Excessive new origin firing underlies selective glioma stem cell cytotoxicity induced by replication stress response inhibition

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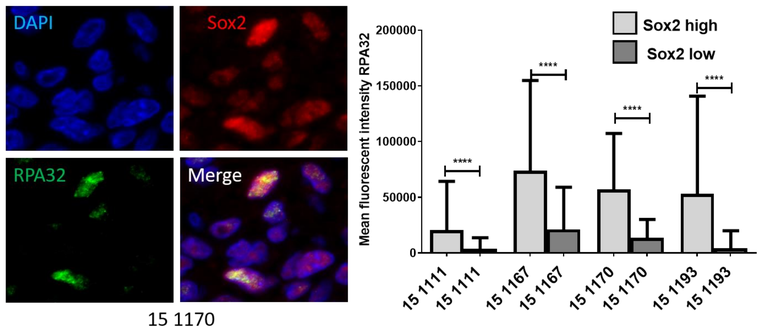
Introduction

- Glioblastoma (GBM) is a deadly cancer associated with local recurrence, driven by treatment-resistant GBM cancer stem cells (GSCs)
- GSC treatment resistance is due to constitutive DNA damage response activation driven by elevated replication stress (RS)



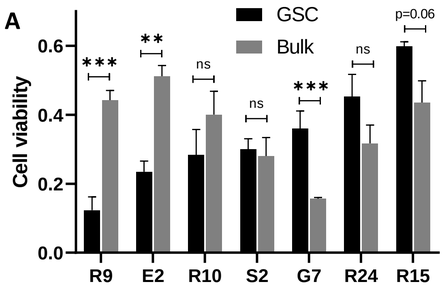
GBM patient tumour immunofluorescence showed RS-marker RPA32 was significantly higher in cell populations with high GSC-marker Sox2 [1].

- RS response inhibition is potently cytotoxic to GSCs
- We investigated response to combined ATR and PARP inhibition (CAiPi) to gain mechanistic insight, for future biomarker development and clinical translation.

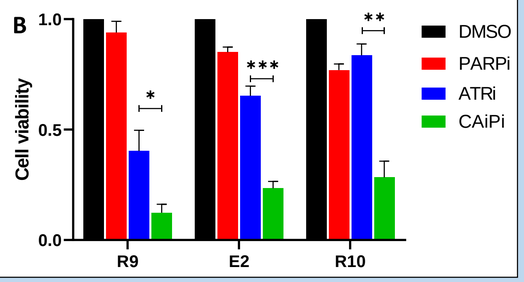
Methods

- A panel of patient-derived GBM cell lines were cultured as stem enriched (GSCs) or stem depleted (bulk), to characterise response to combined ATR inhibition (VE821 5μM) and PARP inhibition (Olaparib 1μM), by CellTiter-Glo viability assay.
- Mechanistic investigations included immunofluorescence of 53BP1 nuclear bodies, DNA fibre analysis to investigate origin firing, PARP trapping studies including the PARPi Veliparib (1μM), and lastly origin firing inhibition with CDK inhibitor Roscovitine.

Figure 1. Characterisation of CAiPi response

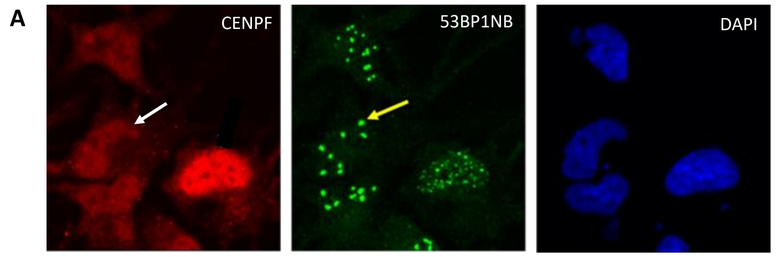


A. Responses to 6-day exposure to CAiPi in a panel of primary paired GBM GSCs vs differentiated progeny were heterogenous. CAiPi was selectively GSC cytotoxic in a subpopulation of tumours; R9, E2 and R10 (ns).



B. CAiPi was significantly more cytotoxic than ATRi single agent activity in GSC-sensitive lines. The combined cytotoxicity from the addition of limited PARPi activity was therefore likely supra-additive effects, highlighting the clinical potential of the drugs.

Figure 2. Increased under-replicated DNA in CAiPi-sensitive cells



A. 53BP1NBs (yellow arrow) are indicative of under-replicated DNA entering S-phase, resulting in lesions sequestered in G1 phase (CENPF negative cells), bound by 53BP1 [2].

B. CAiPi-induced 53BP1NB increased significantly in the GSC-sensitive cell line E2 GSC. More modest increases were observed in relatively resistant R15 and E2 bulk cell lines. The increase in 53BP1NB mirrored the supra-additive effects in E2 GSCs.

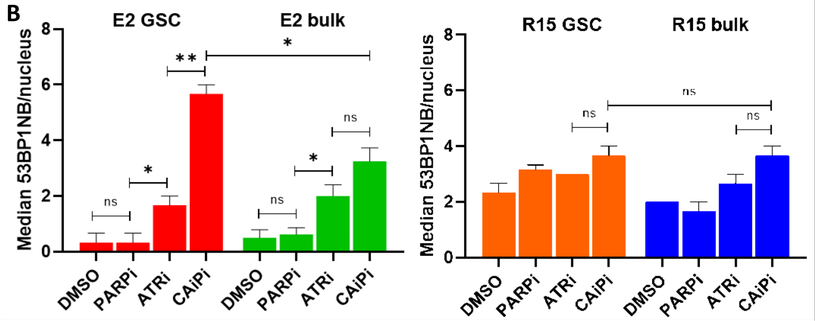
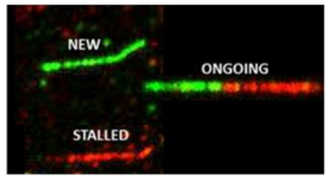
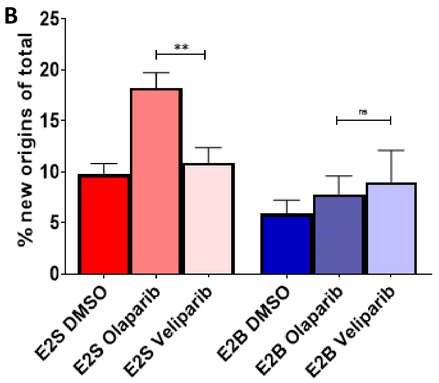


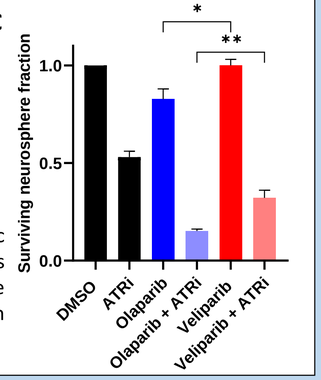
Figure 3. PARP trapping drives excessive origin firing in GSCs



A. DNA fibre assays allowed for quantification of replication structures, by subsequent staining with different fluorescent nucleotide analogues.

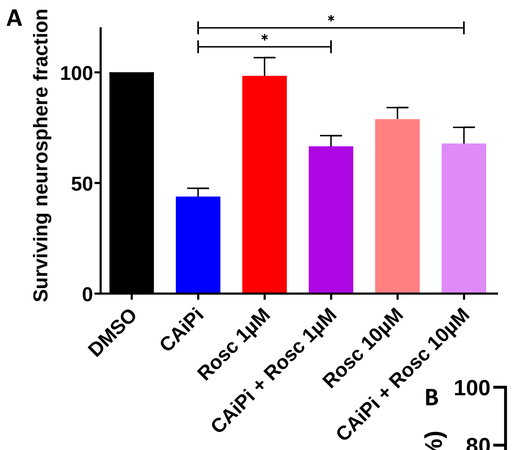
B. One important mechanism of PARPi activity is their ability to trap PARP at sites of damage [3]. Interestingly, the more potent PARP trapper Olaparib induced more new origins than Veliparib, quantified using a DNA fibre assay. This was observed exclusively in GSCs.





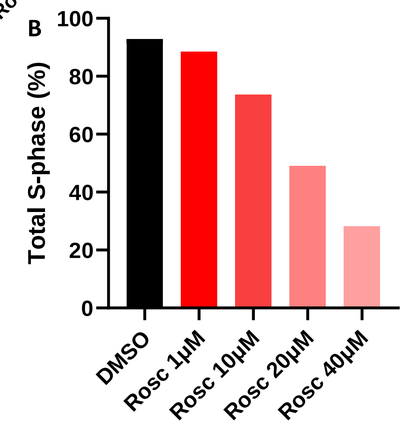
C. Potent PARP-trapper Olaparib increased the cytotoxic effects of combined PARP and ATR inhibition in GSCs more than Veliparib, measured by neurosphere formation. This suggests PARP trapping-induced origin firing may be involved in CAiPi GSC response.

Figure 4. Inhibition of origin firing rescued the CAiPi cytotoxic phenotype



A. Roscovitine has been shown to reduce origin firing [4]. Various concentrations of Roscovitine showed partial rescue of the CAiPi cytotoxic effects in E2 GSC, further suggesting the PARPi-induced increase in origin firing is important for the CAiPi mechanism of response.

B. Roscovitine is a CDK inhibitor, so its effects on cell cycle progression in E2 GSCs were investigated. Lower concentrations of Roscovitine did not significantly block entry into S-phase. Therefore, the rescue phenotype observed at these concentrations was likely due to a reduction in origin firing, as opposed to a complete ablation of replication via S-phase entry blockage.



Conclusions

- Heterogenous responses to CAiPi suggested there are likely complex mechanisms driving sensitivity, but supra-additive effects highlighted the clinical potential of these therapies
- Mechanistic investigations found that selective GSC CAiPi cytotoxicity was induced via dysregulation of replication
- Firstly, through under-replicated DNA entering mitosis
- Secondly, by the novel finding of increased new origin firing in GSCs via PARPi.
- Further mechanistic and omic understanding, and planned in vivo survival studies will lead to the discovery of clinically-useful biomarkers

References

1. Carruthers, R.D., et al., *Replication Stress Drives Constitutive Activation of the DNA Damage Response and Radioresistance in Glioblastoma Stem-like Cells.* Cancer Res, 2018. **78**(17): p. 5060-5071.
2. Lukas, C., et al., *53BP1 nuclear bodies form around DNA lesions generated by mitotic transmission of chromosomes under replication stress.* Nat Cell Biol, 2011. **13**(3): p. 243-53.
3. Murai, J., et al., *Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors.* Cancer Res, 2012. **72**(21): p. 5588-99.
4. Petermann, E., M. Woodcock, and T. Helleday, *Chk1 promotes replication fork progression by controlling replication initiation.* Proc Natl Acad Sci U S A, 2010. **107**(37): p. 16090-5.