Deciphering Molecular Mechanisms Triggering Pediatric Group 3 Medulloblastomas

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Children's HOSPITAL & MEDICAL CENTER | UNMC

Presenter Disclosures

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Off-label uses	Nortriptylline, Simvastatin, Fluoxetine, Sertraline for Anti-neoplastic effects in group 3 medulloblastoma

Objectives

- Understand the clinical manifestations and implications of medulloblastomas
- Acknowledge that these tumors present a unique public health concern for NE residents
- Identify key gaps in the field surrounding aggressive disease
- Discuss novel mechanisms feeding pathogenesis of aggressive tumors
- Demonstrate how exploiting this knowledge can generate alternative anti-neoplastic strategies

Introduction

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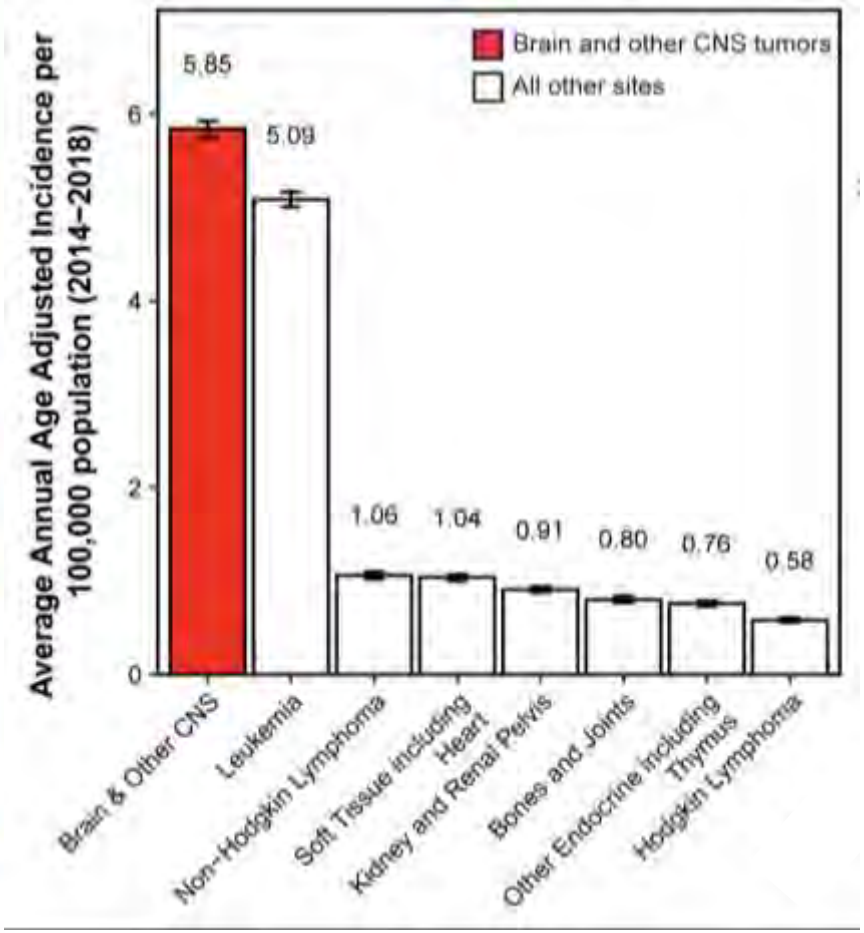
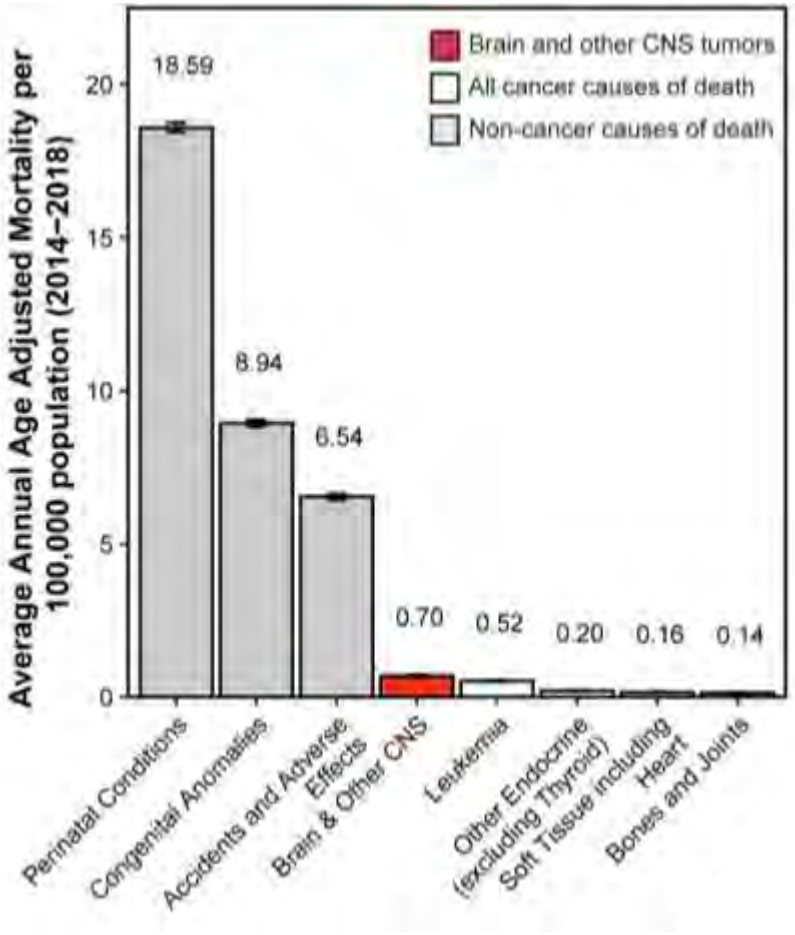
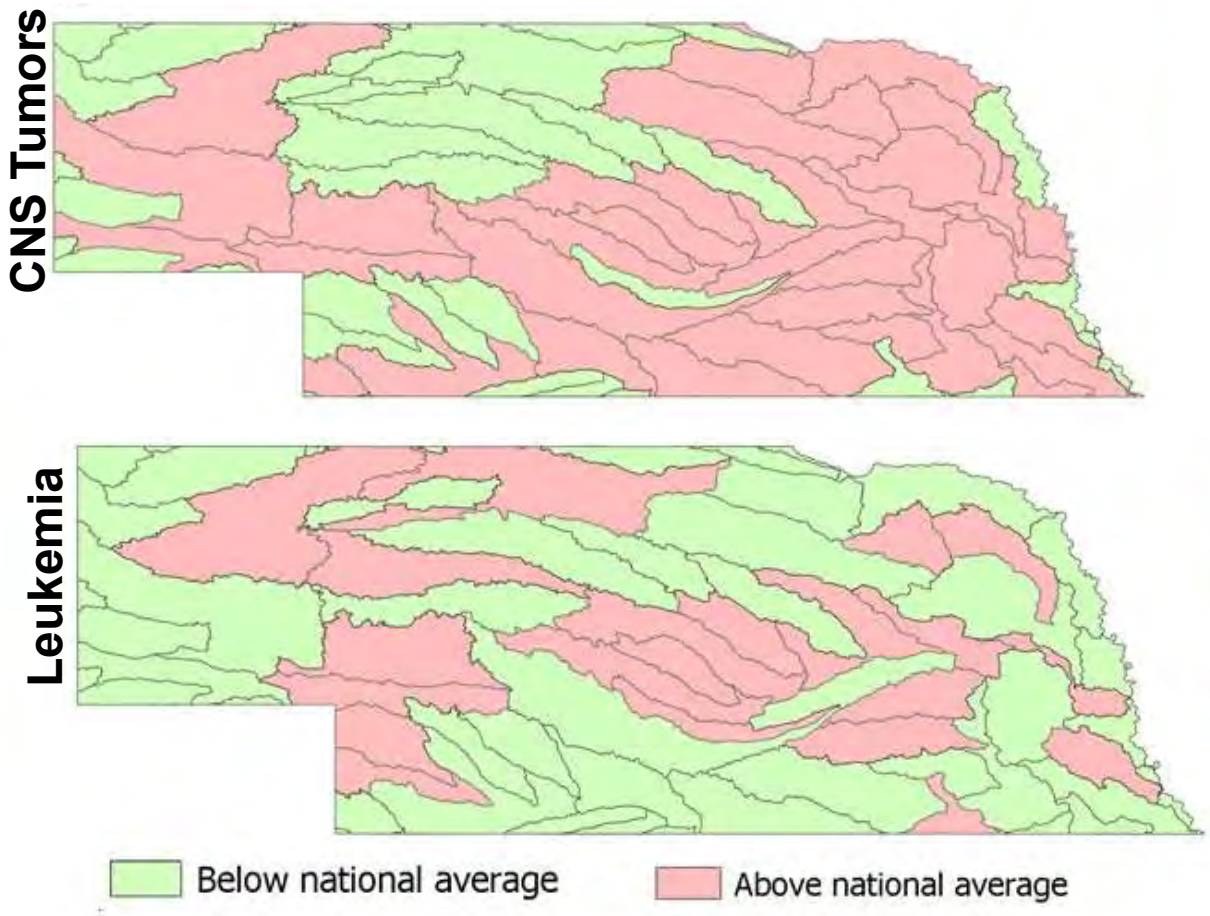
Pediatric Brain Tumors (0-14)





CBTRUS Statistical Report: 2014–2018. *Neuro-Oncol. 2021.*

Nebraska



Farazi et al. *Cancer Epidemiol. 2018.*

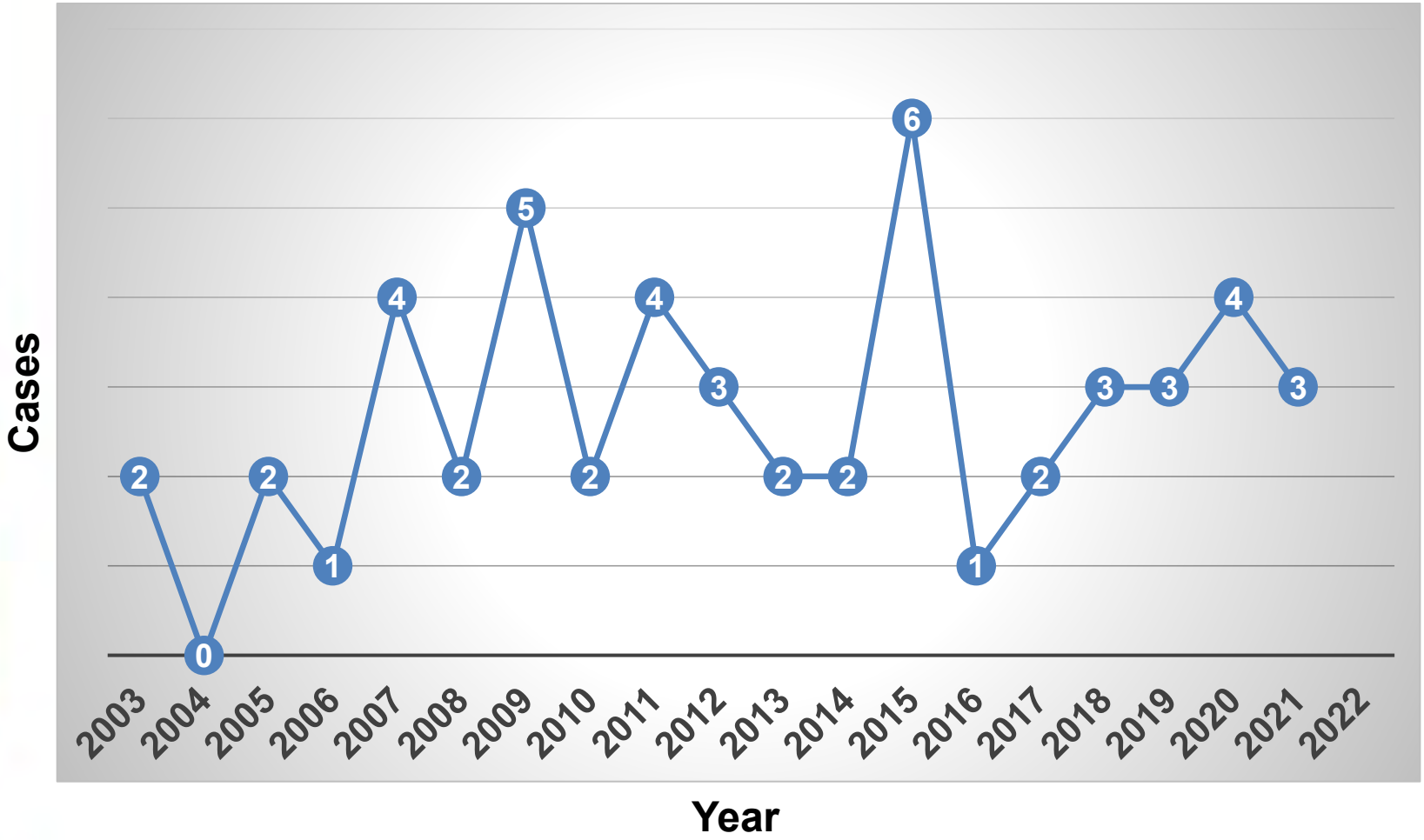
Incidence

NE	US
7.06 per 100,000 (37)	5.85 per 100,000 (3,562)
MB	MB
0.4 per 100,000	0.5 per 100,000
2	297

CBTRUS Statistical Report: 2014–2018. ***Neuro-Oncol. 2021.***

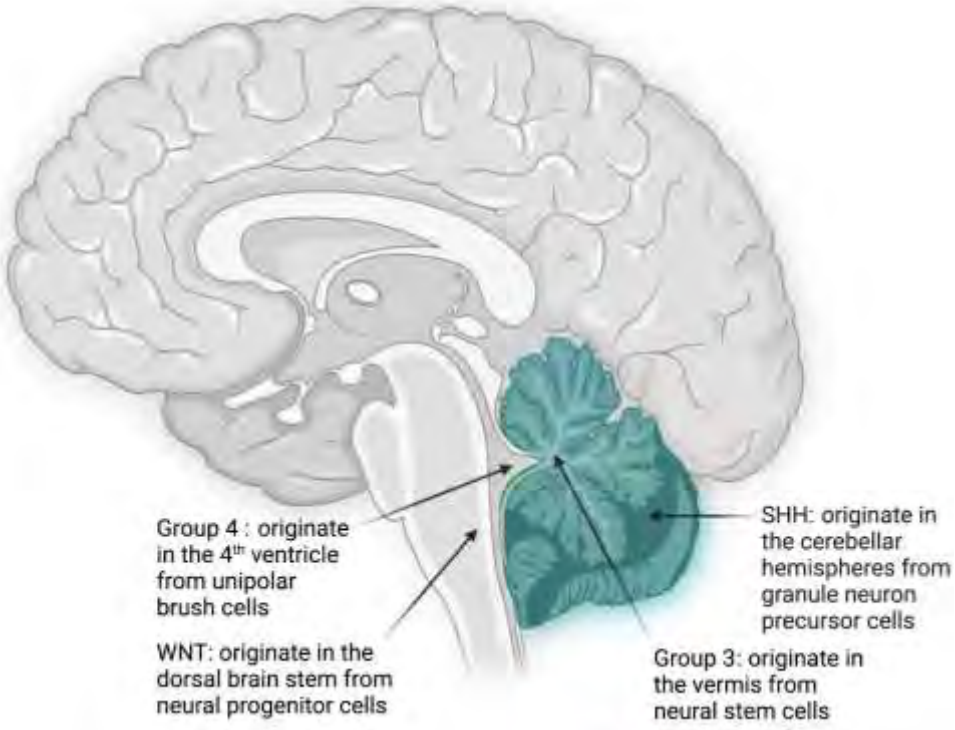
Nebraska (MB)

Pediatric MB cases
Total 51 / 20 years
Median 2/year

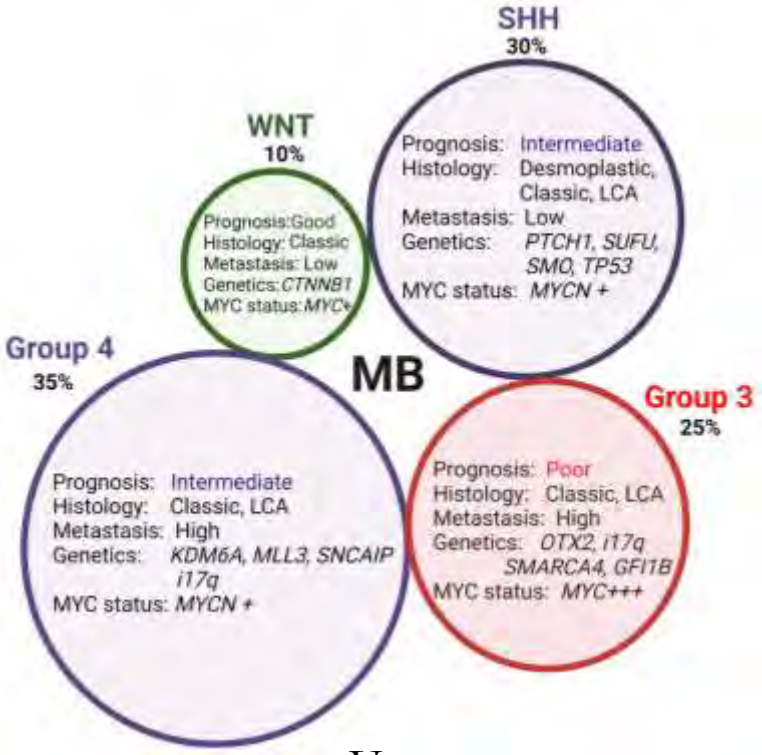


Data provided by Dr. Jie Chen,
UNMC Department of Pathology

Medulloblastoma



Ray and Mahapatra. ***Diagnostics 2022.***

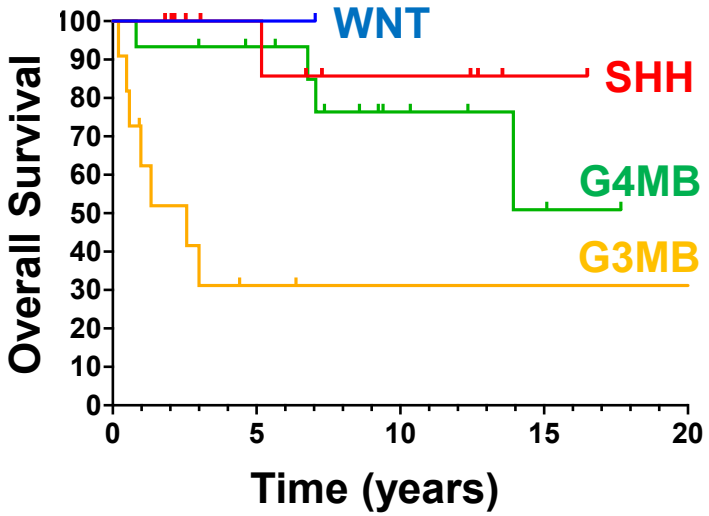


Vs.

Average Risk / High Risk

<u>Diagnosis</u> – DNA methylation, solid tumor fusion analysis, NGS, and germ line mutation

Voskamp *et al.*, *Cancers* 2021



Ranjana et al. ***Brain Path 2020***

NE Cases (GSE148390)

DX	ETHNICITY	AGE	SEX	HISTOLOGY	17*	MYC AMP	METS	RECUR	STATUS
2015	Caucasian	5	M	CLASSIC	-	-	-	-	Alive
2012	Caucasian	14	F	ANAPLASTIC/LARGE CELL	-	-	-	-	Alive
2015	Caucasian	1	M	CLASSIC	-	-	-	-	Alive
2009	Caucasian	0.6	F	CLASSIC	-	-	-	-	Alive
2009	Caucasian	8	M	CLASSIC	-	-	-	-	Alive
2010	Caucasian	0.6	F	NODULAR/DESMOPLASTIC	-	-	-	-	Alive
2015	Caucasian	7	M	NODULAR/DESMOPLASTIC	-	-	-	-	Alive
2006		0.4	F	NODULAR/DESMOPLASTIC	-	-	-	-	Alive
2019	Caucasian	3	M		-	-	-	-	Alive
2019	Caucasian	12	F	CLASSIC	-	+	-	-	Dead
2020	Hispanic	0.6	M	NODULAR/DESMOPLASTIC	-	-	+	-	Alive
2020	Caucasian	0.25	F		-	-	-	-	Alive
2020	Caucasian	1	F	NODULAR/DESMOPLASTIC	-	-	-	-	Alive
2007	Caucasian	1	M	ANAPLASTIC/LARGE CELL	+	+	+	-	Dead
2007	Caucasian	3	M	ANAPLASTIC/LARGE CELL	+	+	+	+	Dead
2009	Caucasian	2	M	CLASSIC	-	-	-	-	Dead
2011	Caucasian	6	M	CLASSIC	+	-	+	+	Dead
2016	Caucasian	7	M	CLASSIC	+	-	+	-	Alive
2017	Caucasian	4	M	ANAPLASTIC/LARGE CELL	+	+	+	-	Dead
2018	Hispanic	3	M	ANAPLASTIC/LARGE CELL	+	-	+	-	Alive
2003		8	M	ANAPLASTIC/LARGE CELL	-	+	-	+	Alive
2009		3	M	ANAPLASTIC/LARGE CELL	+	-	-	+	Dead
2020	Caucasian	6	M	CLASSIC	+	-	+	-	Dead
2021	Caucasian	9	F	ANALPLASTIC/LARGE CELL	-	+	-	-	Alive
2012	Asian	9	M	CLASSIC	-	-	-	-	Alive
2014	African-Am	7	M	ANAPLASTIC/LARGE CELL	-	-	-	-	Dead
2015	Caucasian	2	F	NODULAR/DESMOPLASTIC	-	-	-	+	Alive
2005	Caucasian	12	M	CLASSIC	+	-	-	-	Alive
2013	Caucasian	6	F	ANAPLASTIC/LARGE CELL	+	-	-	-	Alive
2007	Caucasian	13	M	ANAPLASTIC/LARGE CELL	+	-	-	-	Alive
2009	Caucasian	3	F	CLASSIC	+	-	+	-	Alive
2015	Caucasian	6	M	CLASSIC	+	-	-	+	Dead
2015	Caucasian	6	F	CLASSIC	+	-	-	-	Alive
2011	Caucasian	15	M	CLASSIC	+	-	-	-	Dead
2017	Caucasian	6	F	CLASSIC	-	-	-	-	Alive
2018	Caucasian	11	M	CLASSIC	+	-	-	-	Alive
2008		8	F	CLASSIC	+	-	-	+	Dead
2014		5	M	BIPHASIC	-	-	-	-	Alive
2019	Hispanic	8	M	ANAPLASTIC/LARGE CELL	+	-	-	-	Alive
2021	Caucasian	7	F	CLASSIC	-	-	-	-	Alive

WNT (1)

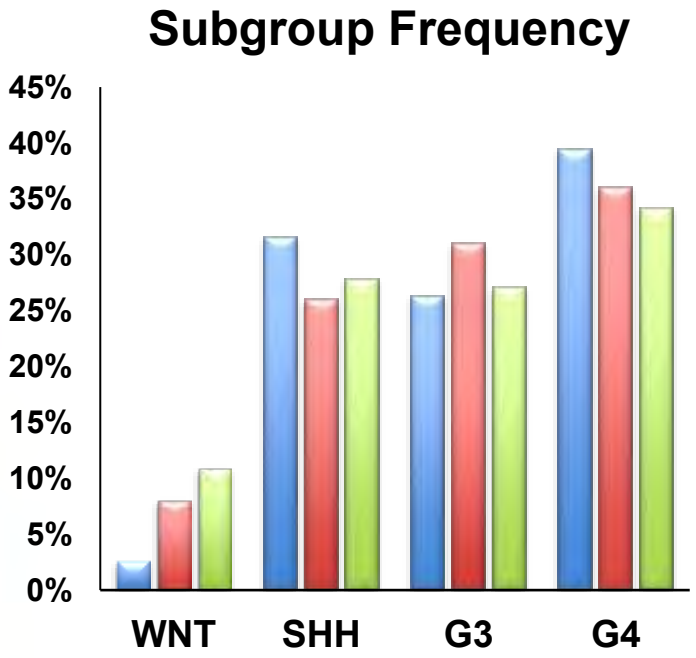
SHH (12)

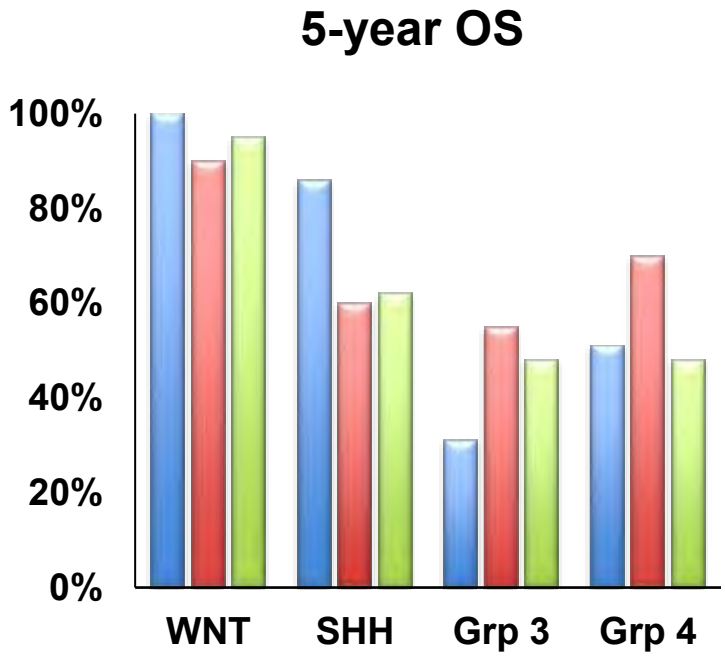
Grp 3 (11)

Grp 4 (16)

Cohort Demographics

Mahapatra (2020): 37 Schwalbe (2017): 438 Kool (2012): 550

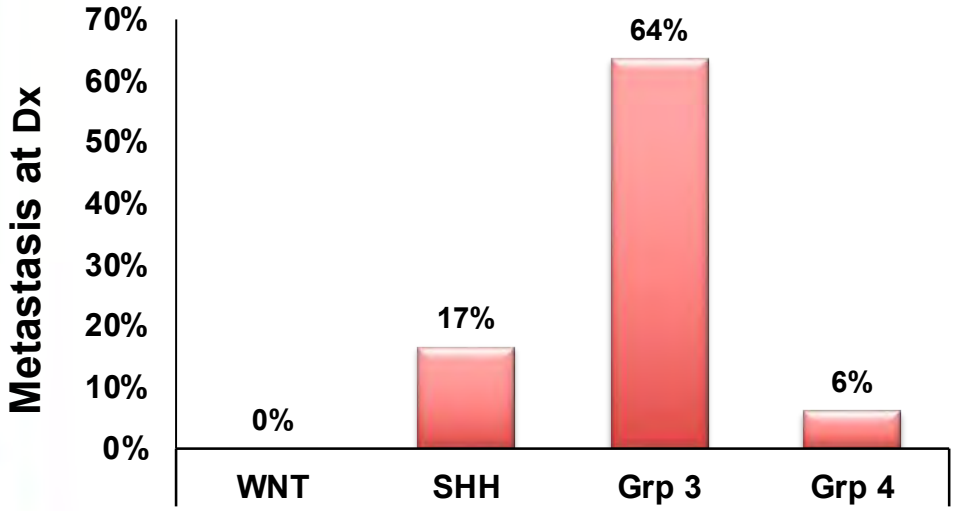


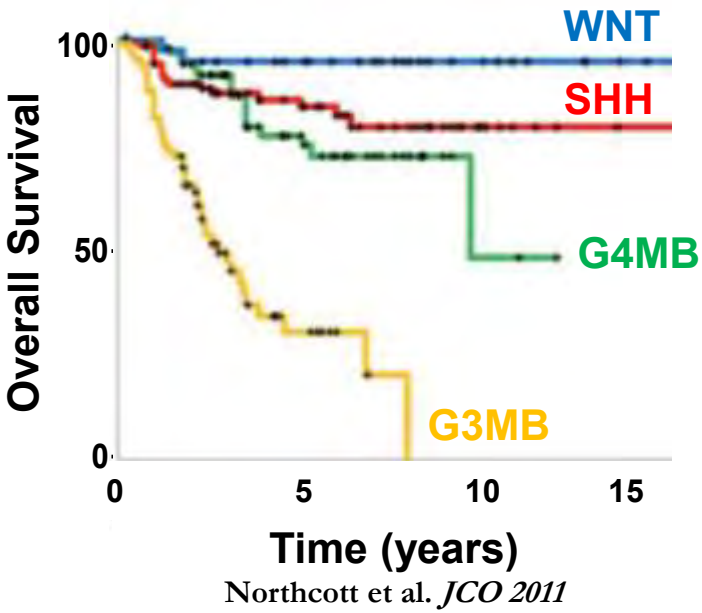


WNT:	3% vs. 10%
SHH:	30% vs. 30%
G3MB:	28% vs. 25%
G4MB:	40% vs. 35%

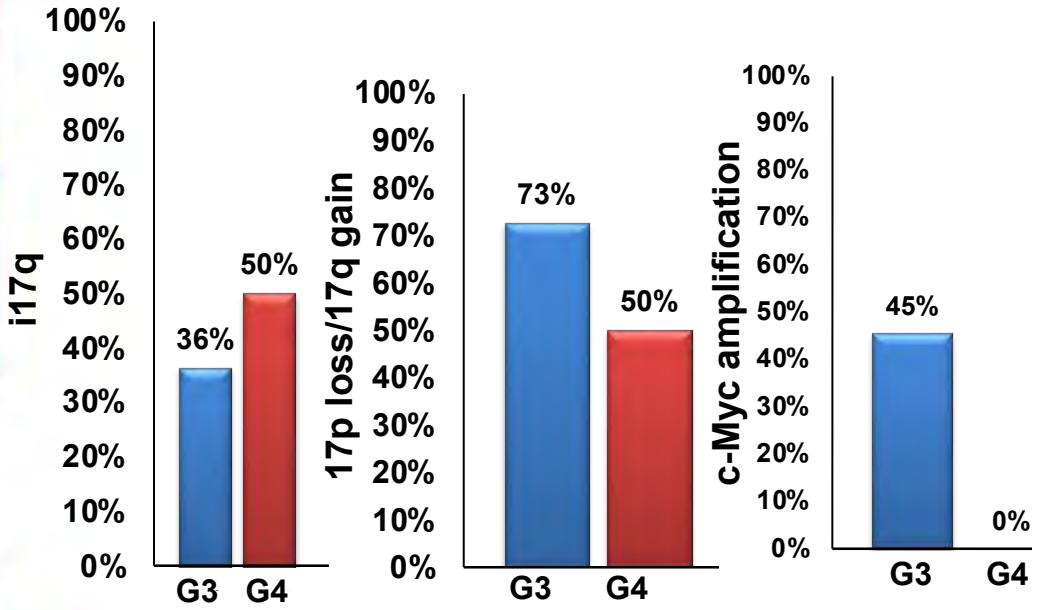
WNT:	100% vs. >95%
SHH:	86% vs. 75-90%
G3MB:	31% vs. <50%
G4MB:	51% vs. 50-75%

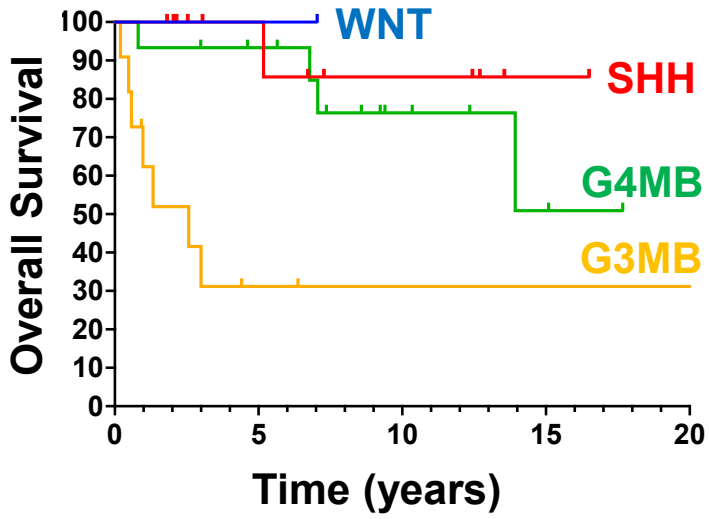
High-risk Features (Mets, i17q, c-Myc)





Northcott et al. *JCO 2011*





Ranjana et al. *Brain Path 2020*

Research/ Knowledge Gap

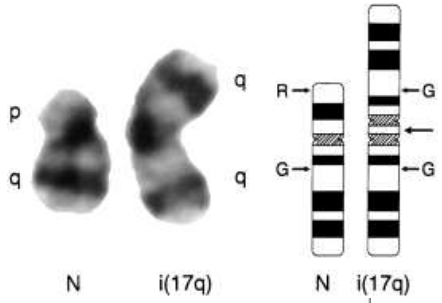
Subgroup	SHH and WNT	Non-SHH/WNT
Pathophysiology	+++	+
Mouse models	+++	+
Novel therapeutics	+++	--
Survival profile	WNT >90%, SHH 75-90%	Grp3 <50%, Grp4 50-75%

Long-term goals:

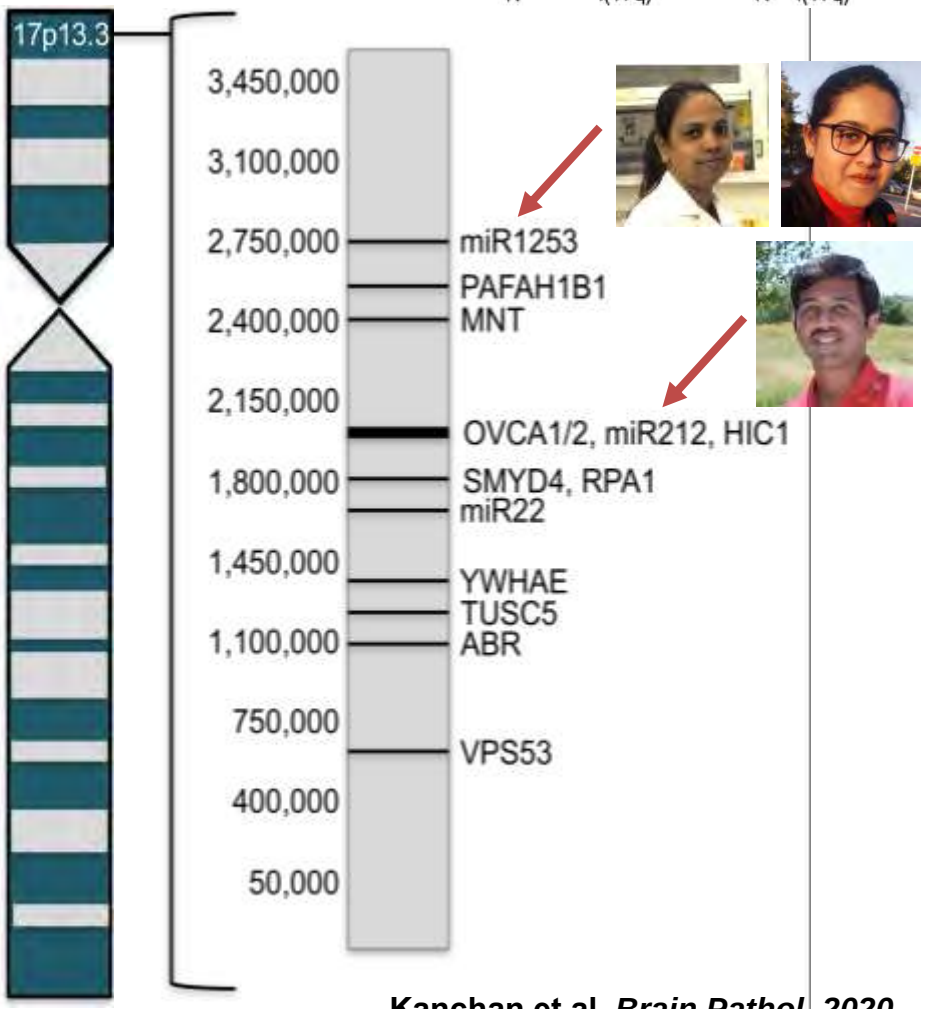
1. To uncover deregulated molecular pathways triggering aggressiveness that can be targeted therapeutically
2. To develop novel treatment strategies that mitigate long-term morbidity/mortality and reduce recurrence

McClure et al. *BJHaem 2002*

Isochromosome 17q



- i17q - the most frequent cytogenetic abnormality in medulloblastoma
- 17p13.3 affected in up to 50% of human MB cases
- Locus houses 14 tumor suppressor genes



Cogen et al. *J Neuro-onc. 1996*
Cogen et al. *Amer J Hum. Gene. 1992*
Hoff et al. *Genom. 2000*

Kanchan et al. *Brain Pathol. 2020*
Perumal et al. *Acta Neuropathologic Comm. 2021*

Targeting deregulated molecular pathways triggering aggressiveness

Iron transport and homeostasis

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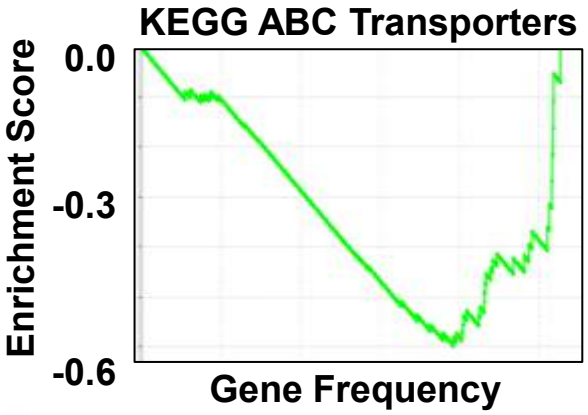
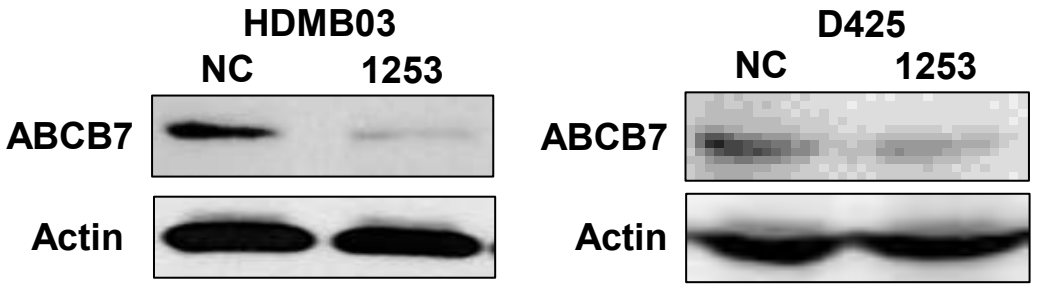
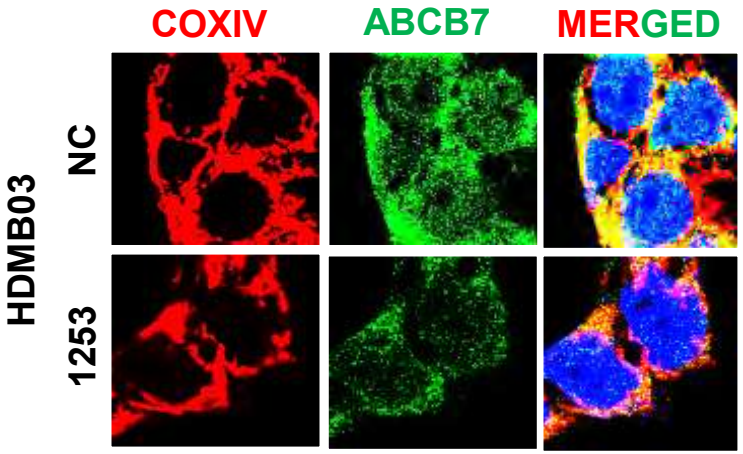
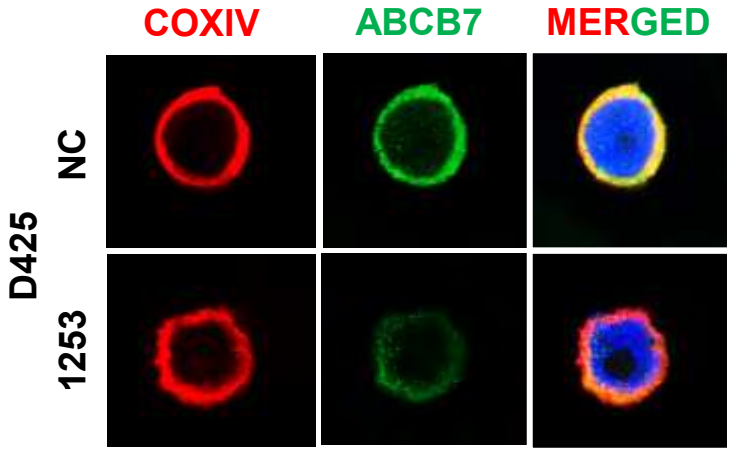
MiR-1253 inhibits ABCB7



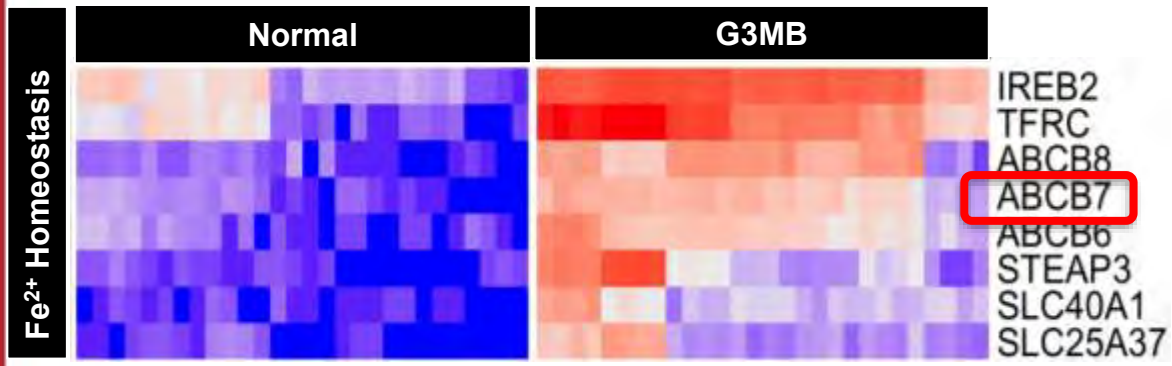
ABC transporters enriched in group 3 MB	Transporters downregulated by miR-1253^{OE} (Log_2 fold $\leq$ -1.0)
ABCA1	ABCA2
ABCA4	ABCA7
ABCC3	ABCA12
ABCG4	ABCA13
ABCA7	**ABCB7**
ABCB6	ABCC9
ABCB7	ABCC13
ABCB8	ABCG8
ABCC1	
ABCD1	
ABCE1	
ABCF1	
ABCF2	

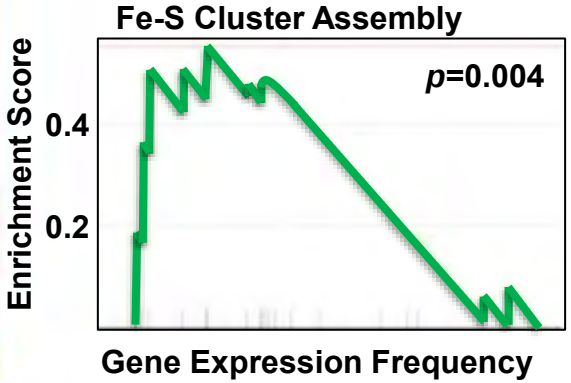






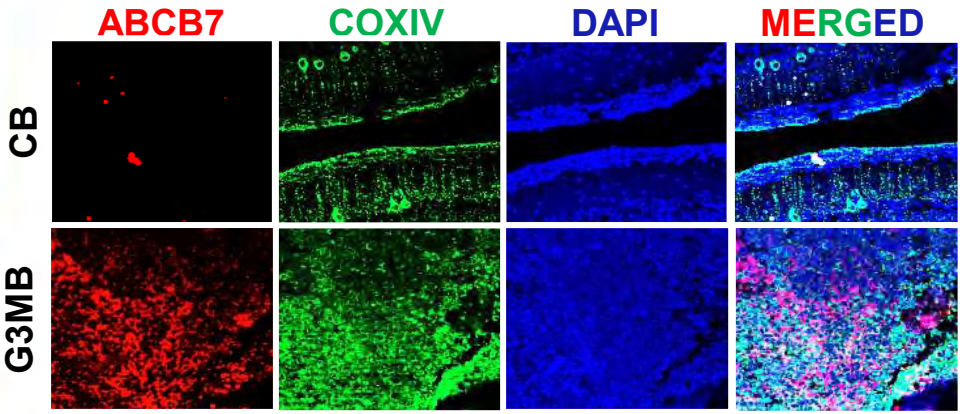
ABCB7 is enriched in G3MB

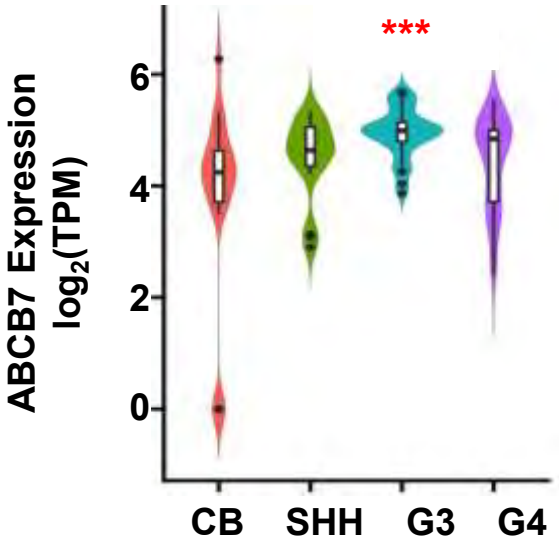


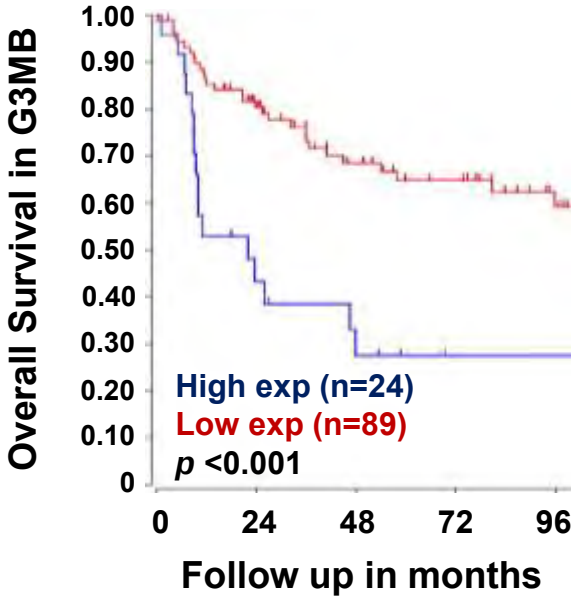


Fe-S clusters
- DNA polymerases
- DNA primases
- DNA helicases
- Electron transport

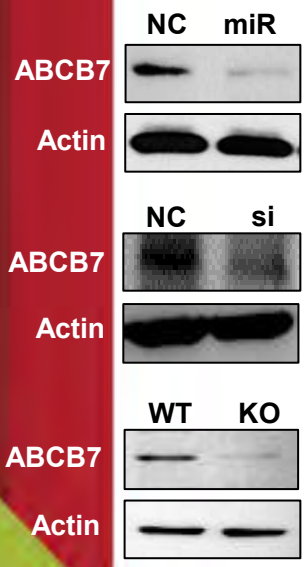
Critical to tumor proliferation

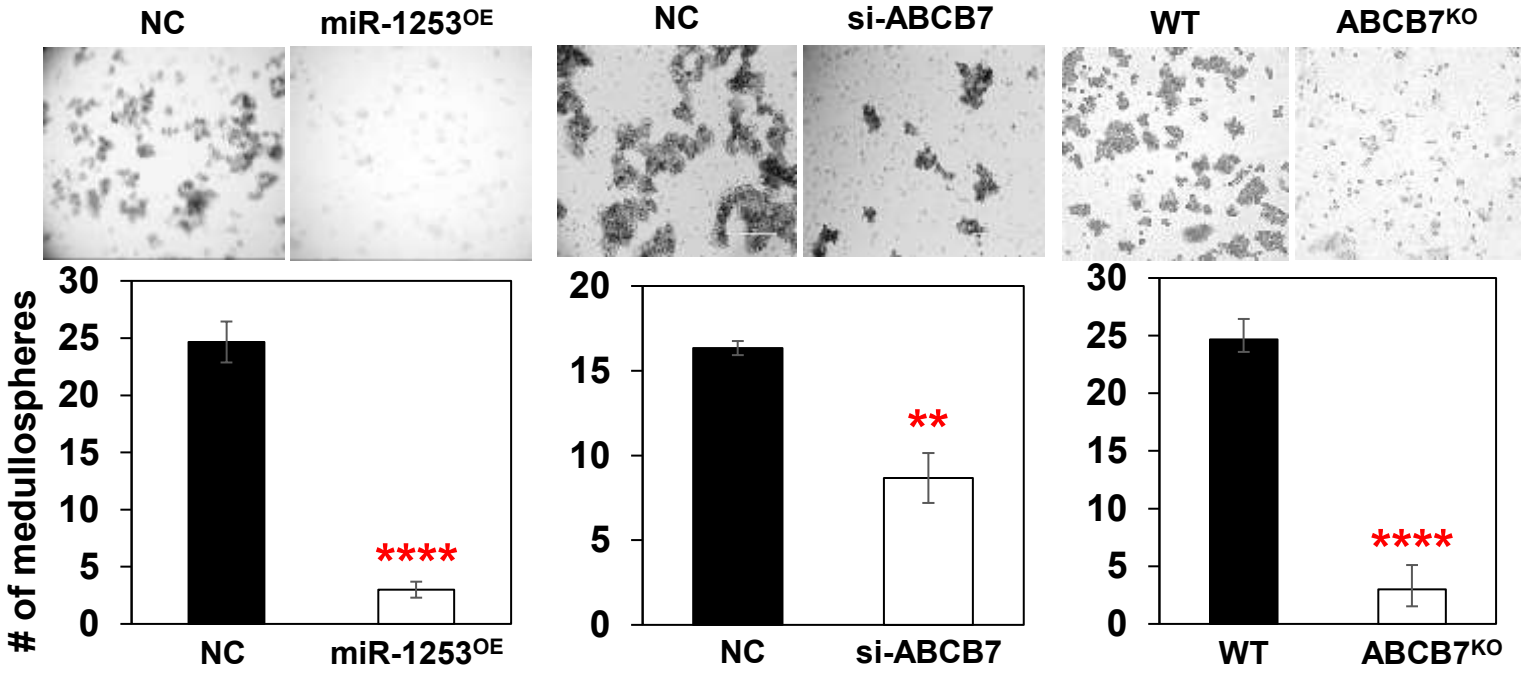


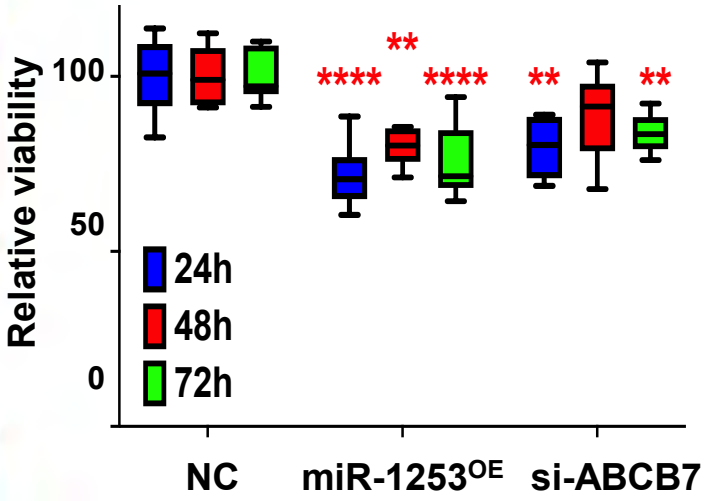


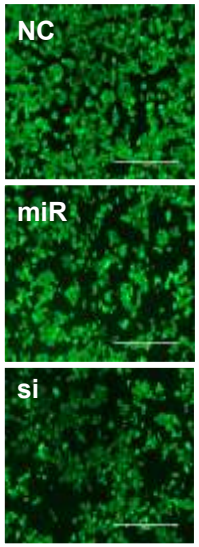


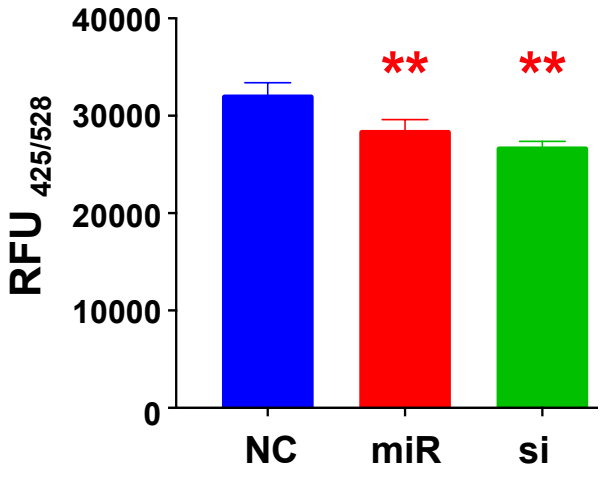
Repressing ABCB7 inhibits cancer cell growth



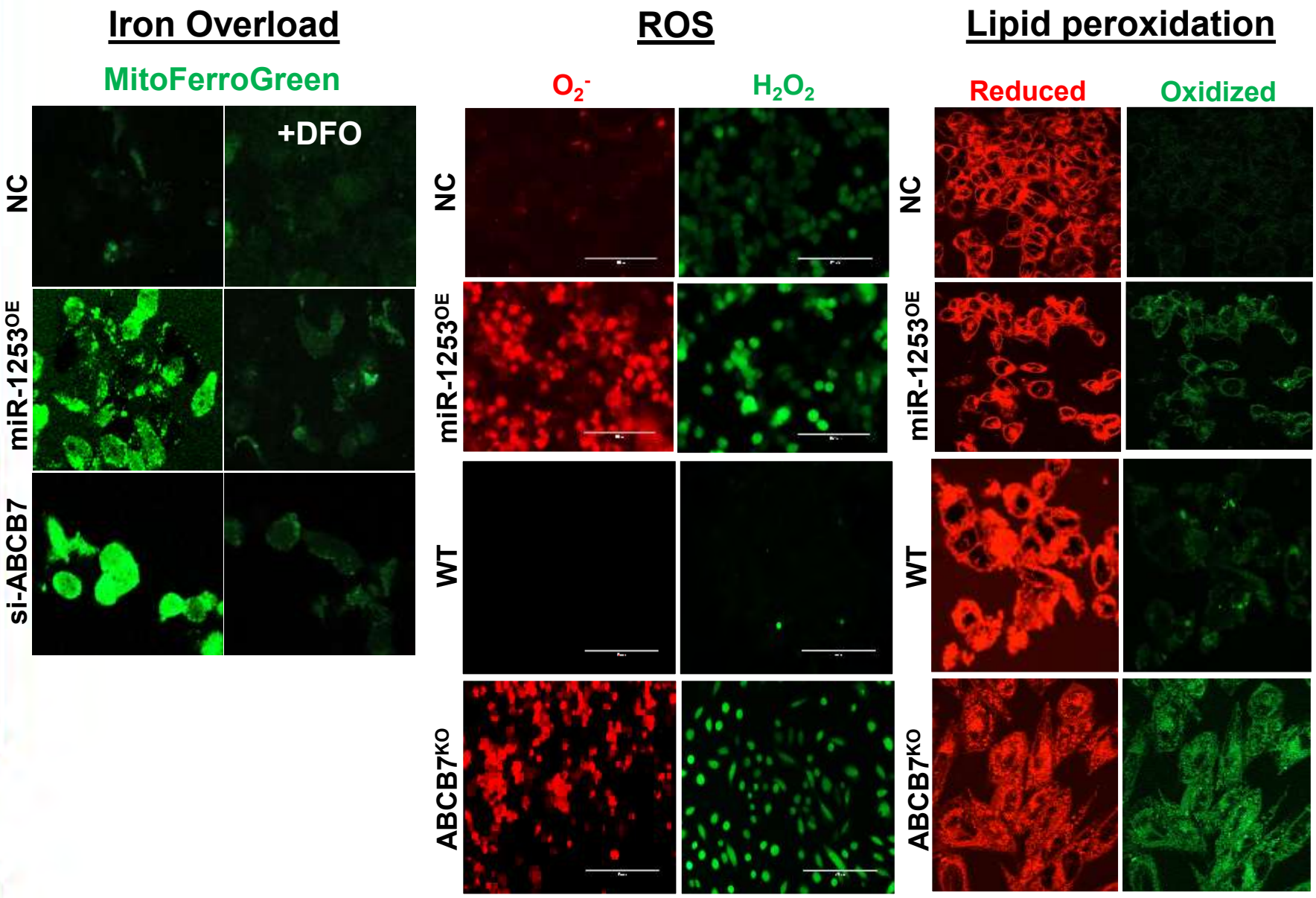




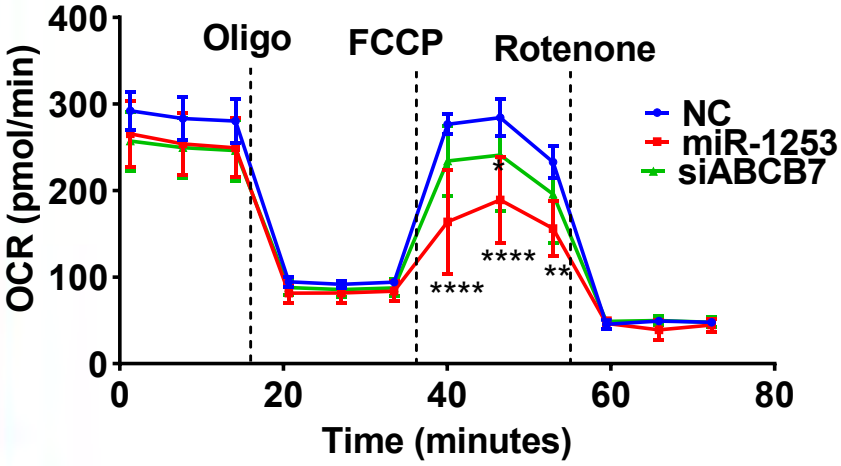


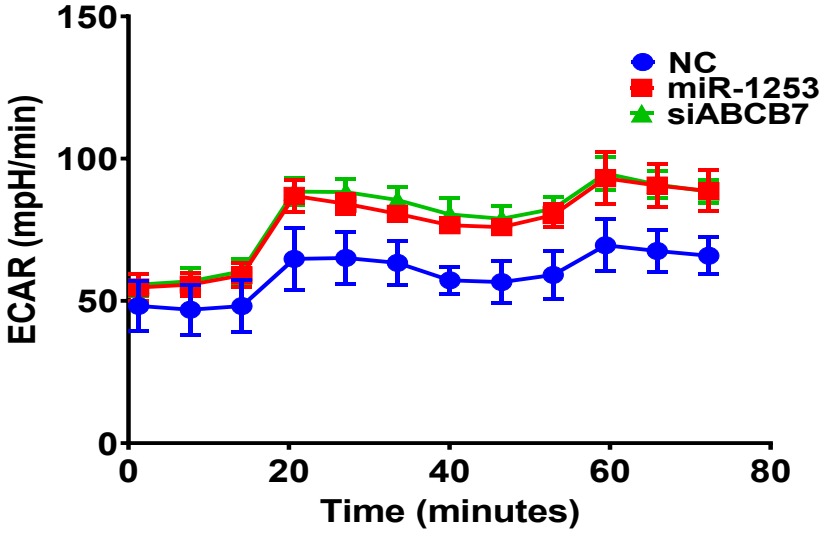


Repressing ABCB7 induces

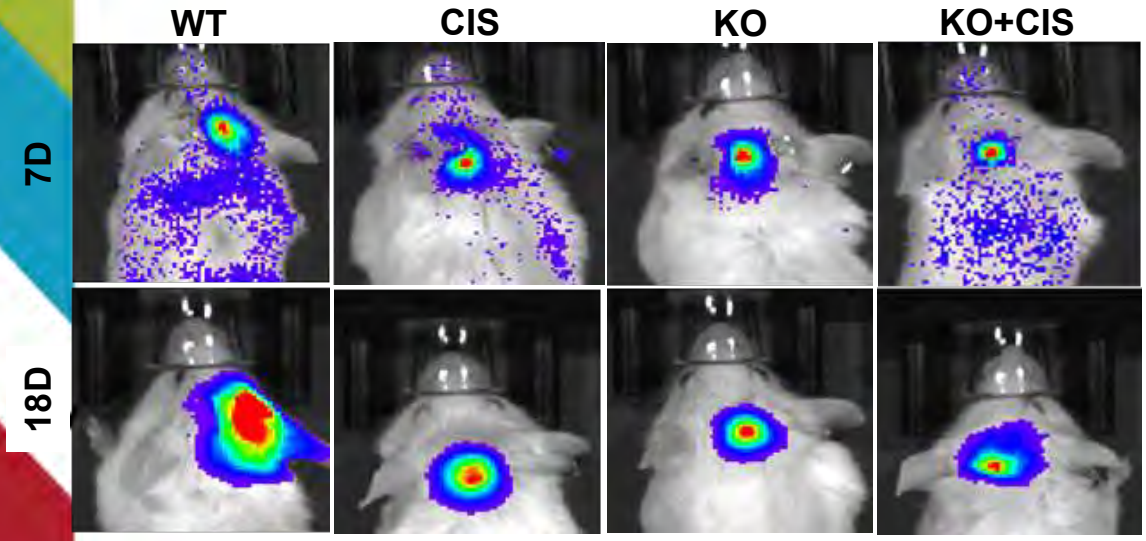


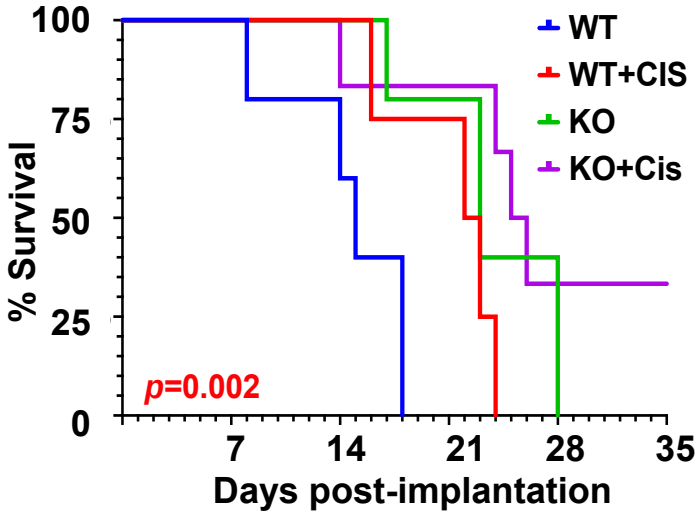
Repressing ABCB7 destabilizes mitochondrial function



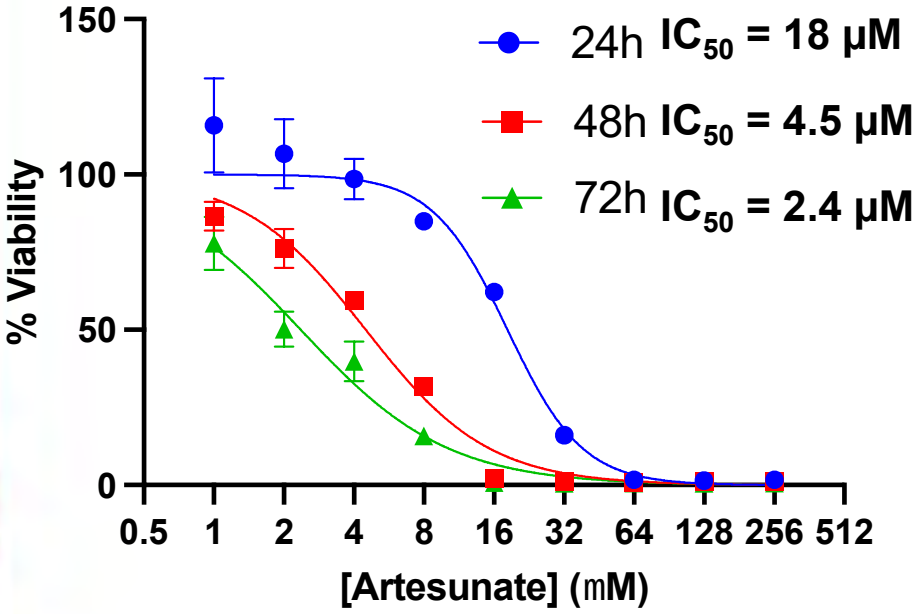


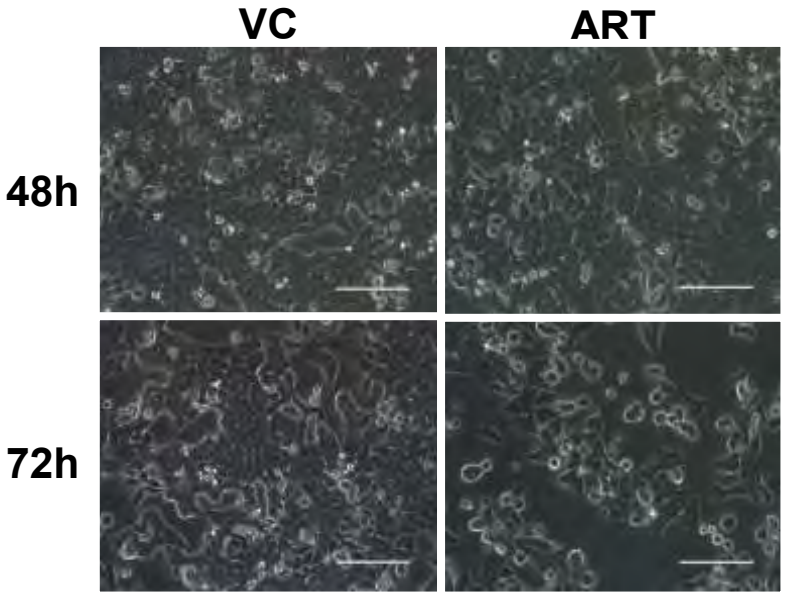
Repressing ABCB7 attenuates G3MB tumor growth

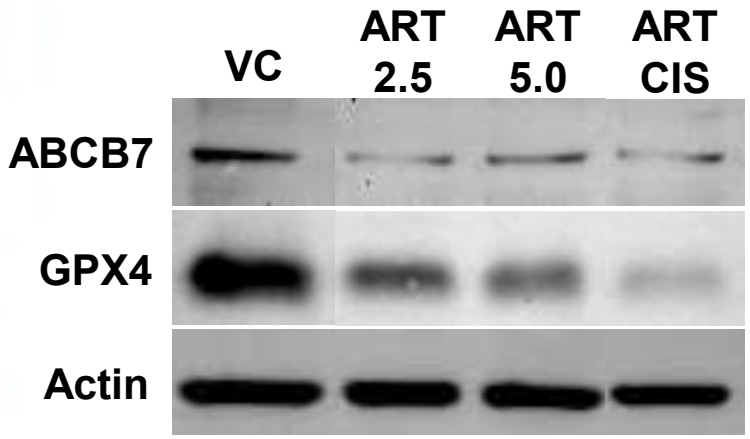


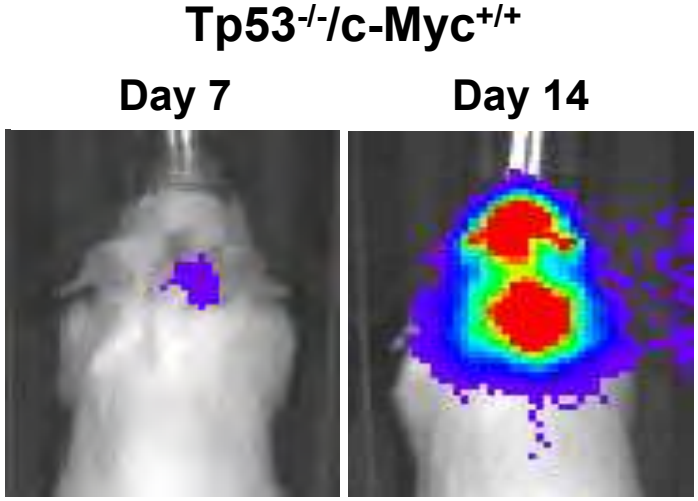


Future Directions









Targeting deregulated molecular pathways triggering aggressiveness

CD276 (B7-H3)

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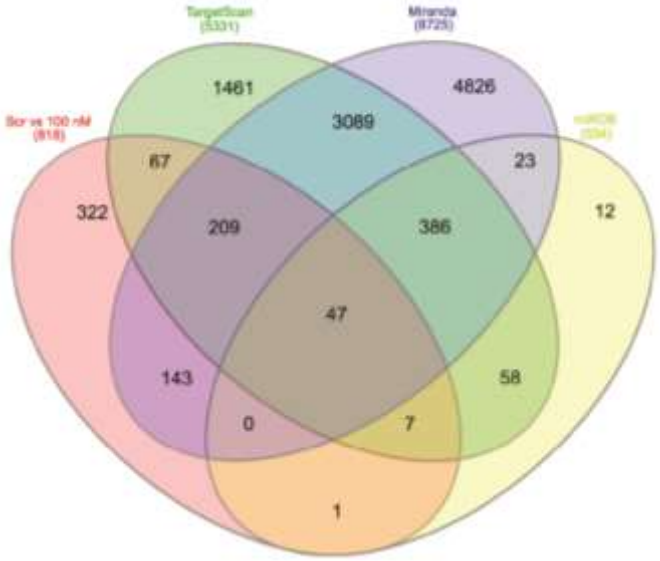
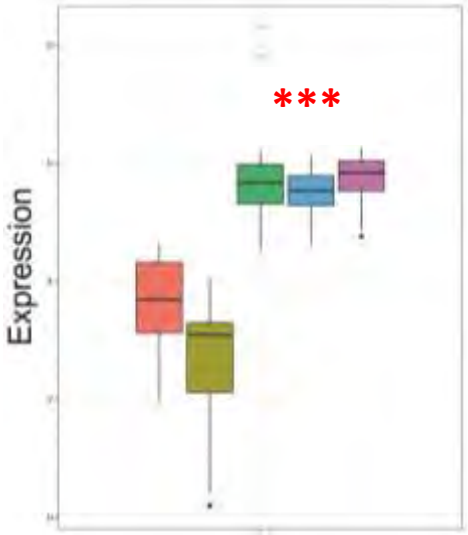
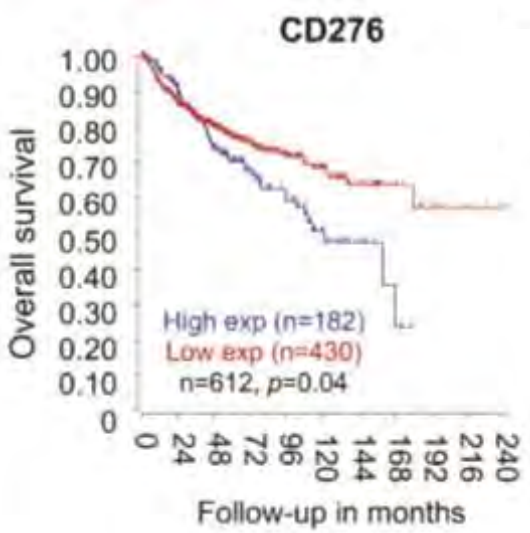
MiR-1253 inhibits CD276 (B7-H3)

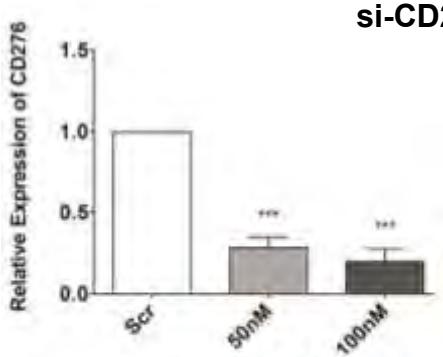


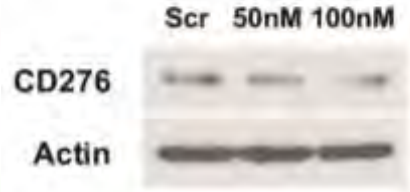


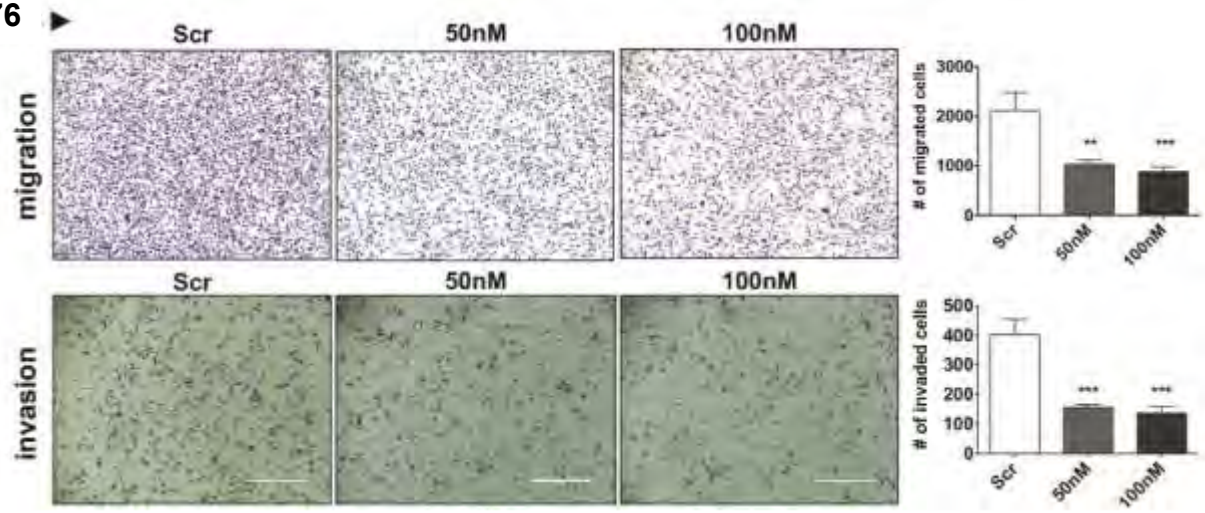
Pedi Adult SHH G3 G4



si-CD276 ▶

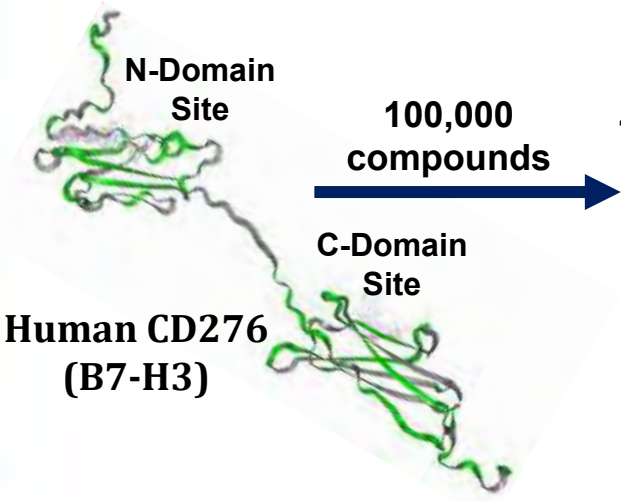






Kanchan et al. *Brain Pathol. 2020.*

Synthetic Inhibitor to B7-H3



100,000 compounds

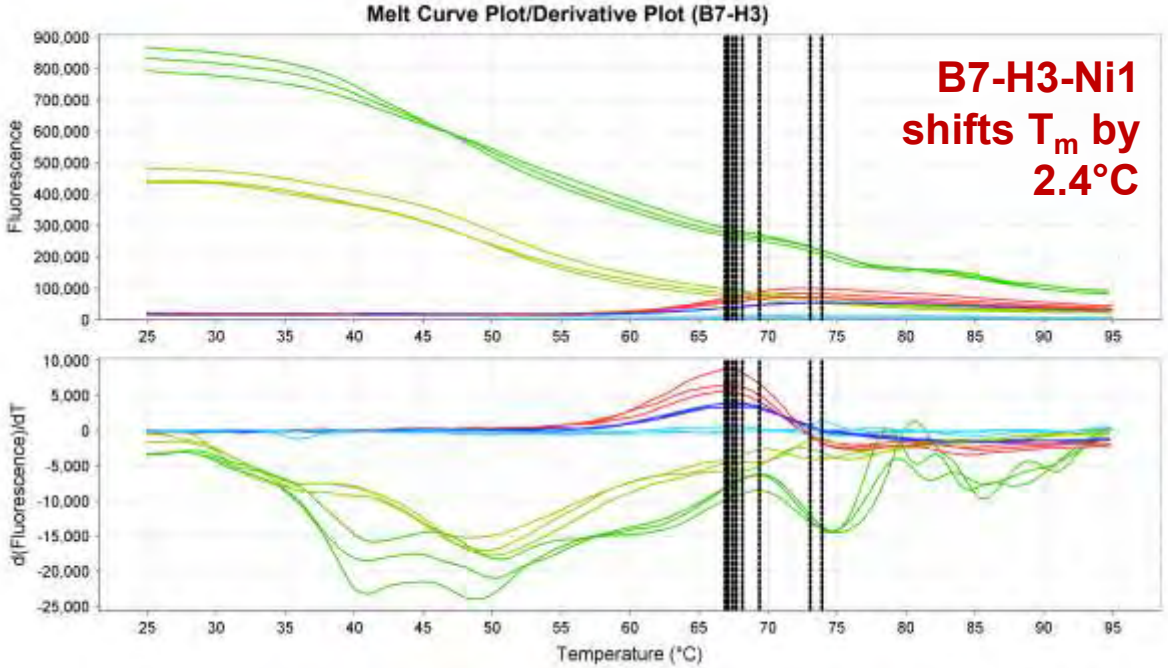
113 hits (0.1%) with docking score -4 or better

Top hit selected from:

- ✓ Docking score -8 or better
- ✓ Percent Human Oral Absorption > 80%
- ✓ Potential CNS Activity = 1
- ✓ Contrasting # of metabolic side reactions

B7-H3-Ni1

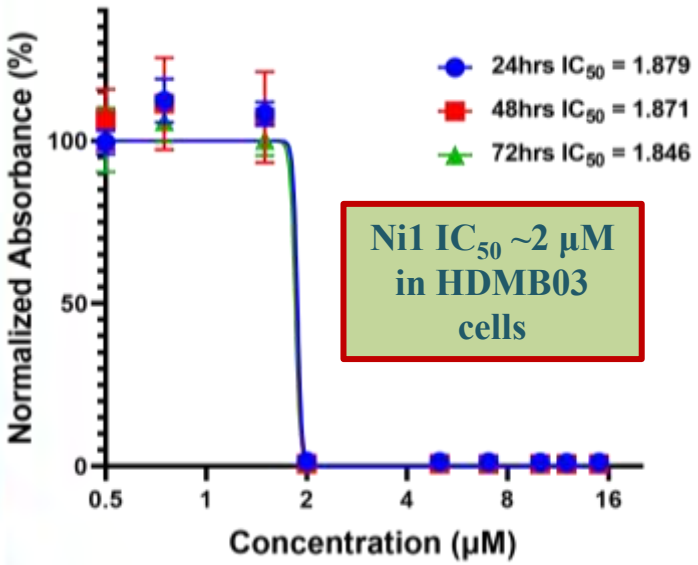
Docking score	Side Rxn	Oral Absorp	CNS
-9.381	6	81%	1



- Control
- B7-H3 + Control
- B7-H3
- B7-H3-Ni1
- B7-H3 + B7-H3-Ni1

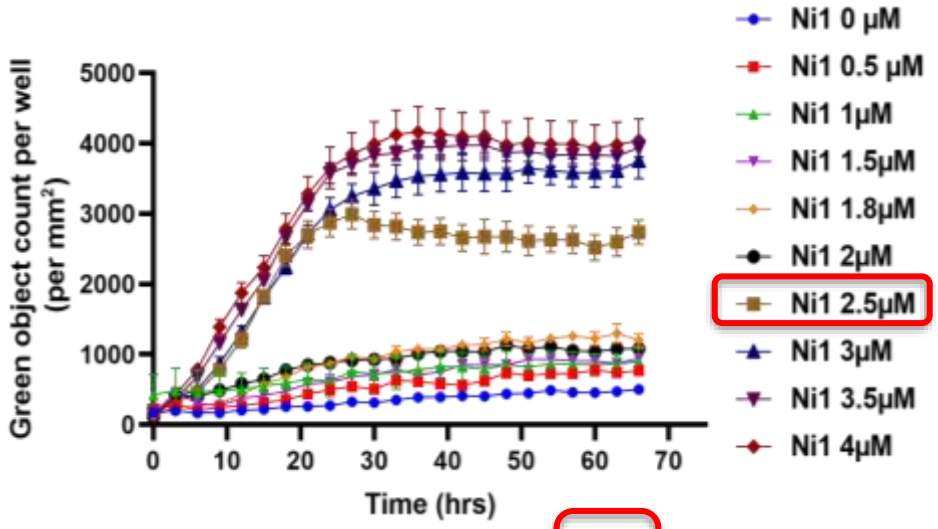
Cytotoxic action of Ni1 at low micromolar concentrations

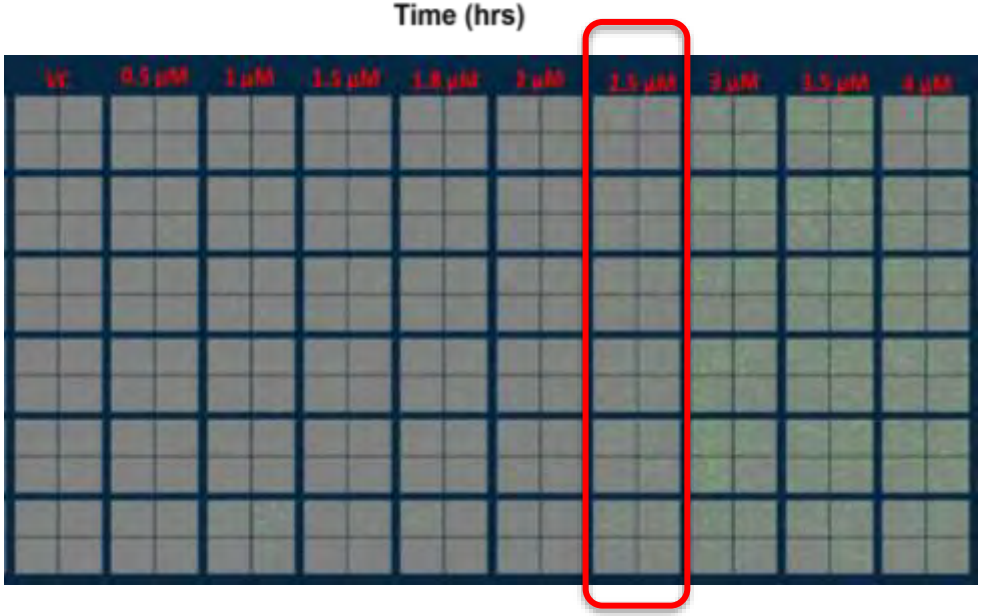
MTT Cytotoxicity Assay



Ni1 IC_{50} ~2 μM in HDMB03 cells

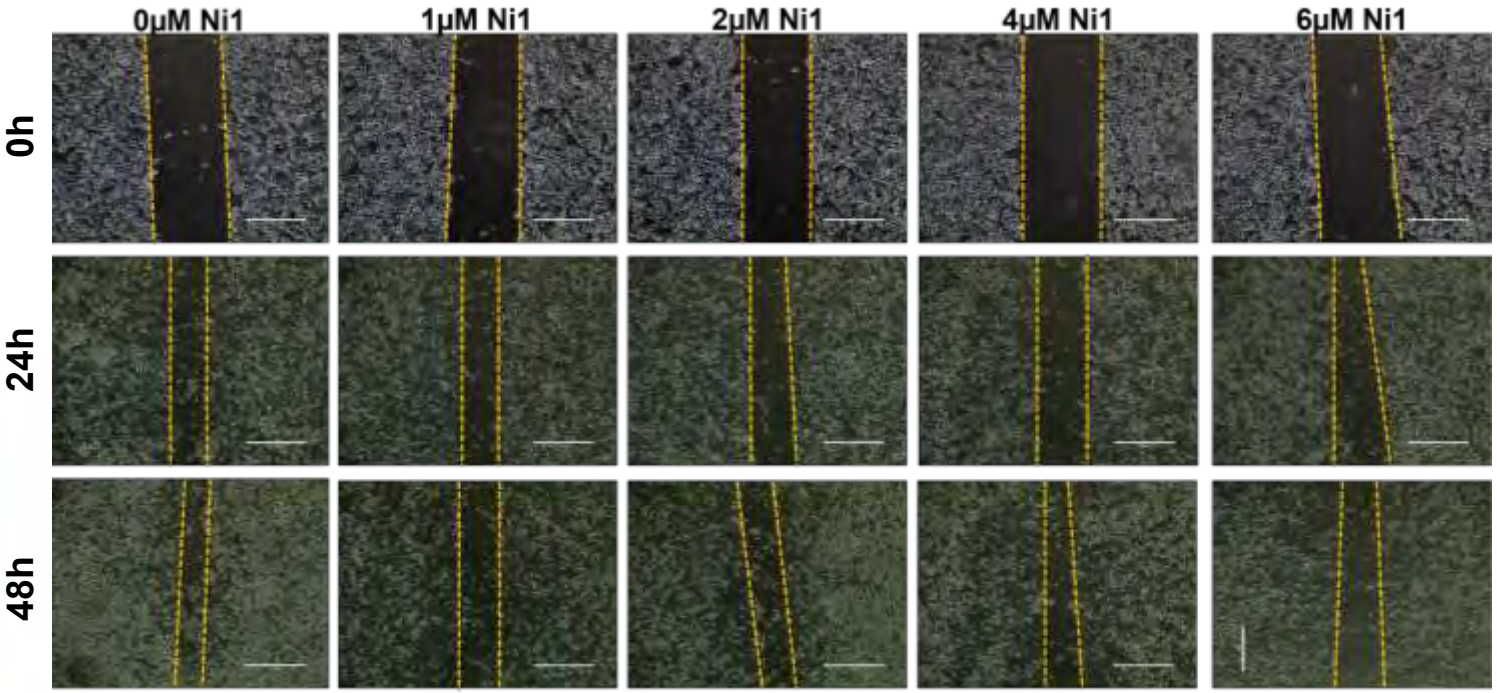
Incucyte® Cytotoxicity Assay

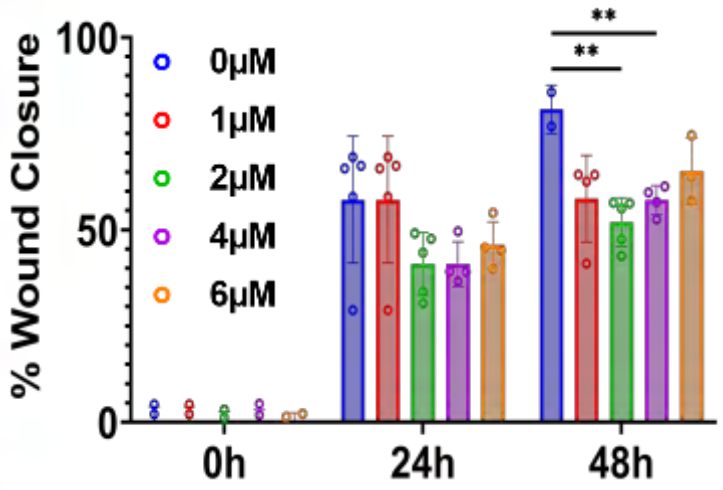


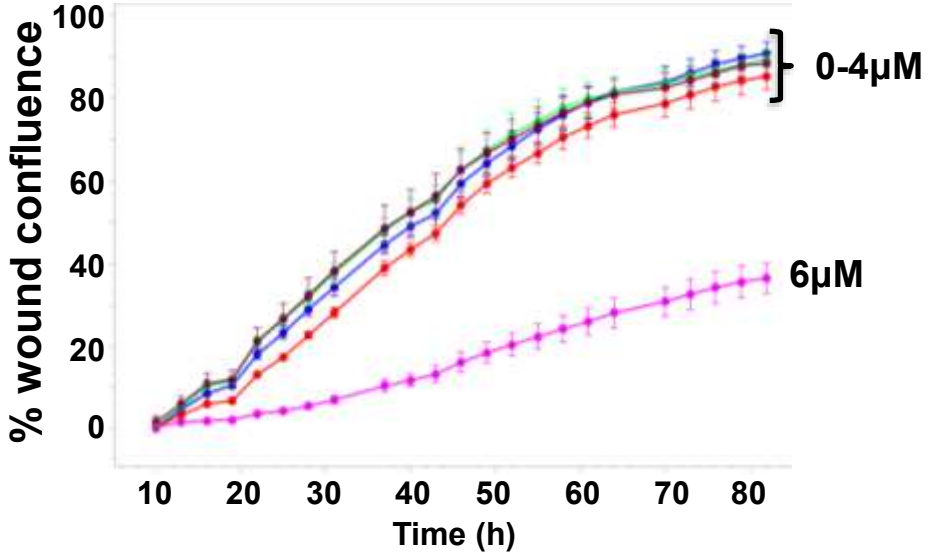


At concentrations higher than 2.5 μM, Ni1 elicited a dose-dependent increase in cytotoxicity in HDMB03 cells.

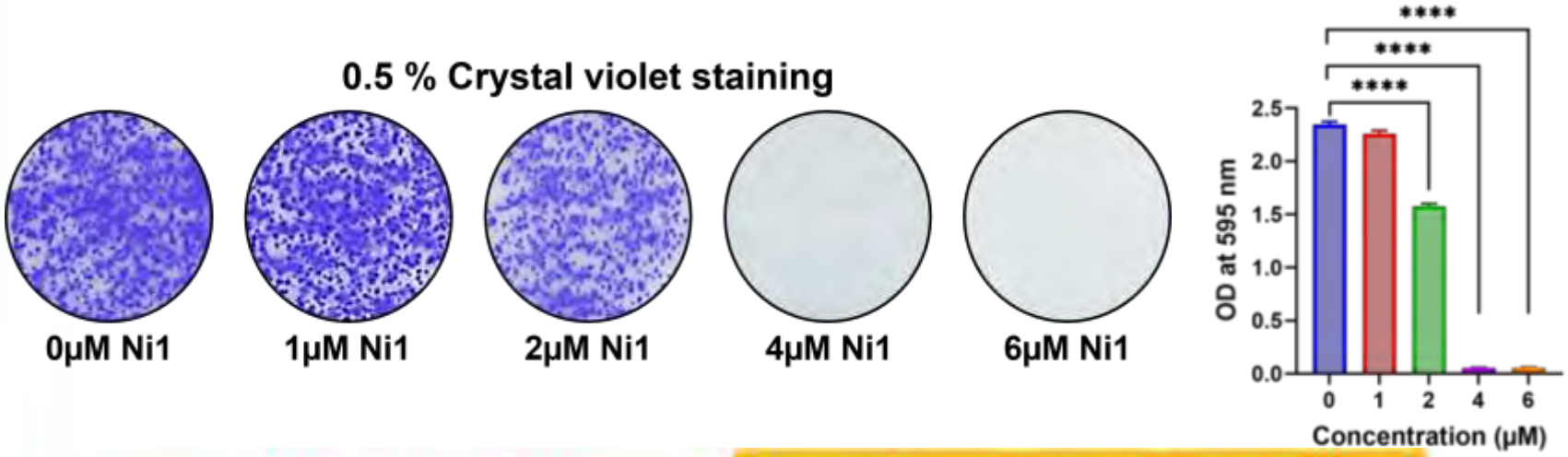
Anti-proliferative action of Ni1

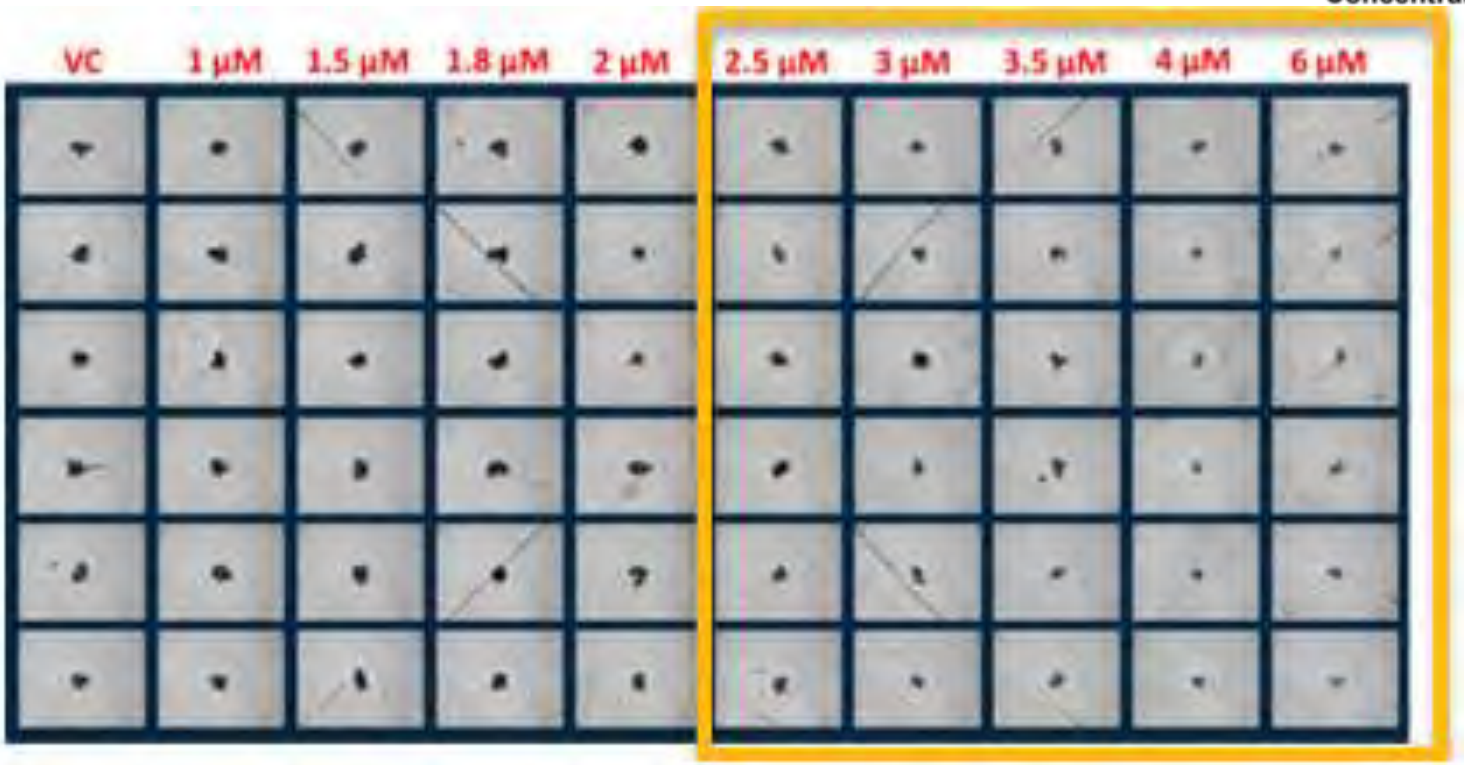




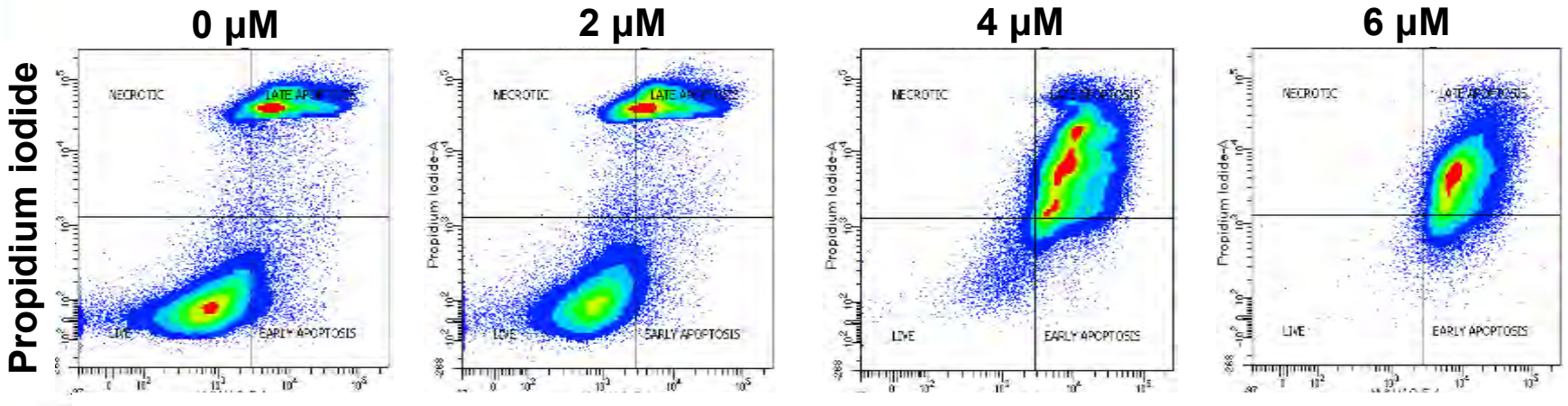


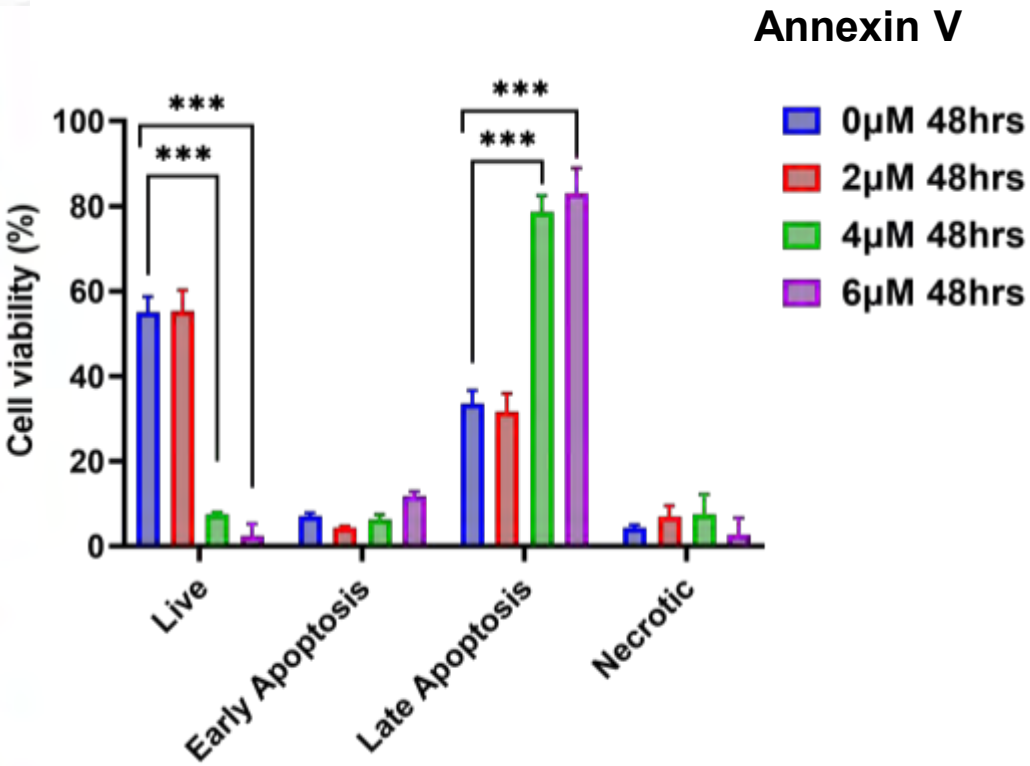
Dose-dependent inhibition on colonogenecity

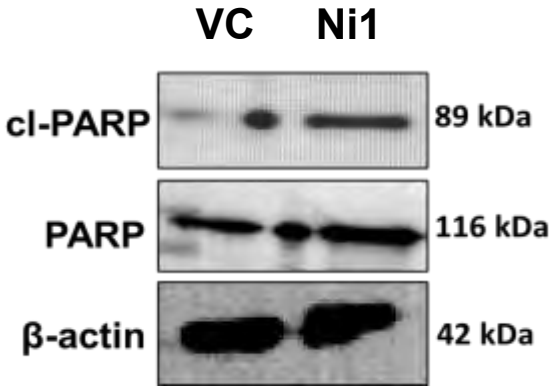




Induction of apoptosis by Ni1



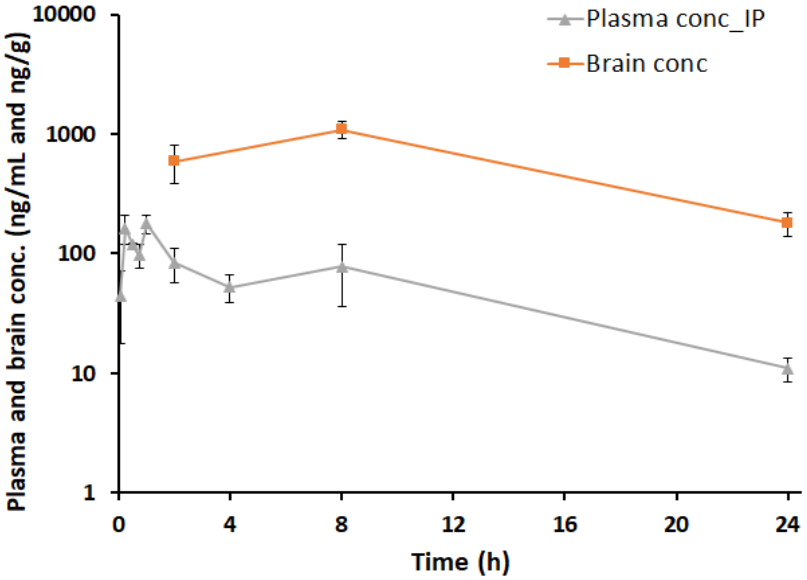




Densitometry Ratio	VC	Ni1
cl-PARP:PARP	0.093	0.367

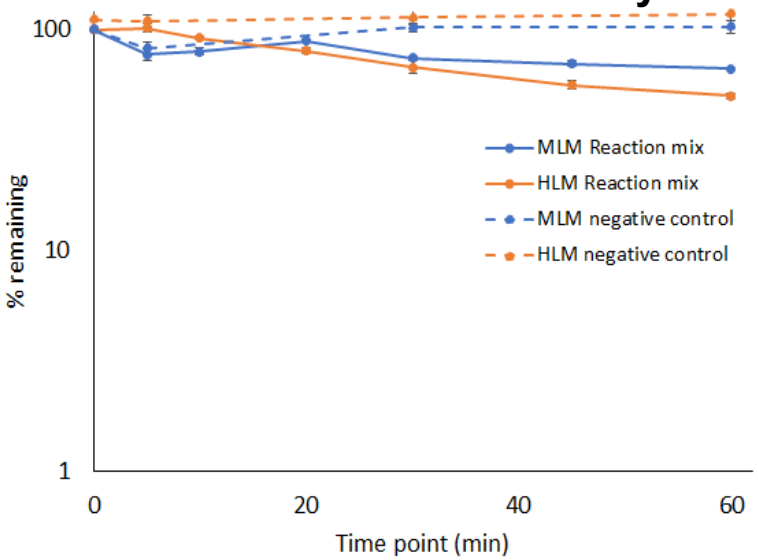
PK/PD/MTD Studies with Ni1

Plasma concentration time profile



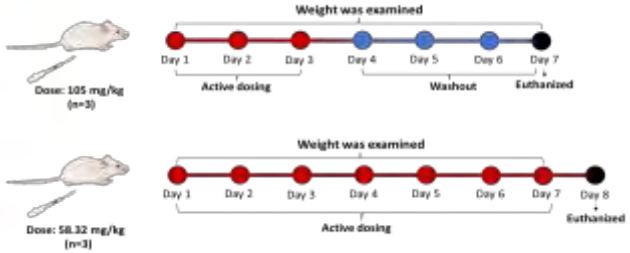
- Ni1 15-fold higher in brain / plasma
- Half-life 8 hours

In vitro mouse and human liver microsomal stability

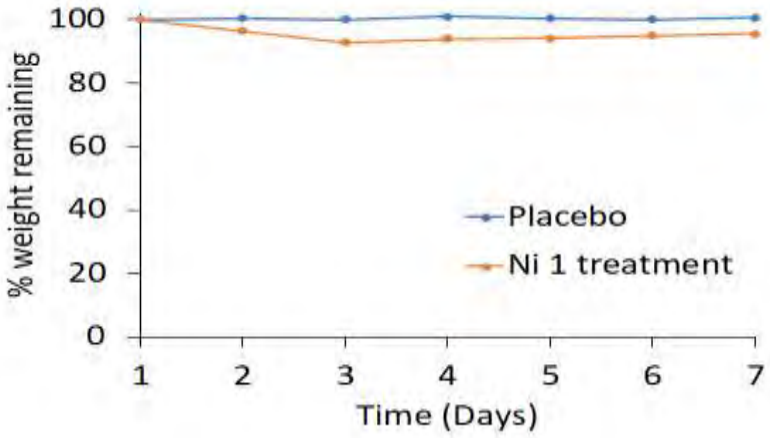


- B7-H3-Ni1 slowly cleared from mouse/ human liver microsomes.

Repeated dose study



- Maximum tolerated dose was 105 mg/kg
- Weight loss of 7.3%



Summary (Objectives re-visited)

- Clinical problem (NE vs. US):
  - Pediatric brain tumors = 7 vs. 5.8 per 100,000
  - Pediatric medulloblastoma = 0.4 vs. 0.5 per 100,000

- Research gaps
  - G3MB >> subgroups in metastasis and recurrence
  - G3MB >> subgroup in mortality

- Novel anti-neoplastic strategies against G3MB
  - Targeting deregulated iron transport in cancer
  - Synthetic inhibitors of B7-H3

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