Disrupting anti-apoptotic BCL-xL sensitizes SHH-driven medulloblastomas to SHH inhibition

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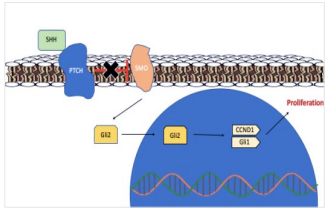
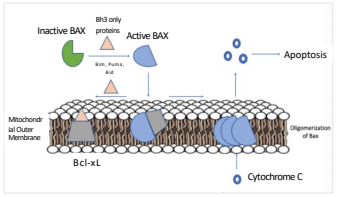
Medulloblastoma Background

- Medulloblastoma is the most common malignant pediatric brain tumor.
- Current treatments result in an ~80% survival rate.
- Issue: therapies still fail 20% of patients and the treatment toxicity can cause long-term neurocognitive deficits.
- Goal: To improve the efficacy of medulloblastoma treatments to increase survival outcomes and decrease treatment toxicity.

Sonic Hedgehog (SHH) signaling drives SHH-subtype medulloblastoma growth



SHH signaling drives medulloblastoma sensitivity to radiation therapy by constitutively activating pro-apoptotic BAX



BCL-xL prevents apoptosis by inhibiting BAX and promotes tumor progression in a SHH subtype medulloblastoma mouse model

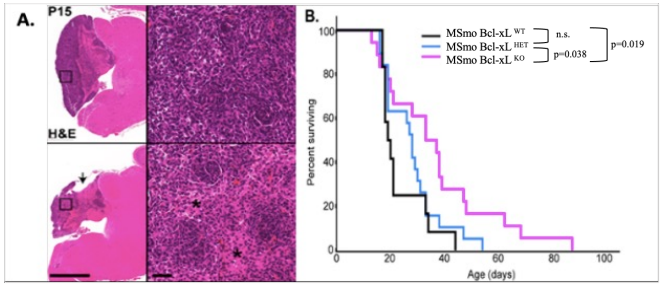


Figure 1: *Bcl-xL* deletion induces focal necrosis and increases survival of medulloblastoma-bearing mice.
(A) Representative images of *Bcl-xL*-intact (top; *MSmo*) and *Bcl-xL*-deleted (bottom; *MSmo/Bcl-xL^{cKO}*) SHH-subtype medulloblastomas in mice. *Bcl-xL*-deleted tumors showed regions of focal necrosis (arrow) and regions of acellularity (*), indicating cell death.

(B) Survival of *MSmo*, *MSmo/Bcl-xL^{HET}*, and *MSmo/Bcl-xL^{cKO}* mice. Deletion of one copy of *Bcl-xL* did not prolong survival, but deletion of both copies of *Bcl-xL* did (p = 0.019).

Hypothesis

Targeting proliferation by inhibiting SHH signaling pharmacologically will sensitize medulloblastomas to *Bcl-xL* deletion.

Experimental Design

Treatment

- Mice injected with 100 mg/kg concentrated POx-Vismodegib from P12-P14.
  - POx-Vismodegib formulation: 8 mg/ml micelle formulation and 10 mg/ml Vismo concentration.
  - Injection volume = (dosage) x (weight in grams)/ (concentration of drug)
- Mice injected every other day until endpoint.

Endpoint

- Injections terminated at P70.
- Humane endpoint=
  - Loss of 15% of body weight overnight
  - Sickly, unresponsive, hunched
- Mouse harvested for IHC study.
  - Brains fixed with 4% PFA

Results

Disrupting BCL-xL sensitizes medulloblastomas to SHH inhibition

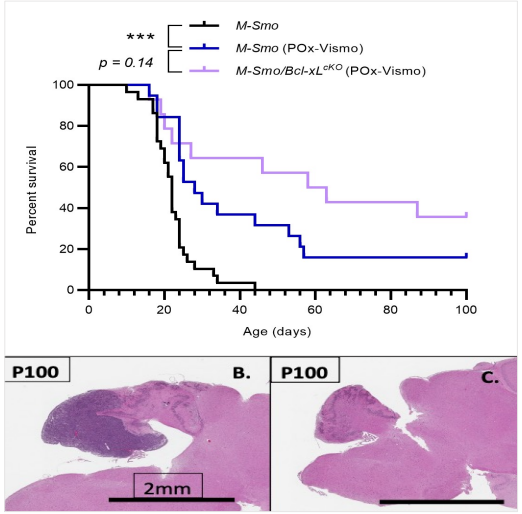


Figure 2: SHH inhibition prolongs survival of SHH medulloblastoma-bearing mice and sensitizes tumors to *Bcl-xL* deletion.
(A)Survival of *MSmo*, *MSmo* (Pox-Vismo), and *MSmo/Bcl-xL^{cKO}* mice. Deletion of both copies of *Bcl-xL* did not prolong survival (p=0.14).

(B-C) Representative H&E stains of tumors from *MSmo* and *MSmo/Bcl-xL^{cKO}* P100 mice. (B) BCL-xL-intact MSmo mice had residual tumors at P100, while (C) *MSmo/Bcl-xL^{cKO}* mice had no residual tumors.

	Sample Size	Median Survival Age	Percent Survival to P100
MSmo (untreated)	29	P22	0 %
MSmo (POx-Vismo)	19	P28	15.7 %
MSmo; Bcl-xL f/f (POx-Vismo)	14	P60.5	35.6 %

Table 1: Sample size, median survival age, and percent survival to P100 of MSmo untreated mice, MSmo POx-Vismo treated mice, and MSmo; Bcl-xL f/f POx-Vismo treated mice.

Conclusions

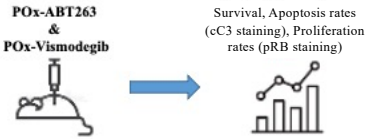
(1) Inhibiting SHH signaling via POx-Vismo prolonged survival of *Bcl-xL*-intact tumor bearing mice.

(2) *Bcl-xL* deletion increased the long-term survival of POx-Vismo-treated tumor mice, as 35.6% of mice with *Bcl-xL*-deleted tumors reached P100, compared to 15.7% for the *Bcl-xL*-intact controls.

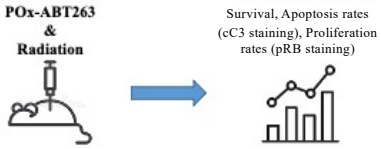
(3) *Bcl-xL* deletion prevents recurrence of POx-Vismo-treated tumor mice, as no residual tumors were found in any *MSmo/Bcl-xL^{cKO}* mice at P100, while all of the P100 *MSmo* mice had residual tumors.

Future Directions

(1) Target both the SHH signaling pathway and BCL-xL pharmalogically.
- POx-ABT263 = inhibits BCL-xL and induces spontaneous apoptosis *in vivo*



(2) Combine BCL-xL deletion, or pharmalogically inhibit BCL-xL using POx-ABT263, with radiation treatment.
- Radiation = targets proliferating tumor cells



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