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 15µM 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 potency 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 in the related kinase ATR). Phosphorylation of CHK1, IC50 7.52µM or DNA-PKcs (auto-phosphorylation of DNA-PKc), IC50 169nM in HT29 cells assessed by ELISA. In U251 GBM cells, 30 µM 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 (10µM WSD-0628-IR 5Gy 1% survival vs 5% survival with IR 5Gy alone, p<0.01), and the SV-40 transformed astrocyte line SVGA (30µM WSD-0628 + IR 5Gy, survival 9.4% vs 15% with IR + 5Gy alone, p<0.01). Evaluation of the pharmacokinetic profile of WSD-0628 in mice after a single 5 mg/kg oral dose reveals a high level of free drug availability in the brain (34%) and in the CSF (50%) with little to no PgP/BCRP substrate liability. An in vivo dose finding study in orthotopic GBM3 PDX yielded significant benefit with WSD-0628 at either 0.5 or 10 mg/kg PO daily when combined with radiation (2Gy QD for 5 days); Median survival for sham RT (2Gd) or RT alone (34g) were significantly different from RT combinations with 5 mg/kg (54g) 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 M23 was well tolerated and provided robust radiosensitizing effects with median survival for the combination treatment of over 180d vs 174 for control and 485 with RT alone (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.

ABSTRACT

ATM SIGNALING

DNA Damage Response Inhibition

DNA Damage Response Inhibition

Target|Cell Line|Assay|IC50 (nM)
---|---|---|---
ATM|GBM43|ELISA|0.42
ATM|DAT|PDE3A (rat/mouse)|7.62
ATM|B|5Gy RT 1h later.

DNA DAMAGE RESPONSE INHIBITION

DNA Damage Repair/Cell survival

ATM

Persistent Damage/Cell death

ATM

SATISFACTORY SAFETY PROFILE

IN VIVO EFFICACY

Figure 1. Adapted from Pouget et al. Antibiotics & Redox Signaling (2018) 20:13

Figure 2. A: DNA damage response was assessed using a dose response of WSD-0628 or DMSO at 0.1µM to 10µM in GBM43 cells. Each cell line was treated with a dose response of WSD-0628 or 1% G4T 1 h prior. Cells were harvested for protein extraction at the times indicated after WSD-0628 treatment and protein expression was analyzed by western blot.

Figure 3. Chronic survival assays. A: U251 and SVGA were plated of optimal cell numbers and treated with WSD-0628 at 4h prior followed by RT 1G. Media was replaced after 24hrs, and colonies were stained ~14 days later. B: M23 orthotopic colonies were transplanted from black tumors cultured in stem cell media, and plated on a matrigel coating before being treated and stained as above.

Table 1. In vitro evaluation of 15µM 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.

REFERENCES, FUNDING, CONTACT


This work was supported by Mayo Clinic, Rochester, MN, and Wayshine BioPharm, Shanghai, China. Contact Ann Miedeck Tuma with questions or comments: tuma.amy@mayo.edu

PHARMACOKINETIC PROFILE

Figure 4. A: Plasma was collected at various timepoints after a single dose of WSD-0628 at 1mg/kg in dog (n=3) or 10mg/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.

FUTURE DIRECTIONS

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

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.