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

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

Fenofibrate accumulates in the mitochondrial membrane fraction. HPLC-based measurement of FF content.
Fenofibrate inhibits mitochondrial respiration in LN229 glioblastoma cells
(mitochondrial stress experiment)

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

A

Oligomycin  
FCCP  
Rotenone  

CTRL  
FF

B

Oligomycin  
FCCP  
Rotenone

CTRL  
FF

C

FF

D

FF

FF5μM  
FF10μM  
FF25μM  
FF50μM

CTRL

FF

E

F

Time (min)

Time (min)

Time (min)

Time (min)
Fenofibrate inhibits mitochondrial respiration at the level of Complex I of the ETC.

- **Complex I (NADH:ubiquinone oxidoreductase)**: Inhibits the entry of electrons from NADH to the electron transport chain.
- **CoQ**: Coenzyme Q (ubiquinone), a component of Complex I, acts as a mobile electron carrier between Complex I and Complex II.
- **Complex II (Succinate:ubiquinone oxidoreductase)**: Converts succinate to fumarate.

**Oxidative Phosphorylation**
- **NAD+/NADH** to **FADH2/FAD+** to **O2**.
- **H+** shuttle between complexes.

**Mitochondrial Respiratory Chain**
- **NADH dehydrogenase** (Complex I) → **CoQ** → **Complex II** → **Complex III** → **Complex IV** → **O2**.
- **FADH2** (from Complex II) enters the electron transport chain at Complex II instead of Complex I.

**Energy Production**
- **ATP** synthesis occurs at Complexes III and IV.

**Regulatory Proteins**
- **AMPK** (AMP-activated protein kinase)
- **SIRT**
- **LKB1**

**Compounds and Pathways**
- **Glutaminolysis**: Breakdown of glutamine to glutamate.
- **Krebs Cycle**: Oxaloacetate, Citrate, α-Ketoglutarate, Fumarate.
- **β-Oxidation**: Fatty acid β-oxidation.
- **Pyruvate-Glycolysis**: Pyruvate to lactate.
- **AOA**
- **Etomoxir**: Inhibits FAO.
- **lonidamine, 2dG, GH**
- **SITR-3/SIRT-1**
- **AMP/ATP ratio**
- **NAD+/NADH ratio**
- **2H+** shuttle between complexes.

**Isolated Mitochondria Study**
- **Succinate + ADP**: A standard substrate for mitochondrial respiration.
- **OCR (Oxygen Consumption Rate)**

**Graphs**
- A: OCR over time with different treatments.
- B: OCR over time with different treatments.
- C: OCR over time with different treatments.

**Legend**
- **FF**: Fenofibrate
- **Rotenone (ROTEN)**
- **FCCP**: FCCP (Fumonisin C1 Carnitine)
- **SITR-3/SIRT-1**: Sirtuins
- **AMPK**: AMP-activated protein kinase
- **AMP/ATP**: Adenosine monophosphate/Adenosine triphosphate
- **NAD+/NADH**: Nicotinamide adenine dinucleotide/Nicotinamide adenine dinucleotide

**Diagram**
- Depicts the mitochondrial respiratory chain, electron transport chain, and major metabolic pathways involved in mitochondrial function.
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 x 10^5) 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.

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](image)

![PP1](image)

Log 

% of control

IC50 = 7.7 µM

IC50 = 31.82 µM
PP1 inhibits Mitochondrial respiration

**LN229**

- **Drug**
- **Oligomycin**
- **FCCP**
- **Rotenone/Antimycin A**

**GBM12TdT**

- **Drug**
- **Oligomycin**
- **FCCP**
- **Rotenone/Antimycin A**

**DMSO**

**PP1**
PP1 triggers inhibition of intracellular ATP and activates AMPK-mediated signaling responses.
PP1-induced anti-cancer effects are glucose dependent

**A** Low glucose (1.0g/L)

![Bar graph showing cell death in low glucose conditions with DMSO, PP1, PP1+Glucose, PP1+Glutamine, and PP1+Pyruvate.

**B**

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

**C**

**PP1 in Low Glucose**

- PP1 IC50 = 7.70 µM
- PP1+GH IC50 = 3.87 µM

**PP1 in High Glucose**

- PP1 IC50 = 40.1 µM
- PP1+GH IC50 = 3.67 µM
Proof of concept efficacy study

However, GH does not cross the BBB!!!
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

<table>
<thead>
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<th>Concentration</th>
<th>IC50 (M)</th>
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<tr>
<td>1.17</td>
<td>1.24</td>
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<tr>
<td>2.19</td>
<td>1.92</td>
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</table>

avg: 1.55 ± 0.47

PP21/23 CNS-MPO = 3.71

PP23/LG

<table>
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<th>Concentration</th>
<th>IC50 (M)</th>
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<tbody>
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<td>0.59</td>
<td>0.56</td>
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</tbody>
</table>

avg: 0.57 ± 0.01

PP21/23 CNS-MPO = 3.71
Is LND capable of improving PP21 anti glioblastoma efficacy??

Normalized OCR Data (Single Injection)

Normalized ECAR Data (Single Injection)

CNS MPO for LND=5.05
GBM12 gliosphere cultures in condition promoting growth of glioma stem cells

![Graphs showing % cell death for DMSO, PP21, and PP21+LND]

- **DMSO**: 
  - IC50 = 18.2 µM

- **PP21**: 
  - IC50 = 68.6 nM

**CNS MPO for LND = 5.05**
New drug candidate, PP211, with highly promising properties

**PP211 Low_glucose**

DMSO = 1.0

IC50 = 12.7 μM

**PP211 High_glucose**

DMSO = 1.0

IC50 = 14.9 μM

**Normalized OCR Data (Single Injection)**

**Normalized ECAR Data (Single Injection)**

OCR (pmol/min) vs Time (minutes)

ECAR (µM/min) vs Time (minutes)
Triple co-culture BBB model membrane

PP211 CNS MOP = 4.5

Artificial BBB penetration

Permeability [cm/s]

- Caffeine
- FF
- PP1
- PP21
- PP211

TEER ($\Omega \times \text{cm}^2$)
PP211 tissue bioavailability and pilot efficacy study

PP211 distribution in tissues

Concentration [µM]

- Blood
- Heart
- Liver
- Kidney
- Spleen
- Brain
- Tumor

3 weeks

4 weeks

6 weeks

DMSO = 1.0
IC50=14.9µM

PP211 High glucose

Concentration
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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Monika Rak, PhD; LSUHSC Cancer Center
Charles Ingraham, MS; LSUHSC Cancer Center
Carlie Bonstaff, PhD; LSUHSC Cancer Center
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