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Abstract # A05

Improving outcome in Homologous recombination competent epithelial ovarian cancer: Hyperthermia and Surgeon's perspective

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Introduction

- We developed a functional assay and showed that 50% epithelial ovarian cancers (EOCs) are homologous recombination (HR) deficient (HRD) and are sensitive to PARP inhibition[1]. HRD patients showed also improved clinical platinum sensitivity (53.8% vs 16.7%), survival (12 month OS-41.7% vs 11.5%) and optimal cytoreduction (80% vs. 62%) rates compared to HR competent (HRC) tumours which represent an **unmet clinical need requiring novel therapeutic strategies for both surgery and chemotherapy.**

- HIPEC (hyperthermic intraperitoneal chemotherapy) has been shown to improve survival in ovarian cancer. Preclinical data indicate that hyperthermia compromises HR, possibly by protein unfolding. Chaperone proteins such as HSP90 are required for re-folding and inhibitors (HSP90i) are being investigated to render these cells HR deficient, and therefore sensitising them to PARPi. [2] There is controversy however over the optimum temperature required to prevent damage to normal tissues and also whether both platinum/PARPi sensitive and resistant cancers will benefit from HIPEC.

- **We hypothesize**
  1. **Hyperthermia compromises HRR function**
  2. **HRC tumours will benefit from targeted HIPEC following primary surgery and HSP90 inhibitors**

Methods

- HRC cell lines (VC8-B2, UWB1.289+BRCA1, A2780) and HRD cell lines (VC8, UWB1.289) were used.

- RAD51 foci, a marker of HRR, and γH2AX foci, a marker of DNA damage, were measured after treatment with heat at 39˚C and 42˚C and HSP90i (17-AAG and NVP-AUY922) by immunofluorescence microscopy and on the levels of RAD51, BRCA1 and BRCA2 by Western Blot (WB).

- Sensitivity to the PARPi (rucaparib and olaparib), in combination with a HSP90 inhibitor and in hyperthermic conditions was measured using clonogenic assays**.**

References

1. Mukhopadhyay A, et al. Clinicopathological features of homologous recombination deficient epithelial ovarian cancers: sensitivity to PARP inhibitors, platinum and survival. *Cancer Research* 2012;72: 5675

2. Choi YE, et al. Sublethal concentrations of 17-AAG suppress homologous recombination DNA repair and enhance sensitivity to carboplatin and olaparib in HR proficient ovarian cancer cells. *Oncotarget* 2014

Results

Figure 1. HSP90 inhibition and incubation at 39^{0}C both resulted in a modest sensitisation to rucaparib

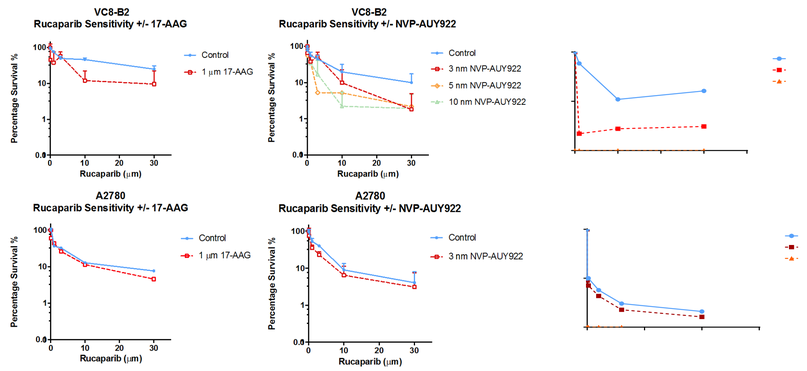
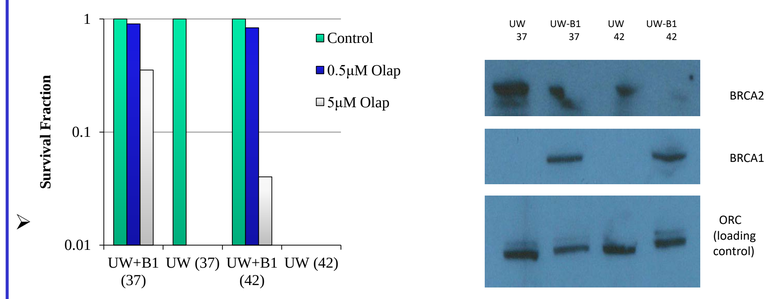


Fig1. Results of clonogenic survival assays and concentration-response to rucaparib in VC8, VC8-B2 cells and A2780 cells, either with HSP90 inhibition or in hyperthermic conditions. 1 hr incubation at 42ºC caused total cell death but at 39ºC, viability was 25% and sensitised V-C8 B2 (HRC) cells, but not V-C8 cells to rucaparib.

Figure 2. Heat at 42ºC sensitized UW+B1 (BRCA 1 competent) cells to olaparib (PARP inhibitor) but UW cells (BRCA1 deficient) all died. WB showed that 42ºC degrades BRCA2 but not BRCA1. (*Helen Bryant, Sheffield, UK)*



Conclusion and future work

- Personalised surgical and chemotherapeutic strategies may be developed for HR stratified EOCs. Primary surgery may be the preferred approach in HRC due to poor chemoresponse; Resources should be optimised to ensure increase in optimal cytoreduction rates and study the factors for surgical resistance in HRC

- Intra-operative hyperthermic treatment and selective HR inhibitors (HSP90) may improve subsequent chemoresponse in HRC (Fig 4). Different profiles of heat sensitisation with rucaparib and olaparib was seen at 39ºC and 42ºC

- Pre-clinical studies on patient tissues before and after HIPEC procedures are proposed in ongoing and future studies as well as animal studies on HIPEC models to consolidate our hypothesis

Figure 3. HSP90 inhibitors reduce RAD51 foci formation in a concentration-dependent manner and levels of RAD51 in WB

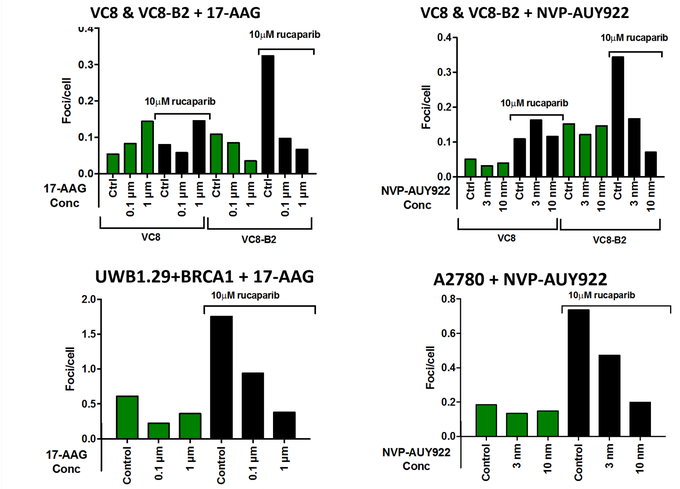


Figure 3A. Average RAD51 foci per cell. A decrease in rucaparib-induced RAD51 foci can be seen across all cell lines with increasing concentrations of HSP90i

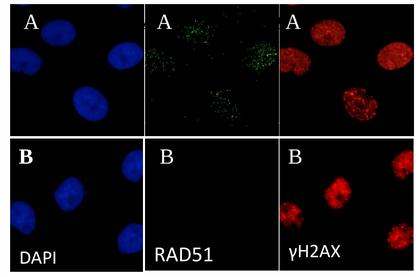


Figure 3B. Immunofluorescence microscopy of UWB 1.29+BCRCA1 cells, showing a decrease in RAD51 with 1 μm 17-AAG (B) when compared with control (A). In all cell lines, rucaparib-induced RAD51 foci decreased on average by 79% with 1 μm 17-AAG and 76% with 10 nm NVP-AUY922.

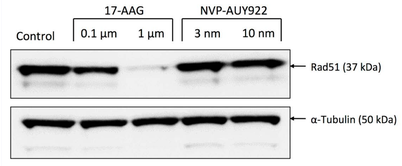


Figure 3C. Western Blot in A2780 cells, with varying concentrations of 17-AAG and NVP-AUY922. RAD51 foci decreased with increasing concentrations of 17-AAG, with almost no expression visible at 1 μm.

Fig. 4