

# Discovery of PRT811, a potent, selective, and orally bioavailable brain penetrant PRMT5 inhibitor for the treatment of brain tumors

Yang Zhang, Hong Lin, Min Wang, Dimitrios Angelis, Michael Hawkins, Dave Rominger, Tom Emm, Venkat Thodima, Juan Luengo, Bruce Ruggeri, Peggy Scherle, Kris Vaddi

Prelude Therapeutics, 200 Powder Mill Road, Wilmington, DE 19803

Contact: yzhang@preludetx.com

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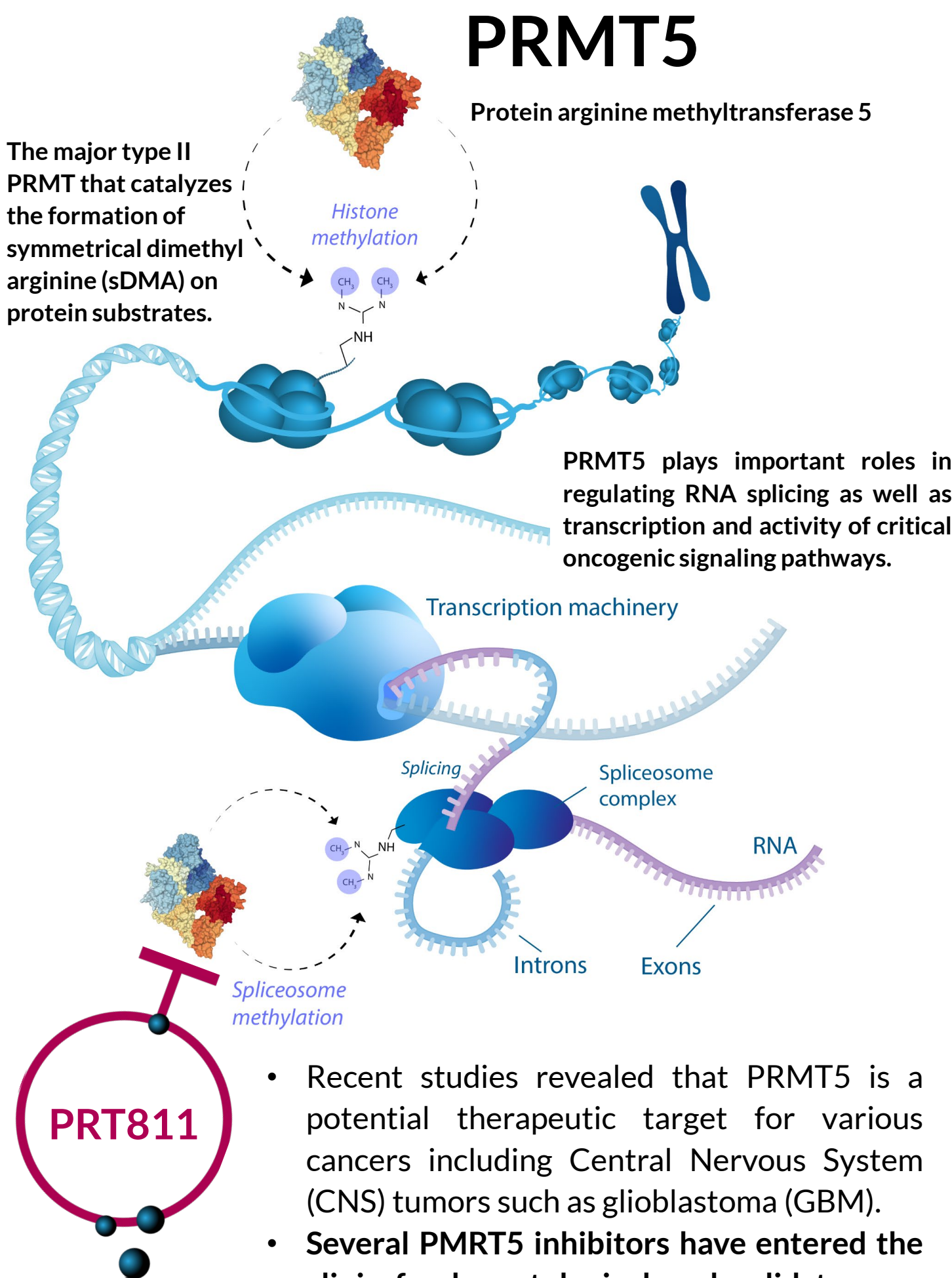
## INTRODUCTION

### PRMT5

Protein arginine methyltransferase 5

The major type II PRMT that catalyzes the formation of symmetrical dimethyl arginine (sDMA) on protein substrates.

PRMT5 plays important roles in regulating RNA splicing as well as transcription and activity of critical oncogenic signaling pathways.

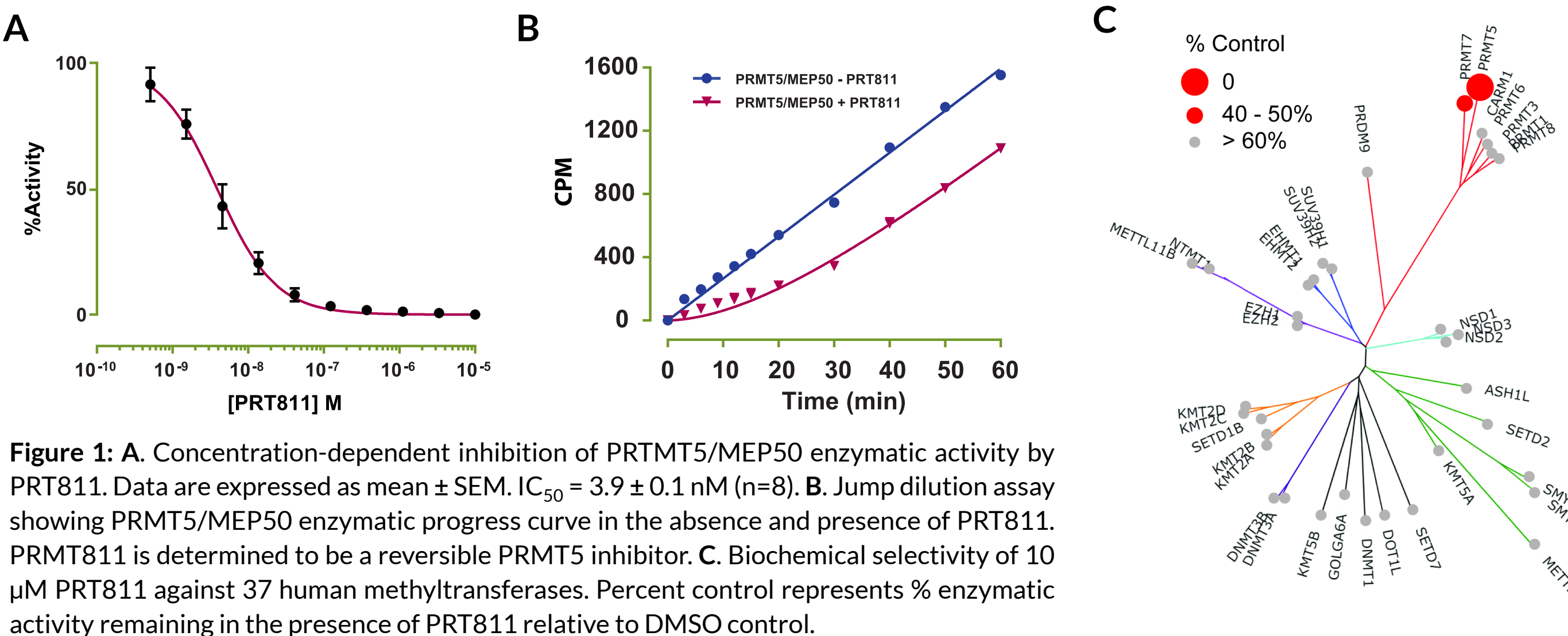


- Recent studies revealed that PRMT5 is a potential therapeutic target for various cancers including Central Nervous System (CNS) tumors such as glioblastoma (GBM).
- Several PMRT5 inhibitors have entered the clinic for hematological and solid tumors, but none have been reported to be brain penetrant.

PRT811 is a potent, selective, and orally bioavailable brain penetrant PRMT5 inhibitor currently under evaluation in a Phase I clinical trial in patients with advanced solid tumors, gliomas, and myelofibrosis (NCT04089449).

## RESULTS

### PRT811 IS A POTENT AND SELECTIVE PRMT5 INHIBITOR



### PRT811 PENETRATES THE BBB IN RODENTS WITH >2-FOLD HIGHER BRAIN EXPOSURE COMPARED TO PLASMA

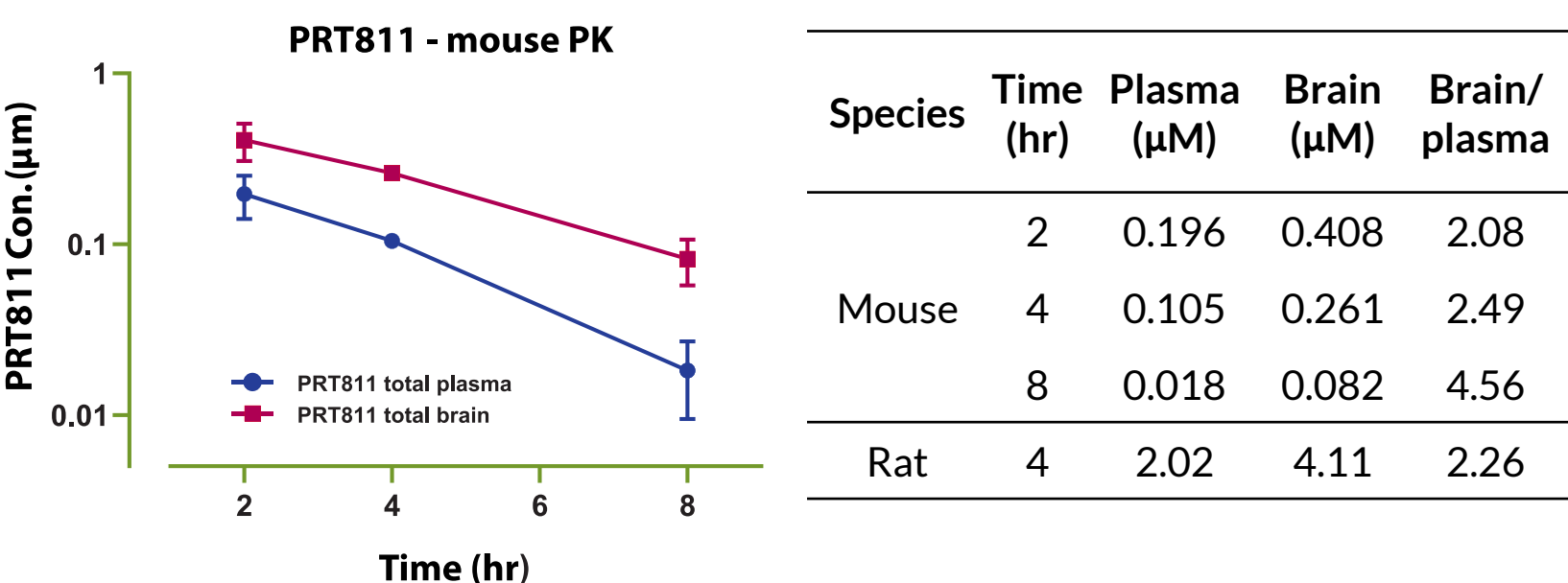


Figure 2. PRT811 pharmacokinetics. Concentration (total) of PRT811 in mouse plasma and brain following oral administration (25 mg/kg). Concentration in rat plasma and brain following IV bolus dose at 2.4 mg/kg followed by a 4-h IV infusion at 5 mL/h/kg. Data are expressed as mean concentration (±SD) in naïve male animals (n = 3 per time point).

### PRT811 INHIBITS sDMA AND CELL PROLIFERATION OF BRAIN TUMOR CELLS REGARDLESS OF THEIR MGMT EXPRESSION STATUS

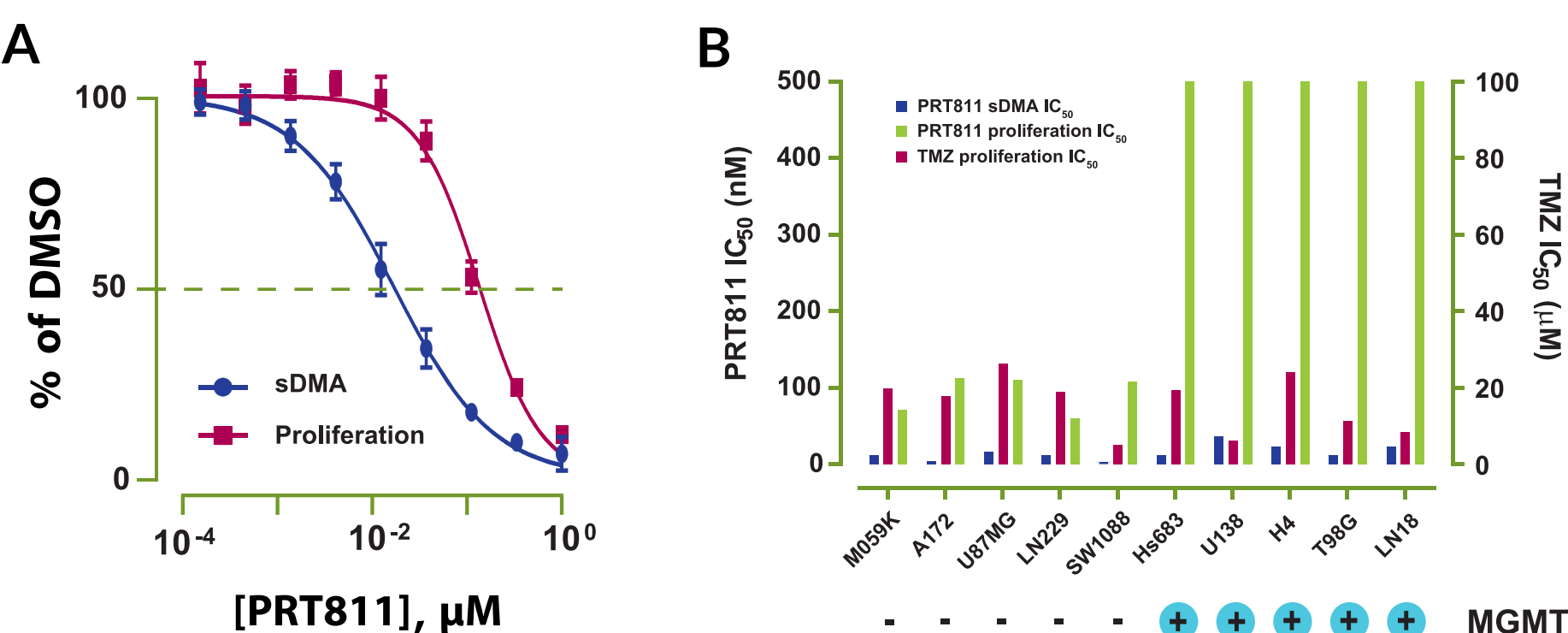


Figure 3: A. Concentration-dependent inhibition of cellular sDMA and cell proliferation by PRT811 in U-87 MG cells. sDMA IC<sub>50</sub>=17 ± 1 nM (n=12); Proliferation IC<sub>50</sub>=134 ± 7 nM (n=12). B. Summary of potencies of PRT811 in inhibiting cellular sDMA and proliferation (PRT811 and TMZ) in a panel of brain cancer cell lines. Cells expressing MGMT are resistant to TMZ, but still sensitive to PRT811. MGMT protein levels in cell lines were validated by WB. -: not expressed; +: expressed.

### PRT811 IS BROADLY ACTIVE AGAINST BRAIN CANCER CELLS AND CANCERS WITH HIGH RATE OF BRAIN METASTASES

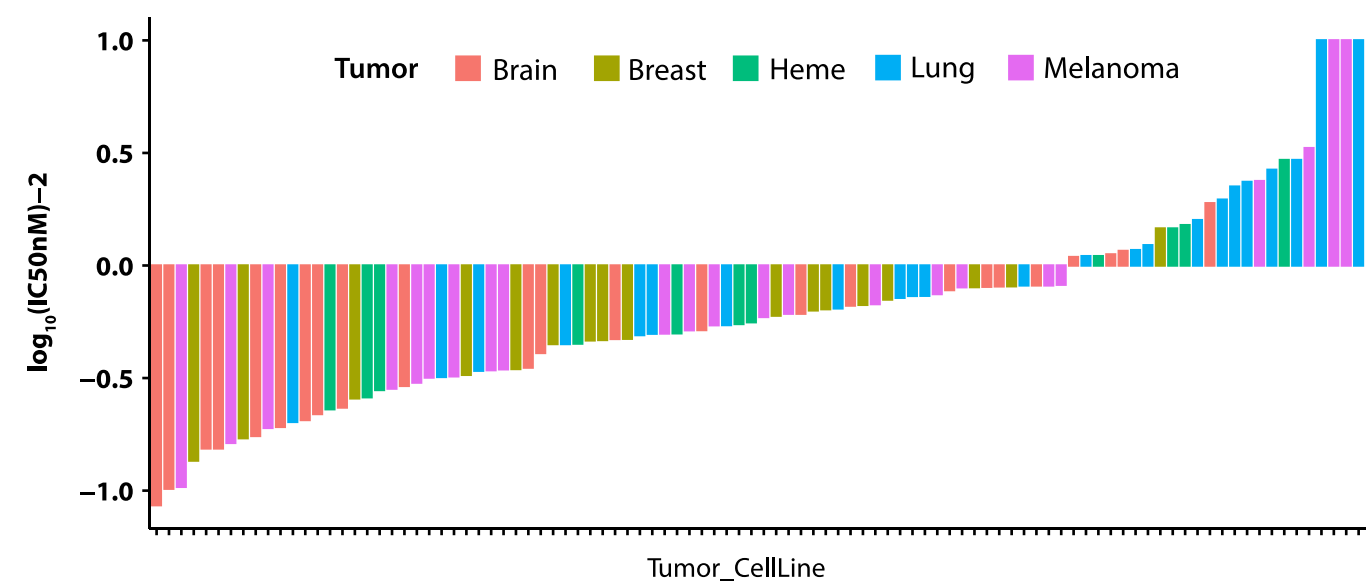


Figure 4: Waterfall plot showing anti-proliferative activity of PRT811 against a panel of 87 cell lines. Cell panel consists of brain cancer cell lines, as well as breast, lung, and melanoma cell lines, the predominant cancer types that metastasize to the brain.

### PRT811 INHIBITS sDMA IN A U-87 MG ORTHOTOPIC MODEL

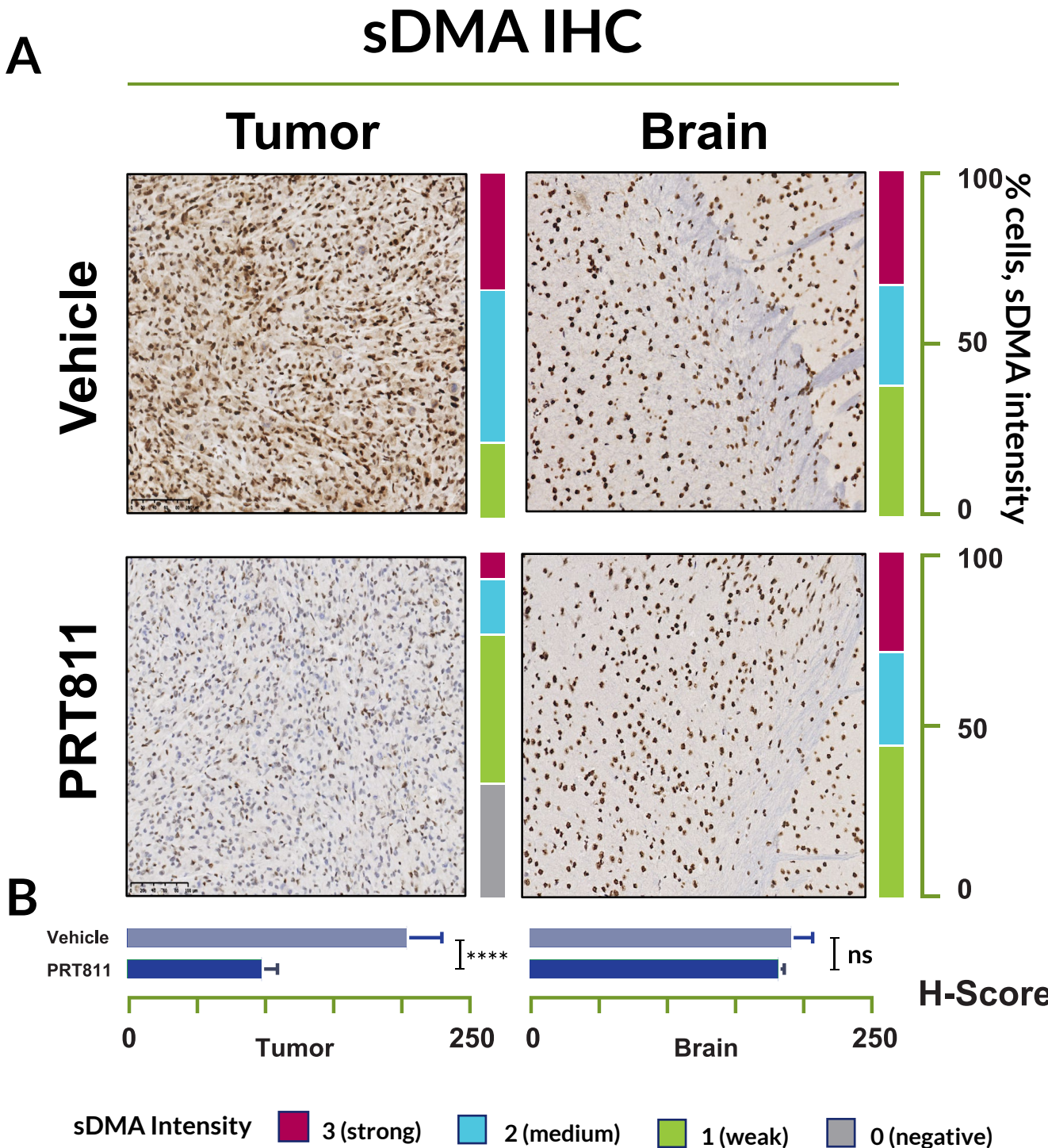
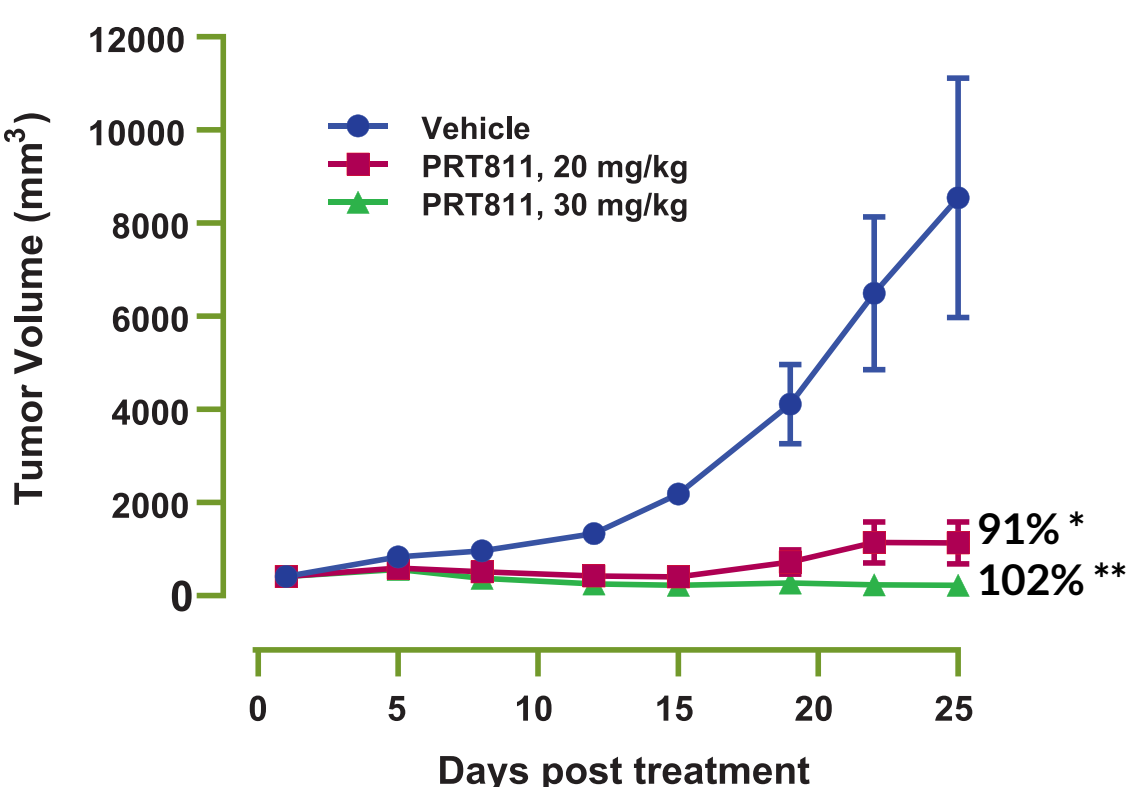


Figure 5. Mice bearing orthotopic U-87 MG tumors were treated with vehicle or PRT811 (80 mg/kg, BID) for a week. Whole brain sections (FFPE) were stained with H&E or sDMA antibody. sDMA H-scores were determined on tumor and normal brain regions in vehicle and PRT811 treated samples. The intensity of IHC staining was scored at four levels, 0 (negative), 1+ (weak), 2+ (medium), 3+ (strong). H-Score = (% at 0)×0 + (% at 1)×1 + (% at 2)×2 + (% at 3)×3. Representative images with intensity distribution (A), H-Score (B), of tumor and brain treated with or without PRT811. \*\*\*\* P < 0.0001, ns: no significance.

### PRT811 IS EFFICACIOUS IN GBM MODELS IN VIVO AND EX VIVO

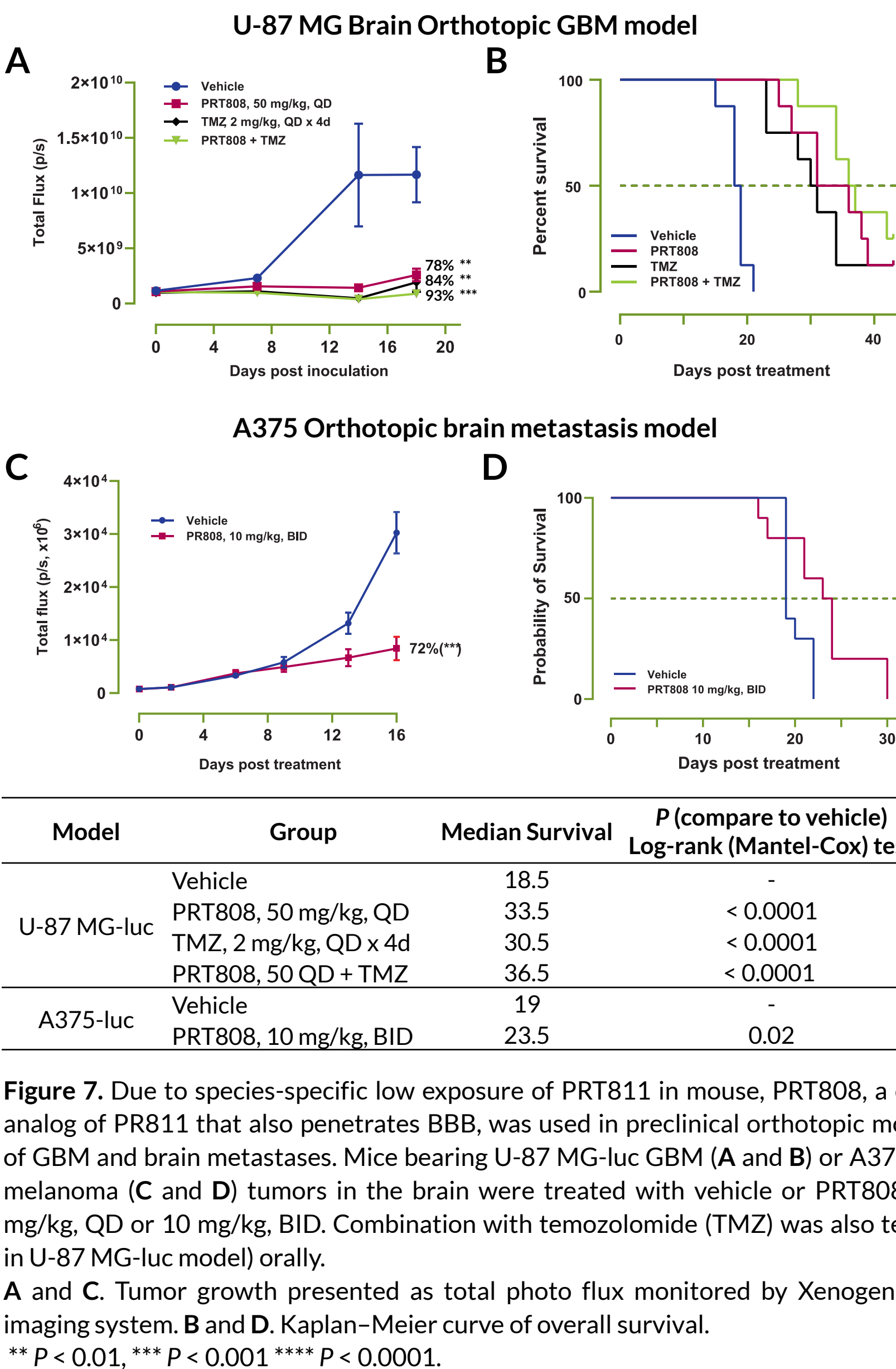
#### U-87 MG GBM model



PDX model	EC <sub>50</sub> (μM)	Max inh. (%)	MGMT
BN0769	0.044	58	U
BN2276	0.094	39	M
BN2287	0.027	27	M
BN2289	0.032	36	M
BN2331	0.018	76	U
BN2338	0.02	55	U
BN3733	0.031	53	M

Figure 6. Left. Nude rats bearing s.c. U-87 MG tumors were dosed orally with 20 or 30 mg/kg PRT811 QD. Significant antitumor activities were observed at both doses (tumor regression for 30 mg/kg). \*: P < 0.05; \*\*: P < 0.01. Student's t test, 2 tailed. N=8/arm. Right. Concentration-dependent inhibition of 7 GBM PDX models by PRT811 in a 3D ex vivo (7-day) format. 6/7 (86%) models responded to PRT811 treatment (cut-off: 30% max inhibition). MGMT status was derived from RNAseq.

### PRMT5 INHIBITION REDUCES TUMOR GROWTH AND EXTENDS SURVIVAL IN ORTHOTOPIC GBM AND BRAIN METASTASIS MODELS



Model	Group	Median Survival	P (compare to vehicle) Log-rank (Mantel-Cox) test
U-87 MG-luc	Vehicle	18.5	-
	PRT808, 50 mg/kg, QD	33.5	< 0.0001
	TMZ, 2 mg/kg, QD x 4d	30.5	< 0.0001
	PRT808, 50 QD + TMZ	36.5	< 0.0001
A375-luc	Vehicle	19	-
	PRT808, 10 mg/kg, BID	23.5	0.02

Figure 7. Due to species-specific low exposure of PRT811 in mouse, PRT808, a close analog of PR811 that also penetrates BBB, was used in preclinical orthotopic models of GBM and brain metastases. Mice bearing U-87 MG-luc GBM (A and B) or A375-luc melanoma (C and D) tumors in the brain were treated with vehicle or PRT808 (50 mg/kg, QD or 10 mg/kg, BID). Combination with temozolomide (TMZ) was also tested in U-87 MG-luc model) orally. A and C. Tumor growth presented as total photo flux monitored by Xenogen IVIS imaging system. B and D. Kaplan-Meier curve of overall survival. \*\* P < 0.01, \*\*\* P < 0.001 \*\*\*\* P < 0.0001.

## CONCLUSIONS

- PRT811 is a potent and selective SAM-competitive PRMT5 inhibitor.
- PRT811 possesses broad anti-proliferative activity in GBM, breast, lung, and melanoma cancer cell lines.
- PRT811 has excellent pharmacokinetic properties in multiple preclinical species with > 2-fold higher brain vs plasma exposure in rodents.
- PRT811/PRT808 are efficacious in preclinical models of GBM and brain metastatic models and inhibit proliferation of GBM PDX models ex vivo (3D).
- PRT811 is currently being evaluated in a Phase I clinical trial for advanced solid tumors, gliomas, and myelofibrosis (NCT04089449).