Platinum therapy resistance is associated with an enrichment of tumour angiogenesis in epithelial ovarian cancer

Nuala McCabe[1,3], Aya El Helali[1,2], Christopher Steele[3], Naomi Dickson[1,3], Lara Dura Perez[1,3], Christina O'Neill[6], Reinhold Medina[6], Laura Knight[3], Charlie Gourley[5], W Glenn McCluggage,[1,4], Denis P Harkin[3], Richard Wilson[1,2], Alan W Stitt[6], Richard D Kennedy[1,2,3]

[1]Centre for Cancer Research and Cell Biology, Queens University Belfast, Northern Ireland, UK; [2]Belfast City Hospital Cancer Centre, Belfast, Northern Ireland, United Kingdom

[3]Almac Diagnostics, 19 Seagoe Industrial Estate, Craigavon, UK; [4]Department of Pathology, Royal Group of Hospitals Trust, Belfast, UK; [5]University of Edinburgh Cancer Research UK Center, UK, [6]Centre for Experimental Medicine, Queen's University Belfast, Northern Ireland, UK.

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Background

Angiogenesis is a key pathological feature of epithelial ovarian cancer (EOC) and anti-angiogenic agents have dominated the field of drug development in EOC. However, only progression-free survival benefit has been observed from use of anti-angiogenic agents in EOC, with no evidence of overall survival benefit. High grade serous ovarian cancer (HGSOC) is the most prevalent form of EOC and is associated with poor outcomes. Currently, there are no clinically approved predictive biomarkers to identify HGSOC patients that will derive benefit from anti-angiogenic therapy.

Aims

The overall aim of this study was to define a novel stratifying approach for selection of EOC patients most likely to benefit from anti-angiogenic therapy. Study objectives were as follows:

- To investigate the relationship between prior exposure to platinum-based chemotherapy and response to anti-angiogenic agents in EOC
- To determine the dominant angiogenesis signalling axis in platinum resistant EOC
- To determine the robustness of platinum resistance as a stratifier for response to anti-angiogenic agents in EOC

1. Platinum Therapy Resistance is Associated with Response to Anti-Angiogenic Agents in Clinical Trials

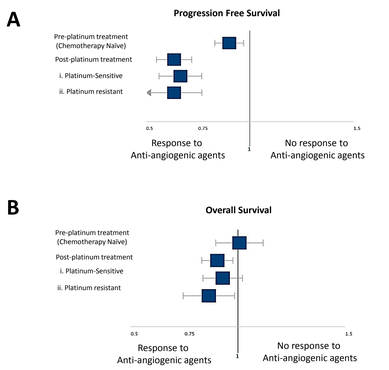
Table 1. Review of phase II/III clinical trials to determine the effect of anti-angiogenic agents in the management of advanced ovarian cancer

Study	Trial Phase	Agent	Target	PFS difference* (Months)	HR-PFS (95% CI)	OS difference* (Months)	HR-OS (95%-CI)
GOG218[#] (Chemotherapy Naïve)	Phase III	Bevacizumab	VEGF-A	Bevacizumab: **+0.9** Bevacizumab-M**: **+3.8**	**Bevacizumab:**0.908 (0.795 to 1.040) *P=0.160*; **Bevacizumab-M:** 0.717 (0.625 to 0.824) *p<0.001**	Bevacizumab:- **0.6** Bevacizumab-M: **+0.4**	**Bevacizumab:** 1.036 (0.827 to 1.297) *P=0.76*; **Bevacizumab-M:** 0.915 (0.727 to 1.152) *p=0.45*
ICON7 (Chemotherapy Naïve)	Phase III	Bevacizumab	VEGF-A	Bevacizumab: **+2.4**	**Bevacizumab:** 0·93 (0·83–1·05) p=0·25	Bevacizumab: - **0.6**	**Bevacizumab:** 0.99 (0.85-1.14) *p=0.85*
AURELIA[#] (Resistant)	Phase III	Bevacizumab	VEGF-A	Bevacizumab: **+3.3**	**Bevacizumab:** 0.42 (0.32 to 0.53) *p<0.001**	Bevacizumab: **+3.3**	**Bevacizumab:** 0.85 (0.66 to 1.08) *p=0.174*
OCEANS[#] (Sensitive)	Phase III	Bevacizumab	VEGF-A	Bevacizumab: **+4.0**	**Bevacizumab:** 0.484 (0.388 to 0.605) *p<0.0001**	Bevacizumab: **+1.8**	**Bevacizumab:** 0.95 (0.77–1.18) *p=0.65*
GOG-213 (Sensitive)	Phase III	Bevacizumab	VEGF-A	Bevacizumab: **+3.4**	**Bevacizumab:** 0.628 (0.534-0.739) *p<0.0001**	Bevacizumab: **+4.9**	**Bevacizumab:** 0.829 (0.683-1.005) *p=0.056*
AGO-OVAR12 (Chemotherapy Naïve)	Phase III	Nintedanib	VEGFR, FGFR, PDGFR	Nintedanib: **+0.6**	**Nintedanib:** 0·84 (0·72–0·98) *p=0·024**	Nintedanib: **+1.2**	**Nintedanib:** 0.99 (0.77 - 1.27) *p=0.9060*
MITO-11 (Resistant)	Phase II	Pazopanib	VEGFR, FGFR, PDGFR	Pazopanib: **+2.86**	**Pazopanib:** 0.42(0.25 - 0.69) *p=0.002**	Pazopanib: **+5.4**	**Pazopanib:** 0.60 (0.32 - 1.13) *p=0.056*
AGO-OVCAR16 (Sensitive)	Phase III	Pazopanib	VEGFR, FGFR, PDGFR	Pazopanib: **+5.6**	**Pazopanib:** 0.766 (0.64-0.91) *p=0.0021**	NR	
ICON6 (Sensitive)	Phase III	Cediranib	VEGFR, FGFR, PDGFR	Cediranib-C***: **+1.2**; Cediranib-C+M****: **+2.3**	**Cediranib-M**: 0·56 (0·44–0·72) *p<0·0001**	Cediranib-C: - **11.1**; Cediranib-C+M: **+5.3**	**Cediranib-C+M**: 0·77 (0·55–1·07) *p=0.11*
Karlan B. Y., et al[#] (Resistant)	Phase II	Trebananib	Ang	Trebananib (10mg/kg): **+2.6**; Trebananib (3mg/kg): **+1.1**	**Trebananib:** 0.76 (0.52-1.12) *p=0.165*	Trebananib (10mg/kg): **+1.6**; Trebananib (3mg/kg): **-0.5**	**Trebananib (10mg/kg):** 0.60 (0.34 - 1.06) *p=0.081*; **Trebananib (3mg/kg):** 0.77 (0.45 - 1.31) *p=0.330*
TRINOVA-1 (Resistant)	Phase III	Trebananib	Ang	Trebananib: **+1.8**	**Trebananib:** 0.66 (0.57-0.77) p=<0·0001*	Trebananib: **+1.7**	**Trebananib:** 0.86 (0.69-1.08)
Matei D., et al (Resistant)	Phase II	Sorafenib	VEGF-2 and 3, PDGFR-β, Flt-3, c-KIT	Sorafenib: **2.1**	**Sorafenib:** 0.70 (0.36 - 1.38)	Sorafenib: **16.33**	**Sorafenib:** 0.66 (0.32 - 1.35)
Herzog T. J., et al (Sensitive)	Phase II	Sorafenib	VEGF-2 and 3, PDGFR-β, Flt-3, c-KIT	Sorafenib: **+3.0**	**Sorafenib:** 1.09 (0.72- 1.63)	NR	

*Compared to control/placebo
**-M = Maintenance
***-C = Concurrent
****-C+M = Concurrent and maintenance

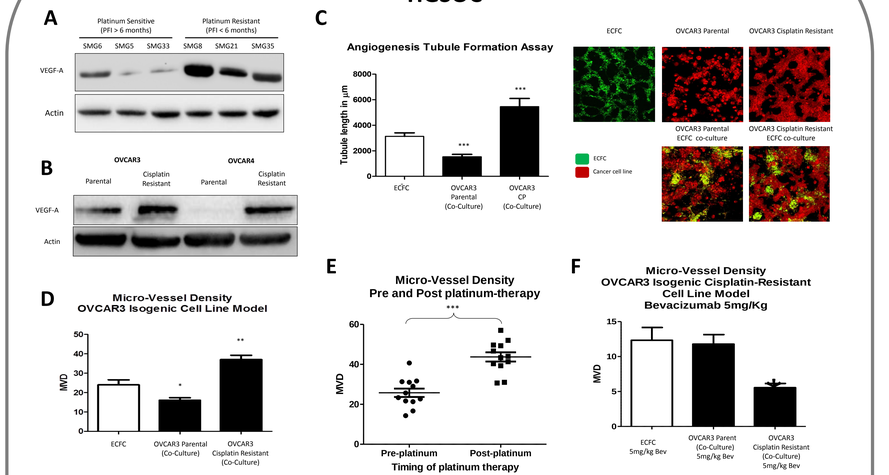
Table 2. PFS and OS benefit from anti-angiogenic agents in EOC relative to platinum sensitivity

Platinum sensitivity	PFS p-value HR (95% CI)	OS p-value HR (95% CI)
Pre-platinum treatment (Treatment Naïve)	0.0065 0.89 (0.813 - 0.9704)	0.9877 1.0 (0.8963 - 1.1176)
Post-platinum treatment	<0.0001 0.63 (0.5501 - 0.7197)	0.0112 0.90 (0.8311 - 0.9766)
Sensitive (PFI >6-12 and >12 months)	<0.0001 0.66 (0.5621 - 0.7681)	0.1223 0.92 (0.8366 - 1.0213)
Resistant (PFI <1 and 1-6 months)	0.0002 0.58 (0.4337 - 0.7689)	0.0289 0.86 (0.7445 - 0.9841)



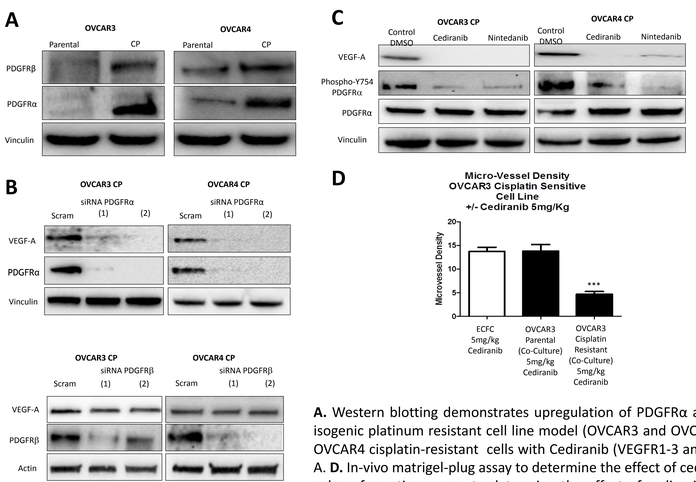
A. Forest plot illustrating the meta-analysis of phase II and III clinical trials demonstrating prolonged PFS benefit with the addition of anti-angiogenic agents in platinum-naïve, sensitive and resistant EOC. **B.** Forest plot illustrating the OS benefit in platinum resistant EOC.

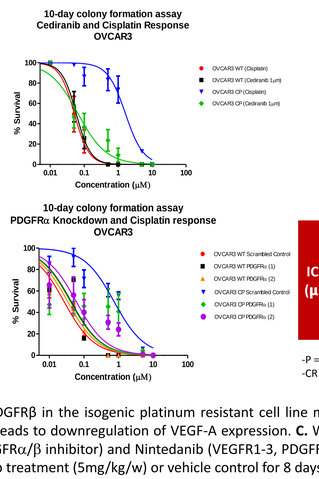
2. Platinum Therapy Selects for an Anti-Angiogenic Phenotype in HGSOC



A. Western blot demonstrating increased VEGF-A expression in 3 platinum resistant ascites-derived primary cells relative to the platinum sensitive primary cells. **B.** OVCAR3 and OVCAR4 HGSOC platinum-resistant cell lines have higher basal VEGF-A expression relative to their parental platinum-sensitive cell lines. **C.** OVCAR3 platinum resistant cell demonstrate increased microtubule length (p= 0.0009)when in co-culture with the Endothelial colony forming cells (ECFC). **D.** *In-vivo* matrigel-plug assay to determine the MVD in the OVCAR3 isogenic cell lines in co-culture with EFCF. The OVCAR3 platinum-resistant cell lines have a higher MVD than the OVCAR3 platinum-naïve pair relative to the ECFC cell lines (p-value: 0.004). **E.** Graph illustrating MVD in the pre and post-chemotherapy paired patient samples. **F.** *In-vivo* matrigel plug assay illustrating that bevacizumab has specificity for the OVCAR3 platinum-resistant cell line which is demonstrated by a reduction in MVD (p-value: 0.0012).

3. PDGFRα is associated with activating the angiogenesis signalling pathway in platinum resistance HGSOC





IC50 (µM)	OVCAR3 –P (Cisplatin)	OVCAR3-P (Cisplatin & Cediranib)	OVCAR3-CR (Cisplatin)	OVCAR3-CR (Cisplatin & Cediranib)
	0.058	0.066	2.591	0.070

-P = parental
-CR = cisplatin resistant

IC50 (µM)	OVCAR3-P AS (Cisplatin)	OVCAR3-P (PDGFRα siRNA 1)	OVCAR3-P (PDGFRα siRNA 2)	OVCAR3-CR AS (Cisplatin)	OVCAR3-CR (PDGFRα siRNA 1)	OVCAR3-CR (PDGFRα siRNA 2)
	0.045	0.052	0.048	1.080	0.437	0.323

-P = parental
-CR = cisplatin resistant

A. Western blotting demonstrates upregulation of PDGFRα and PDGFRβ in the isogenic platinum resistant cell line model (OVCAR3 and OVCAR4). **B.** siRNA of PDGFRα and PDGFRβ in the isogenic platinum resistant cell line model (OVCAR3 and OVCAR4) leads to downregulation of VEGF-A expression. **C.** Western blot following treatment of the OVCAR3 cisplatin-resistant and OVCAR4 cisplatin-resistant cells with Cediranib (VEGFR1-3 and PDGFRα/β inhibitor) and Nintedanib (VEGFR1-3, PDGFRα/β FGFR1-3 inhibitor) leads to downregulation of expression of VEGF-A. **D.** In-vivo matrigel-plug assay to determine the effect of cediranib treatment (5mg/kg/w) or vehicle control for 8 days, in the OVCAR3 paired cell lines in co-culture with EFCF (n=5). **E.** 10-day colony formation assay to determine the effect of cediranib on platinum-resistance in the OVCAR3 and OVCAR4 cisplatin-resistant cell lines (N=3). **F.** 10-day colony formation assay to determine the effect of PDGFRα in the OVCAR3 and OVCAR4 cisplatin-resistant cell lines, relative to the cisplatin-sensitive cell lines (n=3).

Conclusions

- The clinical and pre-clinical data discussed has potentially significant clinical implications in the management of treatment-relapsed HGSOC.
- Platinum-resistance in relapsed HGSOC is an indicator for response to anti-angiogenic agents.
- The novel identification of chemotherapy-mediated selection for an angiogenic phenotype in EOC, through upregulation of the PDGFRα-VEGF-A signalling pathway.
- Targeted inhibition of PDGFRα (using TKI or siRNA knockdown) reverses platinum therapy resistance in EOC.
- This clinical and pre-clinical data supports the use of anti-angiogenic agents in the first and second line setting in patients with innate and acquired resistance to platinum therapy, respectively.

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Contact: **nuala.mccabe@almacgroup.com**