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Glioblastoma mitochondrial respiration as a target for a new class of metabolic compounds capable of crossing the blood brain barrier

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[2] The University of Mississippi, Department of Biomedical Sciences, National Center of Natural Products Research, University, MS 38677;
[3] Department of Chemistry, University of New Orleans, New Orleans, LA 70148.

Disclosure Information

Speaker Name: Krzysztof Reiss

I have the following relevant financial relationships to disclose:

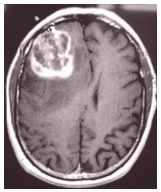
Employee of: Louisiana State University Health Sciences Center, New Orleans
Consultant for: N/A
Speaker's Bureau for: N/A
Grant/Research support from: NIH (P20 GM121288)
Stockholder in: N/A
Honoraria from: N/A

- and -

My additional financial relationship disclosures are:

Discussed here PP compounds are covered by the US patent WO2020/146876 , shared by LSUHSC and UNO. LSUHSC gave exclusive license to WayPath Pharma LLC to develop anticancer drugs based on these compounds. Dr. Reiss is a cofounder of WayPath Pharma.

Glioblastomas



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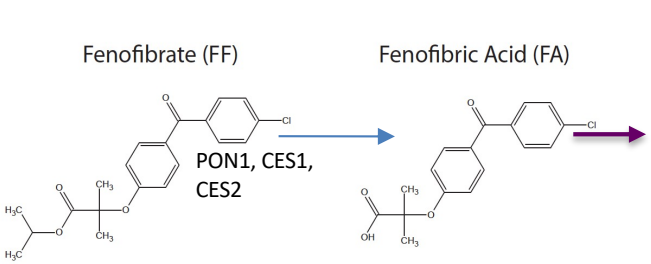
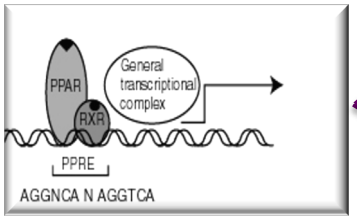
….are fast growing and practically incurable primary brain tumors for which therapeutic options are very limited and median survival time for extensively treated patients is between 14-18 months.

Our new approach to challenge glioblastoma takes advantage of unique anticancer properties of a common lipid lowering drug, Fenofibrate.





Fatty acid β-oxidation

Glycolysis

Inflammation

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

In Cancer Cells which fallow Warburg Effect FF triggers severe energy deficit followed by tumor cell death

However, some effects triggered by FF are difficult to explain 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 (attenuates lateral movement of macromolecules);**
3. **FF inhibits respiration of isolated cardiac and liver mitochondria.**

Fenofibrate accumulates in the mitochondrial membrane fraction and blocks mitochondrial respiration - triggering cellular responses opposite to the expected PPARα metabolic activity

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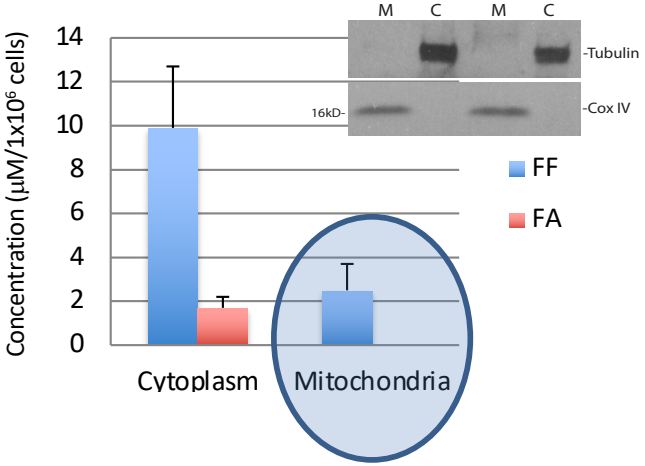
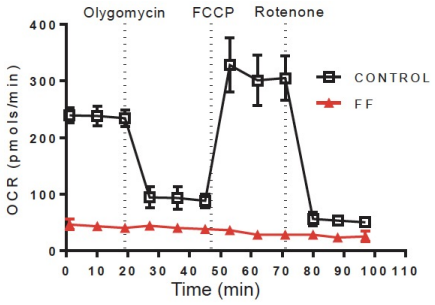
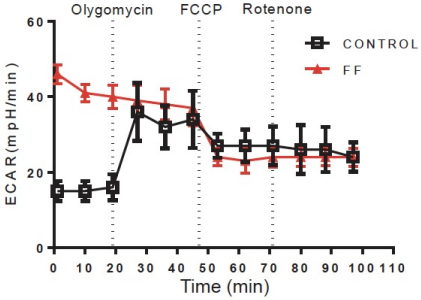
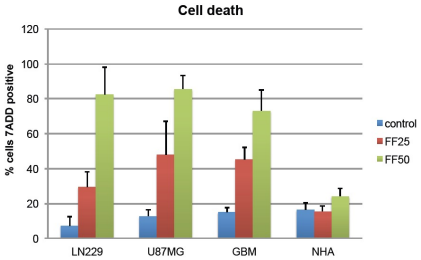
- Subcellular fractionation of FF-treated glioblastoma
- HPLC-based measurement of FF/FA content



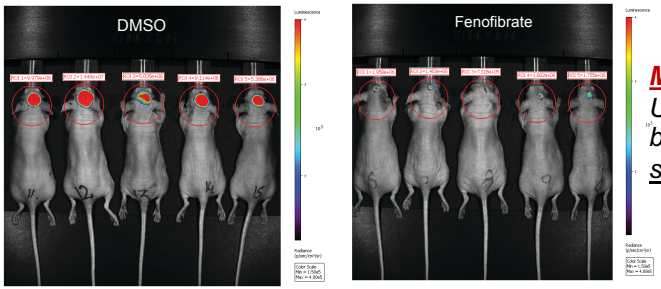
Mitochondrial stress experiment using Seahorse Extracellular Flux (XF) Analyzer







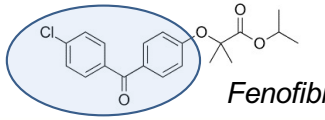
Fenofibrate does not cross BBB, which restricts its anti-glioblastoma potential to intratumoral drug delivery



Measurement of intracranial tumor size following CED-enhanced intracranial injection of FF.
*U-87MG–luc cells (1 x10⁵) were implanted into the brains of immunodeficient mice (Foxn1nu). Tumor-bearing mice were subsequently treated with 5 µl of DMSO (control) or 5 µl of 1mM FF using **CED** system. 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.

Modifications of FF molecular skeleton to improve:



Fenofibrate

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- glioblastoma cytotoxicity
- lipophilicity
- stability in vivo
- BBB penetration

PP21

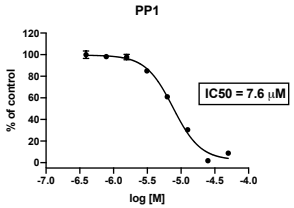
Calc.	*MarvinSketch 19.2*			LogS	PL	LogBB	MPA
ClogP	4.58	-	0.21	-5.89	42.78	-0.18	44.7
ClogD	4.57	-	0.00				
PSA	68.29	-	1.00				
HBD	1.00	-	0.75				
pKa	5.6	-	1.0				
MW	394.86	-	0.75				
CNS MPO			3.71				

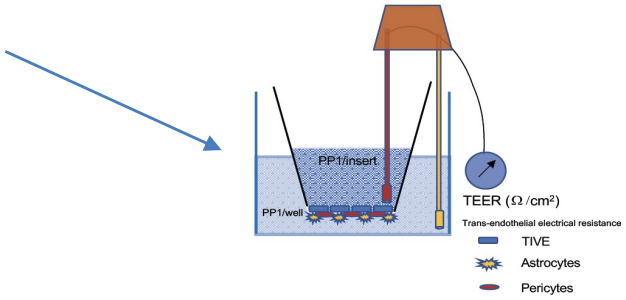
1. We designed over 200 derivatives of FF which share a common chemical skeleton, benzoyl-phenoxy-acetamide (BPA).

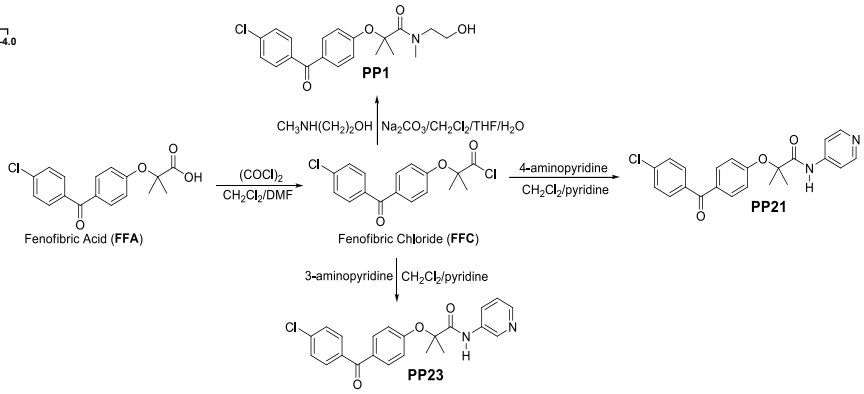
2. In silico calculation of CNS-MPO
[Multiparameter Optimization algorithm (0 – 6)]

3. In vitro cytotoxicity (IC50)

4. Triple co-culture BBB model







CNS-MPO *= Estimated central nervous system multiparameter optimization;* ***ClogP/CLogD*** *= calculated partitioning;* ***PSA*** *= Polar surface area (Å²);* ***HBD*** *= hydrogen bond donor at pH = 7;* ***pKa*** *= estimated acid strength ;* ***LogS*** *= Aqueous solubility;* ***PL*** *= polarizability (Å³);* ***logBB*** *= calculated blood-brain partition; and* ***MPA*** *= Minimal projection area (Å²). All computed molecular calculations were generated by* ***ChemAxon MarvinSketch version 19.20.***

Variants of benzyl-phenoxy-acetamide (BPA) are cytotoxic to glioblastoma and significantly less toxic to NHA

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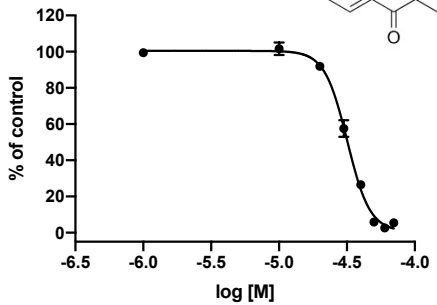
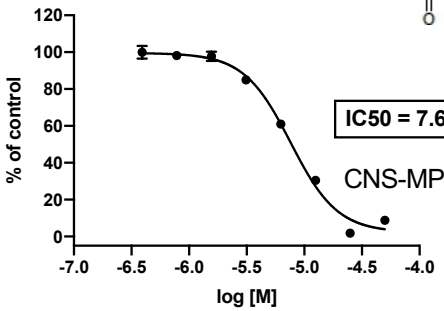
LN229

FF



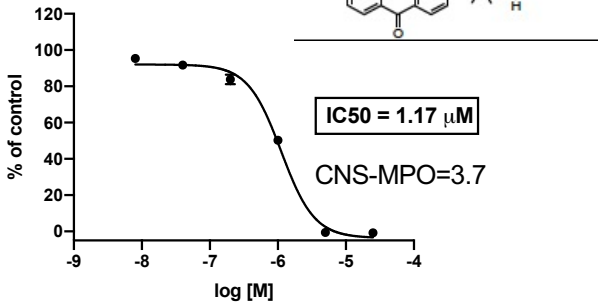
CNS-MPO=2.9

PP1



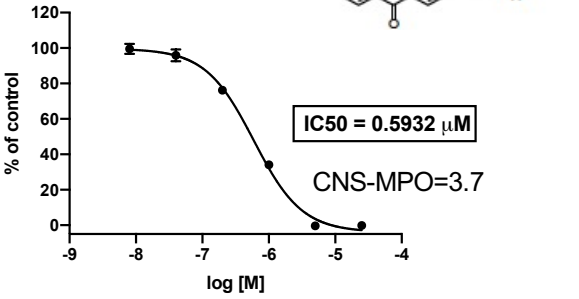
CNS-MPO=3.9

PP21



CNS-MPO=3.7

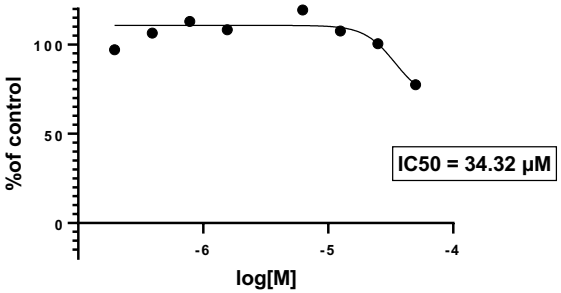
PP23



CNS-MPO=3.7

NHA

PP1



Like FF, selected PP- compounds inhibit mitochondrial respiration followed by immediate increased of glycolysis

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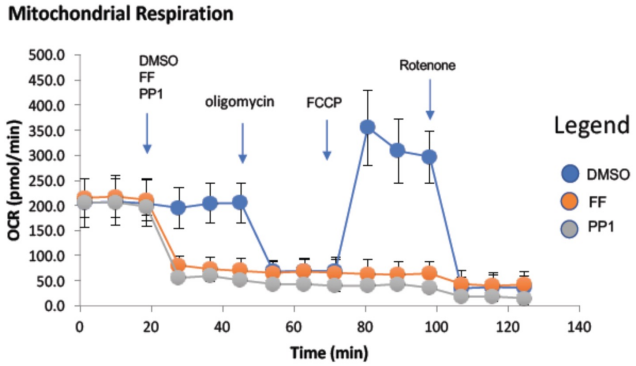

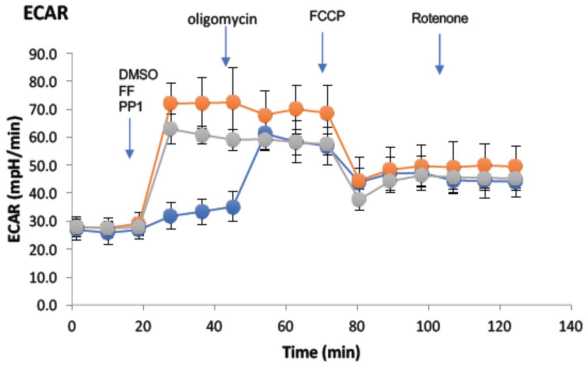

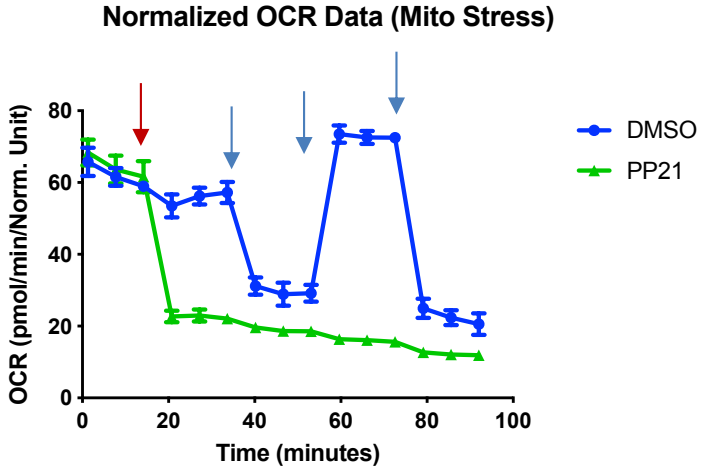

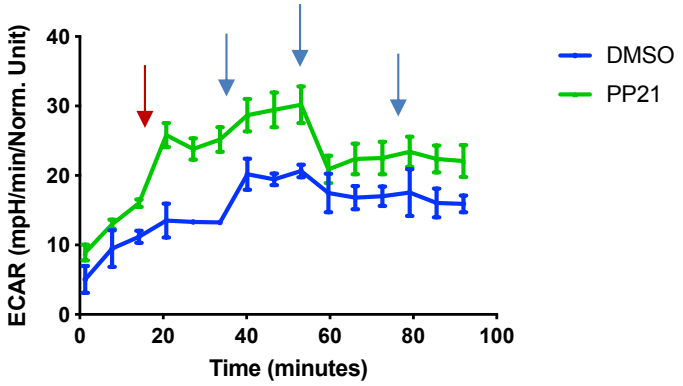

Triple co-culture BBB model membrane

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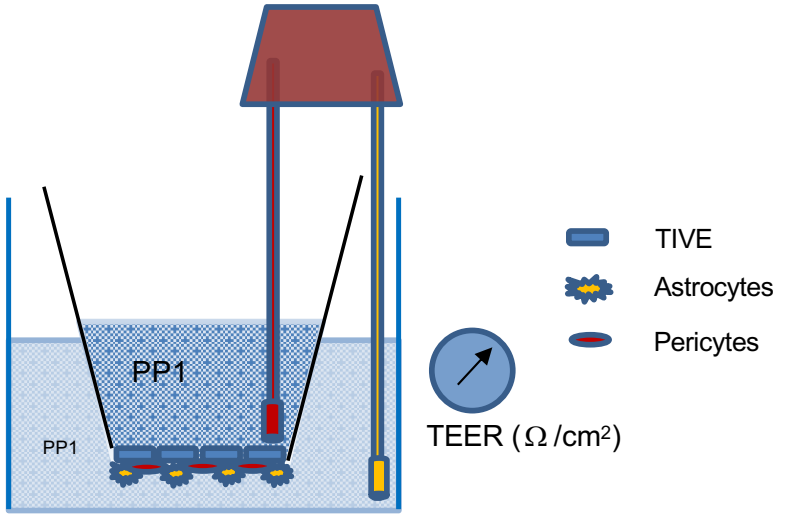

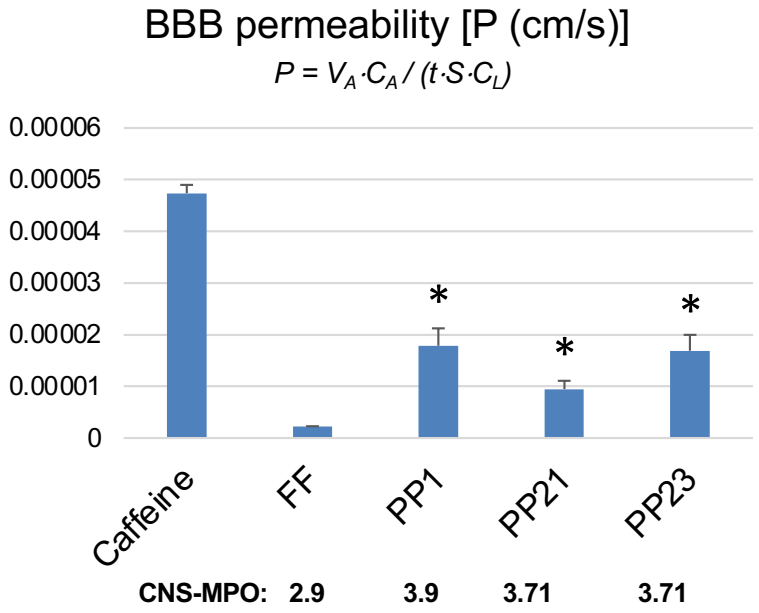

PP1/PP21/23-induced anti-cancer effects are glucose dependent

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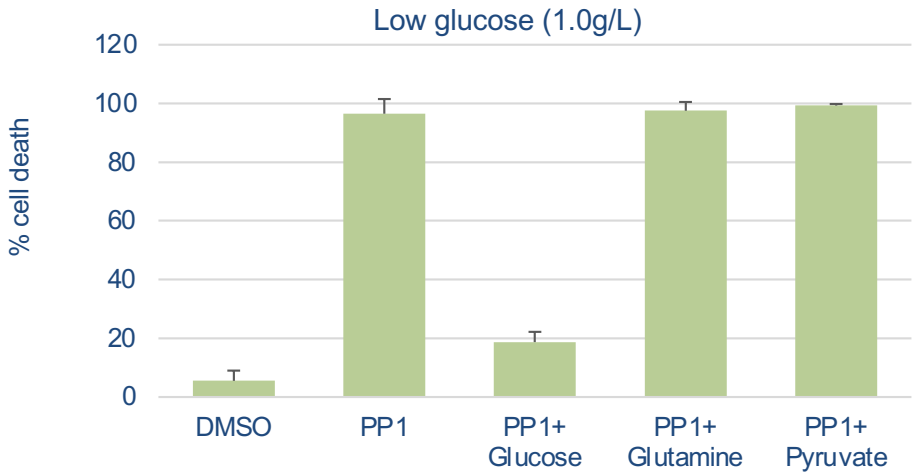
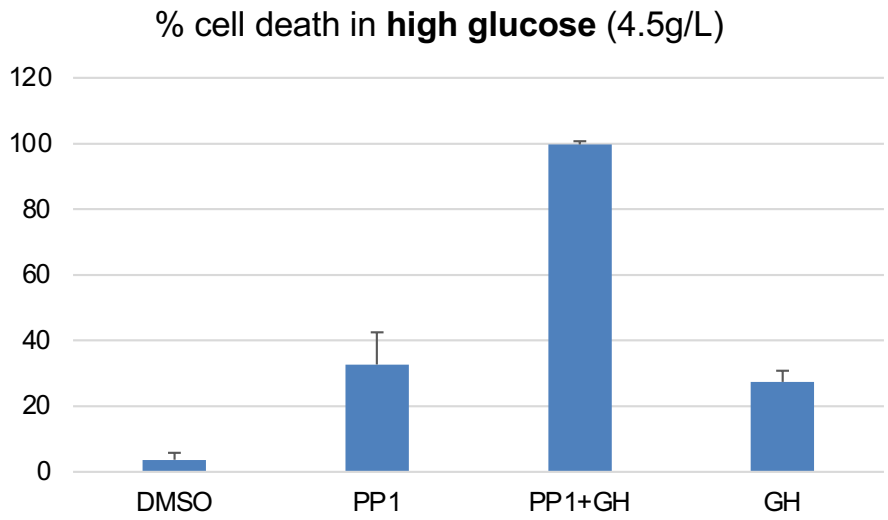


***GH: Gnetin-H** – resveratrol trimer – inhibitor of glycolysis*
In collaboration with Dr. Pier Paolo Claudio



***Paradoxal effects of glycolysis inhibitors** (2dG, LND, ….)*

Cooperative action of PP21 with GH

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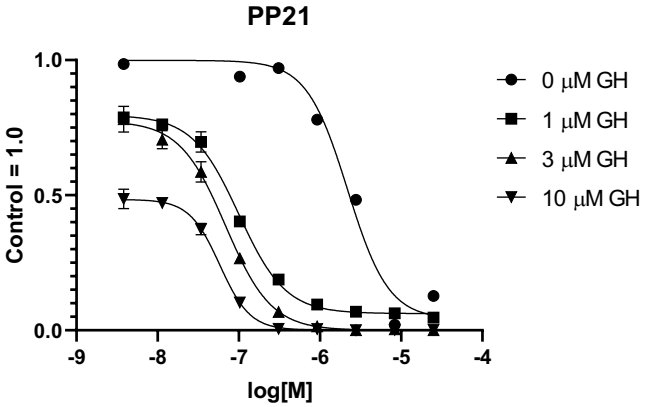
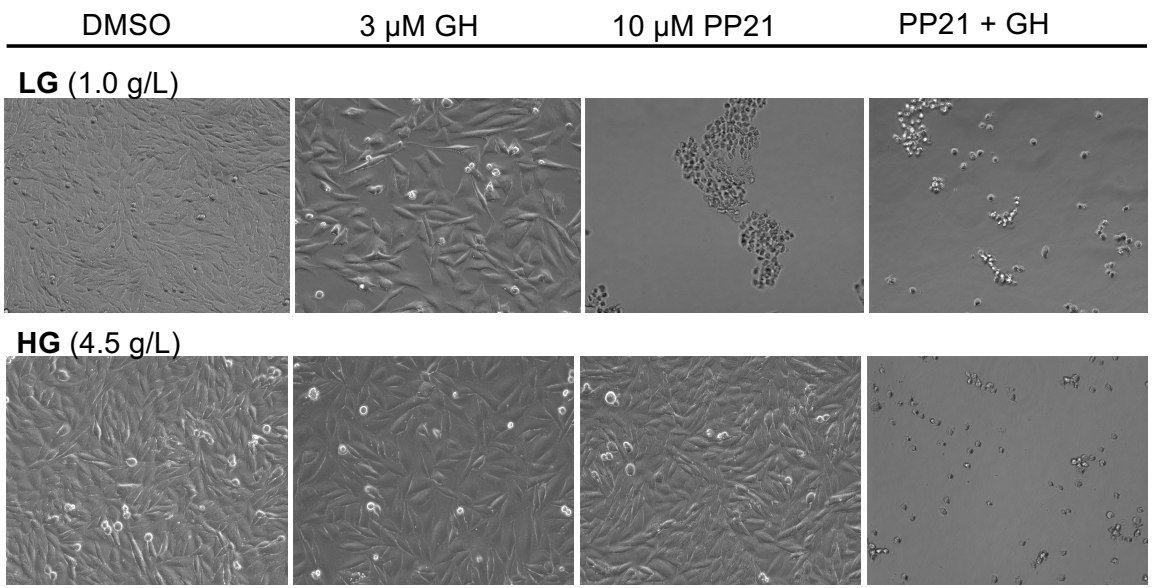


IC50 PP21	2.19 µM
IC50 PP21 (1 µM GH)	100 nM
IC50 PP21 (3 µM GH)	69 nM
IC50 P21 (10 µM GH)	58 nM

LN229 (72h incubation)



4 - 6 hrs following the PP21/GH treatment

Cooperative action of PP21 with GH induces severe energy deficit

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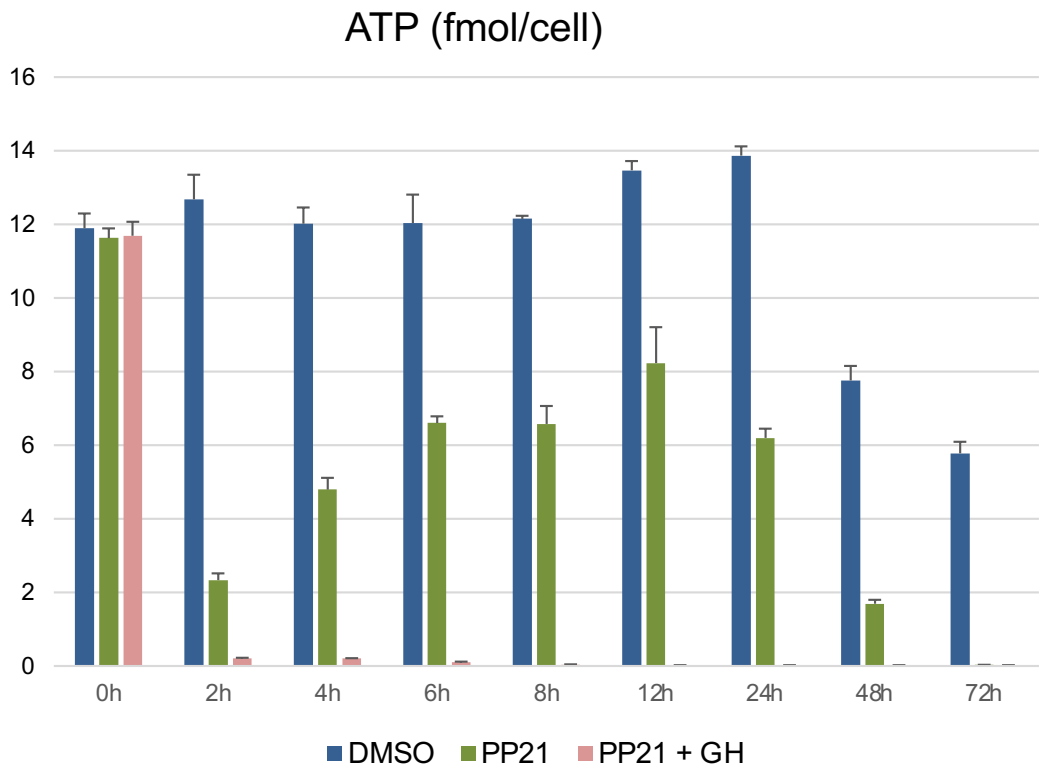

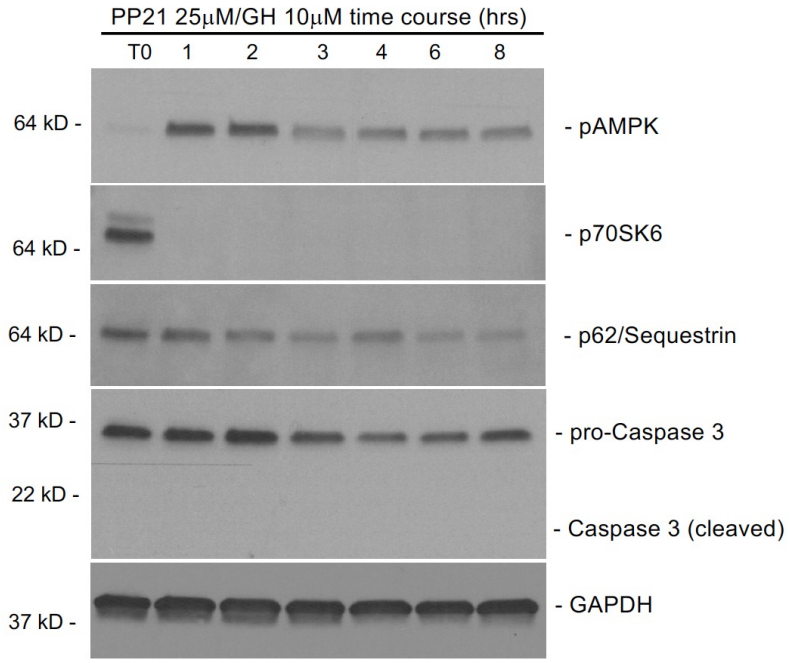

However, GH does not cross the BBB!!!

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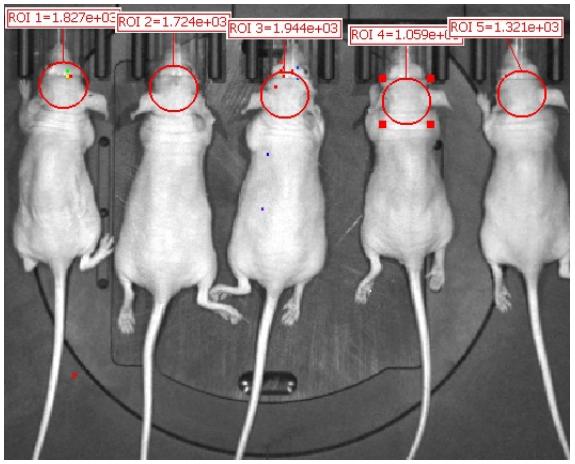
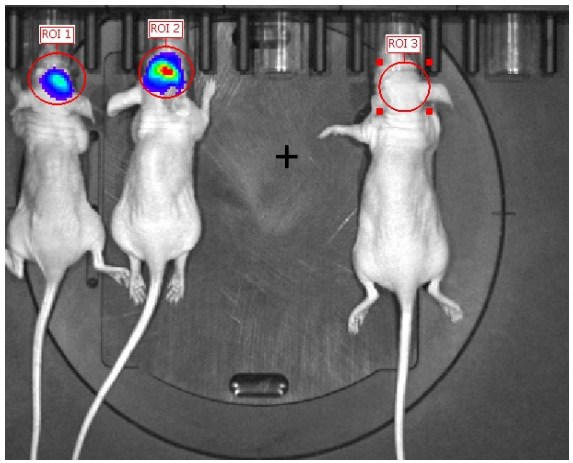
Proof of concept efficacy study

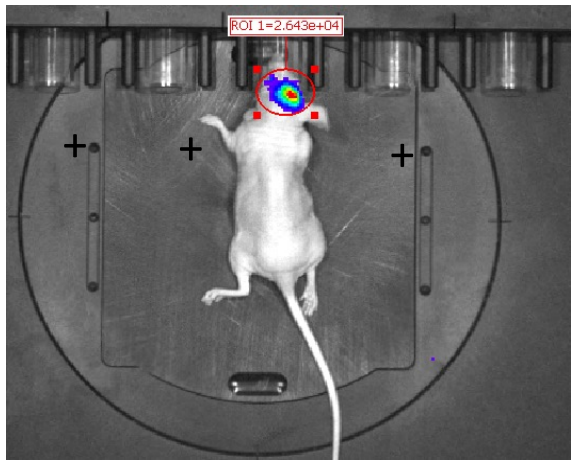
PP1+GH



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 when reached endpoint criteria).

At this point we have three potential options to improve anti-glioblastoma efficacy of our compounds:

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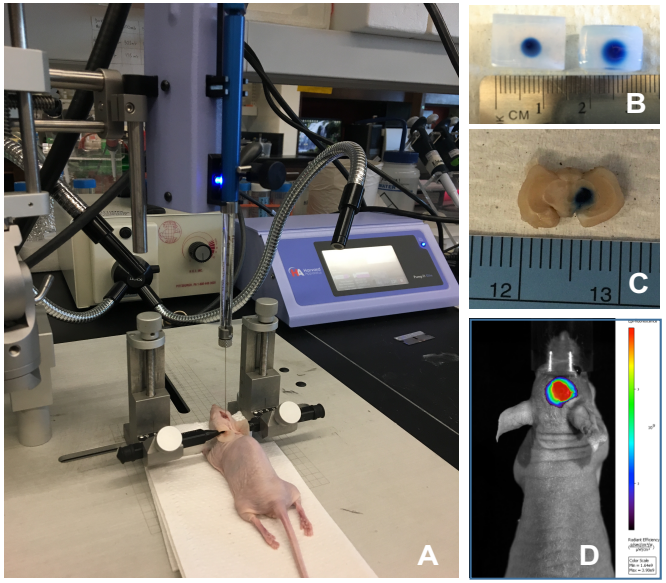
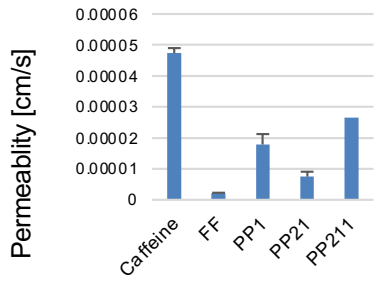
1) Intracranial drug delivery supported by **CED**;
2) Intranasal drug delivery (**PP21+GH);** and/or
3) Keep looking for new PP compound/s with better BBB penetration, lower IC50, and glucose-independent cytotoxicity (**PP211**).

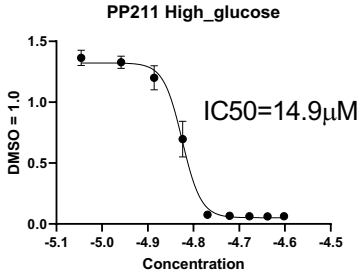
CED system for intracranial glioblastoma



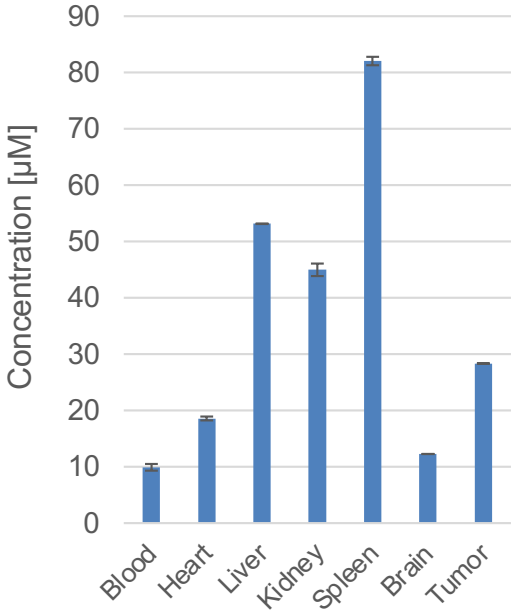
PP211 CNS-MPO= **4.5**

In vitro BBB penetration





PP211 distribution in tissues following oral administration



Scientists involved in this project

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Present and former members of Reiss Lab
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Monika Rak, PhD; LSUHSC Cancer Center
Charles Ingraham, MS; LSUHSC Cancer Center
Carlie Bonstaff, PhD; LSUHSC Cancer Center

Joanna Stalinska, MS; Jagiellonian University Cracow, Poland
Matthew Dean, PhD, LSUHSC Cancer Center
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Dorota Wyczechowska, PhD; LSUHSC Cancer Center
Pier Paolo Claudio, MD; The University of Mississippi
Francesca Peruzzi, PhD; LSUHSC, Cancer Center
Peruzzi Lab.
Cecylia Vittori, PhD; LSUHSC Cancer Center
Celeste Faia, PhD; LSUHSC Cancer Center

Grant support
-2017-2022 – P20 GM121288 (PD/PI: KR) Center for Translational Viral Oncology (CTVO).
-2017-2022 – LSUHSC School of Medicine, Dean Matching Funds (PI:KR)
-2010 - 2015 - P20 GM103501 (PD/PI: AO (KR PI Project #10)
-2002 - 2015 - 2R01 CA095518-06A2 NCI (PI: KR)