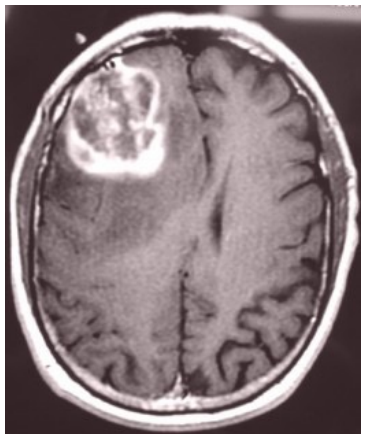


- **Glioblastomas** are fast growing highly heterogeneous primary brain tumor for which therapeutic options are very limited.

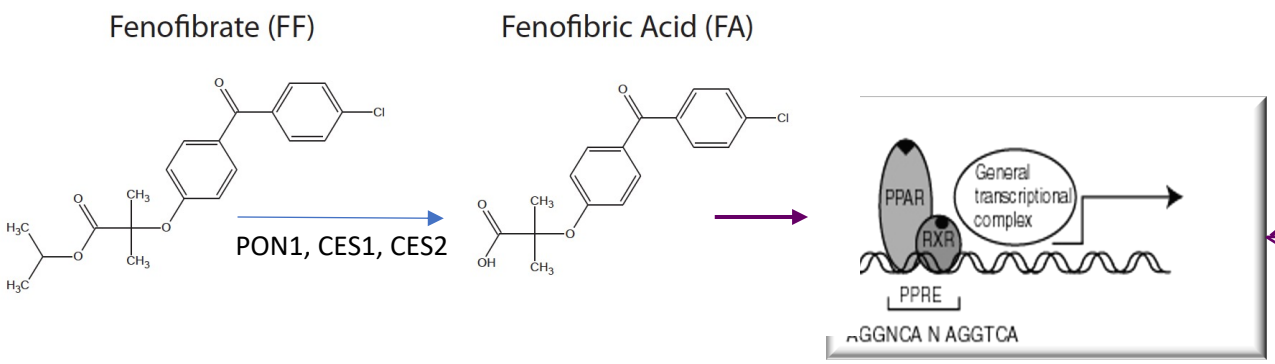
- **On average, about 12,000 new Glioblastoma cases** are diagnosed in the US every year. Median survival time is between 14-16 months for treated and 10-12 months for untreated Glioblastomas. Five-year survival rate is below 8%.

- **Therapeutic options** for newly diagnosed Glioblastoma include aggressive surgery + extensive but highly focused radiation followed by chemotherapy [limited to Temozolomide (TMZ). However, Glioblastomas quickly develop TMZ resistance and recurrent tumors are practically incurable.

- **Clinical trials for recurrent Glioblastomas include** gene- and viro-, and more recently immuno- therapies [immune checkpoint inhibitors, tumor vaccines, and chimeric antigen receptor T cell (CAR T) therapies], which all were extensively tested but failed …..
- In addition, some encouraging results are coming from targeting energy metabolism, (ketogenic diet, Metformin, **Fenofibrate ???**).

What is fenofibrate (FF)?

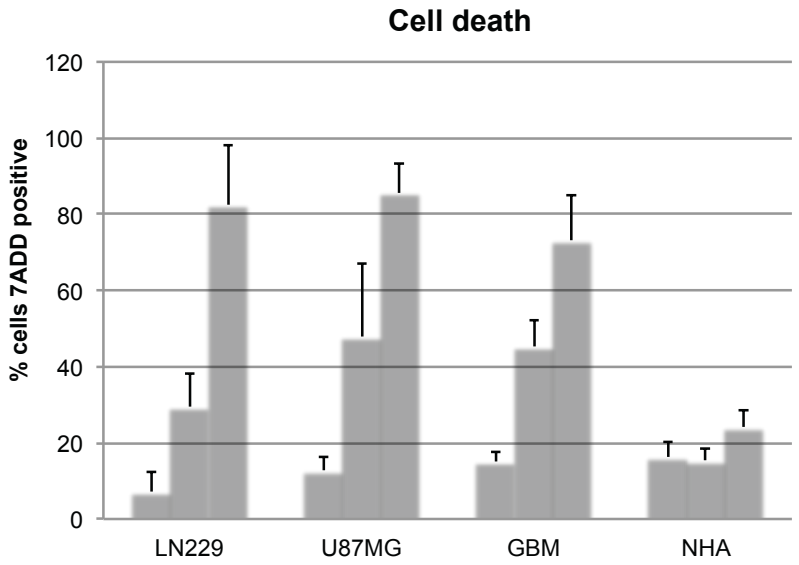
- FF is a member of the fibrate family of anti-hyperlipidemic agents, and is commonly used to combat high cholesterol in patients;
- FF is a pro-drug, which is converted to fenofibric acid (FA) by blood and tissue esterases;
- FA activates nuclear receptor, PPARα (Peroxisome Proliferator Activated Receptor-alpha), stimulating fatty acid metabolism and attenuating glycolysis;
- FF has low systemic toxicity.



In Cancer Cells which fallow Warburg Effect FF triggers severe deficit in intracellular ATP followed by apoptosis

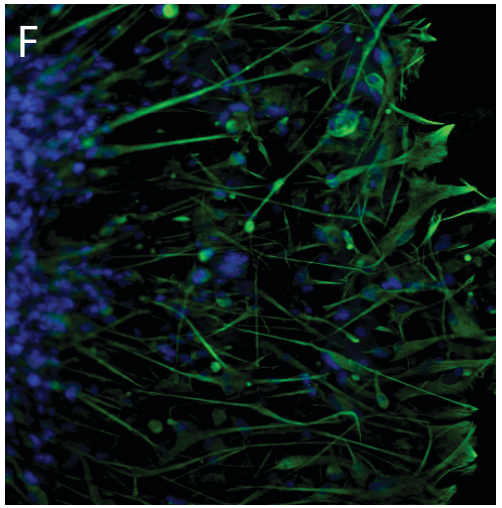
In Normal tissues FF improves energy metabolism of the cell and lowers cholesterol and triglycerides levels in blood

FF is highly cytotoxic to all tested glioblastoma cells triggering delayed but extensive cell death.



Patient-derived GBM spheres (GFAP-positive)

control
FF25
FF50



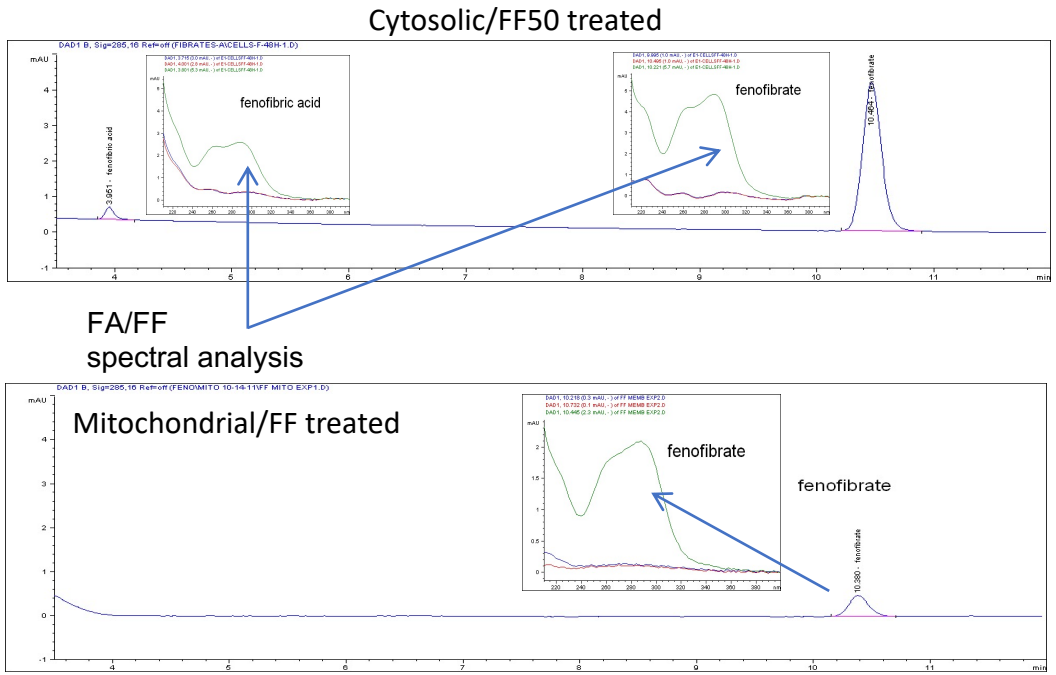
Wilk A, Wyczechowska D, Zapata A, Dean M, Mullinax J, Marrero L, Parsons C, Peruzzi F, Culicchia F, Ochoa A, Grabacka M, **Reiss K**. Molecular mechanisms of fenofibrate-induced metabolic catastrophe and glioblastoma cell death. Mol Cell Biol. 2015 Jan;35(1):182-98. doi: 10.1128/MCB.00562-14. Epub 2014 Oct 20. PubMed PMID: 25332241; PubMed Central PMCID: PMC4295376.

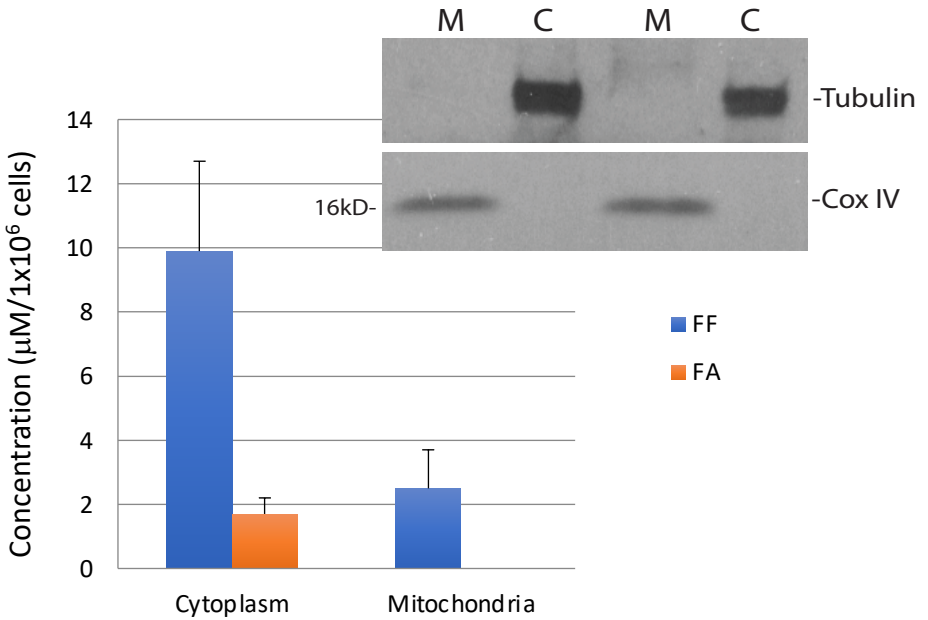
Some of the effects mediated by fenofibrate are difficult to be explained solely by the PPAR-α mechanism:

1. **FA is practically ineffective in killing cancer cells *in vitro*.**
2. **FF (ester) has cholesterol-like effects on biological membranes (rigidifies biological membranes);**
3. **FF inhibits respiration of isolated cardiac and liver mitochondria;**

Fenofibrate accumulates in the mitochondrial membrane fraction

HPLC-based measurement of FF content



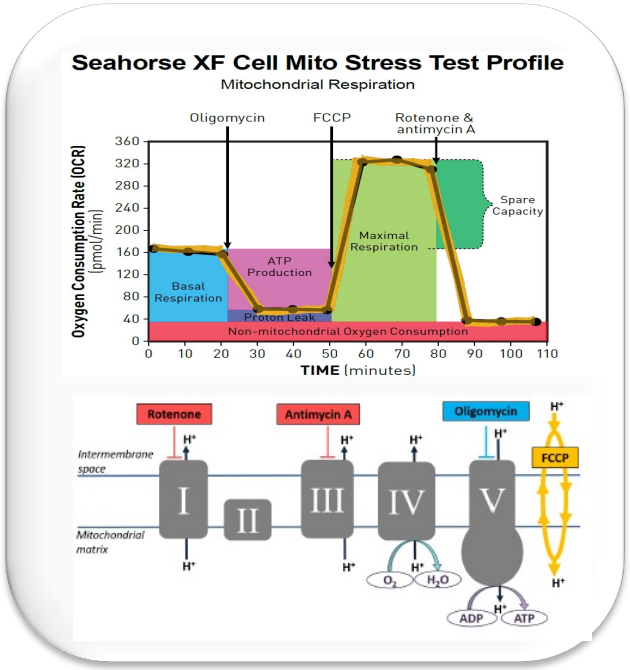


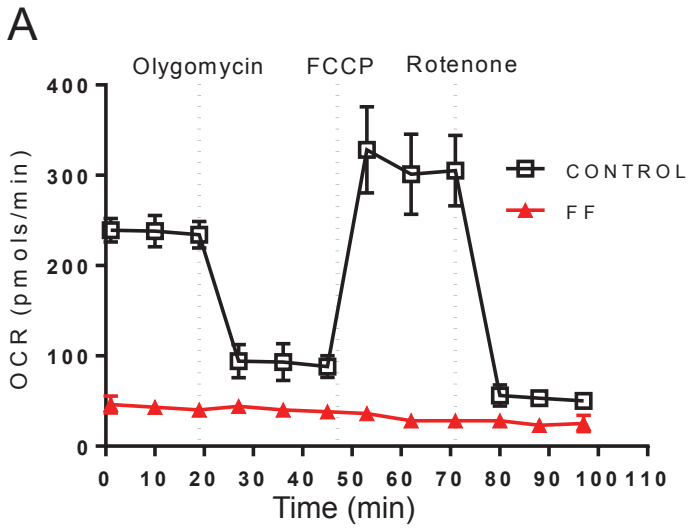
Wilk A, Wyczechowska D, Zapata A, Dean M, Mullinax J, Marrero L, Parsons C, Peruzzi F, Culicchia F, Ochoa A, Grabacka M, **Reiss K**. Molecular mechanisms of fenofibrate-induced metabolic catastrophe and glioblastoma cell death. Mol Cell Biol. 2015 Jan;35(1):182-98. doi: 10.1128/MCB.00562-14. Epub 2014 Oct 20. PubMed PMID: 25332241; PubMed Central PMCID: PMC4295376.

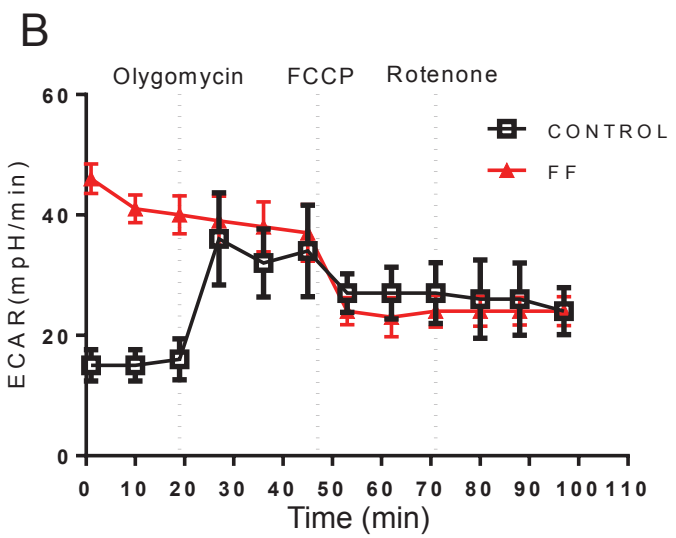
Fenofibrate inhibits mitochondrial respiration in LN229 glioblastoma cells

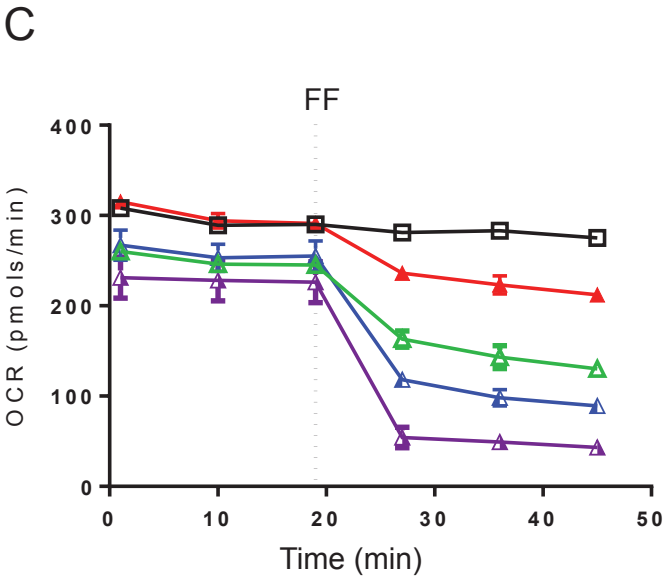
(mitochondrial stress experiment)

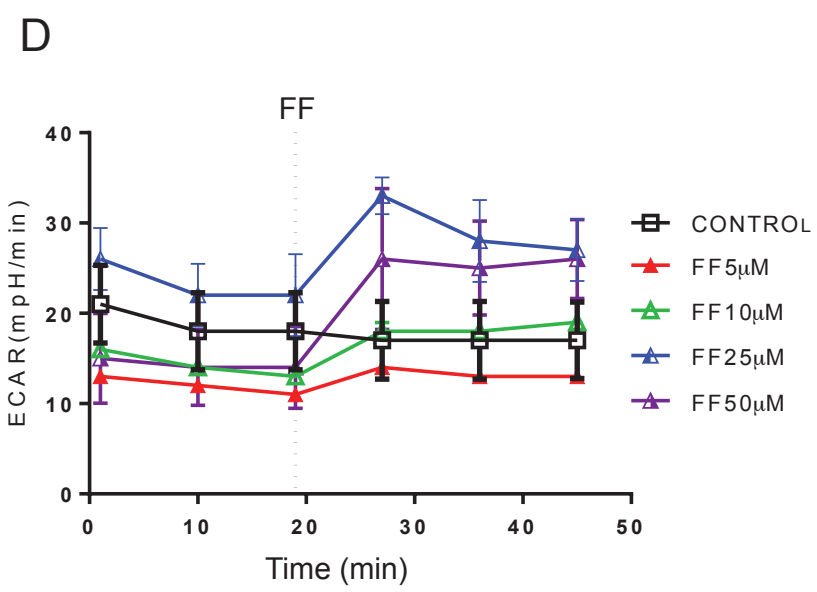
Extracellular Flux (XF) Analyzer – real time measurements of multiple metabolic parameters





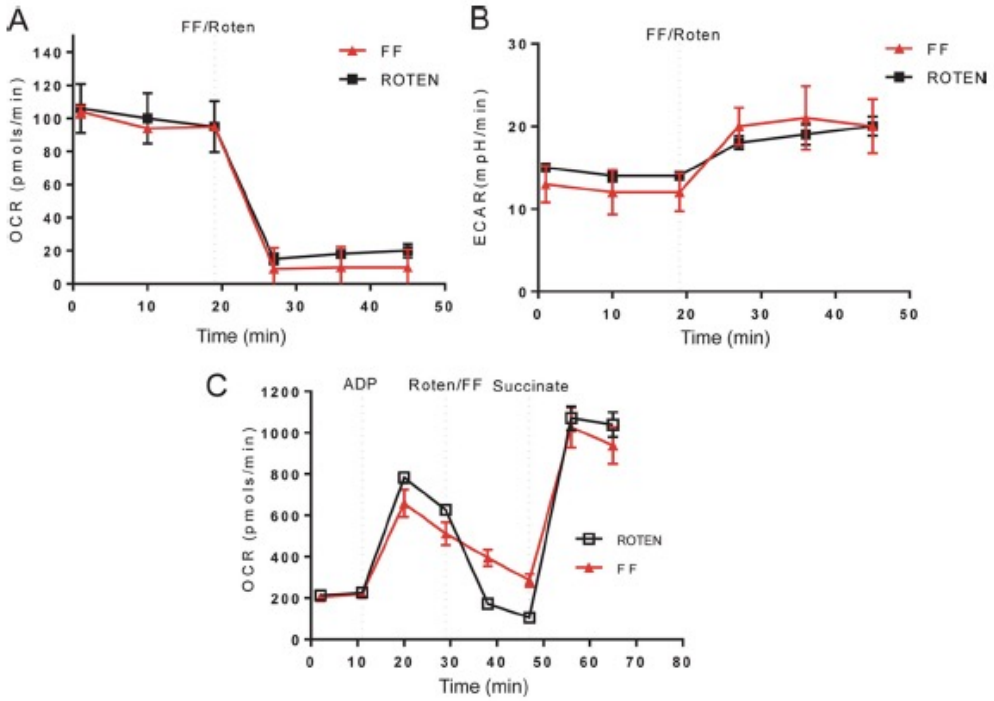




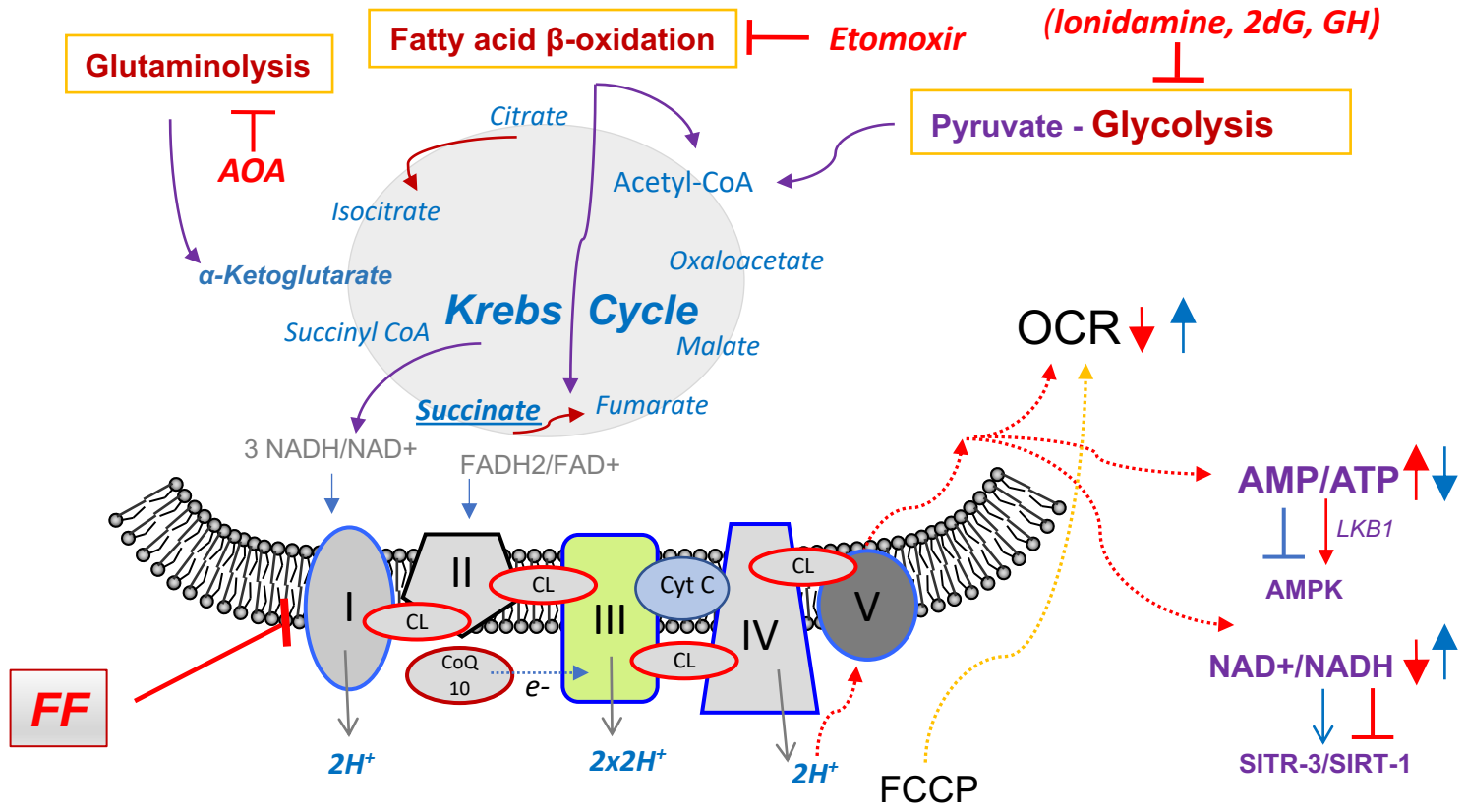


F

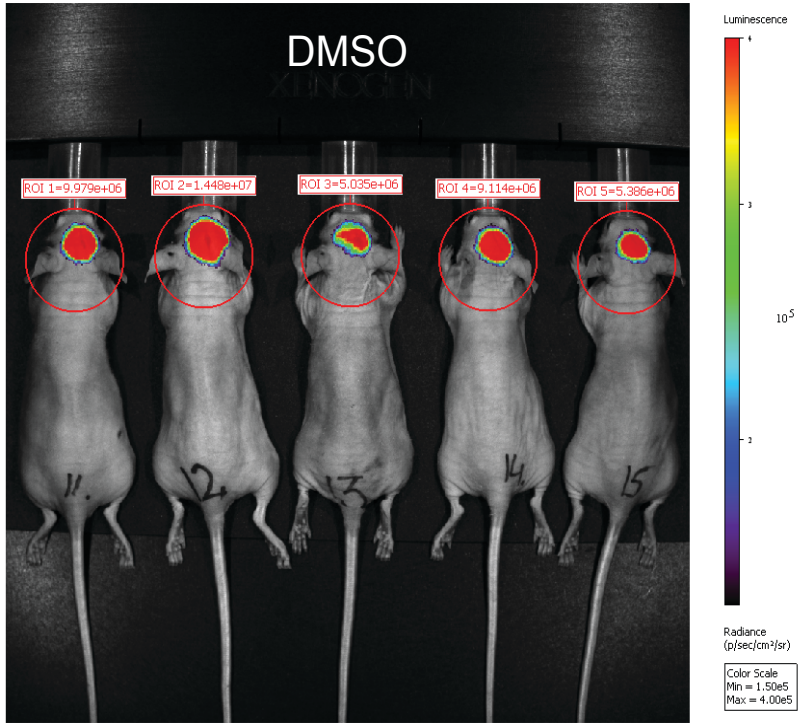
Fenofibrate inhibits mitochondrial respiration at the level of Complex I of the ETC

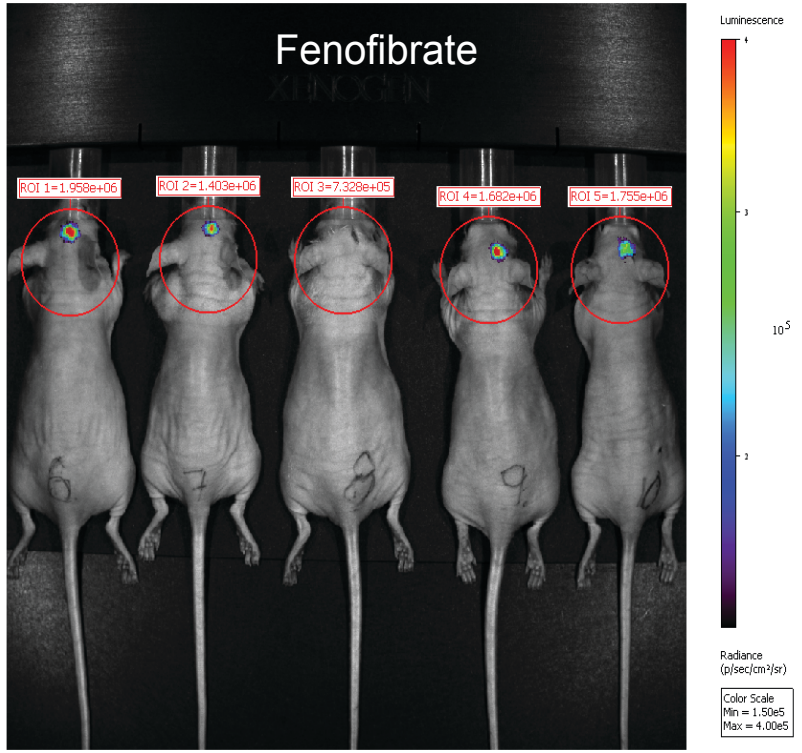


Isolated mitochondria
Succinate + ADP



However, fenofibrate (FF) is unstable in vivo, and does not cross BBB, which lowers its anti-glioblastoma potential

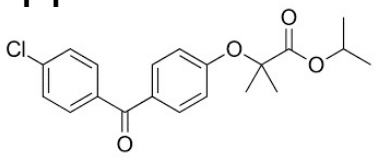




Measurement of intracranial tumor growth after intracranial injection of FF. **U-87MG–luc** cells (1 x10^5) were implanted into the brains of immunodeficient mice (Foxn1nu; Harlan Laboratories). Tumor-bearing mice were subsequently treated with 5 µl of DMSO (control) or 5 µl of 1mM FF in DMSO by injection at the same place where the tumor cells were implanted using CED system **(3 days after initial cell delivery- very small tumors)**. Two weeks later, bioluminescence imaging was performed with Xenogen IVIS 200 system.

Wilk A, Wyczechowska D, Zapata A, Dean M, Mullinax J, Marrero L, Parsons C, Peruzzi F, Culicchia F, Ochoa A, Grabacka M, **Reiss K**. Molecular mechanisms of fenofibrate-induced metabolic catastrophe and glioblastoma cell death. Mol Cell Biol. 2015 Jan;35(1):182-98. doi: 10.1128/MCB.00562-14. Epub 2014 Oct 20. PubMed PMID: 25332241; PubMed Central PMCID: PMC4295376.

FF



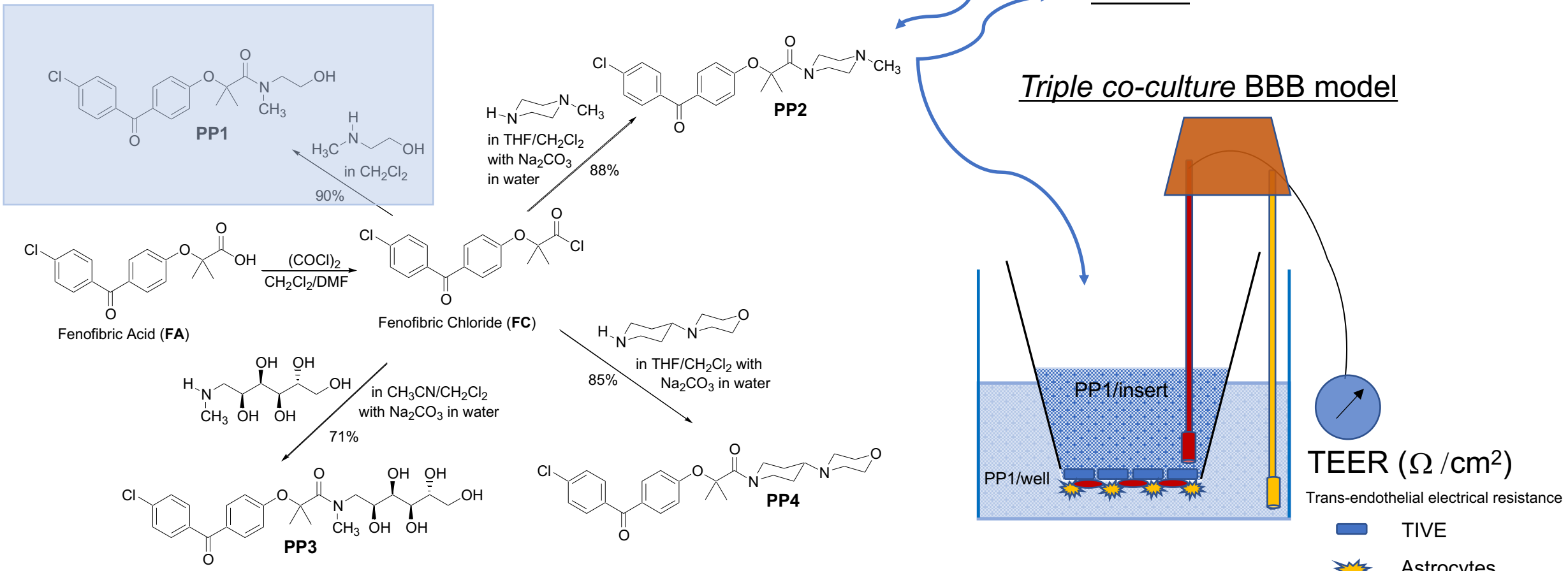
Derivatives of FF: PP1, PP2, PP3, PP4...

*Synthetized by **Dr. Branko Jursic** from UNO*

In silico calculation of CNS-MPO

Multiparameter optimization algorithm (0 – 6)

In vitro cytotoxicity and calculation of IC50



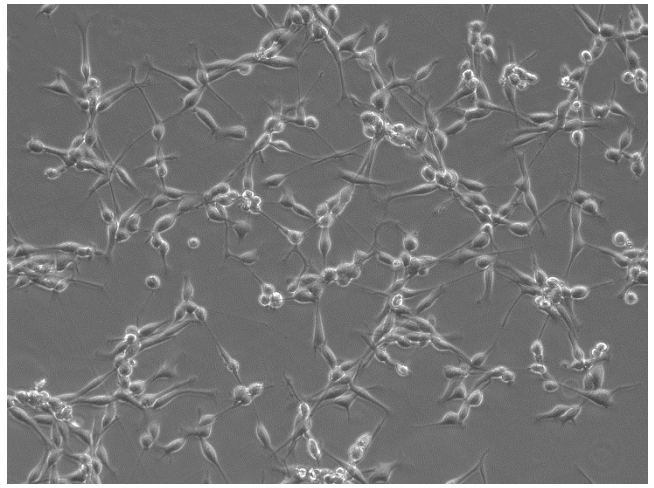
Triple co-culture BBB model

We have synthesized over 200 PP compounds, built on the common structure of benzyl-phenoxy-acetamide (BPA) present in a common lipid-lowering drug, fenofibrate.

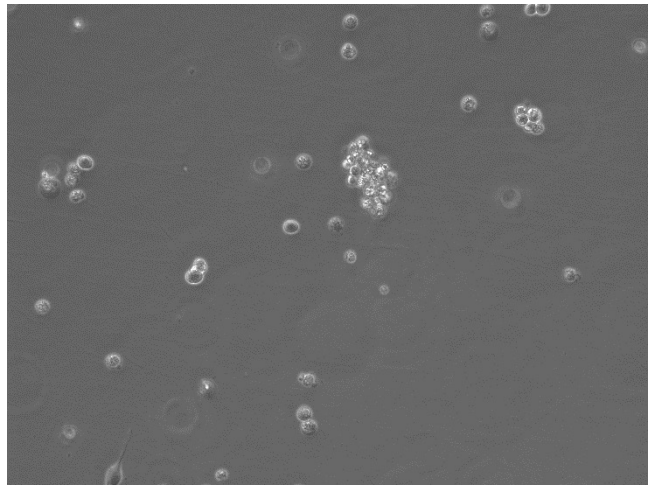
PP1 effects on Patient-derived Glioblastoma cells (GBM12TdT)

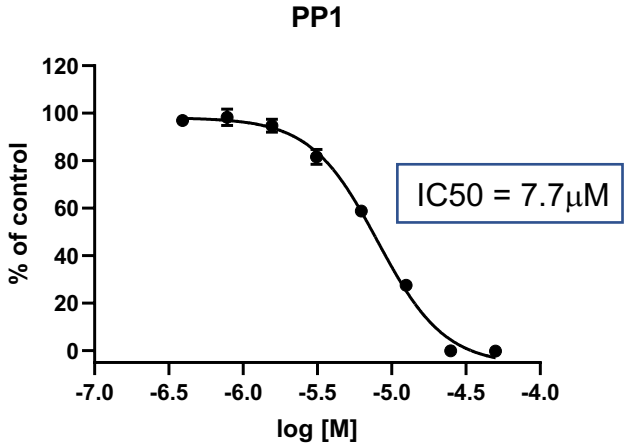
Adherent culture on laminin

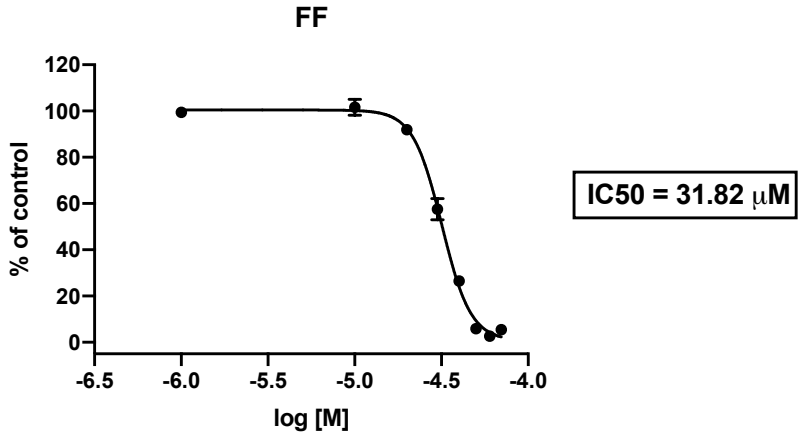
DMSO



PP1

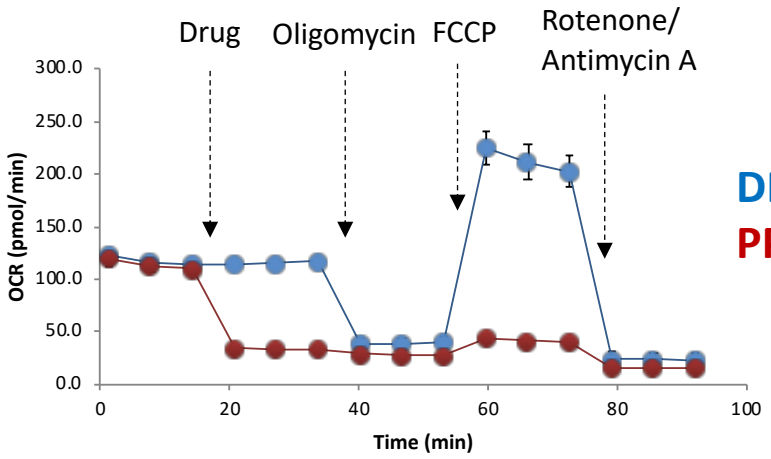






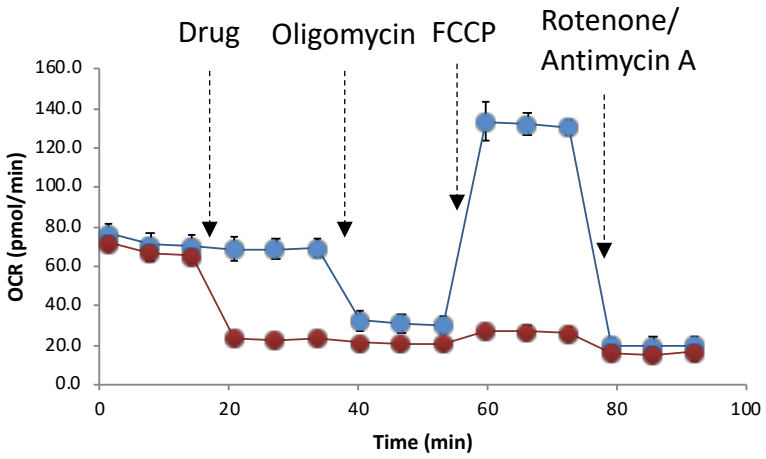
PP1 inhibits Mitochondrial respiration

LN229

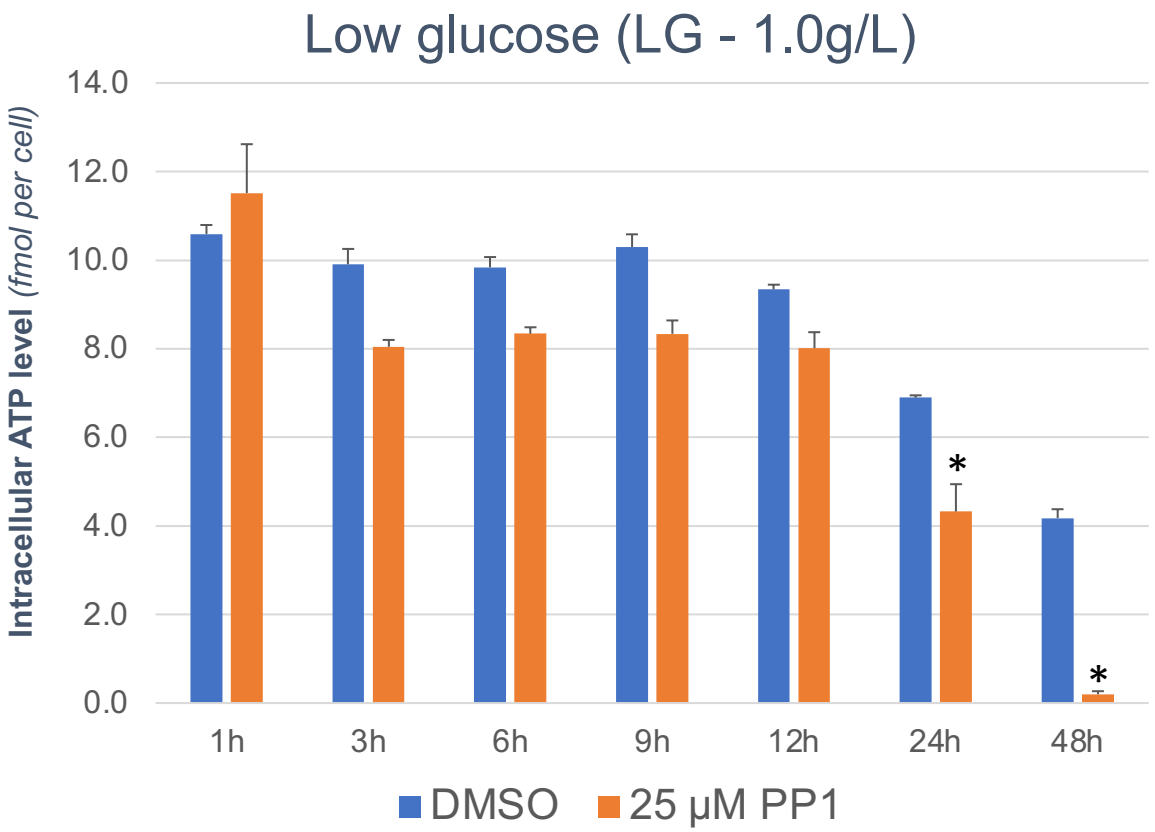


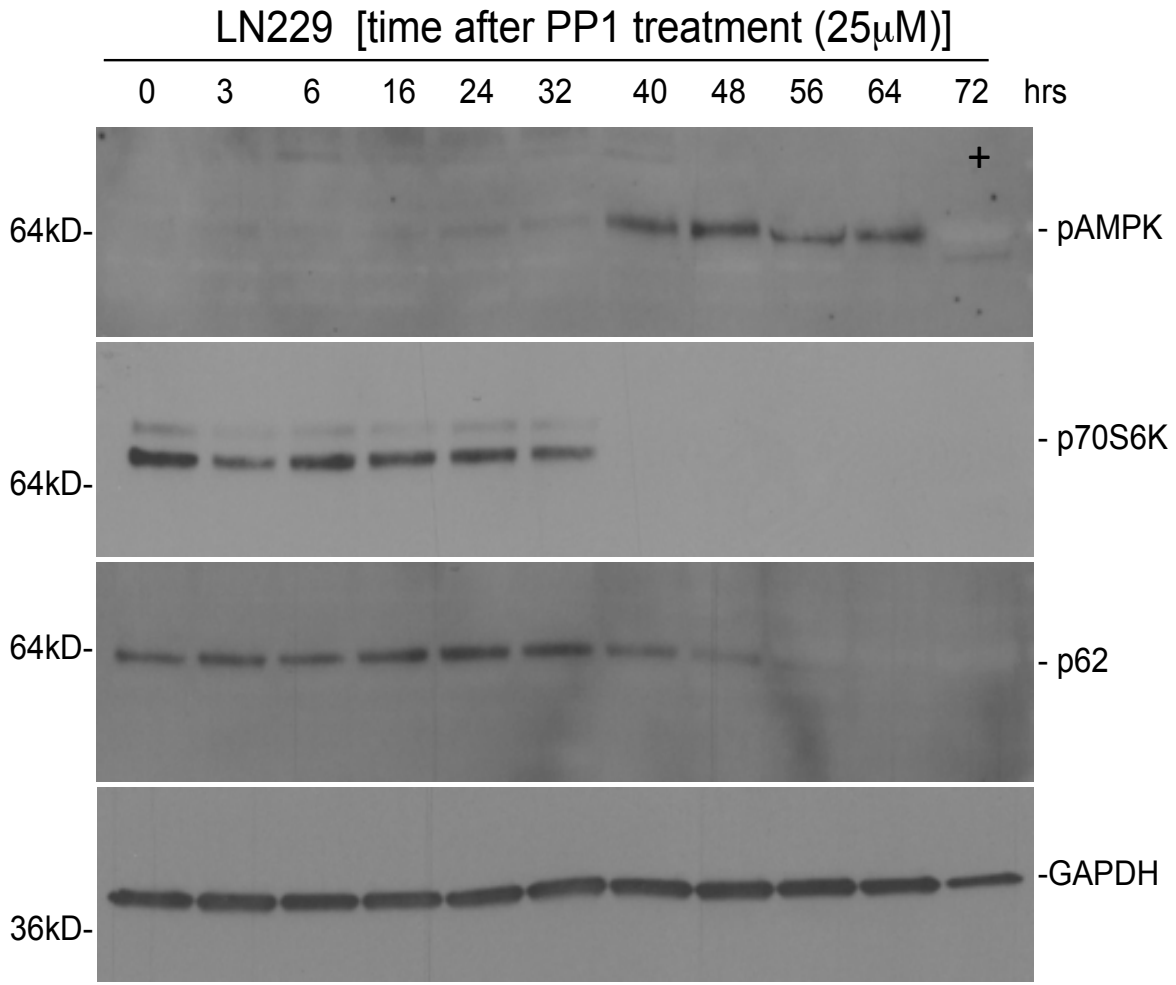
DMSO
PP1

GBM12TdT



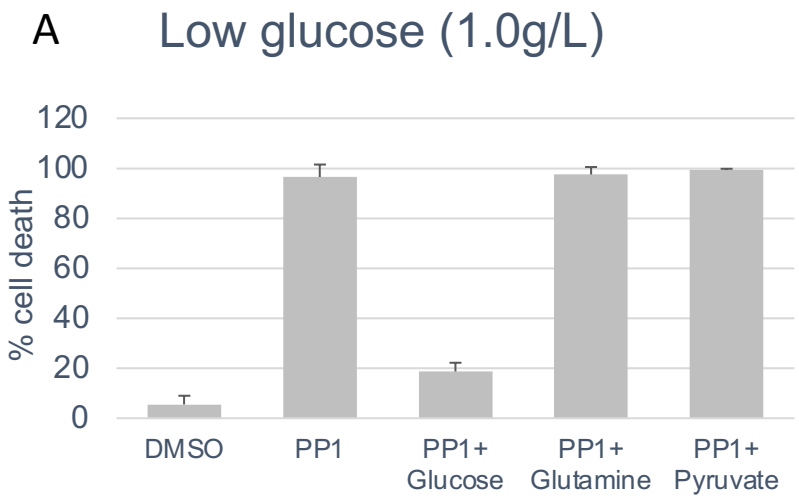
PP1 –triggers inhibition of intracellular ATP and activates AMPK-mediated signaling responses





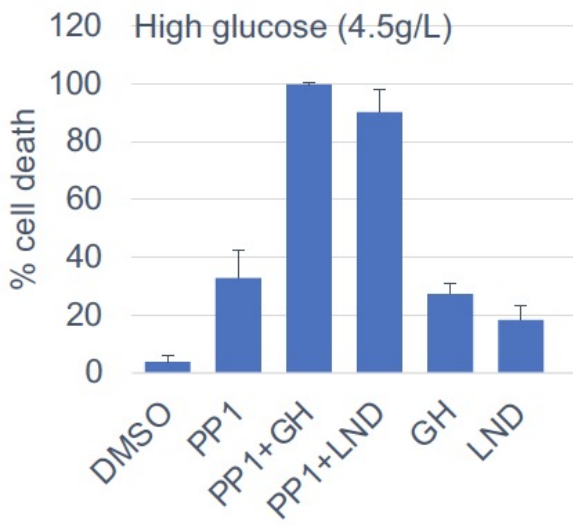
PP1-induced anti-cancer effects are glucose dependent

A

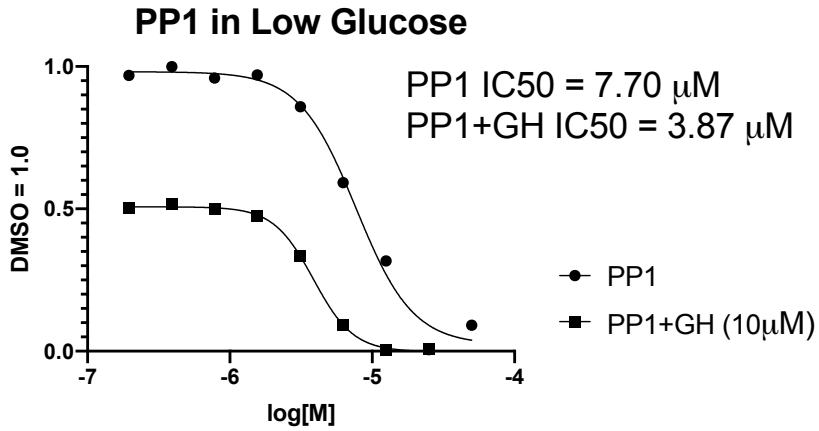


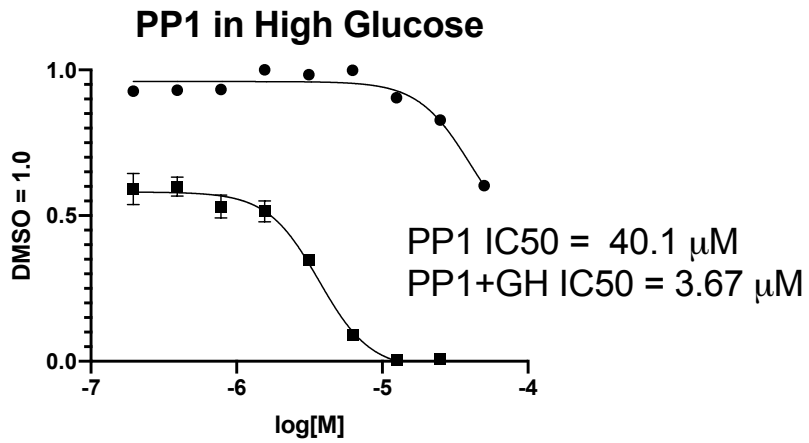
B

**GH: Gnetin-H* – *resveratrol trimer – inhibitor of glycolysis*



C

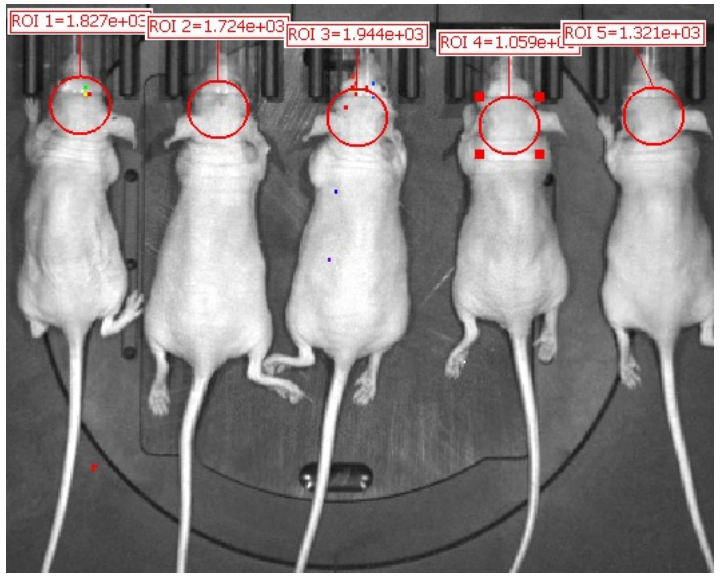




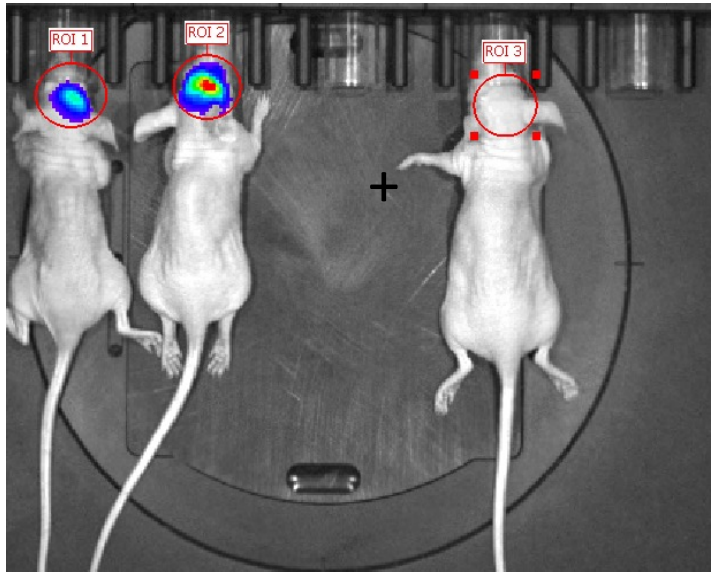
However, GH does not cross the BBB!!!

Proof of concept efficacy study

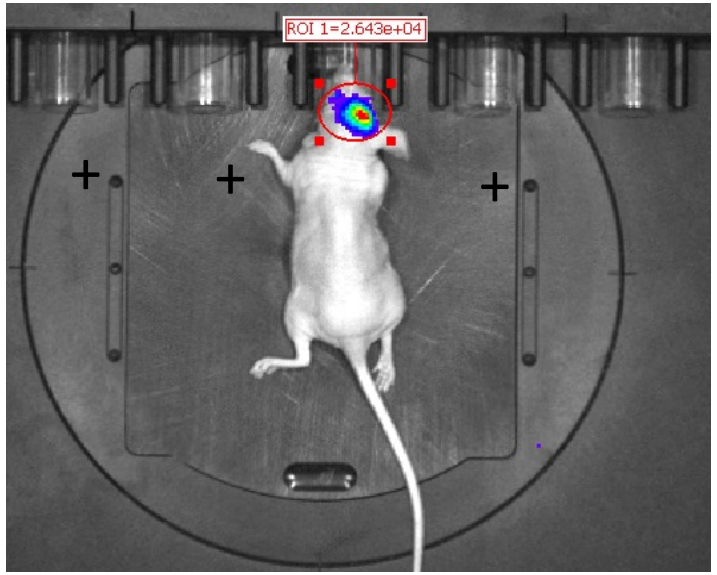
PP1+GH



PP1



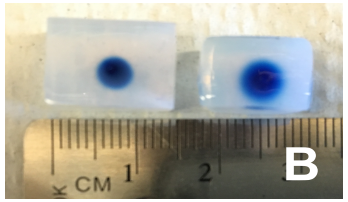
DMSO

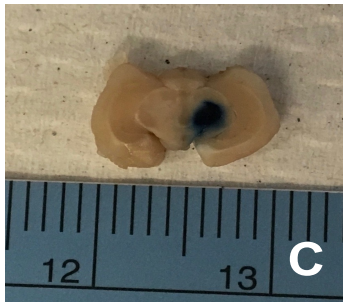


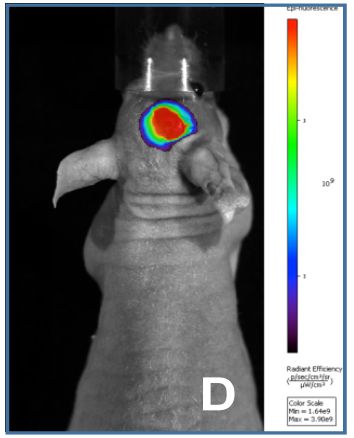
Proof of concept efficacy study. Mice were injected with 5μl of $1x10^5$ of GBM12 patient derived cells in the medium containing 25μM PP1 +/- 10μM GH. 5μl of medium containing 5% DMOS was used as control. Images were taken 6 weeks following initial cell implantation. "+" indicates mice which died before reaching 6 weeks after cell implantation (euthanized because of reaching endpoint criteria).

At this point we have three potential therapeutic options: **1)** intracranial drug delivery supported by CED; **2)** find inhibitor/s of glycolysis, which are capable of synergizing with PP compounds and can penetrate BBB; and/or **3)** keep looking for new PP compound/s with better BBB penetration, lower IC50, and glucose-independent cytotoxicity.

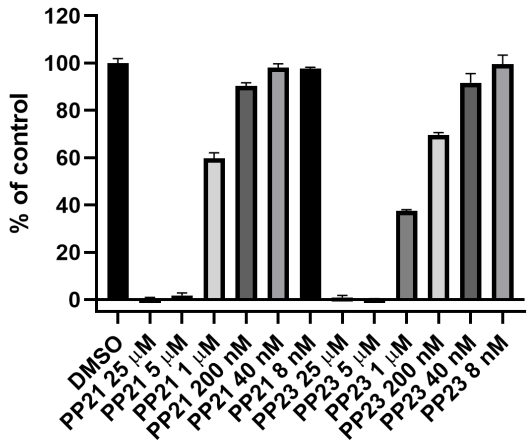
CED-based system of intracranial cell and drug delivery

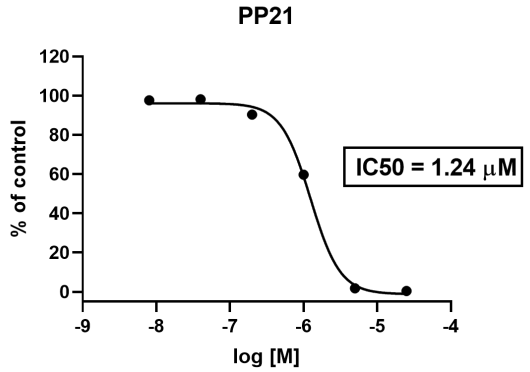




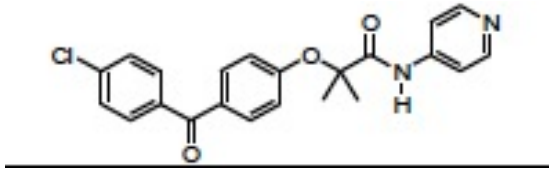


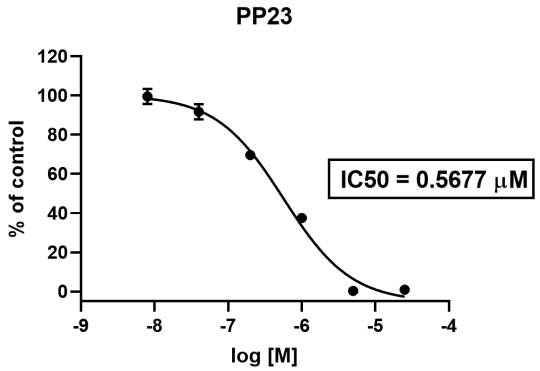
New benzyl-phenoxy-acetamide (BPA) variants: PP21 and PP23



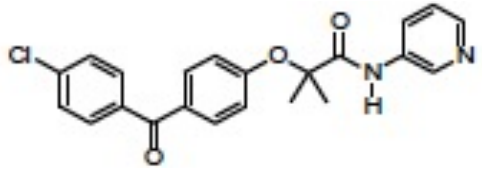


PP21/LG	
	1.17
	1.24
	1.24
	2.19
	1.92
avg	**1.552**
std	0.46986168



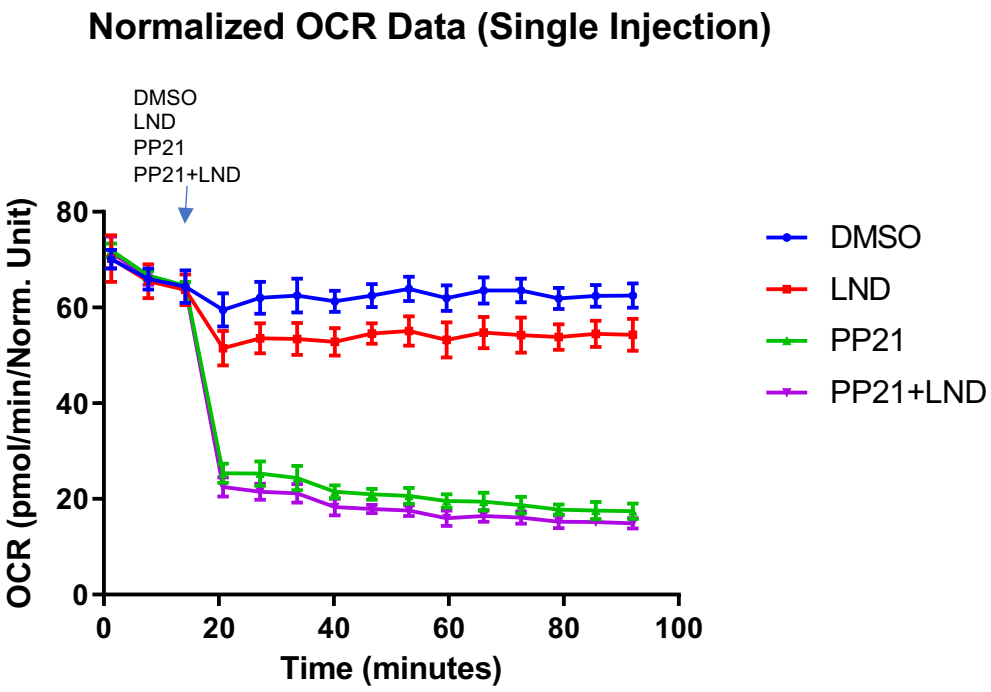


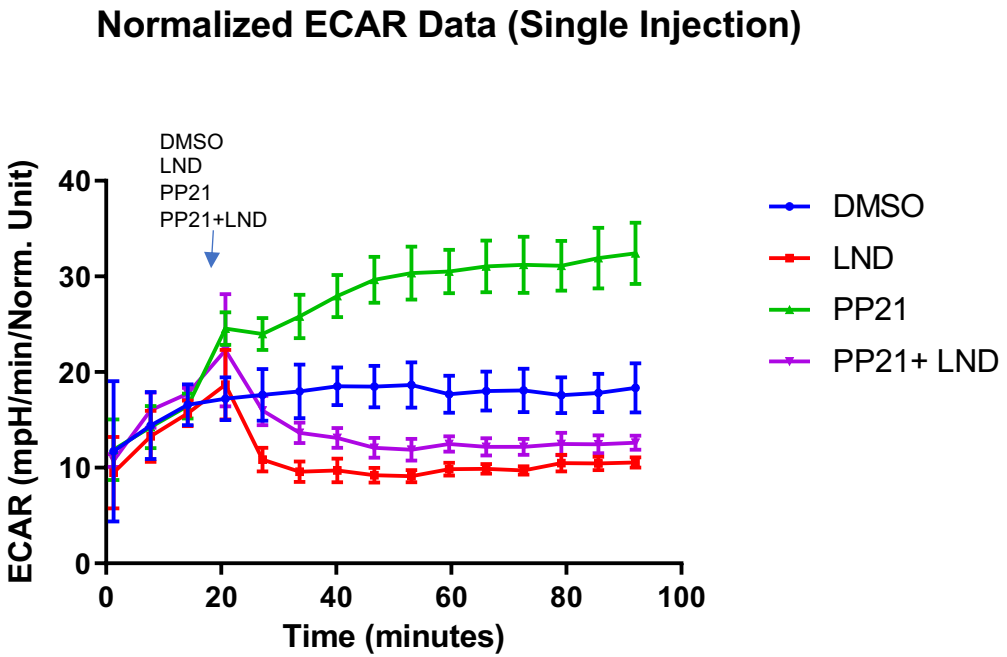
PP23/LG	
	0.59
	0.56
	0.57
avg	**0.57333333**
std	0.01527525



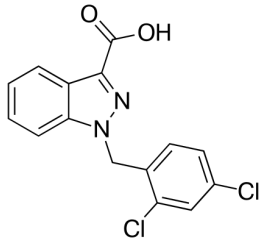
PP21/23 CNS-MPO =3.71

Is LND capable of improving PP21 anti glioblastoma efficacy??

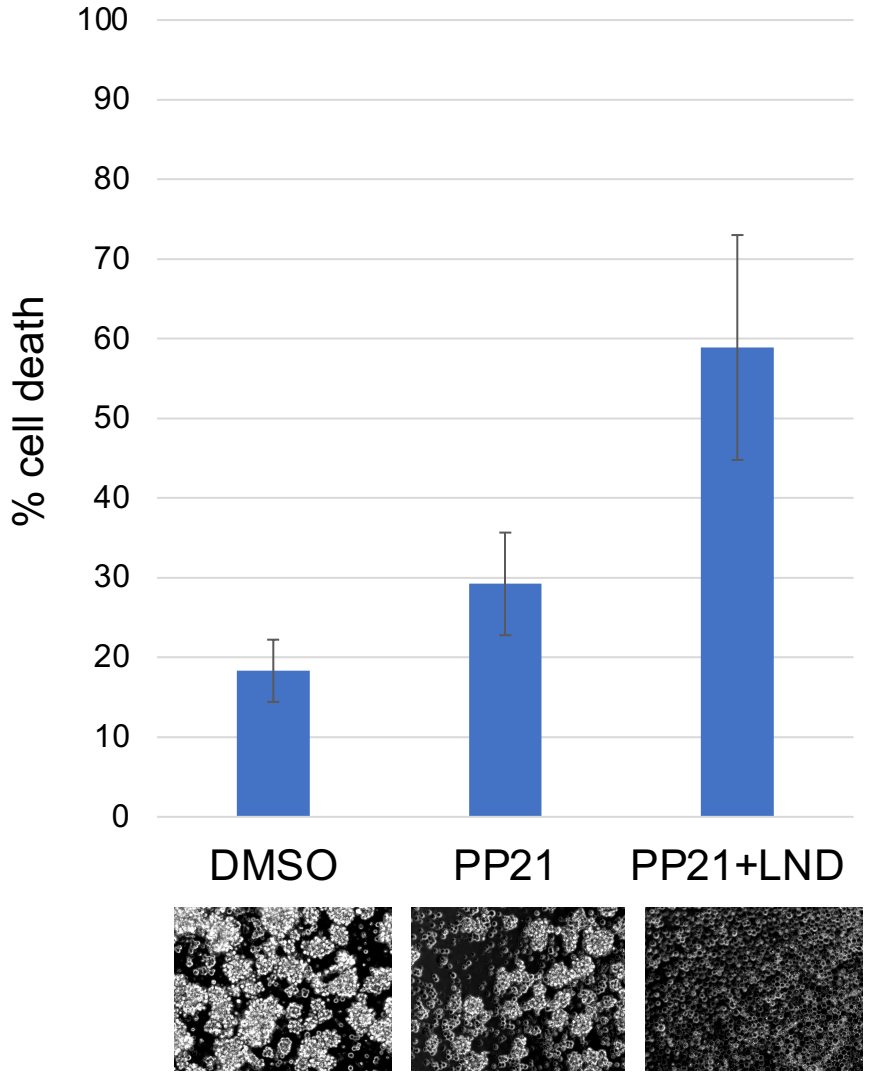


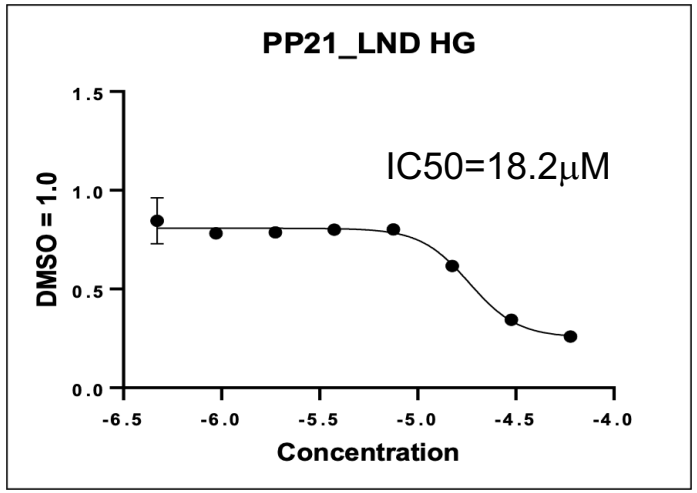


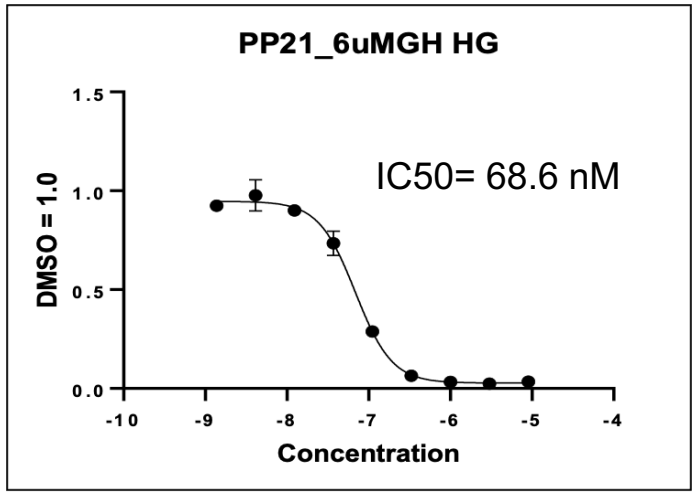
CNS MPO for LND=5.05

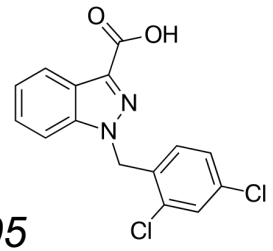


GBM12 gliosphere cultures in condition promoting growth of glioma stem cells



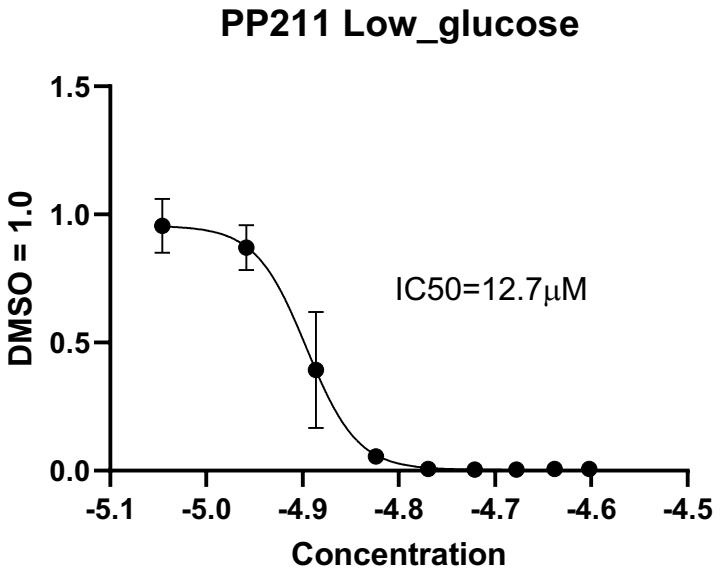


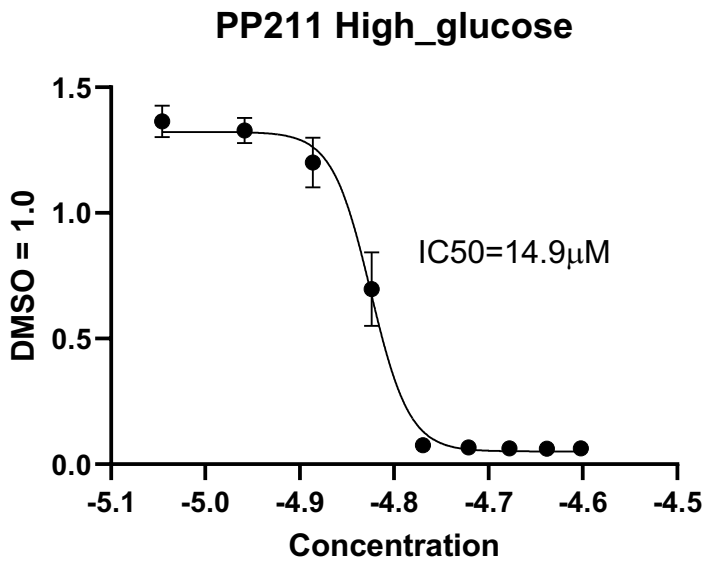


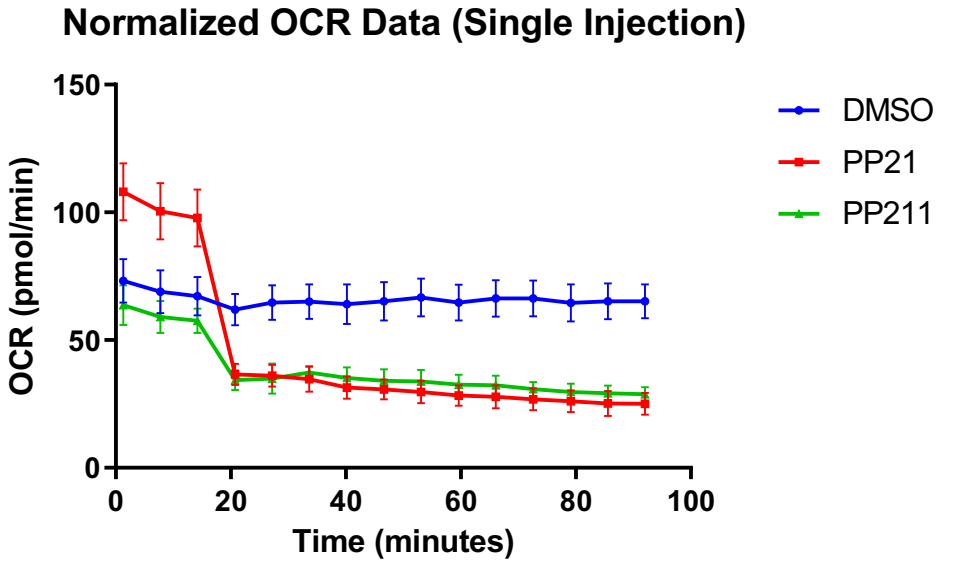


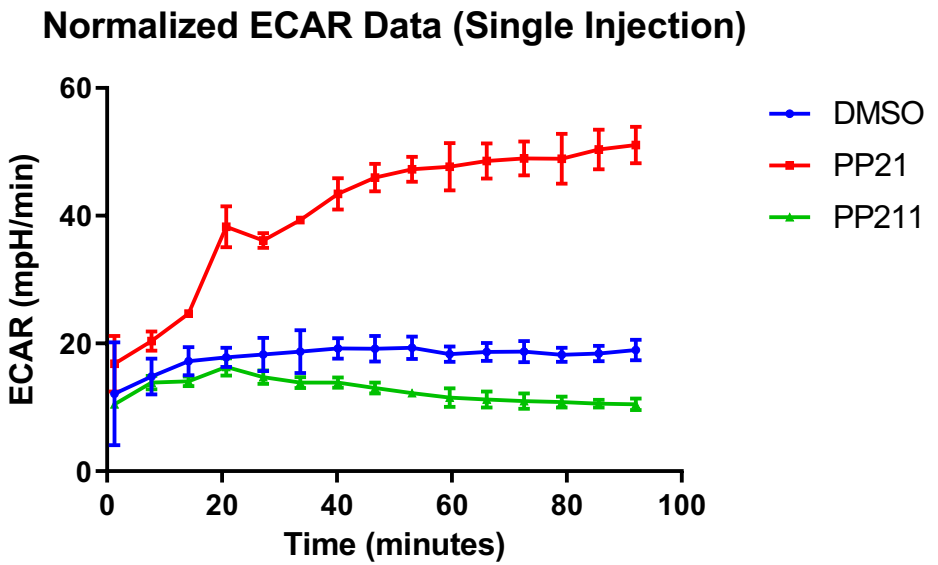
CNS MPO for LND=5.05

New drug candidate, PP211, with highly promising properties



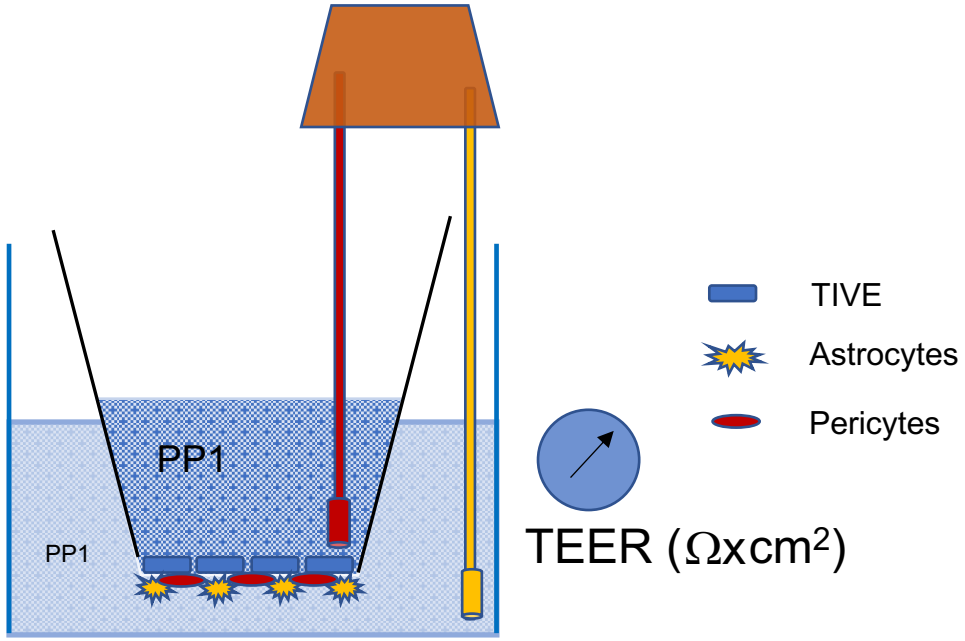


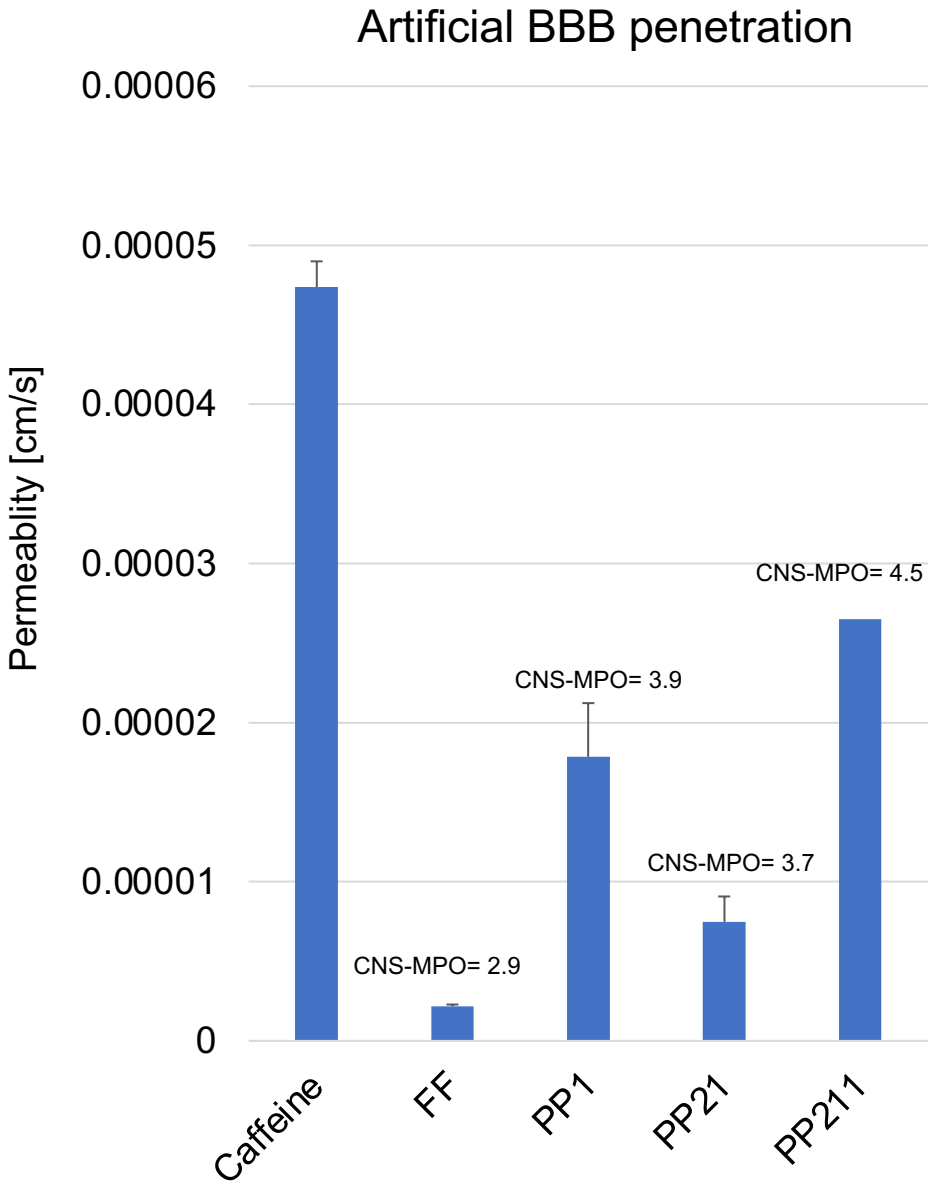




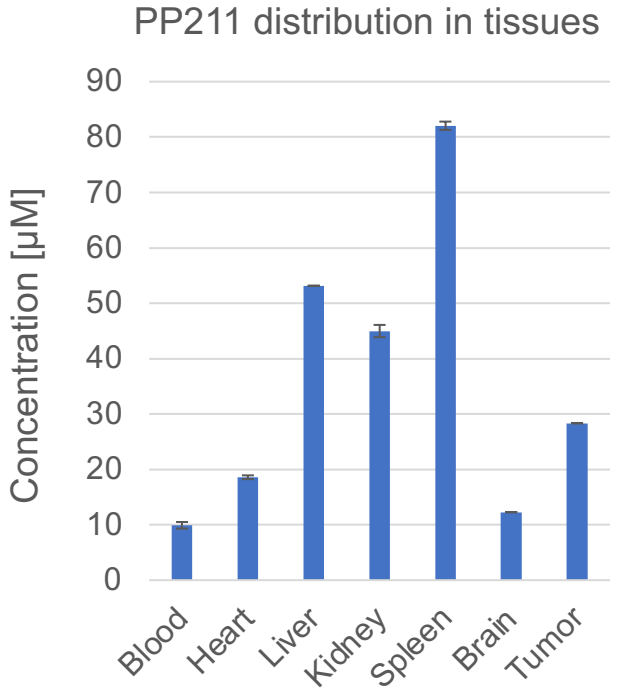
Triple co-culture BBB model membrane

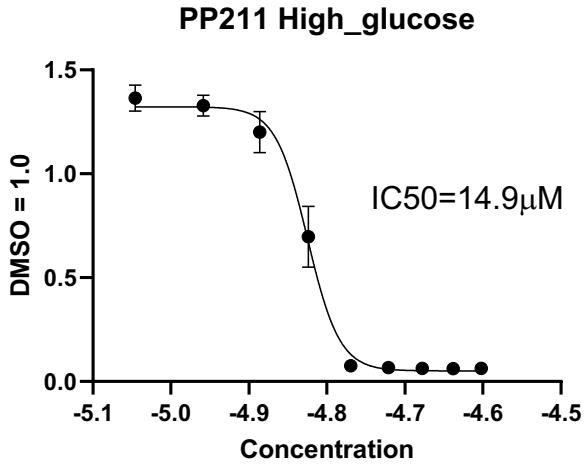
PP211 CNS MOP = 4.5



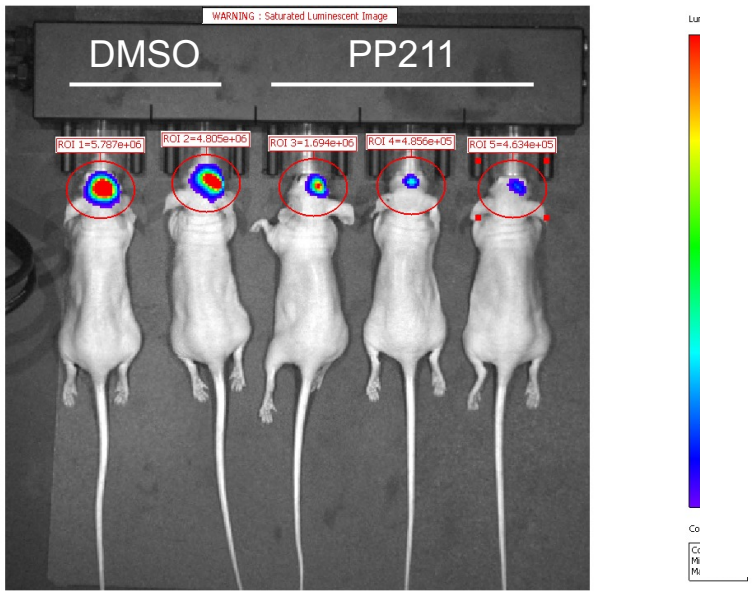


PP211 tissue bioavailability and pilot efficacy study

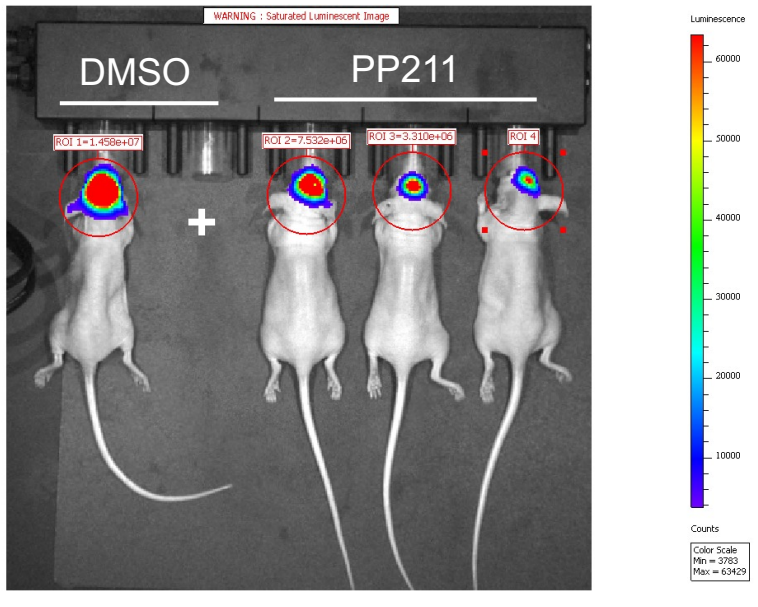




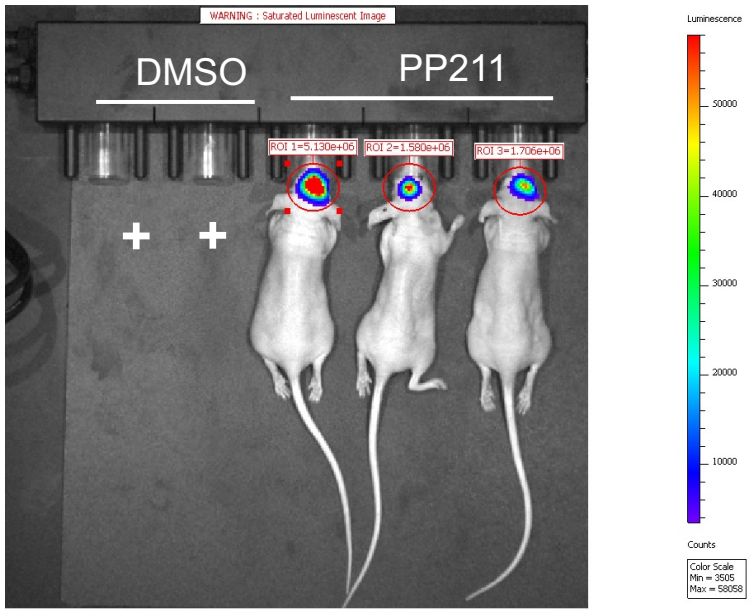
3 weeks



4 weeks



6 weeks



In conclusion:

1. Unprocessed FF (ester) triggers glioblastoma cell death in a PPARα –independent manner.
2. FF inhibits mitochondrial respiration at the level of the Complex I of the ETC.
3. However, FF does not cross the BBB, therefore, FF-based glioblastoma therapy is restricted to the intratumoral drug delivery.
4. Based on the FF molecular skeleton, **BPA**, we have designed, synthesized and tested over 200 FF derivatives and selected few with physicochemical properties indicating high potential for the BBB penetration.
5. In this regard PP1, PP21 and PP23 penetrate the BBB and synergize with the selected glucose inhibitors (GH and LND) to kill glioblastoma cells in the glucose independent manner.

and finally

7. Our new drug candidate, **PP211**, penetrates the BBB and is cytotoxic to glioblastoma cells in high glucose environment !!!!!.
8. Following oral administration PP211 accumulates in the brain tumor tissue at therapeutically relevant levels, supporting our initial anti-glioblastoma efficacy data from patient-derived intracranial glioblastoma model.

Scientists involved in this project:

Present and former members of Reiss Lab

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Charles Ingraham, MS; LSUHSC Cancer Center
Carlie Bonstaff, PhD; LSUHSC Cancer Center

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Francesca Peruzzi, PhD; LSUHSC, Cancer Center

Peruzzi Lab.

Cecylia Vittori, PhD; LSUHSC Cancer Center
Celeste Faia, PhD student; LSUHSC Cancer Center

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-2002 - 2015 - 2R01 CA095518-06A2 NCI (PI: KR)