Abstract 5692

Identification of novel CDK12 inhibitors that synergize with PARP inhibition through induction of ‘BRCAness’ phenotype

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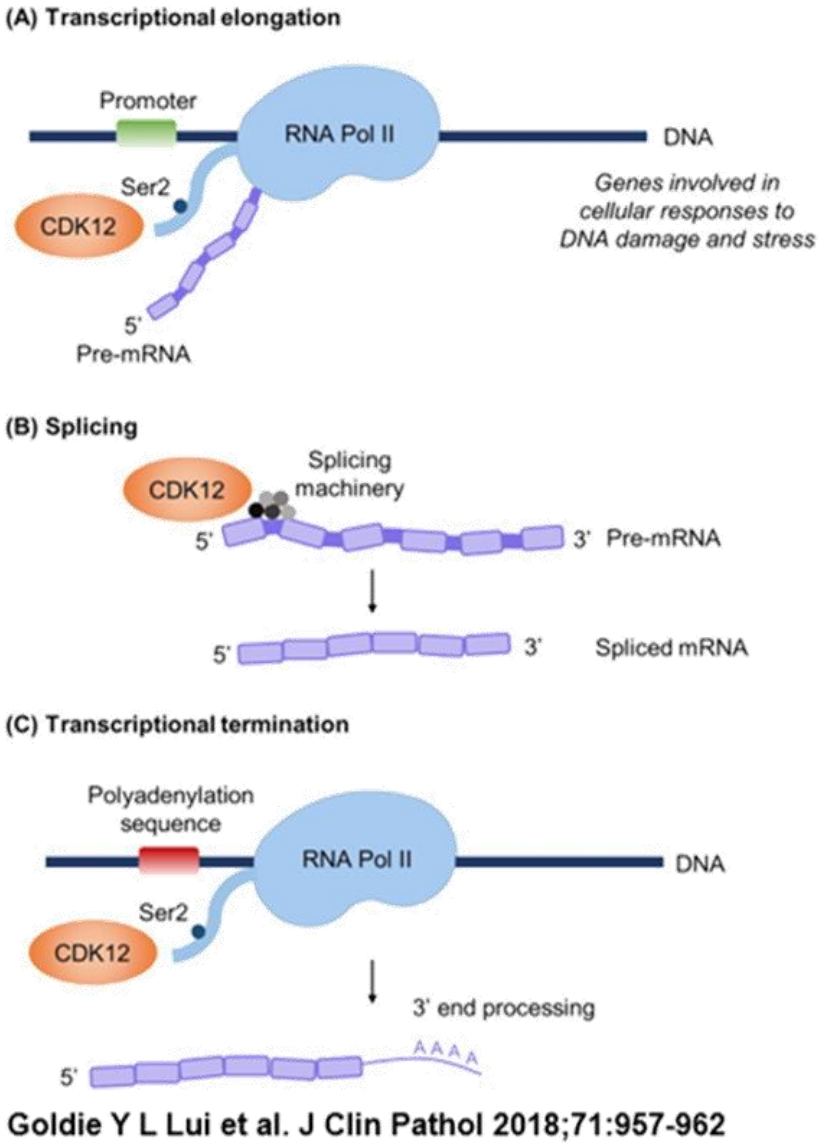
Disclosures

- Shareholder in Carrick Therapeutics & AstraZeneca
- Employee of Carrick Therapeutics

CDK12 is a transcriptional regulator

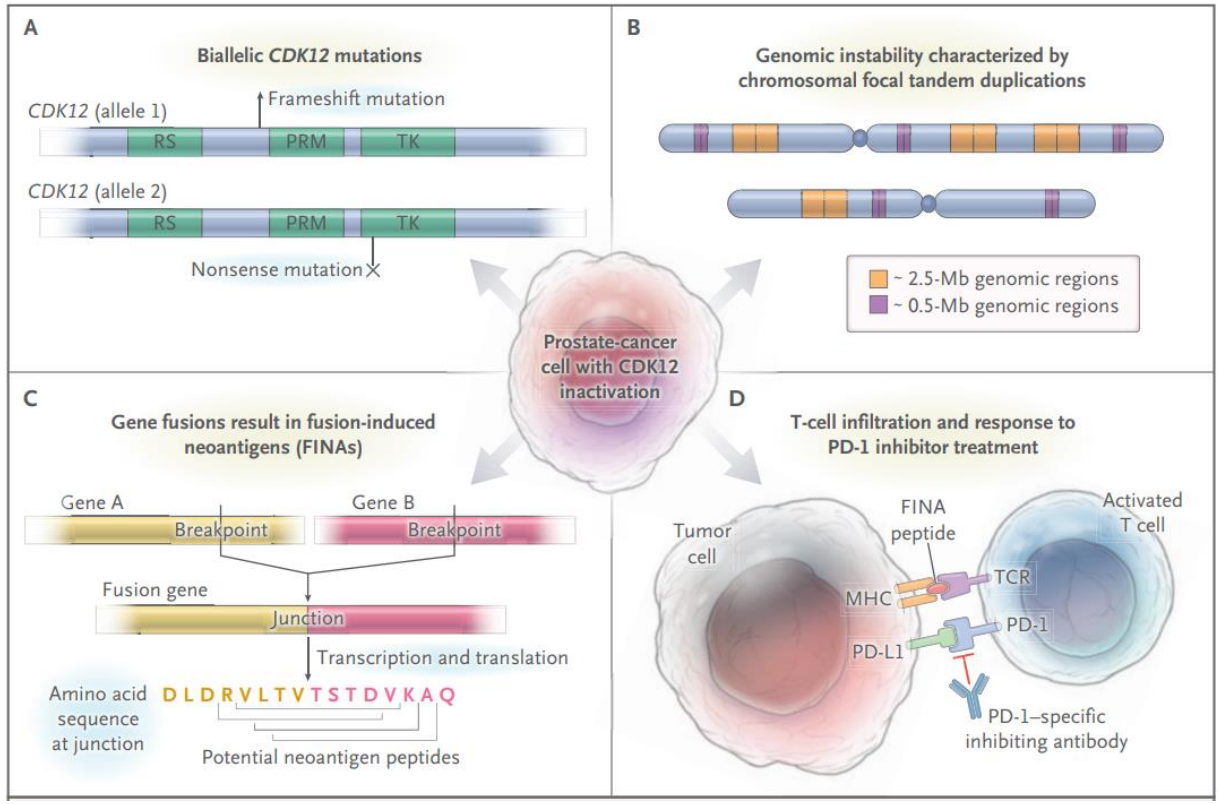


Goldie Y L Lui et al. J Clin Pathol 2018;71:957-962

- CDK12 is a member of the cyclin dependent kinase (CDK) family, but like CDK7 and CDK9 exerts most of its biological effect through regulated transcription
- Effect mediated in part through phosphorylation of Ser2 of c-terminal domain of RNA Pol II
- CDK12 is a transcriptional regulator of DNA-repair genes: **Inhibition of CDK12 induces BRCAness in cells**

Clinical opportunities for CDK12 inhibition

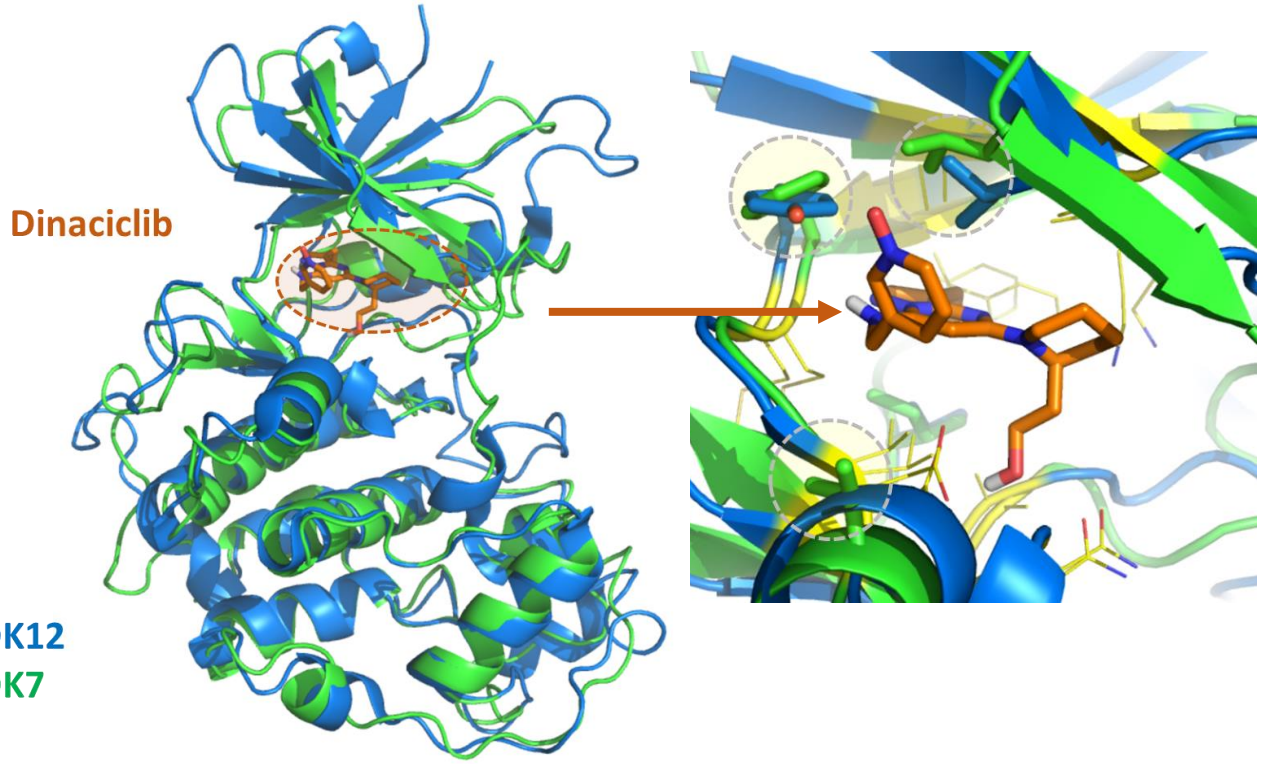
- CDK12i in combination with PARP inhibitors. Expand utility of PARP inhibitors though induction of “BRCAness”
- CDK12i as a treatment in PARP resistant TNBC and ovarian cancer
- CDK12i in combination with immuno-oncology therapy
- CDK12i in CDK12 amplified HER2+ breast cancer
- CDK12i as monotherapy in Ewing’s sarcoma



CDK12 and Programmed Death 1 (PD-1) Inhibition – a Conceptual Model
Antonarakis ES, N Engl J Med 2018; 379: 1087-1089

Identification of selective CDK12 inhibitors

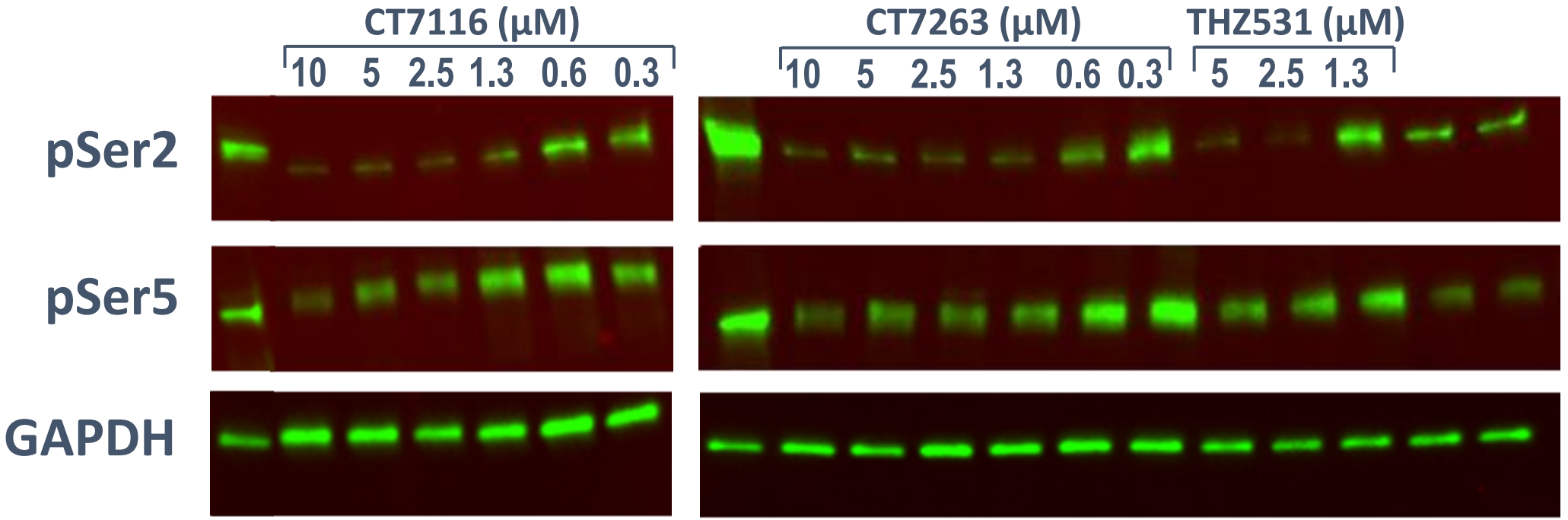
- CT7001 is an orally bio-available, ATP-competitive CDK7 inhibitor currently being investigated in clinical studies for multiple indications
- Structural modelling suggests modifications to CT7001 could yield a CDK12 selective compound
- CT7116 is CDK12 selective analogue of CT7001
  - CDK12 IC_{50} = 50 nM: 60x more potent vs. CT7001
  - CDK7: 13x less potent vs. CT7001
- Kinative cell lysate profiling of CT7116 shows that CDK12, 13, 7 and 10 are the only significantly inhibited CDKs



Kinase	CDK10	CDK12	CDK7	CDK13	CDK11, CDK8	CDK9	CDK5	CDK2	CDK2	CDK4	CDK6	CDK9	CDK5
CT7116 1µM (% inhibition)	96.5	86.9	79.4	78.2	24.6	23.1	6.1	4.1	3.9	-5.3	-22.0	-26.6	-37.2

Inhibition Ser2 phosphorylation of RNA PolII

- CT7116 and a second analogue CT7263 cause selective inhibition of Ser2 phosphorylation of RNA PolII
- Response is comparable to that seen with THZ-1, a reference covalent CDK12/13 inhibitor (*Zhang T, et al, Nat Chem Biol 2016; 12(10): 876-884*)

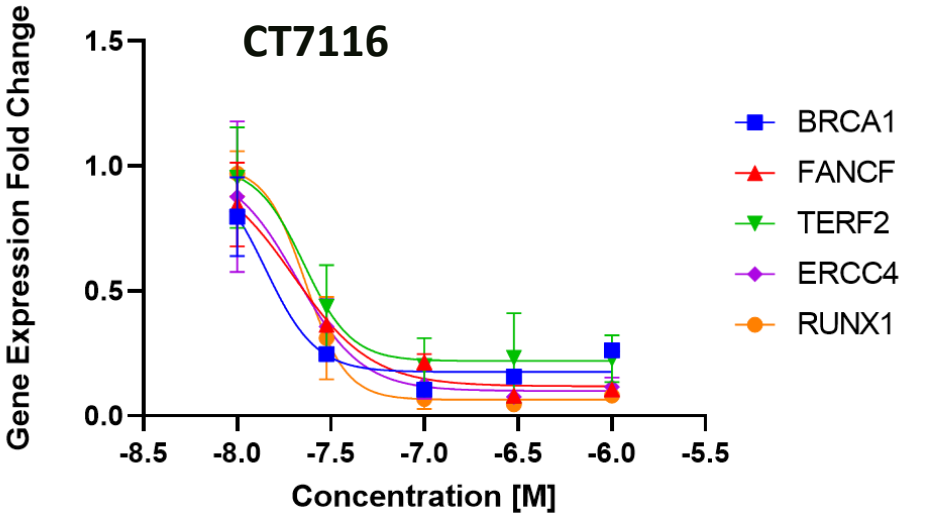


OV-90 cell line; 4hr incubation with compounds

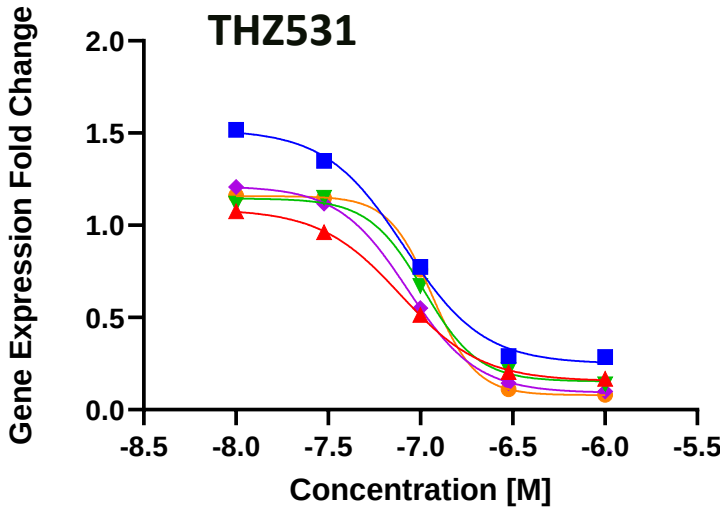
CDK12 inhibition: Effect on DNA repair gene expression

- CT7116 causes acute down regulation of DNA damage repair genes that are known to be regulated by CDK12 loss of function (e.g. BRCA1, FANCF, TERF2) which is distinct from CDK7 inhibition by CT7001

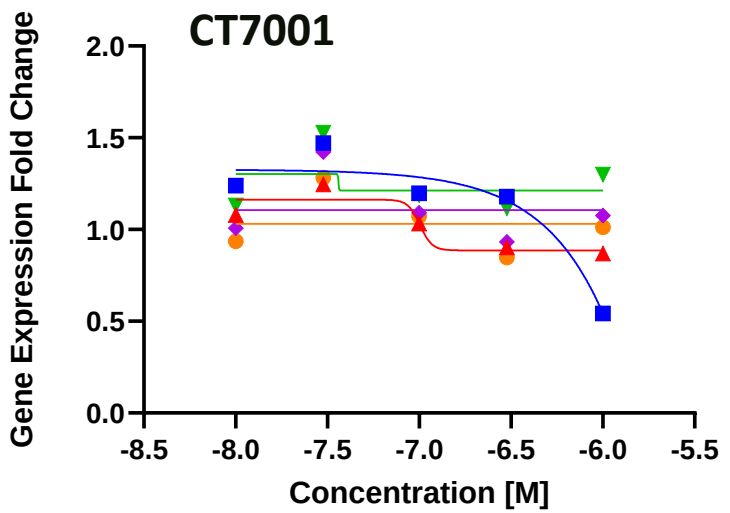
OV-90 cell line; 6hr incubation with compounds



BRCA1 IC_{50} = 14 nM
FANCF IC_{50} = 20 nM
TERF2 IC_{50} = 23 nM
ERCC4 IC_{50} = 21 nM
RUNX1 IC_{50} = 24 nM



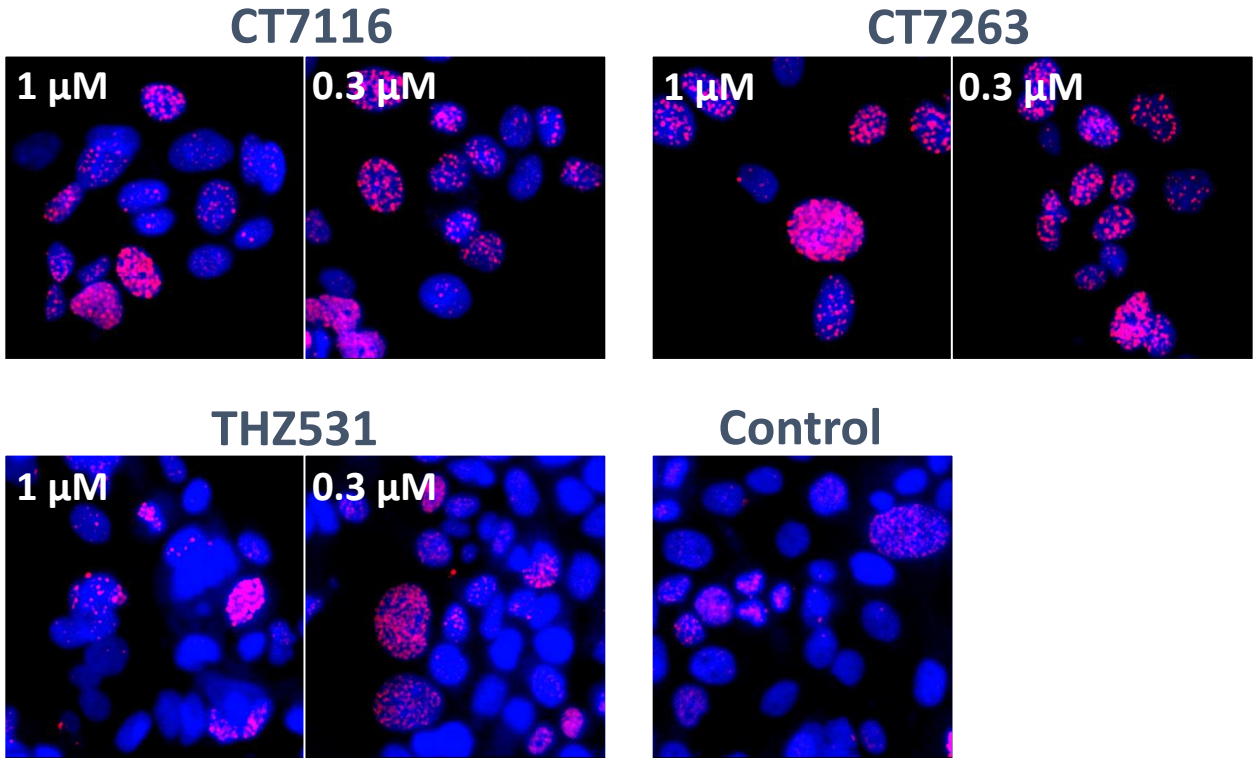
BRCA1 IC_{50} = 82 nM
FANCF IC_{50} = 78 nM
TERF2 IC_{50} = 104 nM
ERCC4 IC_{50} = 85 nM
RUNX1 IC_{50} = 116 nM

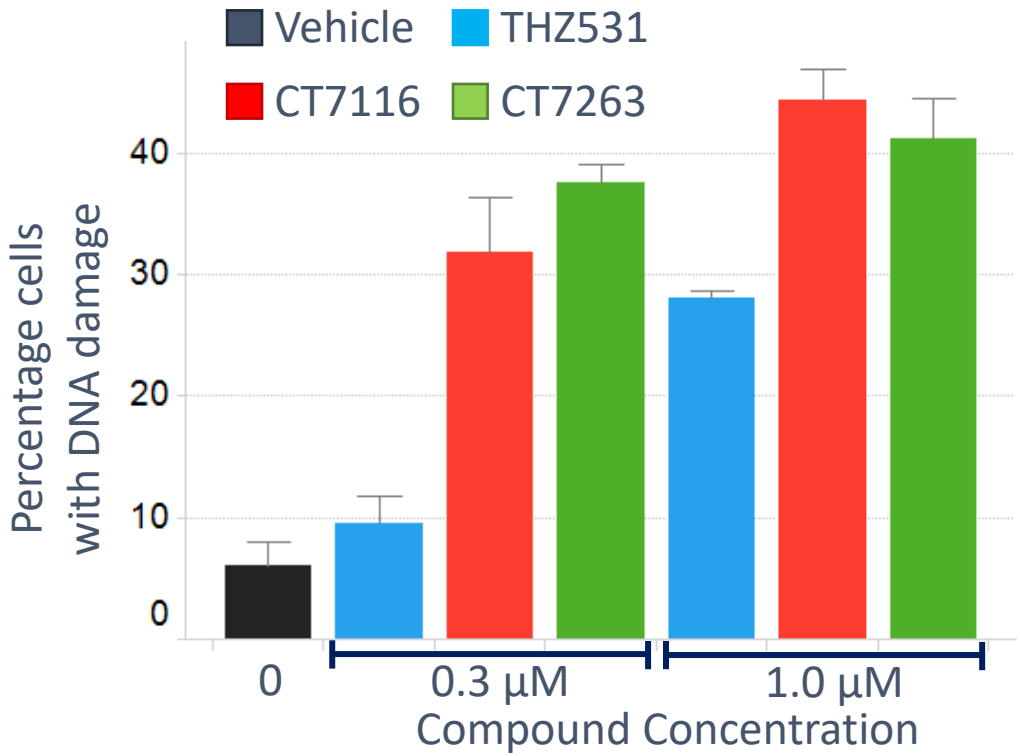


No IC_{50} determined for all genes

Treatment with CT7116 & CT763 leads to cellular DNA damage

- Loss of DNA repair genes leads to accumulation of DNA strand breaks which can be visualised by staining for the histone marker γ-H2AX
- CT7116 and CT7236 show induction of DNA damage in OV90, BRCAwt ovarian cancer cells

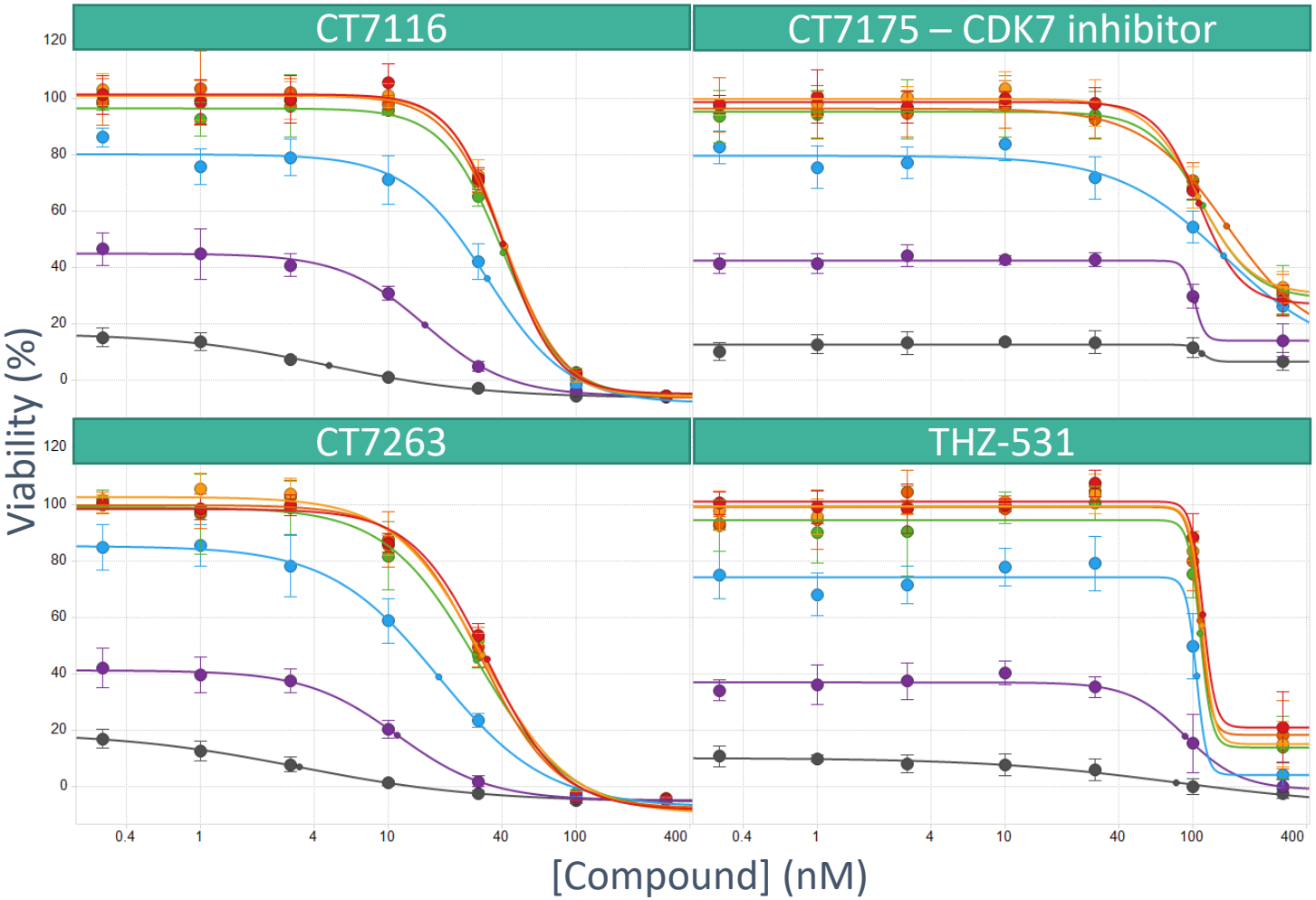




OV-90 cell line; 24hr incubation with compounds

Synergy with PARP inhibition observed with CDK12 inhibitors

OV90 – Ovarian BRCAwt cell line; 10 day exposure

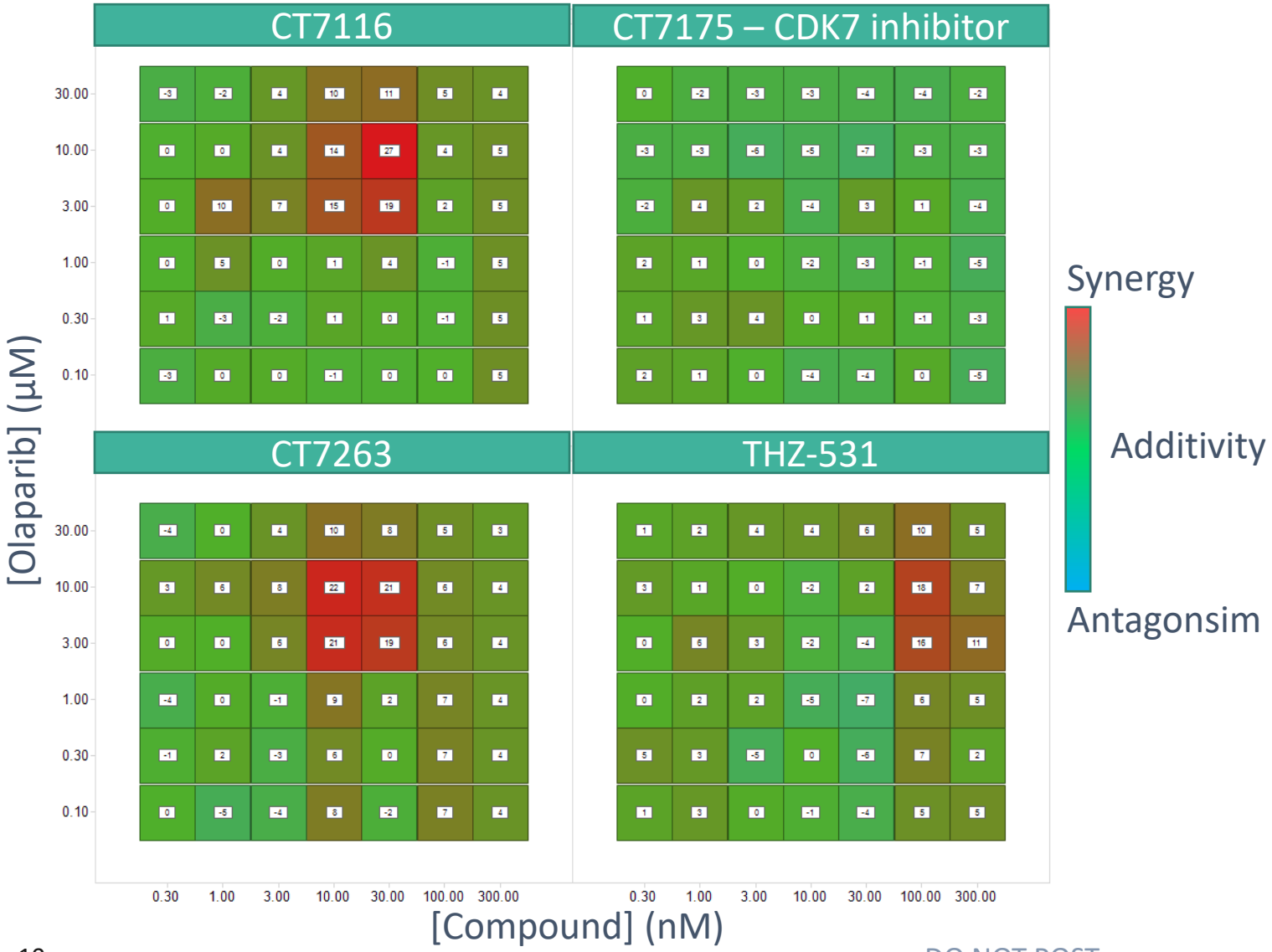


- Viability of cells measured in combination with olaparib
- Shifts in IC_{50} observed with CT7116, CT7263 and THZ531; no shift in IC_{50} with CT7175 a CDK7 selective inhibitor

[Olaparib]	IC_{50} (nM)			
	CT7116	CT7263	THZ-531	CT7175
0 µM	**41**	**33**	112	107
10 µM	**15**	**11**	91	102

Synergy with PARP inhibition observed with CDK12 inhibitors

OV90 – Ovarian BRCAwt cell line; 10 day exposure



- Excess inhibition in dose matrix (using Bliss independence method) shows strong synergy between CT7116 and CT7263 and olaparib
- Additivity observed with CDK7 inhibitor (CT7175)
- **Results are consistent with induction of BRCAness phenotype (homologous repair deficiency) by CDK12 inhibition leading to synergy with the PARP inhibitor**

Summary

- Structural considerations has enabled rational evolution of a clinical ATP-competitive CDK7 inhibitor to a potent CDK12 inhibitor with potential for oral bioavailability
- CDK12 inhibition leads to inhibition of genes involved in homologous DNA repair inducing a BRCAness phenotype
- BRCAness can lead to synergy with PARP inhibitors in a BRCAwt setting, suggesting the opportunity to expand the clinical utility of PARP inhibition where they are currently approved e.g. ovarian, TNBC
- Further work will investigate if BRCAness induced by CDK12 inhibition can lead to the appearance of neo-antigens and increased activity with immuno-oncology agents

Acknowledgements

Sygnature Discovery

- Luke Flatt
- Stuart Thompson
- Michael Cripps
- Robert Workman
- Damien Crepin
- Brett Stevenson
- Mihiro Sunose
- Kam Chohan

Carrick Therapeutics

- Ash Bahl
- Glen Clack
- Stuart McIntosh
- Anthony Brown