WSD-0628, a novel brain penetrant ATM inhibitor, radiosensitizes GBM and melanoma patient derived xenografts

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ABSTRACT

Background: Glioblastoma (GBM) are inherently resistant to radiation therapy (RT), and development of radiosensitizers is one strategy to overcome this limitation. Repair of DNA double strand breaks induced by RT are mediated by the protein kinase Ataxia Telangiectasia *mutated* (ATM).

Objective: In this study, the novel ATM inhibitor WSD-0628 was evaluated in combination with RT using GBM and melanoma models.

Results: *In vitro* evaluation of 10μM WSD-0628 binding to a panel including receptors, ion channels, enzymes, and transporters indicated a satisfactory safety profile with low risk for off-target liability. WSD-0628 potently inhibits ATM-mediated phosphorylation of the DNA damage response protein KAP1 in MCF-7 cells at sub-nanomolar (nM) concentrations (IC50 0.42nM) in comparison to much less potent inhibition of the related kinases ATR (phosphorylation of CHK1, IC50 742nM) or DNA-PKcs (auto-phosphorylation of DNA-PK, IC50 169nM) in HT29 cells assessed by ELISA. In U251 GBM cells, 30 nM WSD-0628 potently inhibited RT-induced phospho-KAP1 and robustly reduced clonogenic survival by 5-fold when combined with 5 Gy irradiation (combination vs RT alone, p<0.01). Similar potent radiosensitizing effects were seen in a melanoma brain metastasis PDX line M12 (10nM WSD-0628+IR-5Gy 1% survival vs 5% survival with IR-5Gy alone. p<0.01), and the SV-40 transformed astrocyte line SVG-A (30nM WSD-0628 + IR-2.5Gy survival 0.04% vs 15% with IR-2.5Gy alone. p<0.01). Evaluation of the pharmacokinetic profile of WSD-0628 in mice 2h after a single 5 mg/kg oral dose reveals a high level of free drug availability in the brain (34nM) and in the CSF (50nM) with little to no Pgp/BCRP substrate liability. An initial *in vivo* dose finding study in orthotopic GBM43 PDX yielded significant benefit with WSD-0628 at either 5 or 10 mg/kg PO daily when combined with radiation (2Gy QD for 5 days); Median survival for sham RT (29d) or RT alone (34d) were significantly different from RT combinations with 5 mg/kg (54d) and 10 mg/kg (73d; p<0.01 for both dose levels), although the higher dose combination was poorly tolerated with body weight loss between 15-20% one week after RT completion. Lower dosing of WSD-0628 (7.5 mg/kg PO, QD) given just before and 24h after a single dose of RT (12.5Gy) in mice with orthotopic M12 was well tolerated and provided robust radiosensitizing effects with median survival for the combination treatment of over 180d vs 17d for control and 49d with RT alone groups (combination vs RT alone, p=0.04).

Conclusion: Collectively, these results suggest a promising role for WSD-0628 in combination with RT in GBM and melanoma metastatic to the brain.

ATM SIGNALING

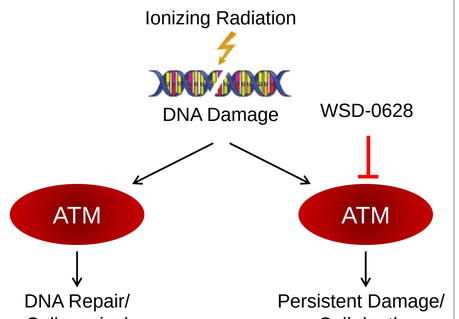


Figure 1. Adapted from Pouget et al. Antioxidants & Redox Signaling (2018) 29:15

SATISFACTORY SAFETY PROFILE

Class	Safety Panel Screening		Assay format	Agonist Mode	Antagonist Mode
	Family	Target		Mean percentage activation (%) at 10μM	Mean percentage inhibition (%) at 10μM
Ion Channel	Sodium channel	Nav1.5	Manual patch-clamp	NA	11.35
	Potassium channel	KCNQ1	Manual patch-clamp	NA	12.83
	Calcium channel	Cav1.2	Ca^{2+} mobilization	N/A	-0.11
GPCR	Adrenoceptors	α1A	Ca^{2+} mobilization	8.05	-10.78
	Acetylcholine	M1	Ca^{2+} mobilization	3.31	5.31
	Cholecystokinin	CCK1	Ca^{2+} mobilization	0.12	11.07
	Histamine	H1	Ca^{2+} mobilization	-0.08	5.83
	5-Hydroxytryptamine	5HT1A	Ca^{2+} mobilization	-1.78	-2.54
	Opioid	OPRD1	Ca^{2+} mobilization	2.02	-3.22
	Vasopressin	AVPR1A	Ca^{2+} mobilization	-0.14	-6.8
	Adenosine	A2A	cAMP assay	-6.62	13.69
	Endothelin	ETA	cAMP assay	-8.54	-8.48
	Dopamine	D1	cAMP assay	2.86	15.16
	Histamine	H2	cAMP assay	7.93	12.59
	Cannabinoid	CB1	cAMP assay	10.51	-23.48
	Dopamine	D2S	cAMP assay	4.72	-12.22
	5-Hydroxytryptamine	5HT1B	cAMP assay	-0.04	-6.64
Transporter	Dopamine Transporter	DAT	Neurotransmitter uptake	NA	-2.13
	Norepinephrine Transporter	NET	Neurotransmitter uptake	NA	-42.23
	Serotonin Transporter	SERT	Neurotransmitter uptake	NA	-1.14
Kinase	TK	LCK	MSA	NA	46.35
Enzyme	Cholinesterase	AChE	AchE(human) FI	NA	-13.44
	Monoamine oxidase	MAO-A	Lum	NA	16.35
	Phosphodiesterase	PDE3A	IMAP	NA	35.58
	Cyclooxygenase	COX1	FI	NA	19.28

Table 1. *In vitro* evaluation of 10uM WSD-0628 binding to a panel including receptors, ion channels, enzymes, and transporters indicated a satisfactory safety profile with low risk for off-target liability.

DNA DAMAGE RESPONSE INHIBITION

A

Target	Cell Line	Assay	IC50 (nM)
pKAP1	MCF-7	Western	0.42
pCHK1	MCF-7	Western	762
p-DNA-PKcs	HT29	ELISA	169

B

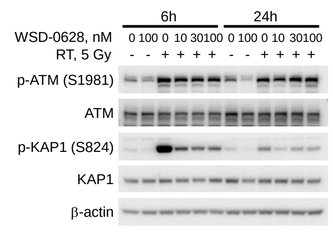


Figure 2. A - DNA damage response was assessed using a dose response of WSD-0628 by in-cell western blot or ELISA. **B -** The established GBM U251 cell line was treated with a dose response of WSD-0628 +/- 5Gy RT 1 h later. Cells were harvested for protein extraction at the times indicated after WSD-0628 treatment and protein expression was analyzed by western blot.

RADIOSENSITIZATION

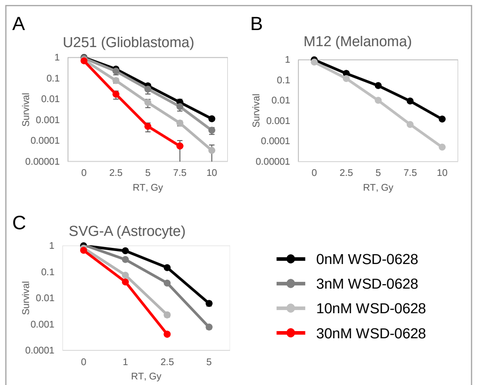
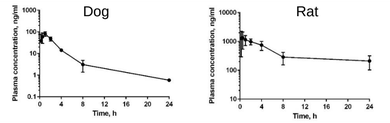


Figure 3. Clonogenic survival assays. **A,C -** U251 and SVG-A were plated at optimal cell numbers and treated with WSD-0628 4h later followed by RT 1h later. Media was replaced after 23 hrs, and colonies were stained ~14 days later. **B -** M12 short explant cultures were harvested from flank tumor, cultured in stem cell media, and plated on a matrigel coating before being treated and stained as above.

PHARMACOKINETIC PROFILE

A



B

CNS penetration	
Kp,uu, brain (rat/mouse)	0.44/0.3
Kp,uu,csf (rat/mouse)	1.07/0.37
Protein Binding	
Plasma (%free)	3.28/1.95/2.85/11.08
Brain (%free) Human/Rat/Mouse/Dog	NA/2.16/2.69/NA
***In vivo* PK, Rat/Dog**	
CL (mL/min/kg)	6.05/61.2
Vss (L/kg)	0.87/4.1
T1/2 (hr)	22/2.52
F (%)	37.2%/41.6%
Efflux transporter (1E-6 cm/s)	
MDCKII-Pgp A to B/B to A Efflux ratio(-inhibitor)	20.64/17.66 (0.86)
Caco-2 A to B/B to A Efflux ratio(-inhibitor)	21.73/14.53 (0.67)
Caco-2-BCRP Efflux ratio(-inhibitor)/(+inhibitor)	24.38/16.06 (0.66)

Figure 4. A - Plasma was collected at various timepoints after a single dose of WSD-0628 at 2mg/kg in dog (n=3) or 10 mg/kg in rat (n=3). Concentrations were determined by LC-MS/MS. **B -** Equilibrium dialysis using brain homogenate and plasma protein binding, as well as efflux transporter permeability coefficients were analyzed by LC-MS/MS.

IN VIVO EFFICACY

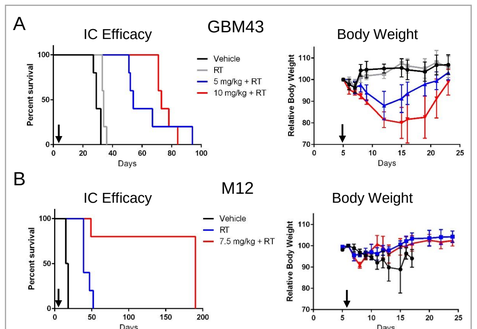


Figure 5. A - 100,000 GBM43 cells were injected intracranially and dosing started 5 days later. WSD-0628 was dosed orally at 5 or 10 mg/kg ~15 minutes before RT and supratentorial RT was delivered using the PXI X-RAD SmART+ irradiation platform at 2Gy/day for 5 days. p<0.01 for both dose levels. **B -** M12 cells were injected as in A and dosing started 6 days later. WSD-0628 was dosed orally at 7.5 mg 1x/day for 2 days with a single 12.5Gy dose of RT given 15 minutes after the first WSD-0628 dose. p=0.04 combination vs RT alone. For both studies, mice were observed daily and euthanized upon signs of morbidity. Arrows indicate start of dosing. Statistics calculated by t-test.

CONCLUSIONS

- The ATM inhibitor WSD-0628 is a non-toxic compound and inhibits the DNA damage response associated with radiation therapy.
- WSD-0628 radiosensitizes Glioblastoma cells as well as Melanoma and human astrocytes.
- WSD-0628 is capable of crossing the blood brain barrier and has minimal efflux liability.
- In patient-derived Glioblastoma and Melanoma intracranial xenograft models, WSD-0628 yielded significant benefit when combined with radiation therapy.

FUTURE DIRECTIONS

- Additional GBM PDX lines will be tested with the combination of WSD-0628 and radiation therapy.
- The pharmacodynamic and pharmacokinetic profiles of WSD-0628 will be analyzed in the GBM PDX intracranial models.
- Clinical trial development in GBM and Melanoma metastasis to the brain is underway with the combination of WSD-0628 and radiation therapy.

REFERENCES, FUNDING, CONTACT

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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