SRA737, a novel Chk1 inhibitor, shows efficacy in *CCNE1*-amplified and MYCN-overexpressing high-grade serous ovarian cancer patient-derived xenograft models

G.Y. Ho[1,2,3], H. Barker[1], C. Vandenberg[1], O. Kondrashova[1], E. Kyran[1], E. Lieschke[1], E. Mathieson[1], Silvia Stoev[1], Kathy Barber[1], O. McNally[2], A. Hamilton[2,3], V. Heong[1], R. Hansen[4], S. Milutinovic[4], B. Strouse[4], M. Hedrick[4], C. Hassig[4], D. Bowtell[3], MJ. Wakefield[1], C. Scott[1,3].

[1]Walter and Eliza Hall Institute for Medical Research, Stem Cells and Cancer, Melbourne, Australia; [2]Royal Women's' Hospital, Oncology, Melbourne, Australia;[3]Peter MacCallum Cancer Centre, Oncology, Melbourne, Australia; [4]Sierra Oncology, Vancouver, Canada.

Introduction

- Oncogene-driven high-grade serous ovarian cancers (HGSOC) with CCNE1 or MYCN pathway activation exhibit defective cell cycle checkpoint control and/or replicative stress and associated with treatment resistance [1,2,3].
- The DNA damage response effector kinase, Chk1, modulates the cellular response to replicative stress and has been shown to be upregulated in these subtypes of HGSOC *(Figure 1)*.
- SRA737 is a novel, potent, highly selective and bioavailable ATP-competitive CHK1 inhibitor[1].
- We explored the efficacy of single agent SRA737 at three different doses and compared it with olaparib, the PARP inhibitor recently approved for HGSOC, in our pre-clinical HGSOC patient derived xenograft models[4] *(Figure 2)*.

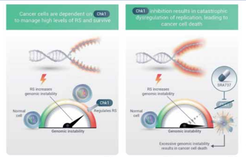


Figure 1: Interaction between Chk1, CCNE1 and replicative stress during proliferation. Inhibition of Chk1 can result in catastrophic genomic instability.

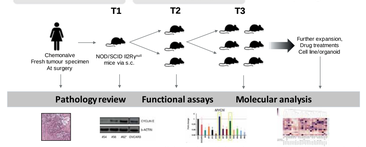
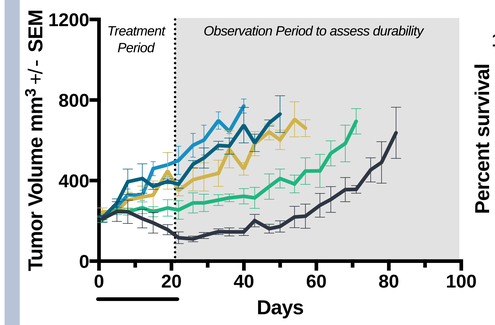
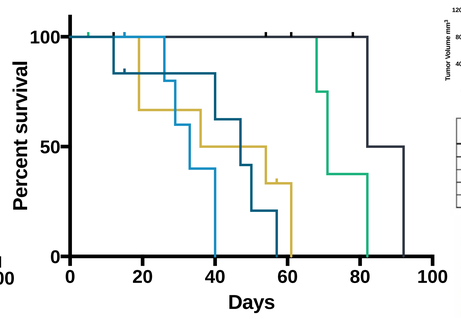
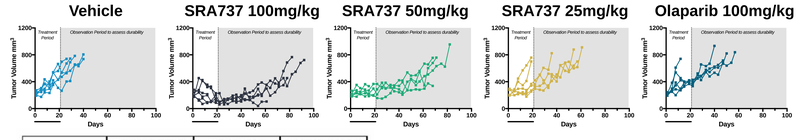


Figure 2: PDX tumours were validated by histology, molecular analysis and functional assays to resemble the original tumour samples, then expanded in NSG mice for *in vivo* drug response analysis.

In vivo efficacy of SRA737 in PDX 134029







Arm	Time to progressive disease (PD) (days)[a]	Median time to harvest (TTH) (days)[b]	p value (compared to vehicle)
Vehicle	7	33	
SRA737 100mg/kg	52	87	0.0027**
SRA737 50mg/kg	24	71	0.0065**
SRA737 25mg/kg	7	45	0.2685 (NS)
Olaparib	7	47	0.0875 (NS)

SRA737 100mg/kg (n = 7)
SRA737 50mg/kg (n = 6)
SRA737 25mg/kg (n = 6)
Olaparib 100mg/kg (n = 6)
Vehicle (n = 6)
** ** **
Log-rank test

- PDX 134029 demonstrated a dose dependent response to SRA737 therapy.
- Treatment with SRA737 at 100mg/kg given daily for 21 days resulted in tumour regression and improvement of median time to harvest from 33 days to 87 days (p = 0.0027).

[a] Time to progressive disease (PD) is defined as the time (in days) from beginning of treatment to an increase in average tumour volume of >20% from nadir or 200mm³ if nadir is less than 200mm³.
[b] Time to harvest (TTH) is defined as the time (in days) from beginning of treatment to day of harvest at 700mm³.

Molecular characteristics of PDX models

HGSOC PDX	DNA repair mutation	Molecular characteristics
134029	Not detected	• *Lin28b* (C5 HGSOC subtype[2]) – high • *BCL2* – high Mutations: • TP53 mutation • Amplification of *CCND2*, *CCNE1*, *EPHB1*, *FGF23*, *FGF6*, *KRAS* and *MCL1*
134111	Not detected	• *ABCA5* and *TUBA1C* – high • *MYC* and *CCNE1* – mid high (RNA seq data) Mutations: • TP53 mutation • *CCNE1* amplification (58x)

Table 1: Molecular characteristics of PDX 134029[1] and 134111.

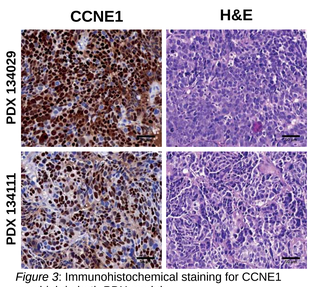


Figure 3: Immunohistochemical staining for CCNE1 was high in both PDX models.

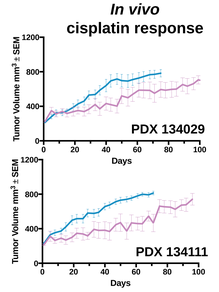
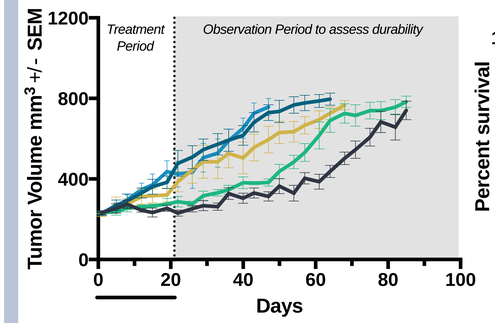


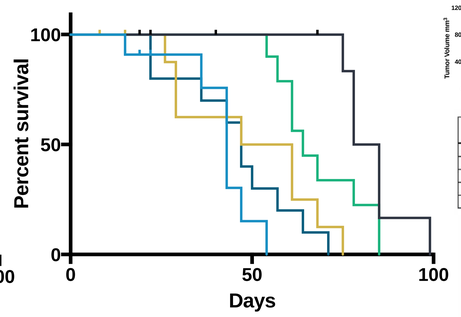
Figure 4: PDX 134029 and 134111 were refractory to platinum based chemotherapy, cisplatin (as defined in Topp et al [4])

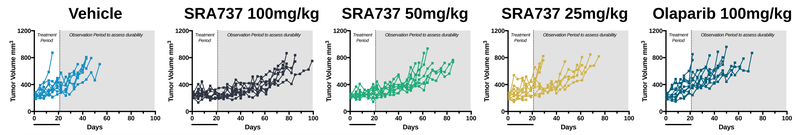
Vehicle
Cisplatin 4mg/kg d1, d8 and d18

- Both PDX models have *CCNE1* amplification with over-expression of *CCNE1* confirmed by IHC and refractory to standard HGSOC treatment, cisplatin.

In vivo efficacy of SRA737 in PDX 134111







Arm	Time to progressive disease (PD) (days)	Median time to harvest (TTH) (days)	p value (compared to vehicle)
Vehicle	7	43	
SRA737 100mg/kg	35	81.5	0.0001***
SRA737 50mg/kg	28	66	0.0001***
SRA737 25mg/kg	7	47	0.1565 (NS)
Olaparib	7	47	0.2685 (NS)

SRA737 100mg/kg (n = 11)
SRA737 50mg/kg (n = 10)
SRA737 25mg/kg (n = 10)
Olaparib 100mg/kg (n = 10)
Vehicle (n = 10)
*** * ***
Log-rank test

- PDX 134111 also demonstrated a dose dependent response to SRA737 therapy.
- Treatment with SRA737 at 100mg/kg given daily for 21 days resulted in significant stablisation, which led to the improvement of median TTH from 43 days to 81.5 days (p = 0.0001).

Effect of SRA737 on tumour biomarkers

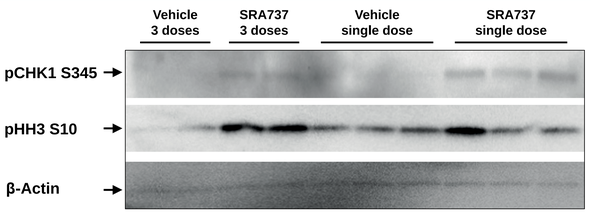
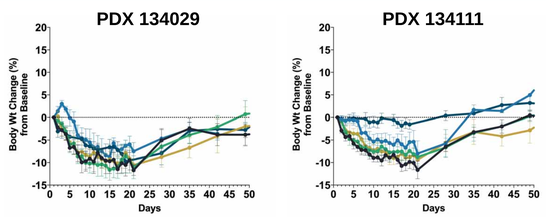


***Figure 5:* Western blot for phosphorylated Chk1 and histone H3 on short term harvest demonstrating on target effect of SRA737 in PDX 134111**

- Phosphorylated Chk1 Ser345 was detected in tumour samples harvested 12 hours following one or 3 doses (once daily) of SRA737 at 100mg/kg confirming on target effects and elevated replication stress induced by SRA737.
- A high level of phosphorylated histone H3 Ser10 following Chk1 inhibition, reflecting potential DNA-damage checkpoint abrogation.

SRA737 tolerability profile in NSG mice



- SRA737 at 100mg/kg, 50mg/kg, 25mg/kg given via oral gavage daily for 21 days was tolerable in tumour bearing NSG mice.
- Weight loss was within the acceptable range, being less than 15% of the starting weight, and was comparable to the weight loss experienced by mice receiving daily oral gavage of vehicle.
- SRA737 was as tolerable as olaparib in PDX 134029, which was given as intra-peritoneal injection.
- Recovery of weight loss was demonstrated post completion of therapy.

Conclusion

- Chk1 inhibition by SRA737 shows promising efficacy in *CCNE1*-amplified and MYCN-overexpressing preclinical PDX models of HGSOC.
- These *in vivo* data demonstrated tumour regression in one and significant tumour stasis in the other, both cisplatin refractory, PDXs following a relatively short duration of SRA737 treatment (21 days).
- These *in vivo* data support the ongoing monotherapy clinical trial of SRA737, which includes the prospective enrollment of patients with these HGSOC subtypes (A Phase 1/2 Trial of SRA737 in Subjects With Advanced Cancer; ClinicalTrials.gov Identifier: NCT02797964).
- Furthermore, these preclinical data suggest that the exploration of potential combination regimens with other compounds, such as low dose gemcitabine and PARPi, is warranted for HGSOC.

Reference

1. Walton M, et al. The clinical development candidate CCT245737 is an orally active CHK1 inhibitor with preclinical activity in RAS mutant NSCLC and Eμ-MYC driven B-cell lymphoma. Oncotarget (7), 2329-2342 (2015)
2. Etemadmoghadam et al. Integrated Genome-wide DNA Copy Number and Expression Analysis Identifies Distinct Mechanisms of Primary Chemo-resistance in Ovarian Carcinomas. Clin Can Res 15 (4), 1417-1427 (2009)
3. Helland, Å. et al. Deregulation of MYCN, LIN28B and LET7 in a Molecular Subtype of Aggressive High-Grade Serous Ovarian Cancers. PLoS ONE 6, e18064–20 (2011).
4. Topp, M et al. Molecular correlates of platinum response in human high-grade serous ovarian cancer patient-derived xenografts. Mol Onc 8, 656-668 (2014).