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Novel Cell Cycle Therapeutic Strategy Against Type 2 Medulloblastoma

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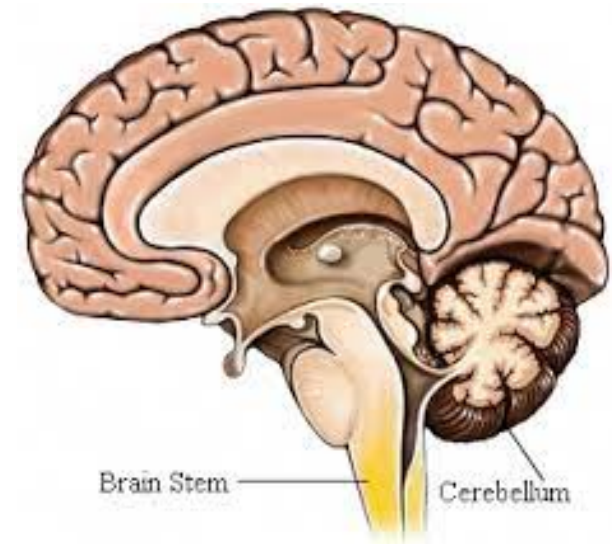
Novel Cell Cycle Therapeutic Strategy Against Type 2 Medulloblastoma

By: Sahar Mouawad

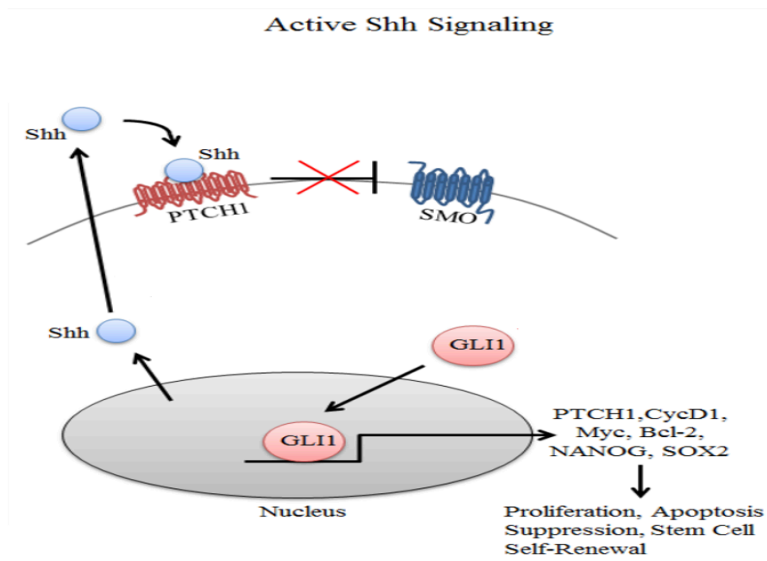


What is Medulloblastoma?

- Medulloblastoma (MB) is a primary central nervous system tumour that occurs in the cerebellum.
- Classified as Grade IV tumour
- Constitutes 16% to 25% of brain cancer cases
- It is common in children between the age of 5 and 9 years old.
- Tumour recurrence in about 30% of children



- Shh – type 2 medulloblastoma
- One of the most prevalent and driven by a pathway involved in stemness of neural cells



Subgroup	WNT	SHH	Group 3	Group 4
% of Cases	10	30	25	35
Age at Diagnosis				
Gender Ratio (M:F)	1:1	1:1	2:1	3:1
Anatomic Location				
Histology	Classic, Rarely LCA	Desmoplastic, Classic, LCA	Classic, LCA	Classic, LCA
Metastasis at Diagnosis (%)	5-10	15-20	40-45	35-40
Recurrence Pattern	Rare; Local or metastatic	Local	Metastatic	Metastatic
Prognosis	Very good	Infants good, others intermediate	Poor	Intermediate
Proposed Cell of Origin	Progenitor cells in the lower rhombic lip	Granule precursors of the external granule layer	Neural stem cells	Unipolar brush cells
Recurrent Gene Amplifications	-	<i>MYCN</i> <i>GLI1</i> or <i>GLI2</i>	<i>MYC</i> <i>MYCN</i> <i>OTX2</i>	<i>SNCAIP</i> <i>MYCN</i> <i>OTX2</i> <i>CDK6</i>
Recurrent SNVs	<i>CTNNB1</i> <i>DDX3X</i> <i>SMARCA4</i> <i>TP53</i>	<i>PTCH1</i> <i>TERT</i> <i>SUFU</i> <i>SMO</i> <i>TP53</i>	<i>SMARCA4</i> <i>KBTBD4</i> <i>CTDNEP1</i> <i>KMT2D</i>	<i>KDM6A</i> <i>ZMYM3</i> <i>KTM2C</i> <i>KBTBD4</i>
Cytogenetic Events ■ Gain ■ Loss	6	3q, 9p 9q, 10q, 17p	1q, 7, 18 8, 10q, 11, 16q i17q	7, 18q 8, 11p, X i17q
Other Recurrent Genetic Events	-	-	<i>GF11</i> and <i>GF11B</i> enhancer hijacking	<i>PRDM6</i> , <i>GF11</i> , and <i>GF11B</i> enhancer hijacking

Age: Infant Child Adult



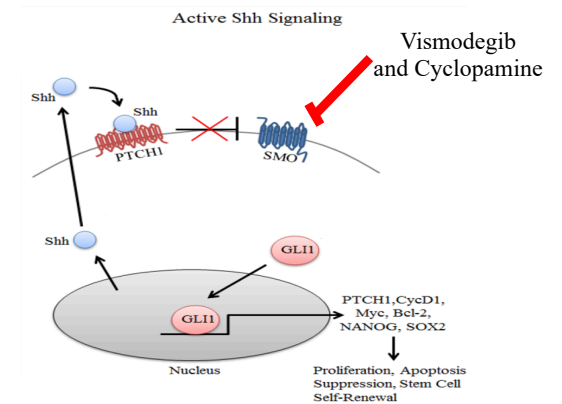
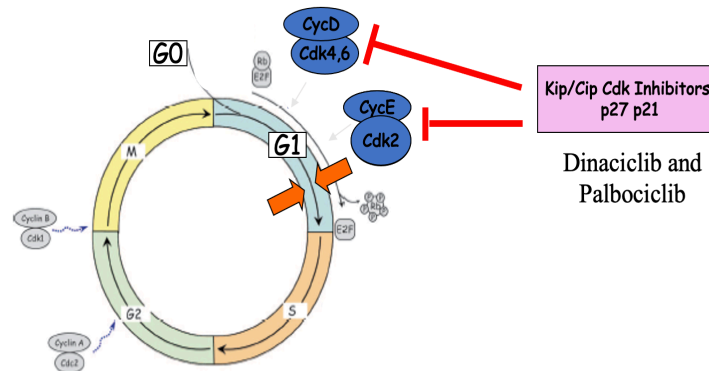
Therapeutic options in MB

Surgery, irradiation
and chemotherapy

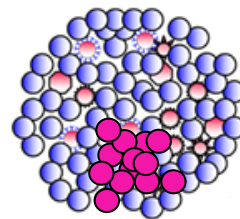
CKIs – Cyclin
Dependent Kinase
Inhibitors

Shh inhibitors

Cisplatin,
Cyclophosphamide
and Temozolomide



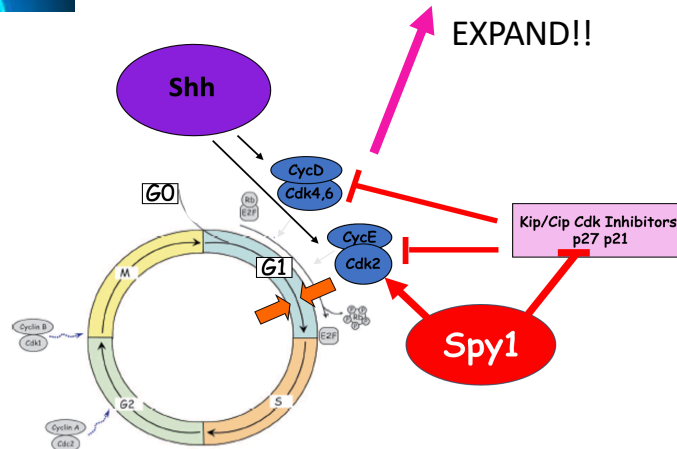
TICs are the treatment obstacle in MB



- Non-TICs
- Potential TICs



Tumour aggressiveness
Therapy resistance
GBM recurrence



What is the role of Spy1 in therapy response of MB?

Objective 1: To study the impact of standard of care chemotherapy on Spy1 expression and its effects in MB.

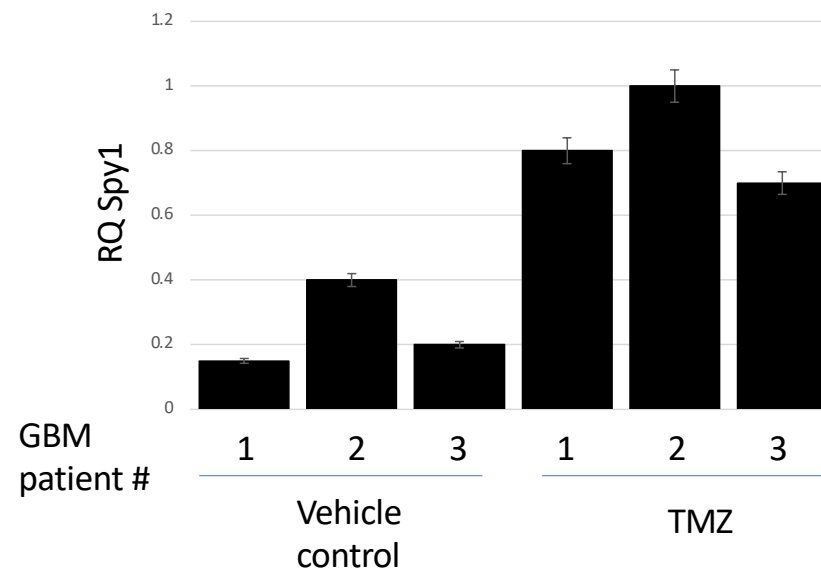
Objective 2: To determine the role of Spy1 in therapy response of MB treated in combination with CKIs and Shh inhibitors.

Objective 3: To investigate the molecular mechanisms regulating the levels of Spy1 in type 2 MB.



Addressing these objectives will allow us to further validate Spy1 as a potential therapeutic target against MB

Spy1 expression levels in response to chemotherapy



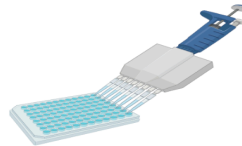
Experimental set up



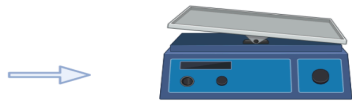
1- Subculture DAOY and ONS-76 cells and seed them on 96 well plate (in 100uL) for 5 different concentrations



2- Add the required amount of chemotherapy vs control (3 replicas for each) , treat for 72 hours by changing media and adding drug every 24 hours



3- After 72 hours add alamar blue and cover the plate in foil.

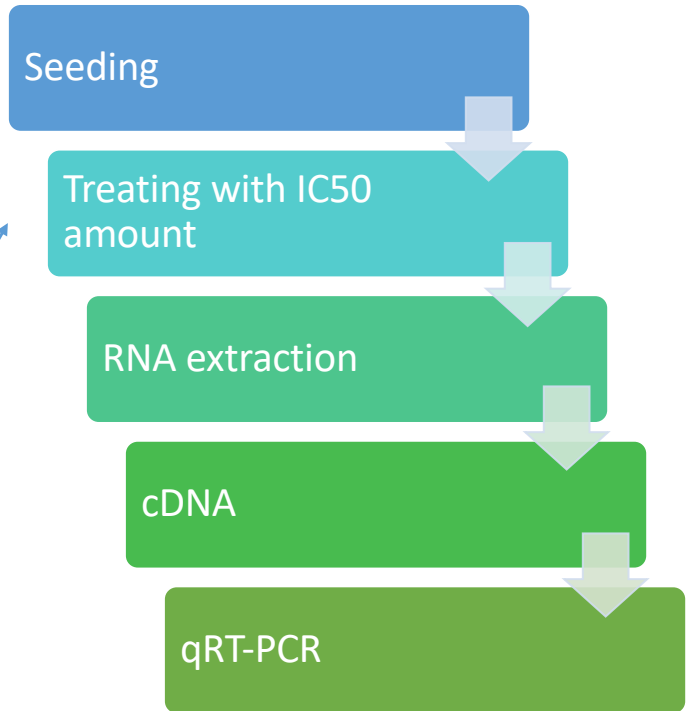
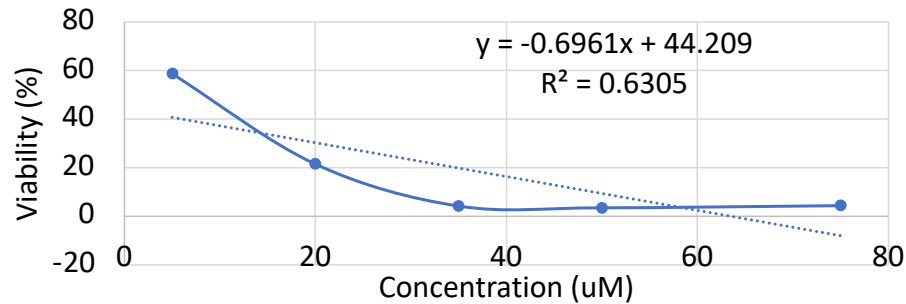


4- Put the plate on the shaker for 2 minutes, take it off and let the plate sit between 1 and 4 hours



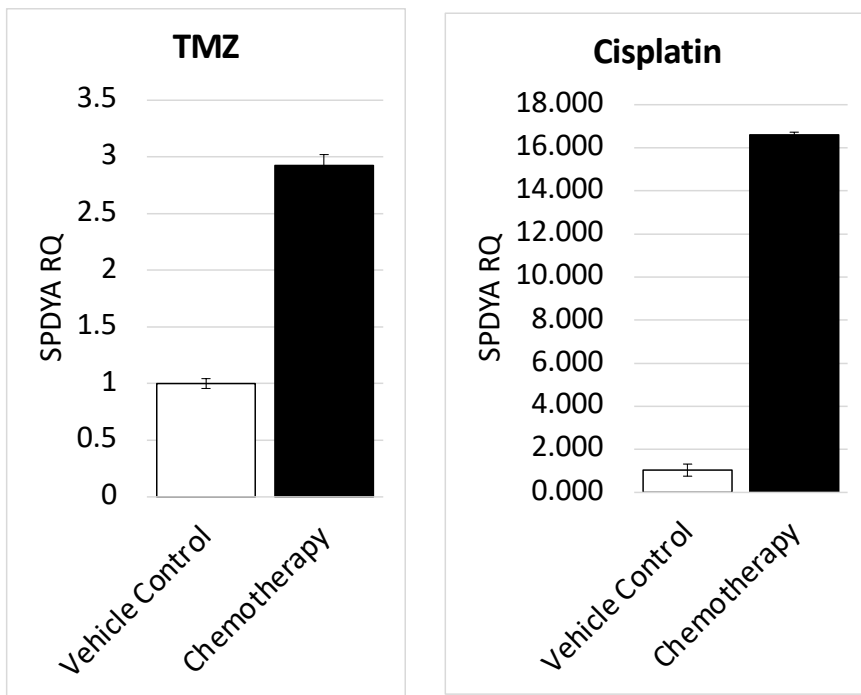
5- Run the results, analyse them and find the IC50 value

ONS-76

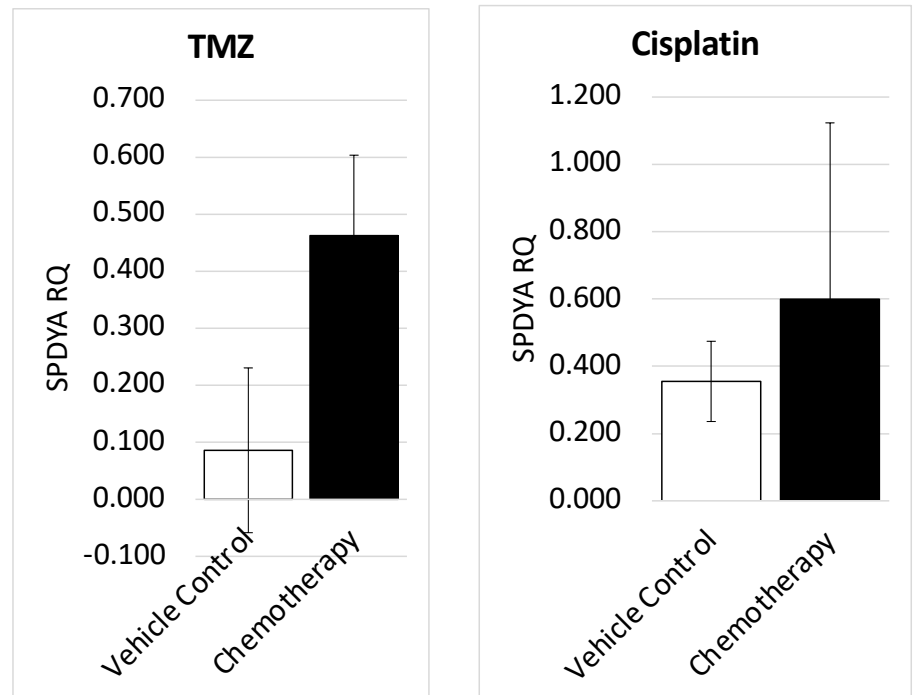


Spy1 expression levels are upregulated in MB cells treated with standard of care chemotherapy

DAOY

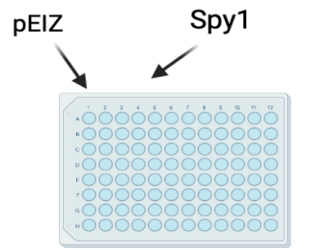


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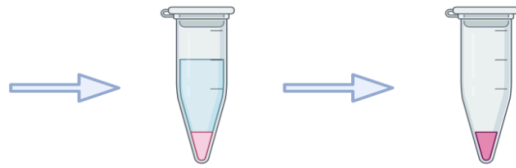


Upregulation of Spy1 in face of the treatment may pose further significant therapeutic challenge

Overexpressing Spy1 using lentivirus

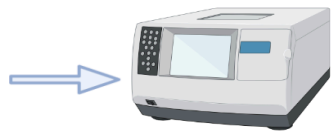


1- Add the lentivirus to the cells (3 replicas for each) leave a couple days then subculture

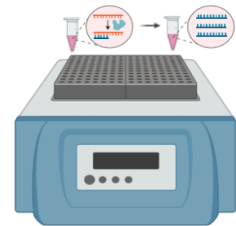


2- After subculturing the cells with the viruses, transfer to eppendorf tubes and spin for 10 minutes at 10000RPM

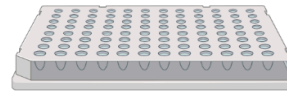
3- After centrifuging take off the supernatant and freeze all tubes at -80°C



4- Perform RNA extraction

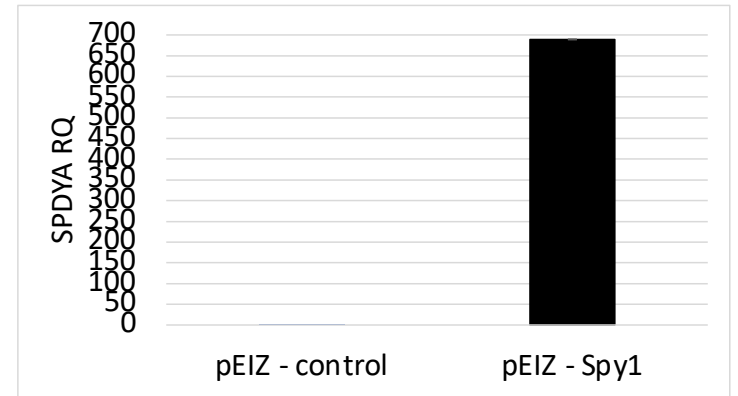


5- Perform cDNA experiment

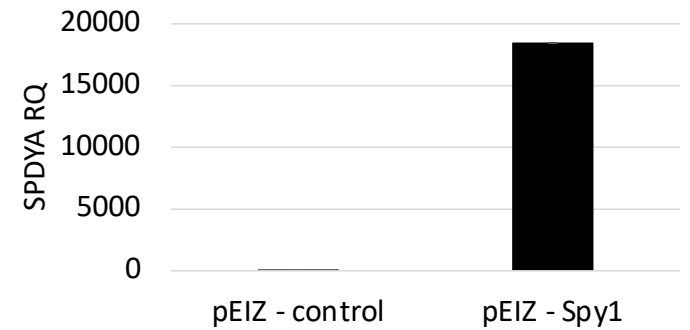


6- Run the qrt-PCR and analyse the results

DAOY



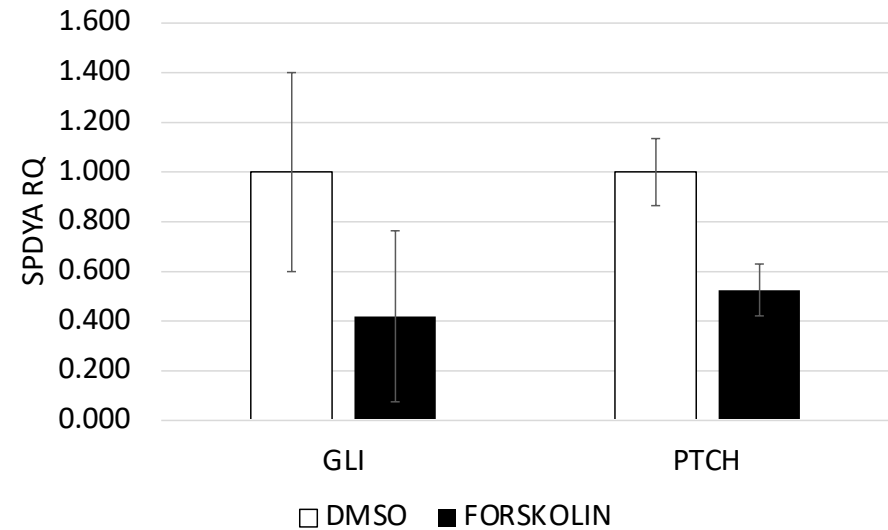
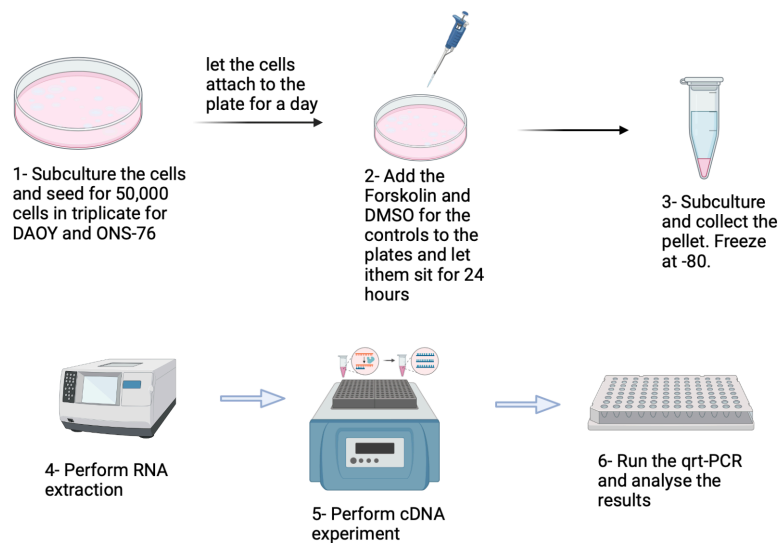
ONS-76



DAOY and ONS-76 cells are successfully infected to overexpress Spy1



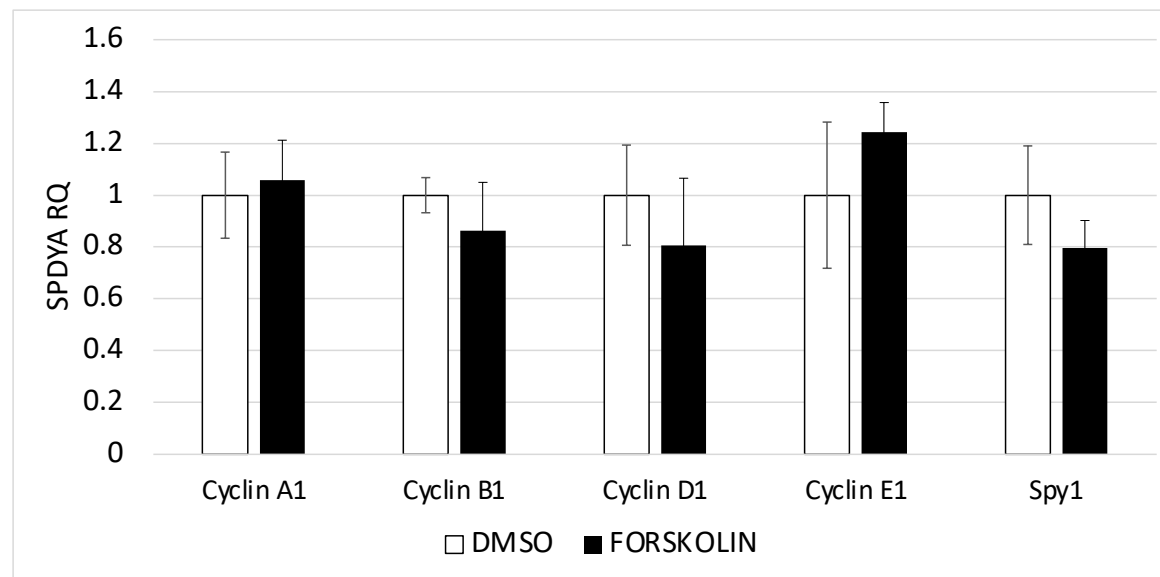
Manipulation of the activity of the Shh pathway



- Treatment with forskolin
- Successfully inhibited the Shh pathway activity

Spy1 mRNA expression levels are downregulated with inhibited Shh pathway

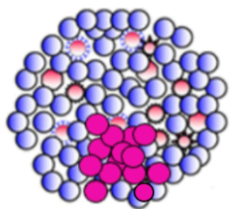
DAOY cells



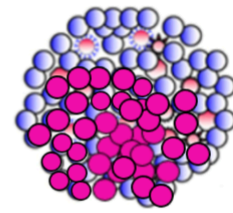
Potential regulation of Spy1 levels by Shh pathway has important implications in effective treatment of MB and needs to be further investigated

● Other MB cells
○ TICs
●

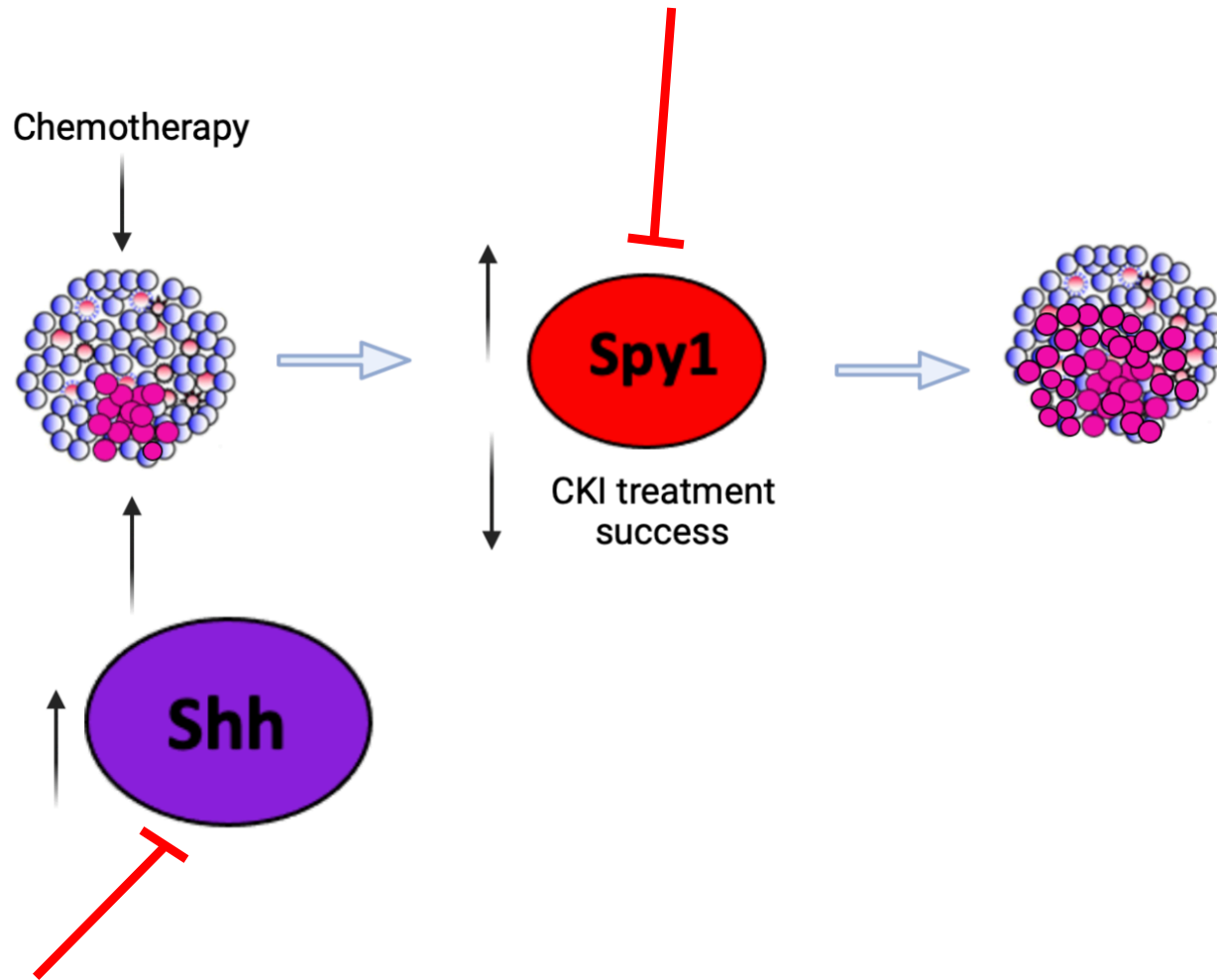
Chemotherapy



CKI treatment success

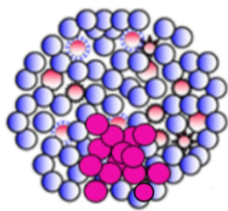


● Other MB cells
○ TICs



● Other MB cells
○ TICs
●

Chemotherapy



CKI treatment success



Treatment success



Recurrence

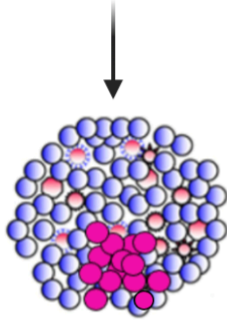


Shh



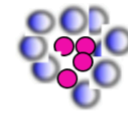
● Other MB cells
● TICs

Chemotherapy



CKI treatment success

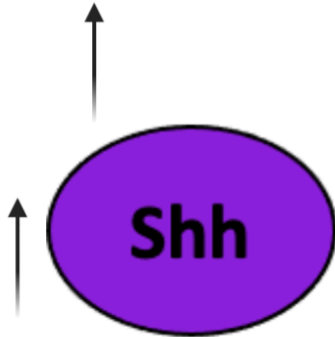
Successful CKI treatment



Recurrence



Patient Survival



Acknowledgments

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- Porter Lab Members



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