The radiosensitizing effects of the novel brain penetrant and potent ATM inhibitor WSD0628 in glioblastoma and melanoma patient derived xenografts

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ABSTRACT

BACKGROUND: Radiation therapy (RT) is an important, non-surgical clinical treatment for glioblastoma (GBM) and brain metastases, but inherent radio-resistance can limit the efficacy in many patients. The Ataxia Telangiectasia Mutated (ATM) protein critically regulates radiotherapy-mediated DNA damage repair pathways, and defects in this kinase can lead to profound radiosensitivity.

OBJECTIVE: In this study, the efficacy of the highly potent ATM inhibitor WSD0628 was evaluated in GBM and melanoma brain metastasis patient derived xenografts (PDXs).

RESULTS: In short term explant cultures of three PDXs, WSD0628 robustly suppressed RT-induced autophosphorylation of ATM at serine 1981 and ATM-mediated phosphorylation of Chk2 at threonine-68 and KAP1 at serine-824, with maximal inhibition at 100 nM. Similarly, RT-induced γH2AX foci were significantly reduced when combined with WSD0628 in GBM43 cells. Consistent with the importance of ATM in the DNA damage response after radiation, WSD0628 significantly increased the radiosensitivity of U251 cells in clonogenic survival assays (0nM vs. 30nM at 5Gy, p<0.01). To evaluate the optimal duration of drug exposure, WSD0628 was removed from U251 cells at various intervals following irradiation with 5 Gy. Using clonogenic survival as a readout, a 10-fold increase in cytotoxicity was observed with an 8 hour drug exposure, with progressively increasing cytotoxicity gains with exposures up to 24 hours. In vivo efficacy of WSD0628 was evaluated in multiple intracranial PDX models. In an initial dose-ranging study, robust radiosensitization was observed in GBM43 treated with 2 Gy x 5 fractions combined with 5 mg/kg WSD0628 (20 day survival extension) or 10mg/kg WSD0628 (39 day with 10 mg/kg; p<0.01 for both dose levels). Moreover, a single dose of 12.5 Gy combined with 10 mg/kg WSD0628 had a profound impact on treatment efficacy in the melanoma brain metastasis M12 PDX: at 180 days post-treatment, all mice in the combination group were electively euthanized, while median survival for sham or RT alone was 17d and 49d, respectively. Histologic analysis identified large intracranial tumors in all sham and RT-only treated mice, but only a small accumulation of melanotic cells without obvious tumor in the combination-treated mice. Immunohistochemical staining of NeuN and GFAP in the combination-treated mice showed preserved neuronal density at the 180-post treatment timepoint and minimal reactive gliosis within the 'tumor scar'. An ongoing study is comparing this single-fraction 12.5 Gy regimen to a 2.4 Gy x 10 fraction regimen alone or in combination with WSD0628.

CONCLUSIONS: Collectively, this study demonstrates the potential for profound radiosensitizing effects of WSD0628 in combination without obvious neuronal toxicity and has provided the scientific rational for the first-in-man study of this combination in recurrent GBM (NCT05917145) at Mayo Clinic.

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ATM SIGNALING

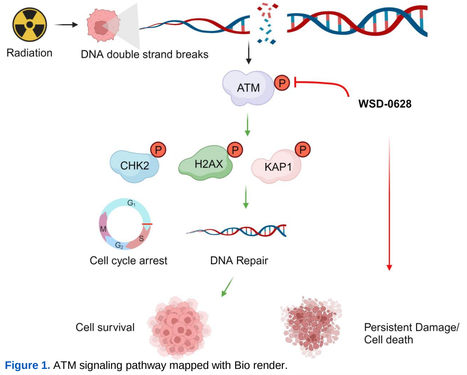

Figure 1. ATM signaling pathway mapped with Bio render.

DNA DAMAGE RESPONSE INHIBITION

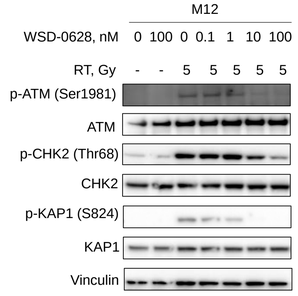

Figure 2. Established M12 cells was treated with a dose response of WSD-0628 30 minutes prior to 5Gy RT. Cells were harvested for protein extraction 4 hours after irradiation and protein expression was analyzed by western blot.

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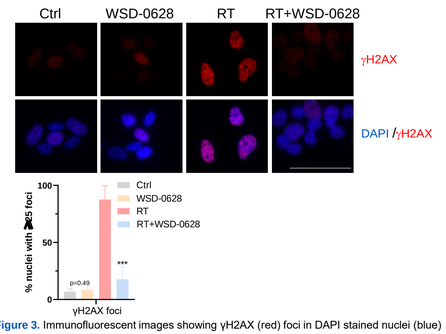

Figure 3. Immunofluorescent images showing γH2AX (red) foci in DAPI stained nuclei (blue) of GBM43 cells treated as indicated and processed for immunostaining 1 hour after irradiation, bar graphs (lower panel) show mean % nuclei with >25 foci ± SEM from 3 independent experiments and compared by two-sample t-test. Scale bar 50 μm .

RADIOSENSITIZATION

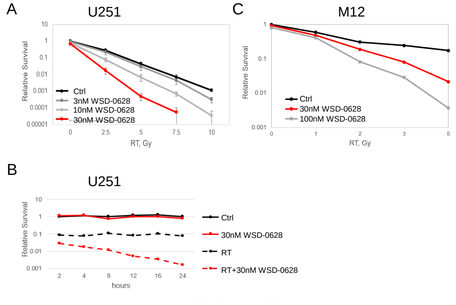

Figure 4. Radiosensitizing effects of WSD0628. **(A)** U251 clonogenic survival. Cells treated with different WSD0628 concentration prior to different irradiation and removed after 24 hours. **(B)** 30 nM WSD-0628 subsequently removed from the media at indicated times after 5Gy irradiation. **(C)** M12 cells were incubated with drug 1 hour prior to irradiation and removed after 24 hours.

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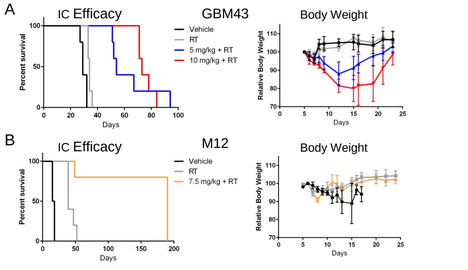

Figure 5. (**A**) 100,000 GBM43 cells were injected intracranially and dosing started 5 days later. WSD-0628 was dosed orally at 5 or 10 mg/kg −15 minutes before RT and supratentorial RT was delivered using the PXI X-RAD SmART+ irradiation platform at 2 Gy/day for 5 days. p<0.01 for both dose levels. (**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 WSD-0628 dose. p=0.04 combination vs RT alone. For both studies, mice were observed daily and euthanized upon signs of morbidity. Statistics calculated by t-test.

IMPACT IN NORMAL BRAIN TISSUE

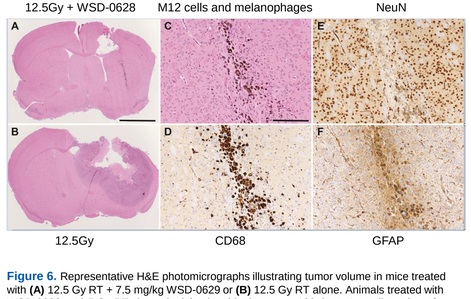

Figure 6. Representative H&E photomicrographs illustrating tumor volume in mice treated with **(A)** 12.5 Gy RT + 7.5 mg/kg WSD-0629 or **(B)** 12.5 Gy RT alone. Animals treated with WSD-0628 + 12.5 Gy RT showed minimal residual tumor at 190 days. A small number of residual melanoma cells **(C)** were admixed with numerous melanin-laden macrophages (melanophages), highlighted by **(D)** CD68 immunostain. The adjacent uninvolved basal ganglia showed preservation of neurons with minimal reactive gliosis, highlighted by **(E)** NeuN and **(F)** GFAP stains. Scale bar 2 mm **(A-B)** or 20 μm **(C-F)**.

CONCLUSIONS

- ATM Inhibitor WSD-0628 is a novel brain penetrate compound that inhibits the DNA damage response associated with radiation therapy.
- WSD-0628 radiosensitizing Glioblastoma cells and melanoma cells.
- In patient-derived intracranial xenograft models of Glioblastoma and Melanoma, WSD-0628 produced significant efficacy in combination with radiation therapy.
- In evaluations of long-term surviving mice treated with a combination of radiosurgical dose (12.5 Gy) and WSD-0628, no significant CNS pathologic changes were observed.

FUTURE DIRECTIONS

- The pharmacodynamic and pharmacokinetic profiles of WSD-0628 will be analyzed in the GBM PDX intracranial models.
- The clinical trial developing WSD-0628 in combination with radiation therapy for the treatment of GBM and Melanoma metastatic to the brain is ongoing.

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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