AZD1390 radio-sensitizes p53-mutant GBM by disrupting homology-directed DNA repair

Abstract # 2598

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ABSTRACT

The ATM inhibitor AZD1390 disrupts cellular responses to ionizing radiation (IR) and is a potent radiosensitizer being tested in clinical trials. In this study, the effects of AZD1390 on radiation sensitivity and DNA damage repair pathways were evaluated in glioblastoma (GBM) cells and patient derived xenografts (PDXs).

AZD1390 (30 nM and higher) suppressed IR (5 Gy)-induced phosphorylation of ATM-Serine^{1981} and downstream phosphorylation sites on KAP1, Chk2 and H2AX in U251 cells and multiple PDXs. Consistent with enhanced DNA damage, AZD1390 accentuated IR-induced G2/M arrest in U251 (80.6% with AZD1390/IR vs. 64.6% with IR alone, p<0.01), GBM43 (61.9% vs. 25.7%, p=0.01) and GBM39 (40.9% vs. 25.4%, p< 0.01) after 48 hours of incubation. Moreover, in a clonogenic survival assay, AZD1390 sensitized U251 cells to 5 Gy IR (0.24% survival with AZD1390/IR vs. 2.3% with IR alone, p=0.01). In a reporter-based analysis of DNA repair capacity, ATM inhibition resulted in a 40 to 60% reduction in homologous recombination (HR) and modest but significant decrease in micro-homology mediated end joining (MMEJ) and gap fill-in synthesis in U251 cells but had no effect on non-homologous end joining, translesion synthesis, nucleotide or base excision repair pathways. Comparing effects of AZD1390 on repair in GBM14 (TP53-wt) and GBM43 (TP53-mutant), similar results were observed except that decreased MMEJ was seen only in GBM43 (0.03± 0.01% vs. 0.07± 0.01% in control, p=0.002). Intriguingly, HR disruption by RAD51 knockdown sensitized GBM43 but not GBM14.

The efficacy of AZD1390 ± IR was studied *in vivo* in 10 PDXs. IR was delivered to orthotopic tumors using opposed lateral 225 kVp beams. AZD1390 (20 mg/kg PO) was given just prior to each daily radiation dose (2 Gy x 5 fractions). AZD1390 monotherapy was mostly ineffective, IR alone was reasonably efficacious with an average 1.8 ± 0.1-fold-increase in survival relative to sham radiation (survival ratio) across all 10 models. IR/AZD1390 treatment resulted in significant survival extension relative to IR alone in 6 of 10 models. Analysis of the survival benefit of combination therapy compared to IR alone across the entire cohort of PDXs was statistically marginal (average survival ratio 2.3± 0.3 vs. 1.8 ± 0.1 with IR, p=0.08). However, when stratified by TP53 status, combination therapy was significantly more effective than IR (mean survival ratio 2.3 ± 0.1 vs. 1.6 ± 0.2 with IR alone, p=0.02) in TP53-mutant PDXs, where all 5 models benefited. In contrast, TP53-wt group had no benefit (mean survival ratio 2.2 ± 0.5 vs. 2.0 ± 0.2, p=0.61), GBM39 was only TP53-wt PDX that benefited from the combination.

In conclusion, AZD1390 is an effective radio-sensitizer that causes disruption in HR and potentially other DNA repair pathways. Interestingly, *in vivo* radiosensitizing effects are mostly restricted to TP53-mutant GBM PDXs. Understanding mechanisms of resistance in the context of different TP53 backgrounds remains an important future direction.

ACKNOWLEDGEMENTS

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AZD1390 DISRUPTS ATM SIGNALING & DNA REPAIR

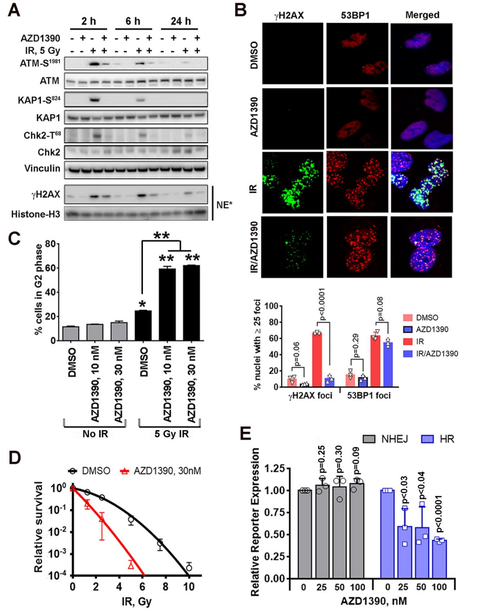

Figure1. AZD1390 sensitizes U251 to IR *in vitro*. A) Western blots showing effects of AZD1390 on RT induced DNA damage signaling. Cells treated with 30nM AZD1390 ± 5Gy IR were lysed at 2, 6, or 24 hours after IR treatment. **B)** Immunofluorescent images showing 53BP1 (red) or γH2AX (green) foci in nuclei (blue, DAPI) of U251 cells. Cells were treated with DMSO, 100 nM AZD1390, 5 Gy IR or AZD1390/IR combination and incubated for 1 hour prior to immunostaining, bar graphs (bottom panel) demonstrate % nuclei with >25 foci for each treatment. **C)** Bar graphs showing quantification of G2/M arrested cells after 24 hours of treatment with (or without) 5 Gy IR ± 30 nM AZD1390, *p<0.01, **p<0.001. **D)** Radio-sensitizing effects of AZD1390 in U251 cells. Graphs represent a clonogenic survival assay for U251 cells treated with increasing dose of IR ± 30nM AZD1390. **E)** Bar graphs showing basal NHEJ or HR capacity and effect of varied concentrations of AZD1390, as assessed by pathway specific reporter plasmids. Data (normalized to control) presented is mean ± SEM.

RADIO-SENSITIZING EFFECTS IN PDX-DERIVED CELLS

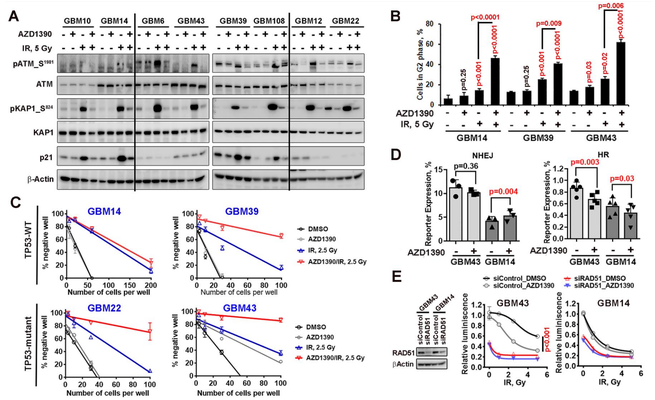

Figure 2: AZD1390 sensitizes GBM PDX cells to IR. A) Western blots showing effect of AZD1390 on ATM signaling in primary cells treated with AZD1390 ± 5 Gy IR and lysed 6 hours after irradiation. **B)** Bar graphs showing quantification of G2-arrested cells 48 hours after treatment with (or without) AZD1390 ± 5 Gy IR. **C)** Graphs showing survival assessed by limiting dilution clonogenicity in absence (or presence) of AZD1390 ± 2.5 Gy IR. **D)** Basal NHEJ or HR activity determined by plasmid reporters in GBM43 and GBM14 cells treated with or without AZD1390,.**E)** Effects of RAD51 knockdown (shown in western blots, left).on AZD1390-mediated radiosensitization in GBM43 (graphs in center) and GBM14 (graphs on right). Shown in graphs are measurements of ATP by Cell-Titer-Glo (CTG) assay, as a marker of relative cell survival and growth.

THERAPEUTIC ACTIVITY IN GBM

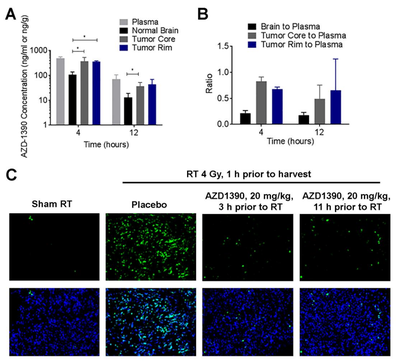

Figure 3. Pharmacokinetic and pharmacodynamic effects of AZD1390. A) Bar graphs showing distribution of AZD1390 (measured by LC-MS) in plasma, normal brain or indicated regions of orthotopic GBM12 xenografts harvested from mice at 4 or 12 hours after dosing with 20 mg/kg AZD1390; *p<0.05. **B)** Kp in normal brain, tumor core or tumor rim. **C)** Mice treated with 5 Gy RT (3 or 11 hours after single dose of 20 mg/kg AZD1390) were euthanized 1 hour after RT and intact brains harvested and flash frozen in OCT. Tissues were then sectioned and immunostained for γH2AX (green) in the tumor region and nuclei counter-stained with DAPI (blue).

THERAPEUTIC ACTIVITY IN GBM

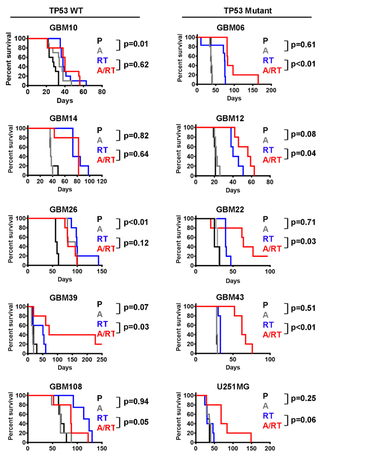

Figure 4: Evaluation of AZD1390 in orthotopic GBM xenografts. Kaplan-Meier plots showing survival over time for animals treated with placebo (P) or 20 mg/kg/d AZD1390 (A) ± 2 Gy RT for 5 consecutive days. Mice were monitored until moribund and results were compared by log rank.

SUMMARY OF *IN VIVO* STUDIES

GBM model	TP53	Median survival, days				Survival ratio#	
		P	A	RT	A+RT	RT/P	A+RT/P
U251	R273H	33.5	36	46	68*	1.4	2.0
GBM6	R273C	36.5	37	73	81*	2.0	2.2
GBM12	X187_splice	21	22	40	56*	1.9	2.7
GBM22	R273C	25	29	40	64*	1.6	2.6
GBM43	F270C	28	28	33	62*	1.2	2.2
GBM10	WT	28	34*	38	40	1.4	1.4
GBM14	WT	37	37	73	82	2.0	2.2
GBM26	WT	57.5	88*	99	81	1.7	1.4
GBM39	WT	19	16	51	72*	2.7	3.8
GBM108	WT	67	66	113*	87	1.7	1.3

Table 1: Summary of TP53 status and median survival by treatment for each PDX model

MUTANT TP53 ASSOCIATED WITH THERAPEUTIC ACTIVITY

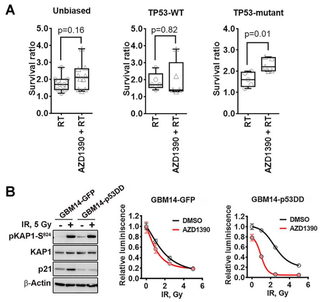

Figure 5: TP53 mutation status as predictor of treatment response. **A)** Boxplots showing comparison of response to RT vs AZD1390/RT, ratio of median survival for the treatment relative to placebo (survival ratio) were compared for all (unbiased, left), for the TP53-WT (center) or TP53-mutant (right) PDXs. **B)** Effect of constitutively-expressed dominant-negative TP53 (p53DD) vs. GFP in GBM14, a TP53-WT model, on IR-induced KAP1 phosphorylation, p21 levels are used as a measure of p53 transactivation (left); response to treatment with (or without) 30 nM AZD1390 ± increasing dose of IR delivered by Cs^{137} irradiator, as assessed by CTG assay (right).

SUMMARY & CONCLUSIONS

- AZD1390 suppressed IR-induced phosphorylation of ATM and its downstream targets, culminating in decreased HR activity.
- AZD1390 promoted IR-induced G2/M arrest and compromised cell survival more efficiently in TP53-mutant GBM lines.
- Consistent with adequate CNS distribution and targeting of ATM signaling, AZD1390 dosed at 20 mg/kg suppressed RT-induced γH2AX in orthotopic GBM12 xenografts.
- An AZD1390 and RT combination was remarkably effective in a subset of orthotopic GBM models; with the statistically significant response restricted to TP53-mutant group, suggesting that TP53-status can be a biomarker of therapeutic response.
- While the role of TP53 in AZD1390-mediated sensitization remains unclear, the dominant negative action of mutant TP53 could render vulnerability to AZD1390-mediated sensitization.