Modeling pharmacokinetics and efficacy of the ATM inhibitor WSD0628 in GBM and melanoma metastasis PDX models

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ABSTRACT

BACKGROUND

Radiation is a cornerstone of glioblastoma (GBM) treatment, however, the majority of tumors progress within the radiation field due to inherent radiotherapy (RT) resistance. The integration of highly potent radiosensitizers, such as the ATM inhibitors AZD1390 or WSD0628, could reverse resistance and significantly improve local control of these tumors. However, the achievable brain tumor exposure of these inhibitors may be limited by drug exposure in skin and mucosa, leading to enhanced radiation toxicity.

OBJECTIVE

To support the clinical development of WSD0628, we developed pharmacokinetic (PK)-efficacy models to help interpret ongoing PK analyses.

RESULTS

A model based on *in vitro* cell studies was based on the in vitro observations that 30 nM WSD0628 provided maximum ATM inhibition and the extent of radiosensitization was directly related to the duration of drug incubation after irradiation, up to a maximum of 24 hours. The unbound WSD0628 concentration, measured by rapid equilibrium dialysis, was 66% of the total concentration in cell culture media, and was used to define an optimal radiosensitizing target of 20 nM free WSD0628 for 4 to 24 hours. Previous PK analyses showed that the free, unbound fraction (f_u) of WSD0628 in plasma and brain tumor tissue are 0.02 and 0.054, respectively, and the brain tumor-to-plasma ratio is 0.32 in GBM43 patient-derived xenografts (PDXs). From these data, a total drug level of 1160 nM in plasma is predicted to achieve unbound drug levels above the 20 nM WSD0628 target unbound concentration. To validate this prediction, parallel *in vivo* dose-ranging studies were performed in orthotopic PDXs. Mice with orthotopic GBM43 were randomized to treatment with RT (8 Gy x1) alone or in combination with a range of WSD0628 doses. RT combined with 0.25 mg/kg and 1 mg/kg WSD0628 had no impact on survival, compared to RT alone, while median survival increased by 1.3-fold for RT combined with 2.5 mg/kg, 1.7-fold with 5 mg/kg, and 3.2-fold with 10 mg/kg WSD0628. A similar dose-response was observed in an M12 melanoma brain metastasis PDX. Based on PK modeling of these drug doses, 2.5 mg/kg dosing will maintain total WSD0628 plasma levels above 1160 nM for 11.9 hours, 5 mg/kg for 23.6 hours, and 10 mg/kg for 40 hours.

CONCLUSIONS

Taken together, we have developed a model to predict efficacious dosing of a brain tumor based on the free drug hypothesis, which allows integration of multiple aspects of *in vitro* modeling with measured drug levels in animal models to predict total plasma levels associated with robust sensitizing effects. Assuming similar tissue to plasma partitioning in human GBM, this model could be directly applied to interpret the ongoing Phase 1 PK analysis of WSD0628 in recurrent GBM.

DEVELOPING CLINICAL RADIOSENSITIZING STRATEGIES

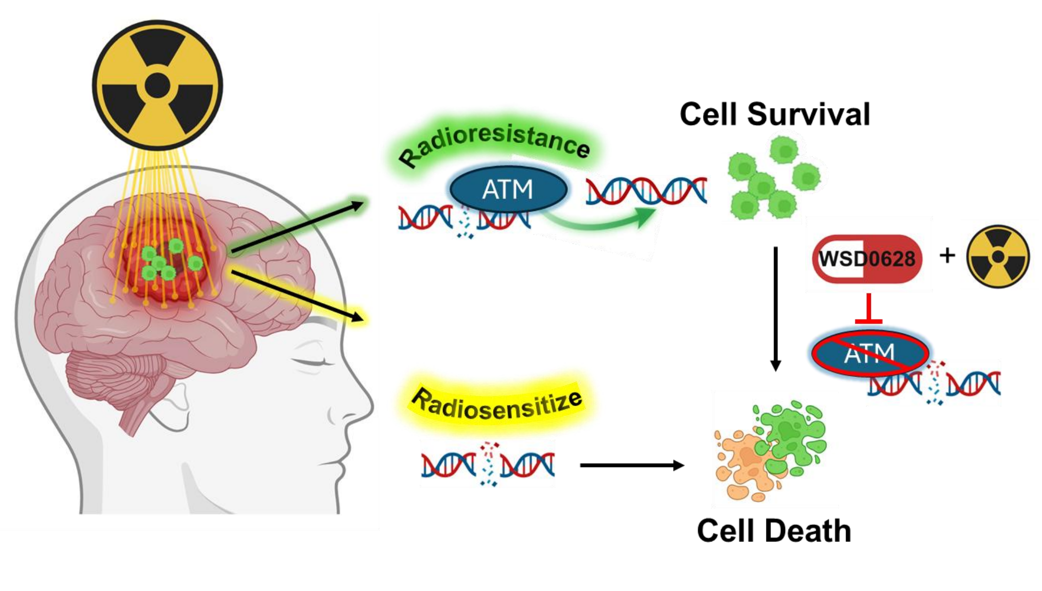


Figure 1. Model of radiosensitization vs resistance in GBM. Image adapted using BioRender.

INHIBITION AND SENSITIZATION

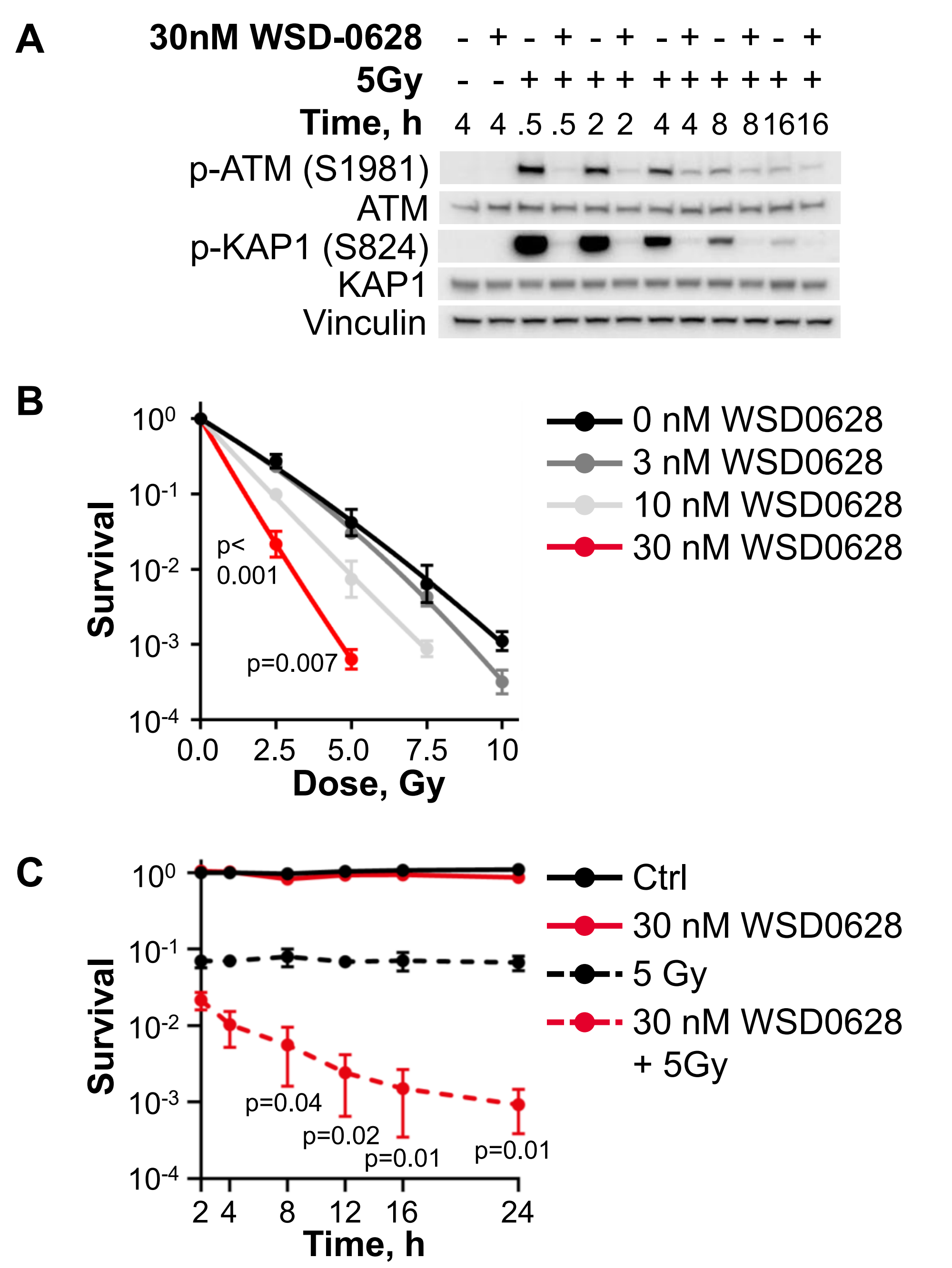
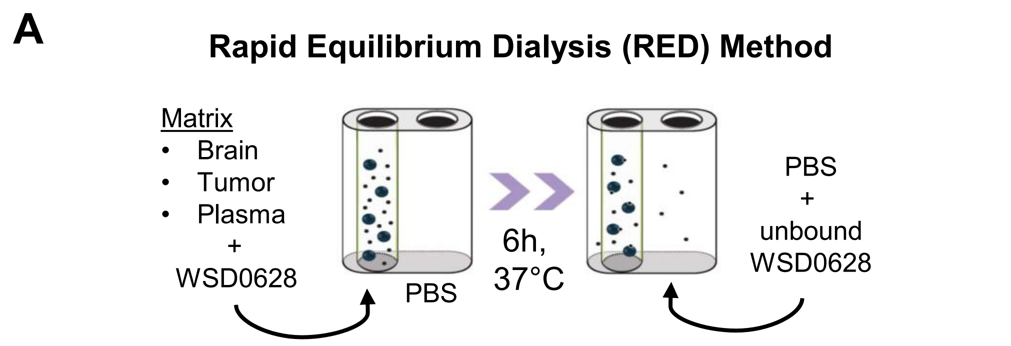


Figure 2. U251 cells treated 30m before RT at dose and times indicated. **A.** Western blot analysis **B.** Clonogenic survival after a 24h treatment. **C.** Clonogenic survival after incubation with WSD0628 for times indicated. Adapted from Xue et al[1].

UNBOUND DRUG AVAILABILITY

A



B

Matrix	Unbound WSD0628 fraction (f_u)
Mouse plasma	0.020 ± 0.002
Mouse brain	0.019 ± 0.002
Human plasma	0.0172 ± 0.001
Human brain (white matter)	0.0294 ± 0.002
Human GBM tumor	0.054 ± 0.002
DMEM Media (10% FBS)	0.655 ± 0.019

C

GBM43 WSD0628 levels

Matrix	Total, nM	Unbound, nM
Plasma	10596.3 ± 1926.5	211.9 ± 36.3
Brain	2874.4 ± 642.6	54.6 ± 12.2
Tumor	3346.0 ± 608.7	180.7 ± 32.9
Tumor/Plasma: 0.32		

Figure 3. A. RED method schematic for determining drug free fraction. **B.** Free fraction of WSD0628 determined by RED and measured by LC-MS/MS. **C.** WSD0628 concentrations in GBM43 tumor-bearing mice 2 h after a single dose of 7.5 mg/kg. Adapted from Rathi et al[2].

CALCULATING *IN VIVO* EFFICACIOUS CONCENTRATION

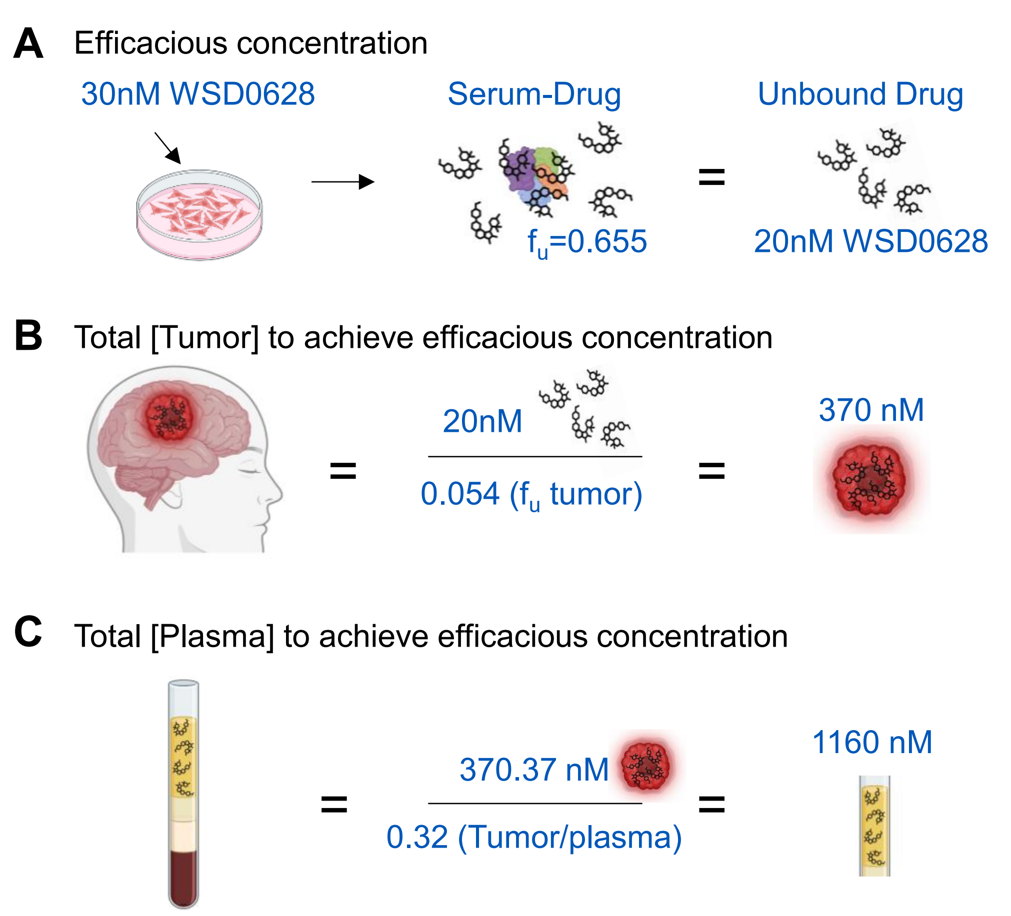


Figure 4. Schematic workflow illustrating (**A**) estimation of efficacious concentration, (**B**) required total tumor concentration, and (**C**) required total plasma concentration. Image adapted using BioRender.

IN VIVO EFFICACY WITH WSD0628 + RT

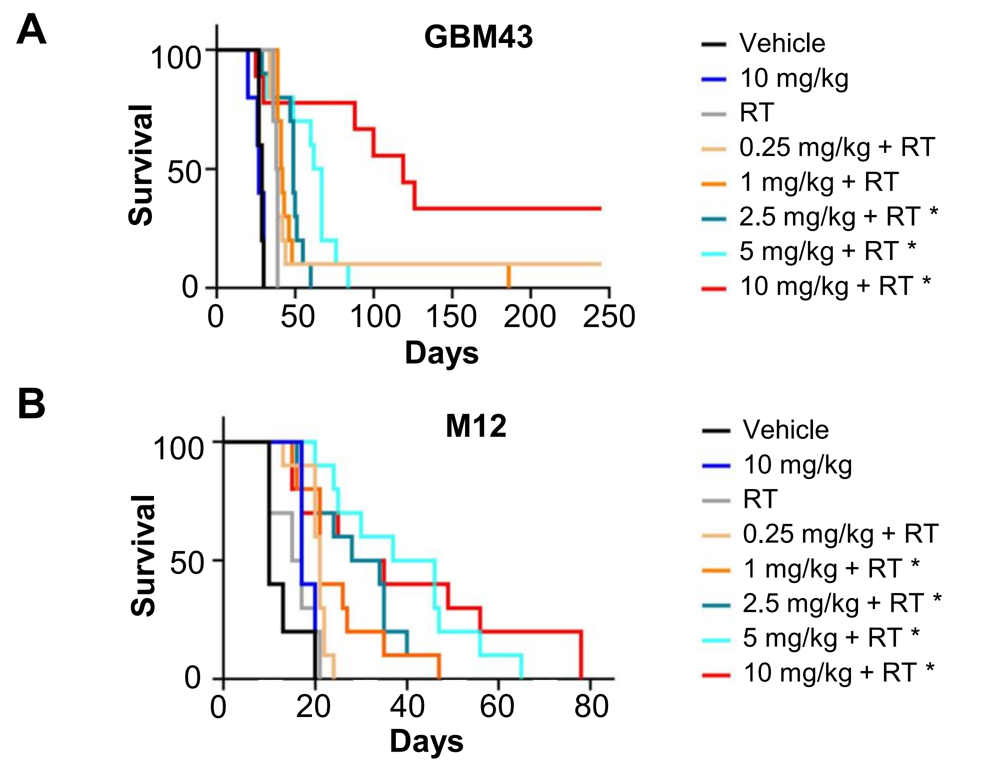
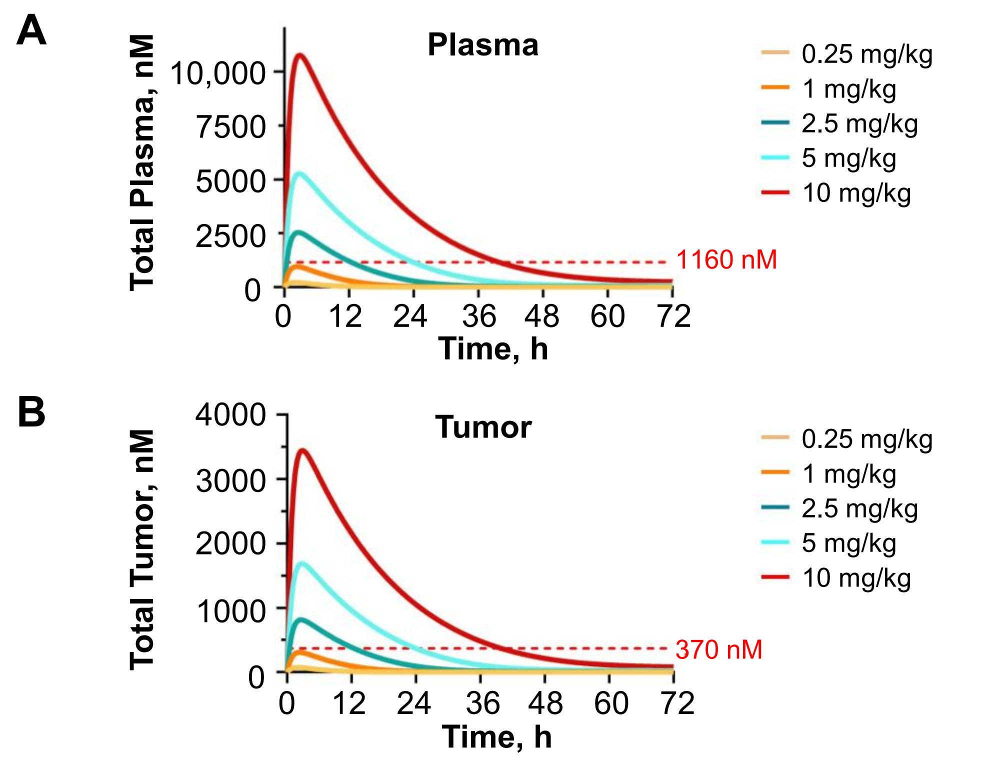


Figure 5 Mice with (**A**) GBM43 (glioma) and (**B**) M12 (melanoma metastasis) intracranial tumors were treated with a single dose of WSD0628 and 8Gy as indicated. Weights were taken daily, and mice were euthanized at moribund. * $p<0.01$ compared to RT alone.

PHARMACOKINETIC MODELING



C

Dose	Brain		Tumor	
	AUC (μM*h)	Time ≥ 20 nM, h	AUC (μM*h)	Time ≥ 20 nM, h
0.25 mg/kg	0	0	0	0
1 mg/kg	0	0	0	0
2.5 mg/kg	0	0	7.3	11.9 (0.5 – 12.4)
5 mg/kg	9.1	7.0 (1.14 – 8.14)	23.3	23.6 (0.3 – 23.9)
10 mg/kg	40.7	20.7 (0.5 – 21.2)	62.2	39.6 (0.2 – 39.8)

Figure 6. Predicted plasma (**A**) and tumor (**B**) concentration-time profiles using GBM43/M12 efficacy data incorporating free fractions, tissue-to-plasma ratios, and published PK data (Rathi et al[2]).
C. WSD0628 exposure and duration above target concentration.

CONCLUSIONS

- The ATM inhibitor WSD0628 radiosensitizes GBM and brain metastasis cells *in vitro* and in intracranial mouse models.
- Determination of free drug availability in plasma, brain, and tumor in animal models can predict efficacious drug levels in humans.
- Efficacious concentration data will be applied to assess the ongoing Phase 1 PK analysis of WSD0628 in recurrent GBM (Clinical Trial NCT05917145).

FUTURE DIRECTION

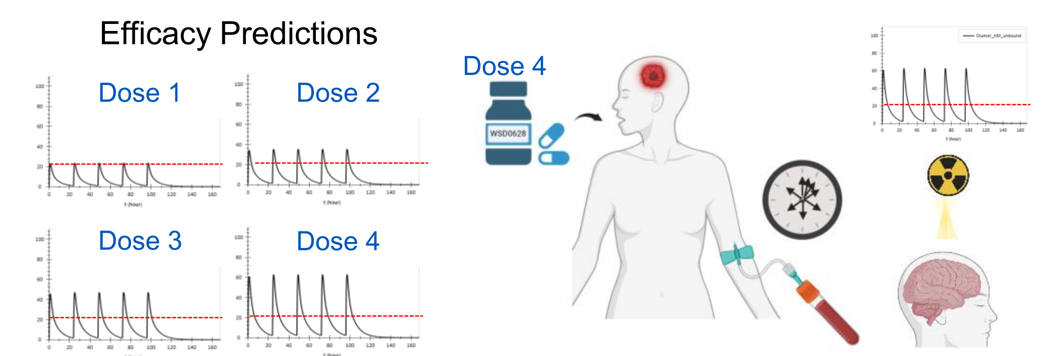


Figure 7. Pharmacokinetic-pharmacodynamic-efficacy modeling using *in vitro* and *in vivo* methods can be applied to determine efficacious drug concentrations in humans. Image adapted using BioRender.

REFERENCES, FUNDING, CONTACT

1. Zhiyi Xue, **Ann C. Mladek**, et al. The novel brain penetrant ataxia-telangiectasia mutated inhibitor WSD0628 provides robust radiosensitization of brain tumor patient-derived xenografts. *Neuro-Oncology*, 27(9): 2313–2325, September 2025.
2. Sneha Rathi, et al. Preclinical Systemic Pharmacokinetics, Dose Proportionality, and Central Nervous System Distribution of the ATM Inhibitor WSD0628, a Novel Radiosensitizer for the Treatment of Brain Tumors. *J Pharmacol Exp Ther* 390:260–275, August 2024.
3. Mayo GBM PDX National Resource Website: https://www.mayo.edu/research/labs/translational-neuro-oncology/mayo-clinic-brain-tumor-patient-derived-xenograft-national-resource/about

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