Realising the Therapeutic Potential of Dinaciclib in Gynaecological Cancers

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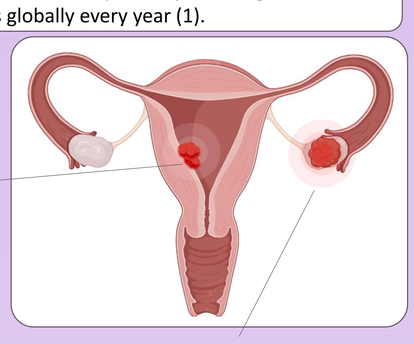
Clinical Challenge

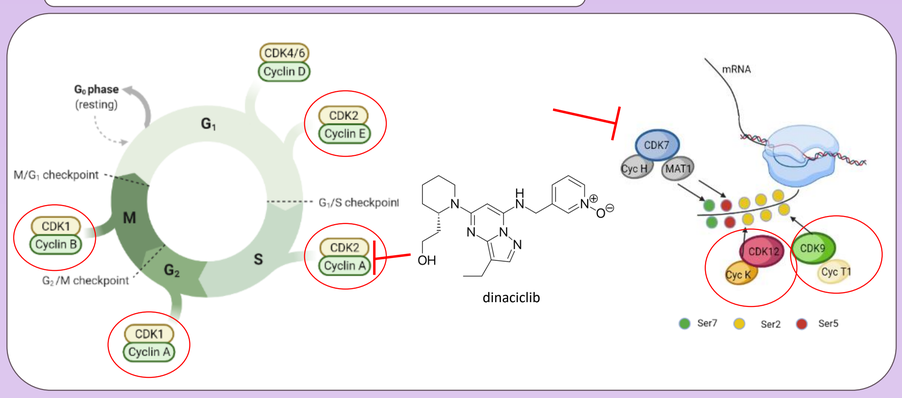
Endometrial and **ovarian** cancers (*EC & OC*) are the 4th and 6th most prevalent cancers in women in the UK, respectively resulting in approximately 270,000 deaths globally every year (1).



EC: Hormone dependent, type I EC has a positive prognosis, however type II EC (10% of cases) is often diagnosed at an advanced stage it's **5-year survival rate is just 55%** (2).

OC: Responsible for approximately 200,000 deaths annually, OC is typically diagnosed late and cancers often reoccur in a platinum resistant form, culminating in a **5-year survival rate of below 40%** (3).

Cyclin Dependent Kinase Inhibitors



Cyclin Dependent Kinases are integral to vital cellular processes including cell cycle progression and transcription and together with partner cyclin proteins are commonly aberrantly expressed in EC and OC.

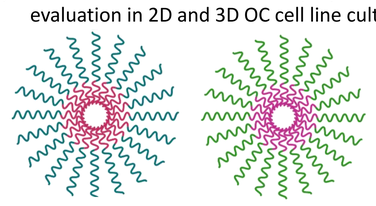
Dinaciclib is a pan-CDKi of CDKs 1, 2, 5, 9 and 12. CDKs 1 and 2 are involved in cell cycle progression, while CDKs 9 and 12, through phosphorylation of RNA Pol II at Ser2 of it's carboxy terminal domain (CTD), are essential to the transcription of the majority of genes (4).

Hypothesis and Presented Data

Despite promising preclinical data, dinaciclib has failed to progress in clinical trials for solid tumour indications. We postulate that the disconnect between preclinical and clinical efficacy is a result of a short circulation times and dose limiting toxicities and that the clinical efficacy of dinaciclib could be improved through encapsulation of a nano-carrier.

Here we:

1) Confirm dinaciclib's bimodal mechanism in EC and OC cell lines.
2) Evaluate dinaciclib's efficacy in Type I vs Type II patient derived EC cells
3) Evaluate dinaciclib's efficacy in platinum sensitive vs resistant patient derived OC cell lines
4) Test the fabrication of two polymeric nanoparticles (NP1 and NP2) with early stage evaluation in 2D and 3D OC cell line culture

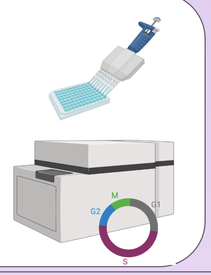


For more on Nanomedicine in EC and OC follow this QR code:

Methods

Viability assays:
Cell viability of adherent cultures was measured using the RealTime-Glo™ MT Cell Viability Assay (Promega). Dose-response curves and LD50 values are for 72h treatments. Cell viability of spheroid/aggregate cultures was measured using CellTiter-Glo® 3D Cell Viability Assay (Promega) following 72 h treatments.

Cell cycle analysis:
Cells were treated with dinaciclib (40 nM), or vehicle control for 24h, ahead of fixation by paraformaldehyde (4%) and nucleic acid staining using Hoescht 33342. Images were taken using an IN Cell 2000 (GE Healthcare). Nuclei were segmented using CellProfiler™ and the integrated intensity of Hoescht staining was used as a measure of relative DNA content. The Watson Pragmatic was applied to integrated intensity histograms to quantify cells per cell cycle stage.



Additional methods used including cell culture, spheroid formation, primary cell isolation, drug treatments, immunoblotting, PCR and nanoparticle fabrication and characterisation can be found in these studies:

Nanoparticle Characterisation

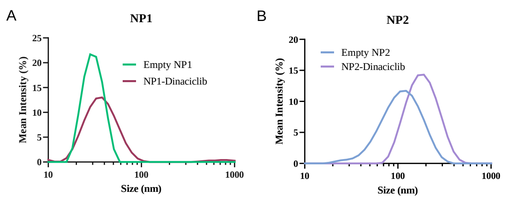


Figure 10 Dynamic Light Scattering histograms of empty and dinaciclib loaded (A) NP1 and (B) NP2. As detailed in the table below, the hydrodynamic diameter (D_H) of empty NP2 is approximately 3x larger than NP1 and as expected, dinaciclib-loaded particles have increased size.

Table 5 Characterisation of NP1 & NP2

	NP1 (empty)	NP1-Dinaciclib	NP2 (empty)	NP2-Dinaciclib
D_H* (nm ± SD)	29.1 ± 2.1	36.9 ± 14.1	105.0 ± 81.0	167.9 ± 64.0
Polydispersity	0.243	0.361	0.246	0.133
Zeta Potential Index (mV± SD)	0.1 ± 0.3	N.A.	-0.8 ± 0.0	N.A.
Encapsulation Efficiency	N.A.	76 %		85 %

* D_H = Hydrodynamic Diameter

Bimodal mechanism of dinaciclib in EC and OC

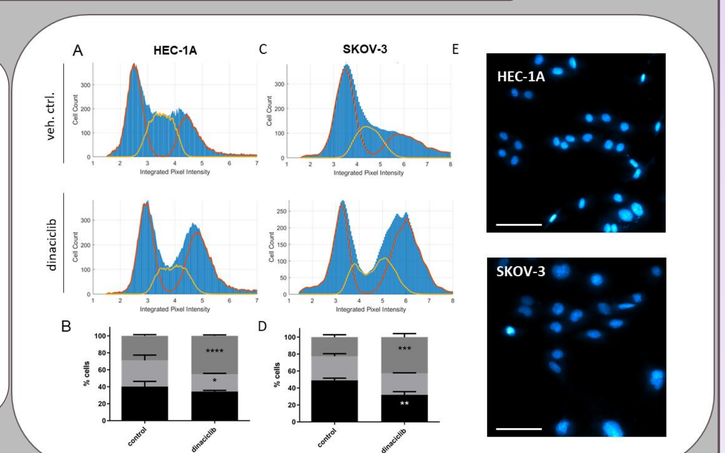


Figure 3 Dinaciclib (40 nM) arrests Type II EC (HEC-1A) and EOC (SKOV-3) cell lines in G2/M. (A, C) DNA content histograms derived from high content imaging. Proportion of cells per cell cycle stage modelled with Watson Pragmatic model. E) Example images

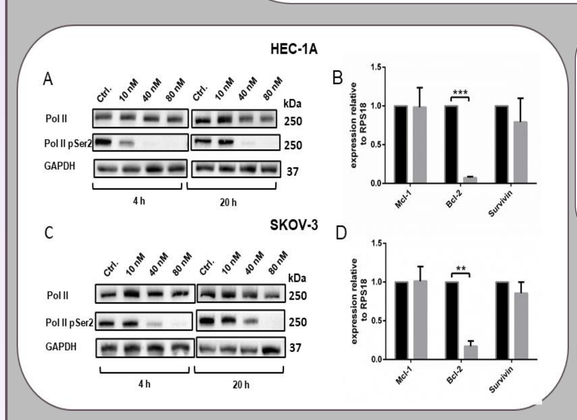
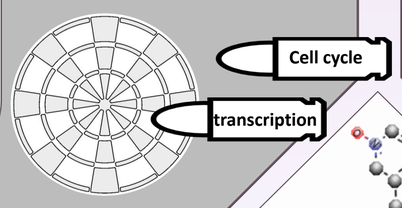
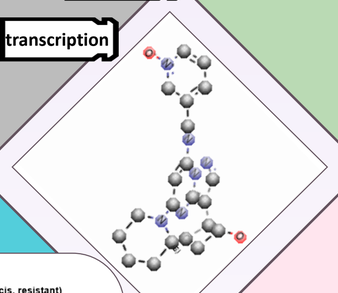


Figure 4 (A,C) Dinaciclib inhibits Pol II phosphorylation at Ser2 of the CTD in HEC-1A (EC) and SKOV-3 (EOC) SKOV-3 cell lines. (B,D) A concomitant reduction is observed in expression of antiapoptotic gene, BCL-2





Dinaciclib efficacy in EC primary cells and cell lines

Figure 5 (a-h) Dose-response curves for dinaciclib cells isolated from tumour samples representing Type I & II EC. i) Comparison dinaciclib sensitivity between EC subgroups show no significant difference. J) ER and p53 staining of tissue samples used to classify tumour samples.

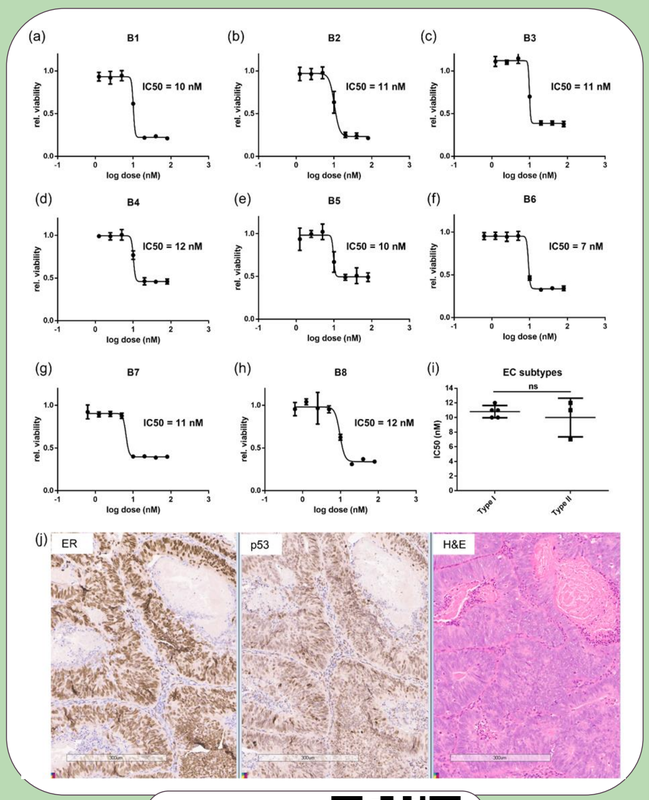


Table 1 Dinaciclib in EC cell lines

Cell Line	LD50 (μM)
Ishikawa (Type I)	0.006
HEC-1A (Type II)	0.009
HEC-1B (Type II)	0.006
Hec-50 (Type II)	0.009

For more on dinaciclib in EC see study:

Dinaciclib efficacy in OC primary cells and cell lines

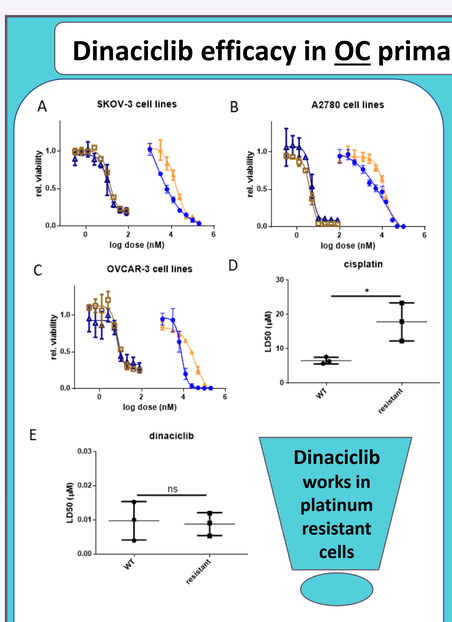


Table 3 Dinaciclib & Cisplatin in OC WT and Cis (cisplatin resistant) pairs.

Cell Line	Cisplatin IC50 (μM)	Dinaciclib LD50 (μM)
SKOV-3 WT/Cis	8/23	0.015/0.012
OVCAR-3 WT/Cis	6/18	0.010/0.090
A2780 WT/Cis	6/12	0.004/0.005

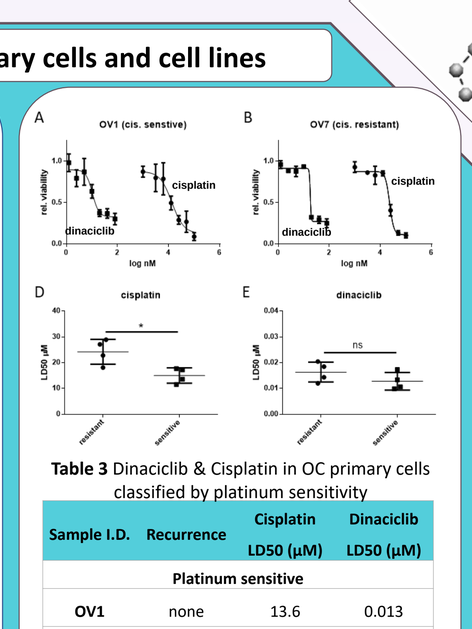


Table 3 Dinaciclib & Cisplatin in OC primary cells classified by platinum sensitivity

Sample I.D.	Recurrence	Cisplatin LD50 (μM)	Dinaciclib LD50 (μM)
Platinum sensitive			
OV1	none	13.6	0.013
OV2	none	11.5	0.017
OV3	none	9.80	0.017
OV4	none	17.7	0.011
Platinum resistant			
OV5	3 months	27.1	0.020
OV6	4 months	28.9	0.012
OV7	3 months	22.8	0.018
OV8	4 months	18.2	0.014

Figure 6 Dinaciclib is equally efficacious in WT and Cis (cisplatin resistant) cell line pairs .(A-C) Dose response curves: ▲, ●= WT; ▲, ■= Cis. (D,E) Comparison of cisplatin and dinaciclib LD50s in cell line pairs

Figure 7 Dinaciclib is equally efficacious in cisplatin sensitive and resistant primary cells. (A,B) Example dose response curves of dinaciclib and cisplatin in primary cells (C,D) Comparison of cisplatin and dinaciclib LD50s

Dinaciclib-Nanoparticles: preliminary efficacy data

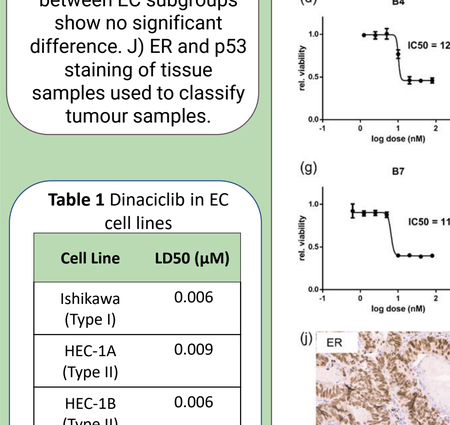


Table 4 NP1 & NP2 72 h LD50s in 2D and 3D culture

Cell Line	NP1 (μM)	NP2 (μM)
SKOV-3WT	00.025	0.005
SKOV-3cis	0.037	0.007
SKOV-3WT (3D)	N.A.	0.11
SKOV-3cis (3D)	N.A.	0.11

Figure 8 Dinaciclib loaded polymeric particles NP1 and NP2 are highly efficacious in SKOV-3WT and Cis cells in 2D (adherent) culture. (A-D) dose-response curves following 72h particle treatments (normalised to controls). Concentrations refer to quantity of encapsulated dinaciclib in treatment volume.

Figure 9 NP2 was also effective in SKOV-3WT/cis spheroids/aggregates. (A, B) Dose response curves of NP2 in 3D culture. C) Brightfield images of control and NP2 (1 μM) treated spheroids/aggregates 72h post-treatment. SKOV-3 WT spheroids are reduced in size, while SKOV-3cis aggregates appear more dispersed

Conclusions

- Dinaciclib exhibits a **bimodal mechanism in EC and OC** cells inducing cell cycle arrest and inhibiting Pol II phosphorylation resulting in reduced expression of BCL-2.
- Dinaciclib is equally efficacious in **Type I & II** cell lines and patient derived cells of these subtypes suggesting the potential of CDK inhibition in EC therapy.
- Beyond late diagnosis, chemo-resistance in OC is perhaps the greatest clinical challenge. Here we demonstrate dinaciclib is efficacious in OC cell lines and patient derived cells **independent of platinum sensitivity.**
- Dinaciclib was encapsulated in two polymeric particle types with **high efficiency: NP1 and NP2**
- NP1 and NP2 are highly efficacious in SKOV-3 WT/Cis cell lines with NP2 showing very low nM LD50s in these cells. NP2 is also effective in 3D cultures of these cell lines (NP1 data pending).

Open questions, ongoing and future work

- Why is NP2 more effective than NP1? Is it to do with uptake, or drug release?
- Evaluation of NP1&2 dinaciclib release profiles
- As NP1&2 are fabricated from different polymers and have different sizes, uptake of these particles should be compared in a panel of OC cell lines
- Evaluation of particle efficacy in a wider panel of cell lines and primary cells in 2D/3D cultures. If particles prove consistently efficacious, one, or both may be tested in mouse xenografts to characterise PK/PD properties and evaluate efficacy *in vivo*.

References

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