

Reactive Oxygen Species (ROS) modulation as a strategy to improve chemo-sensitivity in homologous recombination stratified epithelial ovarian cancer

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Introduction and Objectives:

- Approximately 50% of epithelial ovarian cancers (EOC) harbor defect in the homologous recombination pathway of DNA repair (HRD) and are sensitive to platinum/ PARP inhibitors (PARPi). However, HR competent (HRC) tumours are chemo resistant; combination modalities are proposed to compromise HR function and restore chemo response.
- We propose a mechanism whereby mechanical/ pharmacological reactive oxygen species (ROS) induction which is modulated by NRF2 activation and therefore requires BRCA1 trapping, will lead to functional HR loss (HRD) and restore chemoresponse in HRC cell lines.
- CM5 (mahanine), a carbazole alkaloid isolated from an Indian Medicinal plant is a potential inhibitor of mitochondrial complex III in ETC by which enhanced Reactive Oxygen Species (ROS) is produced to induce various cellular events for apoptosis. We studied the effect of (CM5), in ovarian cancer cell lines and primary cultures as a pilot project.

Methods:

- We showed ROS generation with CM-5 (mahanine) in dose dependent manner in cell lines.
- MTT cytotoxicity assay was performed using increasing dosage of CM-5 in in HRC (UWB1.289 +B1) and HRD cell lines with and without PARP inhibitor rucaparib. Western blot assay was performed to study nuclear/ cytoplasmic distribution of NRF2 and BRCA1 after treatment with CM5.
- Primary cultures were developed from ascites (PCAST) from consecutive patients of EOC (WT1 and PAX8 positive on immunocytochemistry) undergoing primary surgery; Growth inhibitory effect of CM-5 was studied.

Results

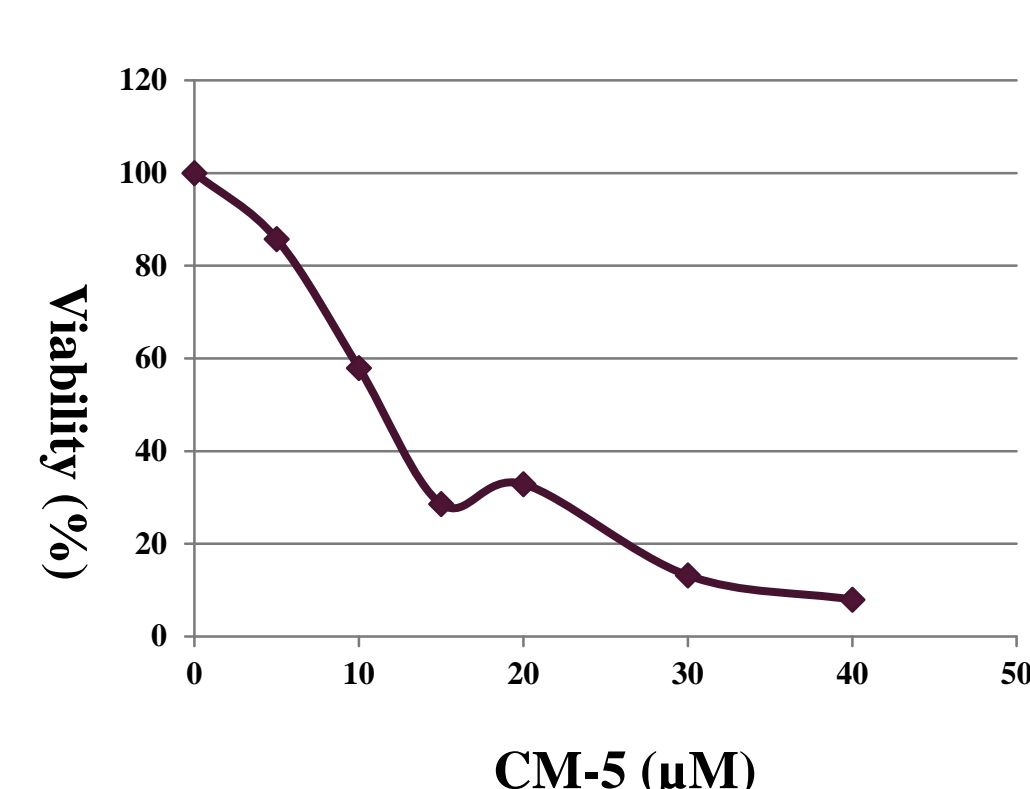


Fig 1A. Anti-proliferative activity by CM-5 in BRCA1 negative (HRD) ovarian cancer cells (UWB1.289) by MTT assay

IC50 = 12 μM

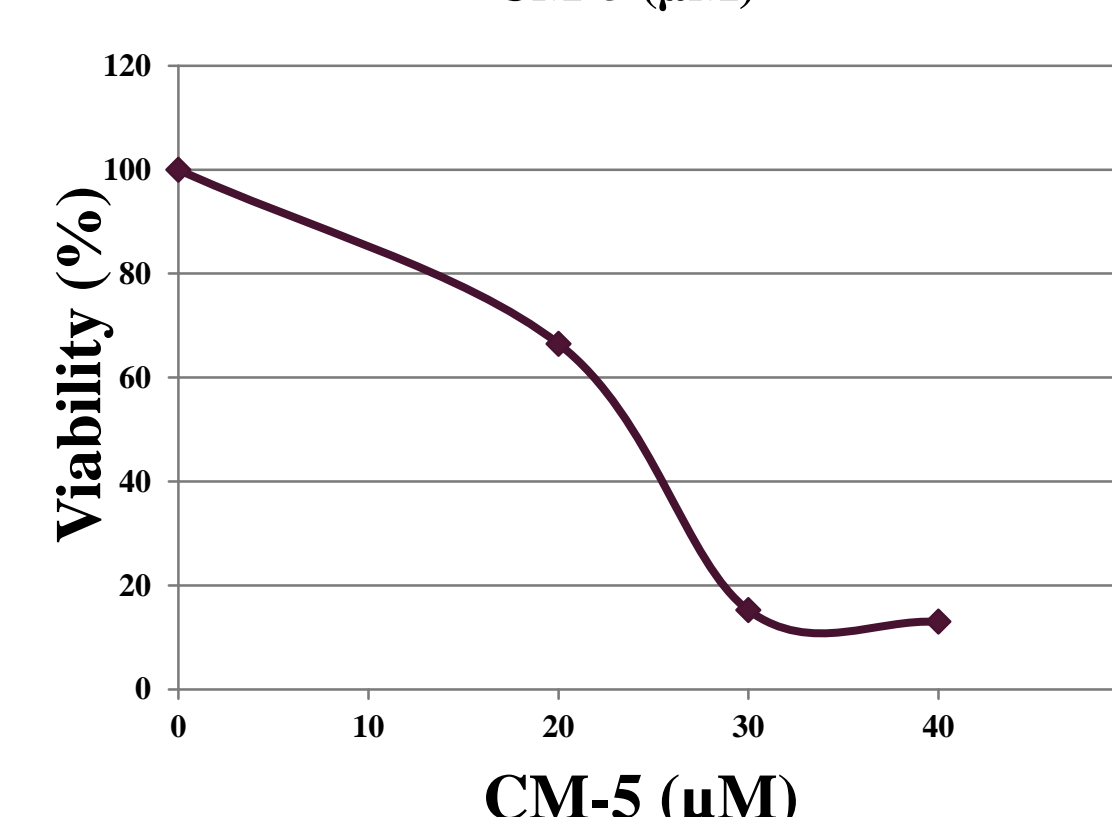


Fig 1B. CM-5 exhibited anti-proliferative activity even in UWB1.289+BRCA1 (HRC) cells by MTT assay

IC50 = 23 μM

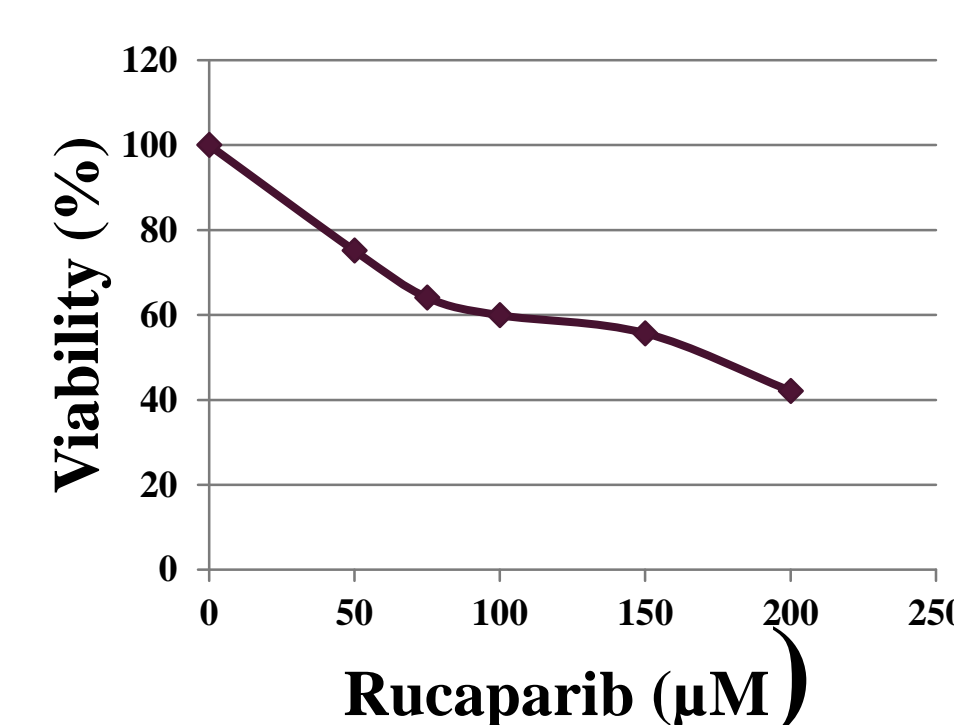


Fig 1C. Rucaparib anti-proliferative activity in UWB1.289+BRCA1 cells by MTT assay

IC50=160 μM

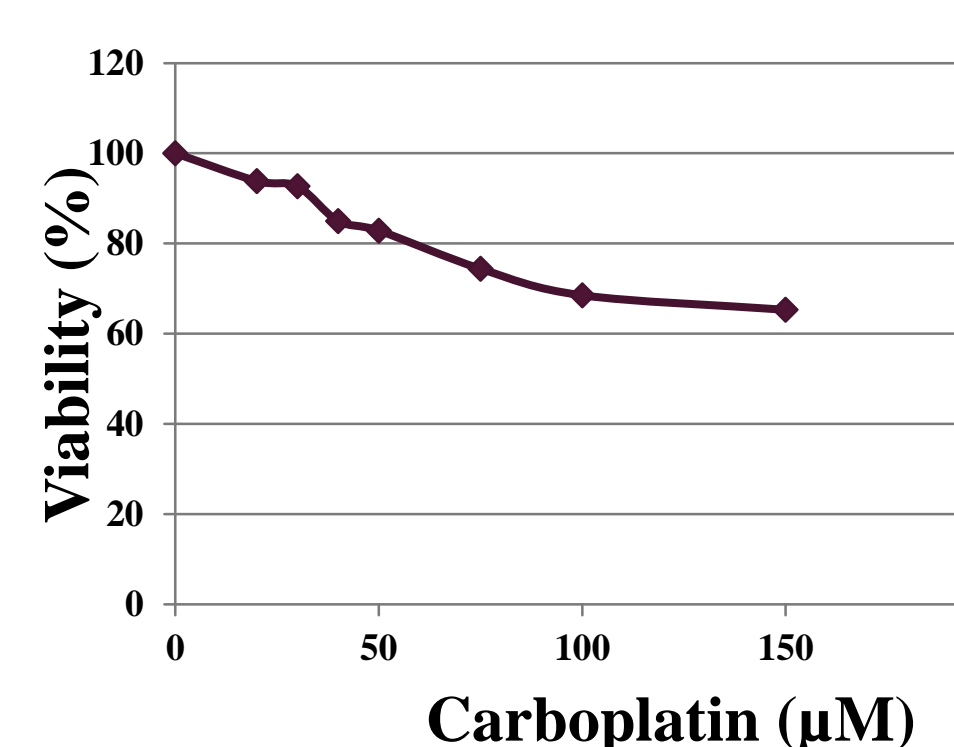


Fig 1D. Anti-proliferative activity of Carboplatin in UWB1.289+BRCA1 cells by MTT assay

IC50= >150 μM

Fig 2. Increased cell death with CM5 after combination Rucaparib

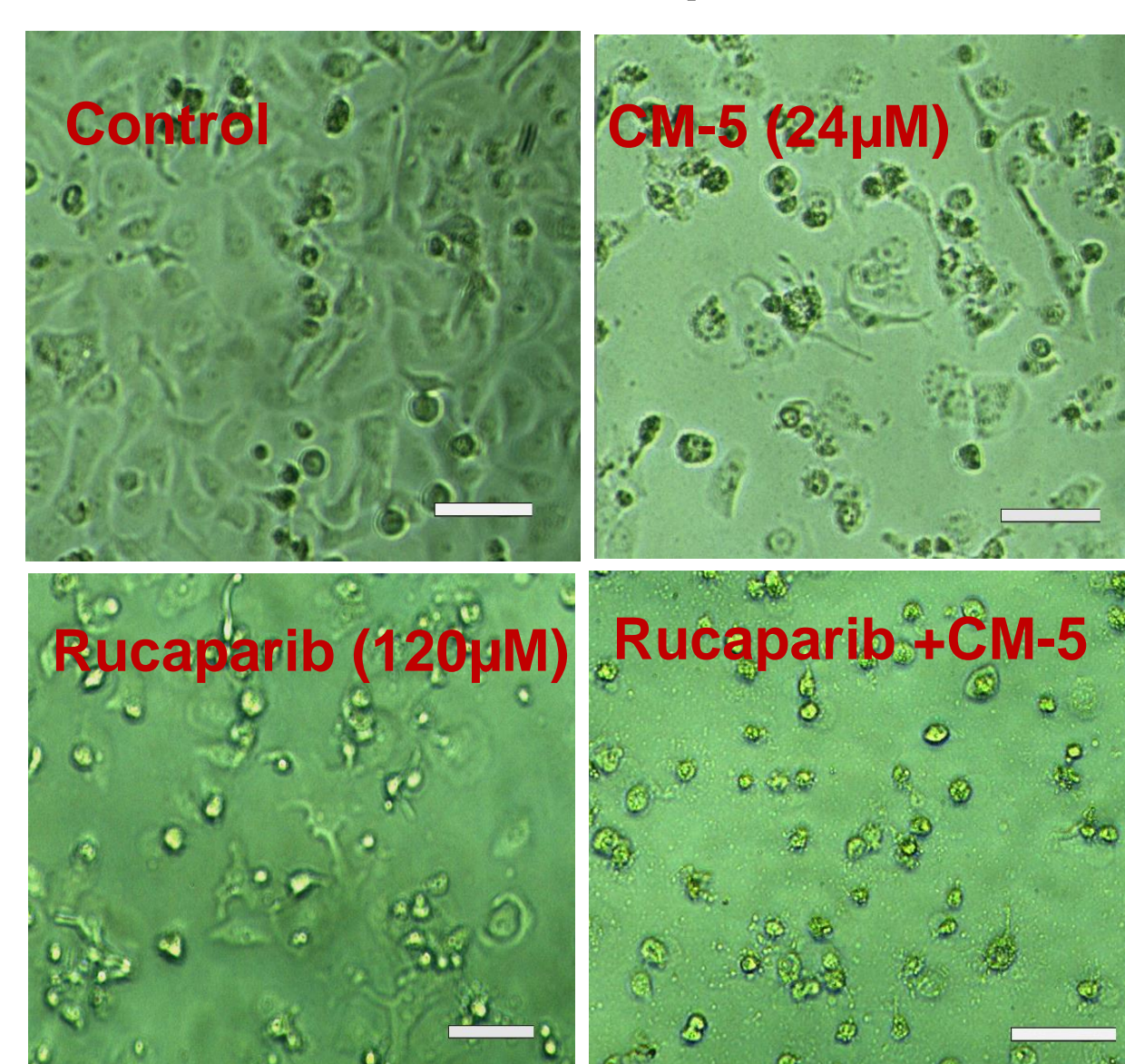


Table 1. Rucaparib (120 μM) induced 20% more cell death in combination CM-5 (24 μM) with in UWB1.289+BRCA1 cells In MTT assay 48hrs

CM-5 (μM)	Rucaparib (μM)			
	0	60	120	160
0	100	87.68	42.33	29.9
12	114.58	89.58	36.57	19.2
24	58.879	39.85	21.24	13.37
36	14.74	15.01	15.85	17.4

Table 2. CM-5 induced higher ROS in BRCA1 positive ovarian cancer cells in time dependent manner ROS level persist even after 4 hours

CM-5 (30 μM) treated UWB1.289 +BRCA1 cells		
Time in hours	MFI (H2DCF-DA)	Relative %
0	6,570	100
0.5	12711	193.4
1	16549	251.8
2	19366	294.7
3	20234	307.9
4	13096	199.3

Fig 3. CM-5 induces apoptosis in BRCA 1 positive Ovarian cancer cells in dose dependent manner ~ 3 fold enhanced apoptotic cell death after 24h treatment with CM-5

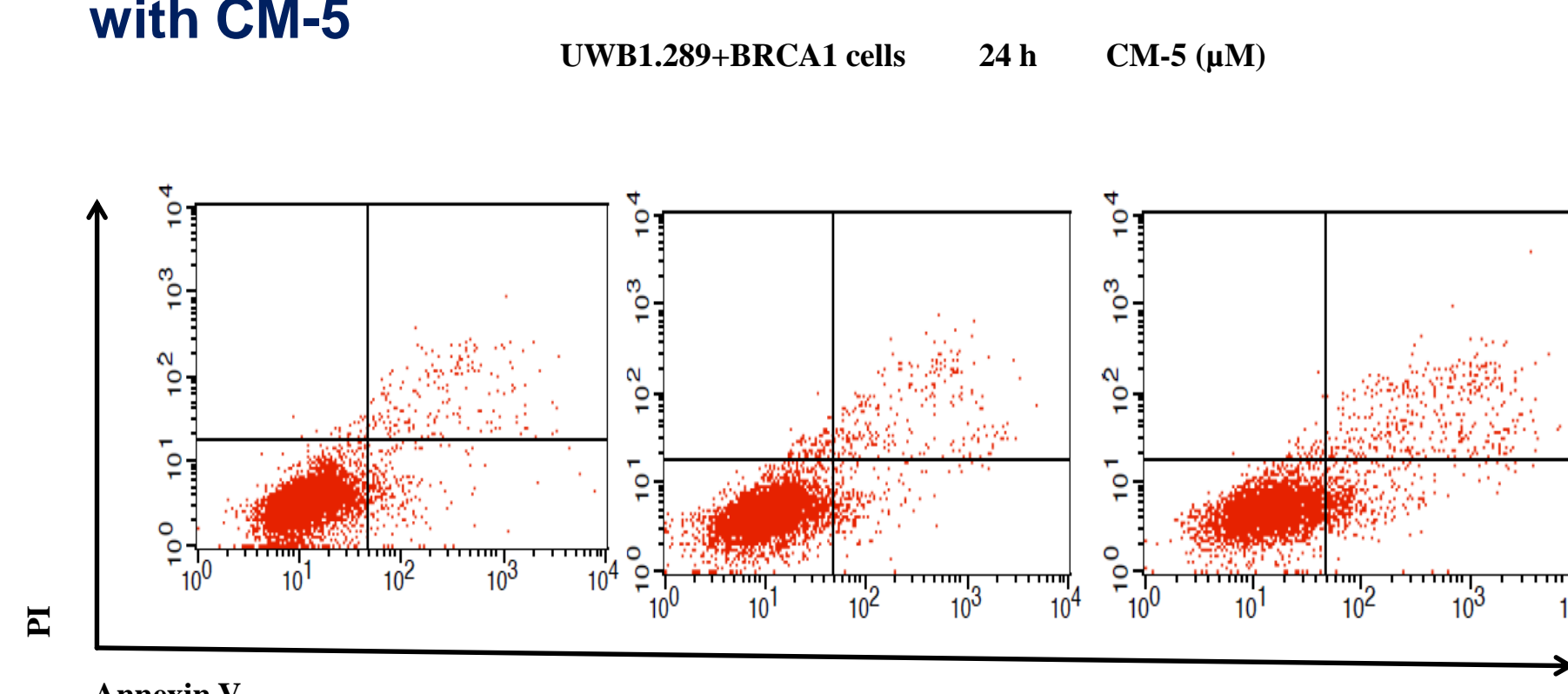
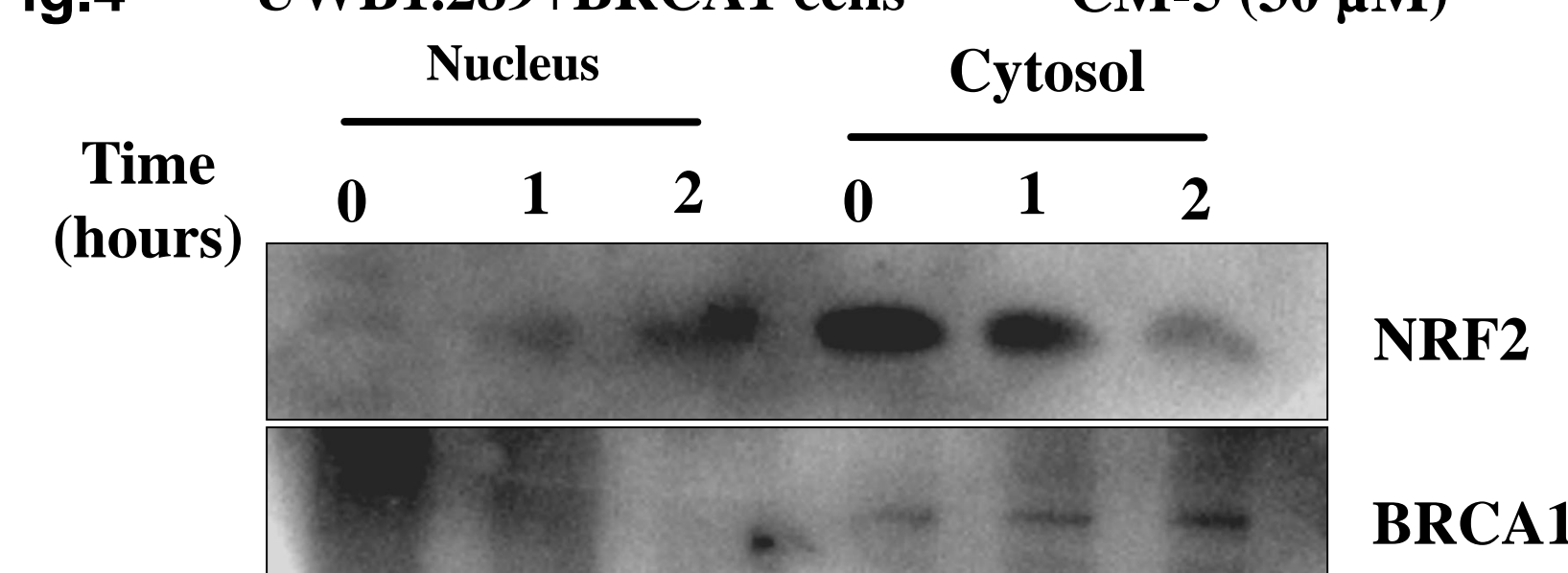


Fig.4 UWB1.289+BRCA1 cells CM-5 (30 μM)



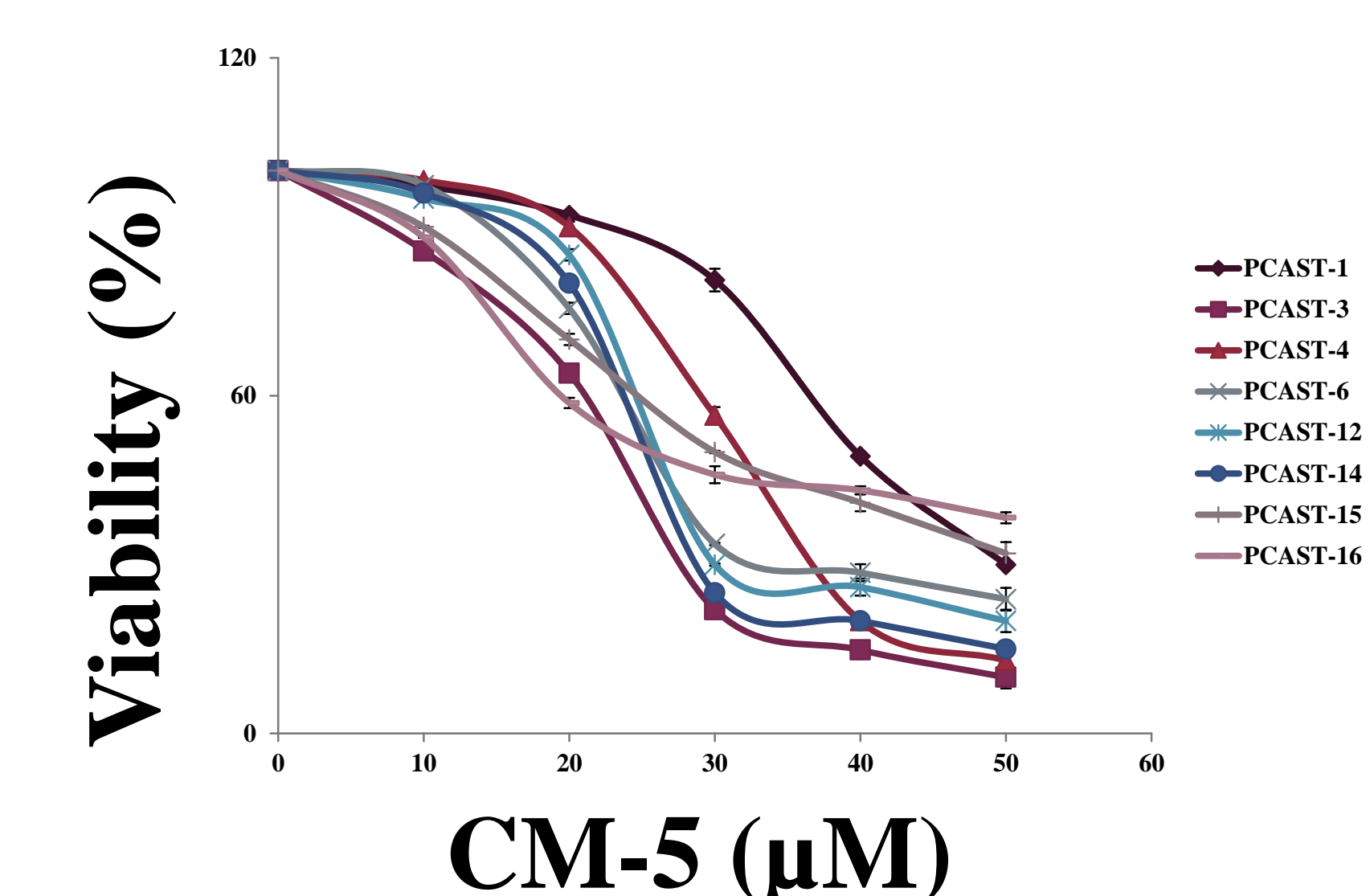
- The expression levels of NRF2 is higher in cytosol of BRCA1 positive cells whereas BRCA1 level is higher in the nucleus
- CM-5 induced translocation of NRF2 from the cytoplasm to the nucleus in time dependent manner
- BRCA1 level is higher in cytosol in CM-5 treated cells

Acknowledgement: Cell lines and rucaparib were obtained from NICR, Newcastle University through a collaborative DST-UKIERI grant jointly held by Dr Asima Mukhopadhyay and Prof Nicola Curtin

Table 3. Clinicopathological features of 8 patients and IC50 of CM-5 in ascetic fluid cultures

Serial no	Stage and histology	Platinum resistance at 6 months	IC 50 CM-5
PCAST-1	IIIC HGCS	no	34.7 ± 0.02
PCAST-3	IVC HGSC	yes	19.6 ± 0.5
PCAST-4	IIIC HGSC	no	28.5 ± 0.08
PCAST-6	IIIB (Clear cell)	no	23.2± 0.02
PCAST-12	IIIC (HGSC)	no	23.2 ± 0.01
PCAST-14	IIIC (HGSC)	yes	22.3 ± 0.02
PCAST-15	IIIC (HGSC)	no	30 ± 0.5
PCAST-16	IVB (HGSC)	no	27.4 ± 0.15

Figure 5. Growth inhibitory effect of CM-5 in primary cells isolated from ovarian cancer patients



Conclusions:

- ROS generating agent CM5 shows cytotoxicity in primary cells in EOC and HRC cell lines.
- Proposed future work: To study ROS-NFR2-BRCA1 interaction to devise novel combination strategies with PARPi in HRC and HRD EOC primary culture models and using other pharmacological/ surgical ROS generating modalities.

