receptorjem

THC nima dodane vrednosti pri kombinirani terapiji glioblastoma, kar nakazuje, da bi morali ta psihotropni kanabinoid v prihodnjih kliničnih študijah nadomestiti s CBG!

GPR55 in TRPV1 sta močno izražena v GSC in sta povezana s selektivnimi, vendar različnimi nizi genov selektivnih za matične celive.

Inhibicija njunega signaliziranja omogoča večjo **občutljivost GSC na terapijo**

Raven kanabinoidnih receptorjev pri posameznih bolnikih žal v naši študiji ne **napoveduje njihovega odziva na kanabinoide**

RAK DOJKE POGOST Z DOBRO NAPOVEDJO PREŽIVETJA

Rak dojke in kanabinoidi

Incidenca - pojavnost 1.5 mio letno /Slo:1224 (2019)

Umrljivost 500 000 letno /Slo: ca 16 /100,000 prebivalcev

Simptomi

- Otrdlina v dojki
- Aksiliarne metastaze bezgavk, spremembe v dojki, inverzija bradavic, izsedek

Diagnostika in presejalni testi

- Mamografija
- Ultrazvok MRI, PET, samopregledovanje

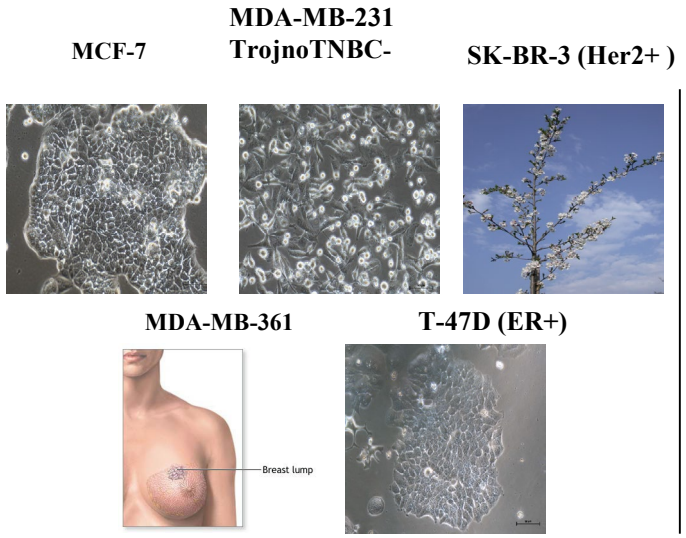
Podtipi:

- **Hormonsko pozitivni (HR+)**
- **Poztivni za rastni factor Her2neu**
- **Trojno negativni**

Zdravljenje

- Biopsija
- Kirurgija (mastektomija, konzervativna kirurgija)
- Radioterapija (5-6 tednov)

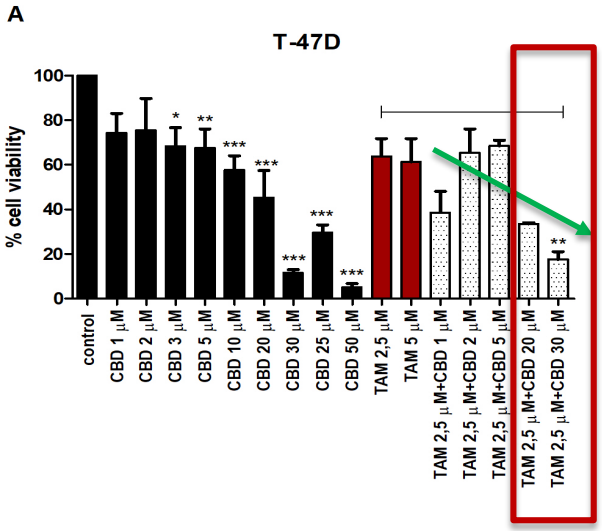
Heterogenost raka dojke



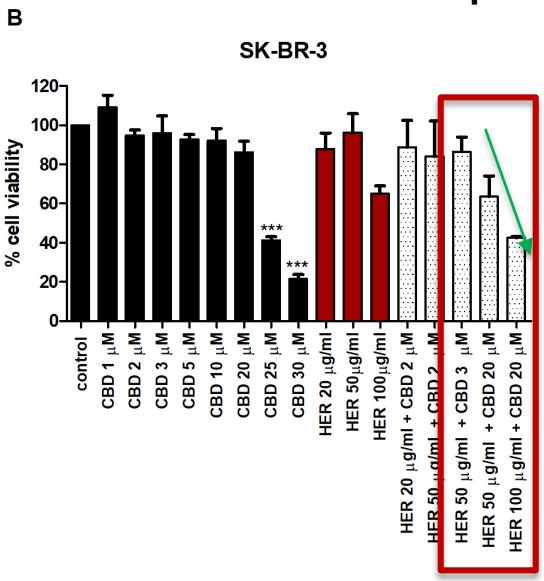
Dobovišek, L., Novak, M., Krstanović, F., Borštnar, S., Turnšek, T. L., & Debeljak, N. (2022). Effect of combining CBD with standard breast cancer therapeutics. *Advances in Cancer Biology - Metastasis*, *4*(January), 100038. https://doi.org/10.1016/j.adcanc.2022.100038

V linijah raka dojke deluje CBD citotoksično v sinergiji s Herceptinom, Tamoksifenom in Cisplatinom

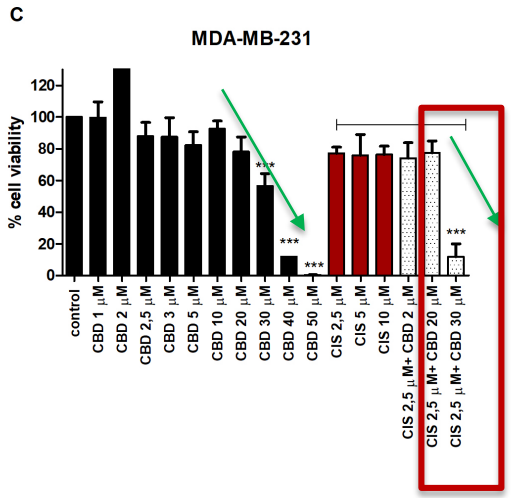
T-47D - Tamoksifen



SK-BR - 3 in Herceptin



MDA-MB-231 . Cisplatin



- **CBD ni oslabil učinka** standardnega zdravljenja s **hormoni** receptorsko pozitivnega raka dojke
- **CBD je povečal učinek Herceptina v HER2 + rakavihm celicah**
- Poleg tega CBD **ni oslabil učinka standardnega zdravljenja trojno negativnega raka dojke s cisplatinom.**

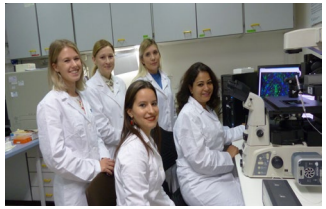
Zaključki

- ✓ Kanabinoidi večiomama upočasnijo rast tumorja in/ali podaljšanje preživetja pri **živalih.**
- ✓ Podobna opažanja **pri človeku (n.pr. CBD za GBM 30 % podaljšanje preživetja), a trenutno podpira to le malo kliničnih študijim a zelo veliko poročil o posameznih primerih.**
- ✓ Niti psihoaktivni THC / niti NOVO predlagani CBG niti CBD **nista univerzalno učinkovita pri dokončem uničenju tumorskih celic**, čeprav dokazano zavirata deljenje in rast, povečata avtofagijo in apoptozo ter neodvisno od teh inhibirata invazijo rakavih celic. **Priporočena je adjuvantna in aditivna teraija s CBD /CBG!**
- ✓ **In vitro je čisti CBD zelo pogosto enako ali bolj učinkovit kot le izvlečki CBD**, ki dokazano nimajo stranskih učinkov.
- ✓ **Izvlečki in čisti THC** je neprimeren za bolnike z **možganskimi tumorji-**
- ✓ **Izvlečki – smole - so neznane sestave in izvora(!) niso priporočljivi za „samozdravljenje“ – vedno nadzor lečečga zadrvnika!?**

DEPARTMENT of GENETIC TOXICOLOGY & CANCER BIOLOGY

Slovenian Research Agency

CANCER BIOLOGY



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MATEJA OBREZ
AJDA SUŠNIK

mgc pharma

ROBY ZOMER

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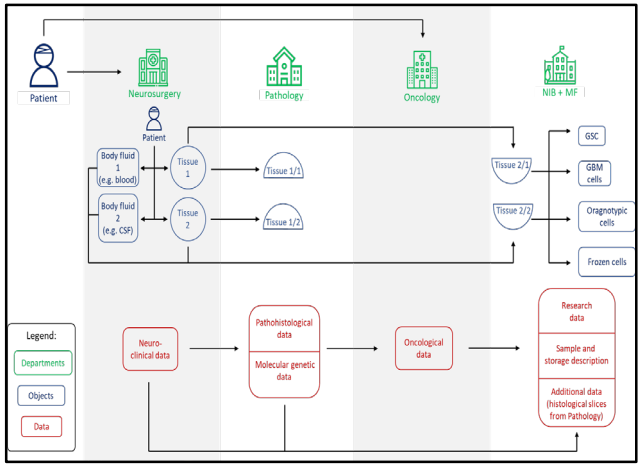
TECHNICON
DAVID MEIRI

Slovenian GLIOBANK



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TRANS-GLIOMA
Progetto standard co-finanziato dal Fondo europeo di sviluppo regionale
Standardni projekt sofinancira Evropski sklad za regionalni razvoj

cells

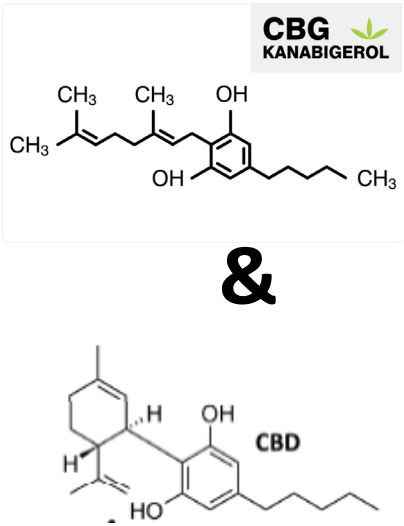
MDPI

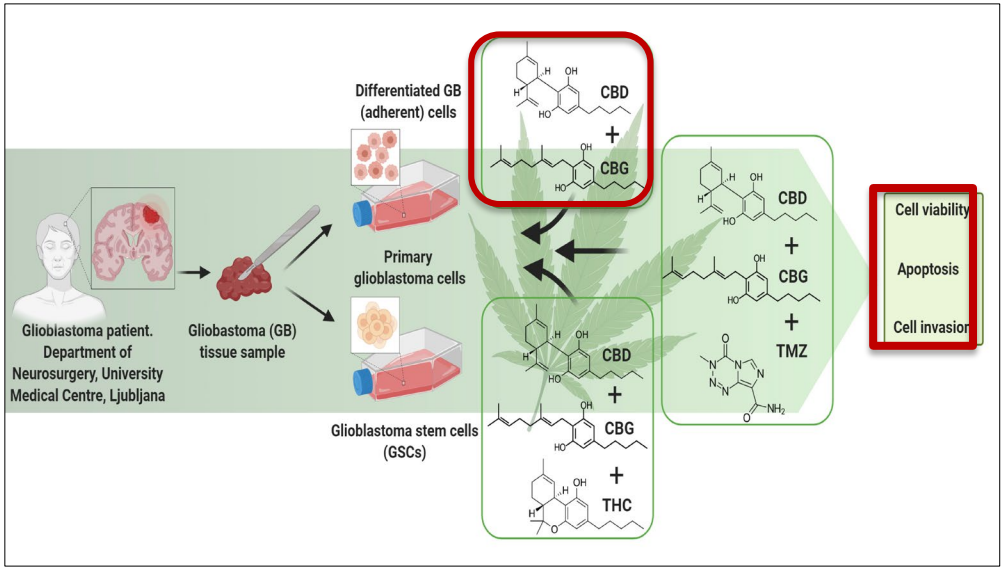
Article

Cannabigerol Is a Potential Therapeutic Agent in a Novel Combined Therapy for Glioblastoma

Tamara T. Lah [1,2,3,*], Metka Novak [1], Milagros A. Pena Almidon [4], Oliviero Marinelli [4], Barbara Žvar Baškovič [1], Bernarda Majc [1,3], Mateja Mlinar [1], Roman Bošnjak [5], Barbara Breznik [1], Roby Zomer [6] and Massimo Nabissi [4]

Summary I

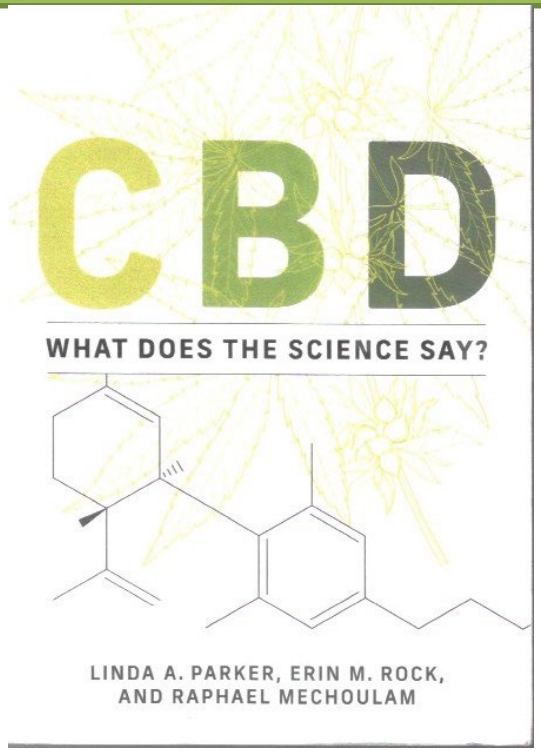


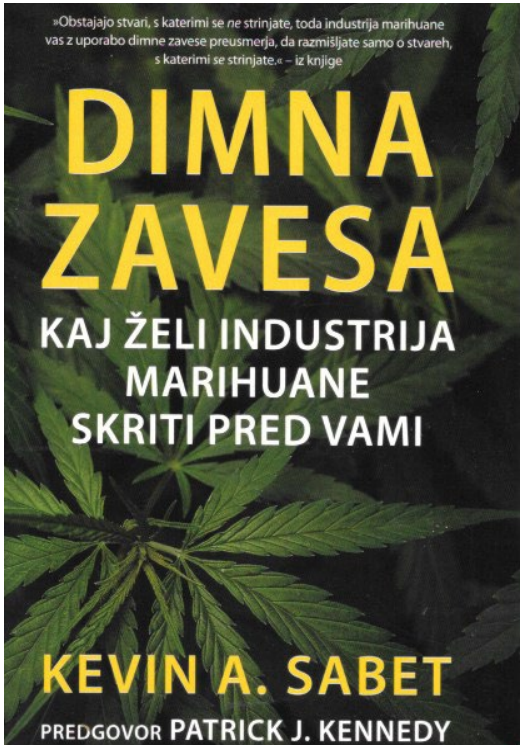


- CBG is non-psychoactive cannabinoid, first used against GBM
- CBD + CBG optimized combination is more toxic to GSCs than GBM cells!
- CBD + CBG & treatment induced GSC differentiation, inhibiting their resistance to IR&TMZ.
- CBG efficiently replaces THC cytotoxicity, induces GBM apoptosis and inhibits invasion.

THANK YOU FOR YOUR ATTENTION

HVALA ZA VAŠO POZORNOST





Zaključki

FDA odobreno je

- ✓ Zdravljenje simptomov – paliativno
- ✓ Anti-memetično delovanje zaradi kemoterapije
- ✓ Povečan apetit zaradi kaheksije
- ✓ Zmanjšanje bolečine, analgetik

E_

- **Ni odobreno: proti-tumorsko delovanje za:**
  - ❖ Zmanjšanje tumorske mase
  - ❖ Zmanjšanje invazije in metastaz
  - ❖ Splošno preživetje- adjuvantna terapija

Problemi

- ➢ **Tumorska heterogenost = personaliziran pristop!**
- ➢ Različne ravni CBN receptorjev
- ➢ Različni genetski podtipi istega raka
- ➢ Matične celice raka - odpornost?!P

- **Potrebne raziskave**
  - ✓ Sinergija ali kontra-indikacija z drugimi tretmaji?
  - ✓ Novi *in vitro* modeli in poskusi s humaniziranimi (GSO) živalmi

✓ **NOVI KANABINOIDI!**

Živalski poskusi in klinične študije

Neuro-Oncology Practice
4(3), 151–160, 2017 | doi:10.1093/nop/npw027 | Advance Access date 18 January 2017

The use of cannabis in supportive care and treatment of brain tumor

Rudolf Likar and Gerhard Nahler

Abteilung für Anästhesiologie und Intensivmedizin, Klinikum Klagenfurt am Wörthersee, Feschnigstrasse 11, 9020 Klagenfurt am Wörthersee (R.L.); CIS Clinical Investigation Support GmbH, Kaiserstrasse 43, 1070 Wien (G.N.)

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Pharmaceutical Medicine
https://doi.org/10.1007/s40290-022-00420-4

REVIEW ARTICLE

Cannabidiol and Other Phytocannabinoids as Cancer Therapeutics

Gerhard Nahler[1]

ANTICANCER RESEARCH *39*: 5797-5801 (2019)
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Concomitant Treatment of Malignant Brain Tumours With CBD – A Case Series and Review of the Literature

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Table 3 Results of THC and/or CBD in animal models of glioma

Treatment	Model	Results	Reference
THC peritumoral, 15 mg/kg/day for 14 days	Human U87MG astrocytoma, s.c. xenograft, mice	~50% reduced tumor growth, increased apoptosis;	66, 67;
CBD peritumoral, ~25 mg/kg/day, 5 days per week for 23 days	U87MG astrocytoma s.c. xenograft, mice	~70% regression at day 18, but ~50% regression at day 23/end	68, 59;
CBD intraperitoneal, 15 mg/kg, 5 days per week for 28 days	U251 glioblastoma cells, intracranial xenograft, mice	~95% decrease of tumor area; in 1/5 mice treated no tumor cells were observed in any of the brain regions analyzed	61;
THC peritumoral, 15 or 7.5 mg THC or 7.5 mg CBD/kg/day, or 7.5 THC + 7.5 mg CBD/kg/day over 14 days	Human glioma U87MG or T98G cells, s.c. xenograft, nude mice	15 mg THC much more effective than 7.5 mg; 7.5 mg CBD/kg was slightly more effective than 7.5 mg THC/kg; THC + CBD was most effective and similar to 15 mg THC /kg; tumor volume was stable on day 14 & 15; further enhancement by combination of cannabinoids with 5 mg TMZ/kg; T98G cells were resistant to THC or TMZ but not to their combination (CBD was not included)	70;
THC intratumoral, total dose 2.5 mg THC or 0.25 mg WIN-55,212-2 / rat over 7 days (~1.5–2 mg THC /kg/day)	Intracerebral C6 glioma model, rats (250-300 g b.w.)	THC was ineffective in 3/15 rats, tumor was completely eradicated in 3/15 rats, survival prolonged in 9 rats; WIN-55,212-2 was similarly effective	71;
CBD +THC (each ~2 mg/kg), intra-peritoneal, on day 9, 13, and 16 after tumor implantation; X-ray (4 Gy) on day 9; CBD-BDS (main: 63.5% CBD, 3.6% THC, 5.2% CBC) or THC-BDS (main: 65.4% THC, 0.4% CBD, 1.8% CBC)	Mouse glioma GL261 cells, orthotopically implanted	>85% decrease of tumor volume and of vascularization on day 21 (animals sacrificed); CBD + THC reduced progression, further enhanced by irradiation (stagnant tumor sizes throughout the experiment); X-rays alone had no dramatic effects	72;

Abbreviations: BDS, botanical drug substance; CBD, cannabidiol; THC, delta-9-tetrahydrocannabinol.

Relativno visoke doze CBD in THC so v živalih signifikantno znižale **med 54 % do 90 %** tumoorski volume ali ga celo eliminirale

Kemoterapija s Temazolomidom in obsevenje **sta učinke še povečala**.

Prenos uporabe **na človeka** je lahlo **drugačen** zaradi imunskega sistema in strukture možganov.

Paliativa se priporoča, ker deluje proti-bolečinsko, proti slabosti in anti-depresivno (CBD), a dvojna vloga THC- ali vzhičeno ali panično odvisno od koncentracije

Klinične študije na glioblastomu- majhno število bolnikov...

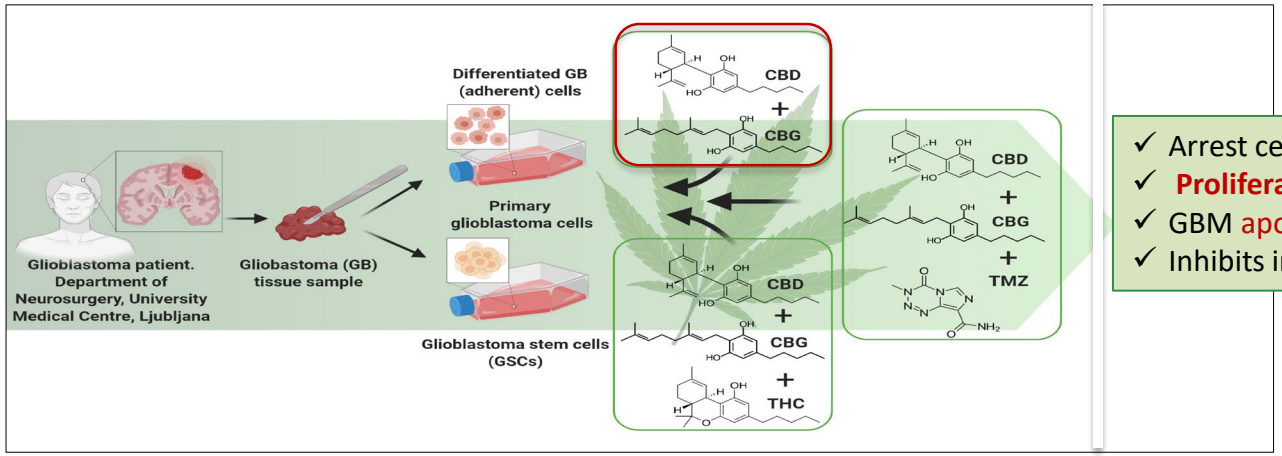
Table 3 Anticancer effects of cannabinergic preparations in man

Disease, no. of patients	Drug	Results	Reference
Glioblastoma multiforme, grade IV, 15 patients	Pure CBD (mainly 400 mg/day PO) in addition to standard therapy (maximum resection of the tumour followed by radiochemotherapy)	Of 15 patients, 7 (46.7%) survived for at least 24 months, and 4 (26.7%) survived for at least 36 months. This is more than twice as long as has been previously reported in the literature. The mean overall survival is currently 24.2 months (median 21 months) and the 1-year survival rate was 87%	[77]
Glioblastoma multiforme , 9 patients (6 were grade IV)	Pure CBD (mainly 400 mg/day PO) in addition to standard therapy (maximum resection of the tumour followed by radiochemotherapy)	Of 9 patients, only 1 had died; mean survival time was 22.3 months (range 7–47 months)	[78]
Brain tumours, 2 patients	Pure CBD with <0.3% THC (case 1: glioblastoma multiforme: 300–450 mg/day; case 2: oligodendroglioma grade III: 100–200 mg/day following resection and chemoradiotherapy	Treatment reduced oedema and inflammation and induced remission (MRI)	[79]
Pilocytic astrocytoma, WHO grade 1; 2 females: case 1 was aged 11 years and case 2 was aged 13 years at diagnosis	Consumption of cannabis of unknown composition via inhalation, on average 3 × weekly during the last 3 years of follow-up	The volume of tumour remnant was calculated using VOXAR volumetric software, and was found to be 1.28 cm^3 at 9 months and 0.27 cm^3 at 6 years post-surgery in the first case, and 3.3 cm^3 at 18 months and 0.28 cm^3 at 6 years postoperatively in the second case; the regular use of cannabis coincided with the time course of radiological tumour regression	[126]
Glioblastoma multiforme, 12 patients with nabiximols, 9 with placebo	Nabiximols (CBD:THC ≃ 1:1, maximum 32.4 mg THC + 30.0 mg CBD) following standard chemoradiotherapy treatment with dose-intense TMZ, as described by Stupp et al. [88]	Median survival in the placebo group was >550 days in the CBD:THC treatment group (not significant) and 369 days in the placebo group; 1-year survival was 83% and 56% in the CBD:THC and placebo groups, respectively ($p = 0.042$)	[89]
Glioblastoma multiforme, 9 patients with progressive tumours	THC solution, 20–40 mg at Day 1, increased for 2–5 days up to 80–180 mg/day, infused into the resection cavity, starting at Days 3–6 after surgery; median duration of an administration cycle was 10 days	5 patients received more than 1 cycle. In 3 of these 5 patients, a temporary reduction of tumour proliferation was observed. Median survival of the cohort from the beginning of THC administration was 24 weeks	[87]

Cannabigerol - neglected cannabinoid??

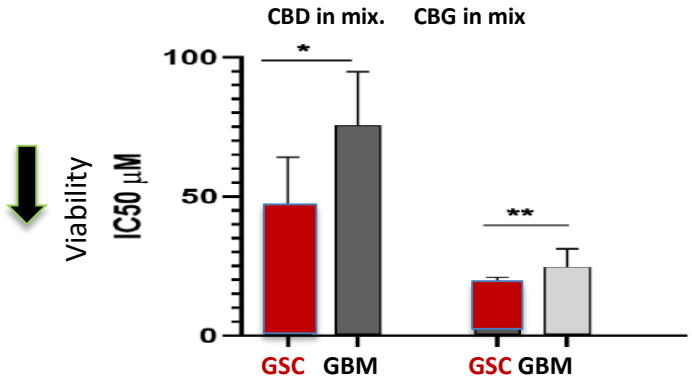
NOVEL cannabinoid CBG effect replacing/ omitting THC on GSCs

Role of cannabinoid receptors ?



- ✓ Arrest cell cycle G0/G1 phase
- ✓ **Proliferation**
- ✓ GBM apoptosis
- ✓ Inhibits invasion

CBG efficiently replaces THC cytotoxicity



More effective inhibition of GSC proliferation was shown at certain CBD:CBG combination!

Summary

- **CBG** represents a new, as yet unexplored possibility of adjuvant treatment of GBM patients, as in some other cancers –similar mechanism as in cholangiocarcinoma *

- **CBG** alone, and added to CBD, most efficiently **eliminates therapy-resistant GBM stem cells,** first by **differentiation upon biding to GPR55 and/or TRPV1 receptors**, followed by triggering apoptosis, possibly in coordinated CB1-activatiin signaling.

- **THC has no added value** in combination therapy for glioblastoma, suggesting that this **psychotropic cannabinoid** should be replaced by **CBG in future clinical studies**!

- **GPR55 and TRPV1 are highly expressed in GSCs and co-related to selective, yet different sets of stemness genes**, The inhibition of their signaling differentiate **GSC to become sensitive to therapy**

- **Cannabinoid receptors level in individual patients do not predict their response to cannabinoids** *