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# THE DNA HYPOMETHYLATING AGENT SGI110 REVERSES THE PLATINUM RESISTANCE OF OVARIAN CANCER MODELS

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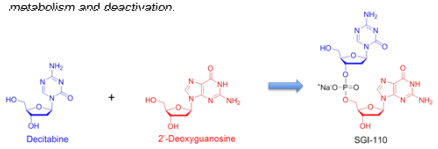
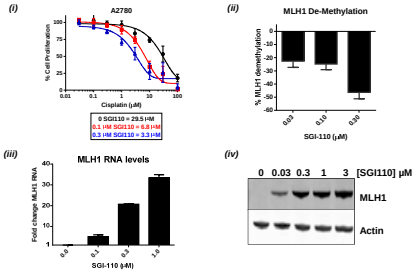
## INTRODUCTION

- Patients with advanced stage ovarian cancer (OC) have a 5-year survival rate of less than 25%. The most common treatment strategy comprises debulking surgery followed by platinum-based chemotherapy. Patients commonly respond to first-line chemotherapy, but >70% relapse, developing platinum-resistance. There is evidence that the acquisition of platinum resistance is associated with the epigenetic silencing of specific genes by DNA methylation.
- SGI110 is a novel second generation DNA hypomethylating agent, which is currently in a Phase II clinical trial in combination with carboplatin, in platinum-resistant recurrent ovarian cancer patients (NCT01696032).
- SGI110 is a dinucleotide of decitabine and deoxyguanosine, which is resistant to modification by cytidine deaminase: a common pathway of decitabine *metabolism and deactivation*.




- Here we demonstrate that SGI110 reverses the cisplatin-resistance of the A2780 OC model, by abrogating the epigenetic silencing of MLH1. We demonstrate SGI110 activity in a panel of OC cell lines. We suggest that epigenetic silencing of ZIC1 is a mechanism of cisplatin resistance in the OAW28 and Ovcar8 cells and demonstrate that it is reversed by SGI110.

## 1. SGI110 REVERSES THE CISPLATIN-RESISTANCE OF A2780 CELLS *IN VITRO*

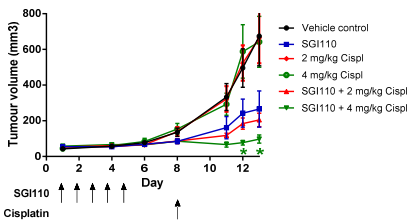



*Cells were treated for 3 consecutive days with SGI110. i. Alamar Blue Proliferation Assay. Cells exposed to cisplatin for 4 days +/- 3-day pre-treatment with SGI110. ii. MLH1 promoter methylation determined by pyrosequencing. iii. RT-PCR analysis of MLH1. iv. Western blot of MLH1 expression.*

SGI110 sensitised A2780 cells to cisplatin *in vitro*. This sensitisation was associated with SGI110-induced MLH1 promoter demethylation and MLH1 re-expression.

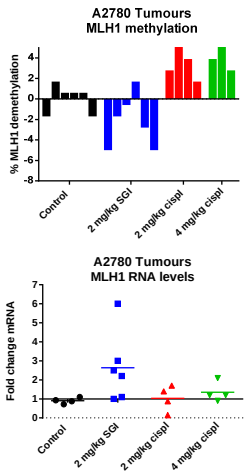
## 2. SGI110 REVERSES THE CISPLATIN-RESISTANCE OF A2780 CELLS *IN VIVO*

### SGI110 sensitises A2780 xenografts to cisplatin




*SCID Beige mice bearing subcutaneous A2780 tumours. 2 mg/kg SGI110 was administered subcutaneously, once daily on days 1-5. A single dose of cisplatin was administered intraperitoneally on day 8, at 2 mg/kg or 4 mg/kg. Both agents were dosed either as single agent or in combination. Data represent mean ± SD (n=8). * p<0.05 vs single agent SGI110.*

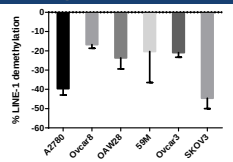
### SGI110 confers MLH1 demethylation and re-expression in A2780 xenografts




*SCID Beige mice bearing subcutaneous A2780 tumours were dosed on days 1-5 with single agent SGI110 (s.c) or on day 1 with single agent cisplatin (i.p). Tumours were excised on day 8. Tumour MLH1 methylation and RNA expression levels were determined by pyrosequencing and RT-PCR, respectively.*

## 3. SGI110 SENSITISES OVARIAN CANCER CELL LINES WITH DISTINCT MECHANISMS OF CISPLATIN RESISTANCE

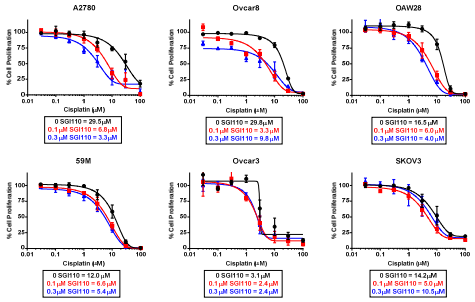
### SGI110 confers LINE-1 demethylation in an OC cell line panel




*Cells were treated for 3 consecutive days with 0.1 μM SGI110. Methylation of global LINE-1 elements were determined by pyrosequencing*

We confirm SGI110 activity across the panel of OC cell lines, as indicated by demethylation of global LINE-1 elements.

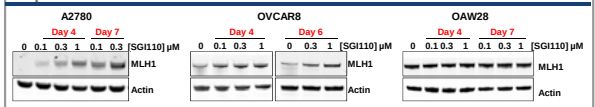
### SGI110 sensitises A2780, OAW28 and Ovcar8 cell lines to cisplatin




*Alamar Blue Proliferation Assay. Cells were exposed to cisplatin for 4 days +/- 3-day pre-treatment with SGI110.*

SGI110 sensitised A2780, Ovcar8 and OAW28 cells to cisplatin *in vitro*. Despite SGI110 activity in 59M, Ovcar3 and SKOV3 cells (LINE-1 demethylation, shown above), SGI110 did not sensitise these cell lines to cisplatin. This suggests that 59M, Ovcar3 and SKOV3 cells do not have an epigenetic mechanism of cisplatin resistance.

### Epigenetic silencing of MLH1 silencing is not responsible for the cisplatin resistance of Ovcar8 and OAW28 cells




*Cells were treated for 3 consecutive days with SGI110 and harvested on day 4, 6 or 7 as indicated.*

MLH1 is not epigenetically silenced in Ovcar8 or OAW28 cells. Therefore, the cisplatin resistance observed in these cell lines is due to distinct epigenetic mechanisms to that of A2780 cells.

### SGI110 induces ZIC1 and DOK2 expression in OAW28 and Ovcar8 cells

We analysed the effect of SGI110 on the RNA levels of potential OC cisplatin resistance biomarkers previously reported by Lum *et al*., 2013.

**Table 1**

| | OAW28 | Ovcar8 |
|---|---|---|
| DOK2 | 41-fold | 65-fold |
| ZIC1 | 13-fold | 11-fold |
| UNG | 0 | 0 |
| OXSR1 | 0 | 0 |
| CENPB | 0 | 0 |
| GTF2B | 0 | 0 |
| ESSRA | 0 | 0 |
| SGPL1 | 0 | 0 |
| GABPA | 0 | 0 |
| TWIST1 | 3-fold | 0 |
| MDH1 | 0 | 0 |
| NR2E1 | 7-fold | 0 |
| NR3C2 | 0 | 0 |
| SOX9 | 3-fold | 0 |
| TOB1 | 0 | 2-fold |
| HIRIP3 | 0 | 0 |
| FZD1 | 0 | 0 |
| APRT | 0 | 0 |
| GSK3B | 0 | 0 |
| MLH1 | 0 | 0 |

*RT-PCR analysis of candidate biomarkers following 3-day treatment with SGI110. Table 1 and 2 show fold-increase in RNA levels in response to 1 μM SGI110 (0 = no increase).*

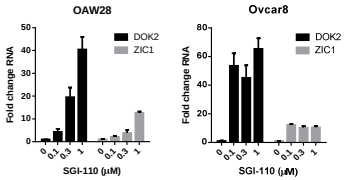


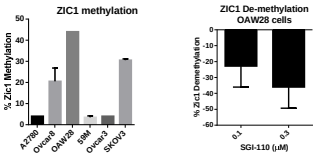

**Table 2 Effect of SGI110 on DOK2 and ZIC1 RNA levels across cell line panel**

| | Ovcar8 | OAW28 | A2780 | SKOV3 | 59M | Ovcar3 |
|---|---|---|---|---|---|---|
| DOK2 | 65-fold | 41-fold | 17-fold | 110-fold | 90-fold | 30-fold |
| ZIC1 | 11-fold | 13-fold | 2-fold | 0 | 0 | 0 |

SGI110 conferred re-expression of DOK2 and ZIC1 in both OAW28 and Ovcar8 cells (Table 1). DOK2 re-expression did not correlate with SGI110-induced sensitisation to cisplatin across the OC cell panel (Table 2). ZIC1 was exclusively re-expressed in the cell lines that were sensitised to cisplatin by SGI110. ZIC1 has previously been identified as a prognostic biomarker in OC (Huang *et al*., 2013)

### SGI110 confers ZIC1 demethylation

*Methylation levels of ZIC1 in untreated cells and SGI110-treated OAW28 cells, as determined by pyrosequencing.*




Ovcar8 and OAW28 cells have relatively high levels of ZIC1 methylation. SGI110 confers ZIC1 demethylation.

## SUMMARY AND CONCLUSIONS

- SGI110 reverses the known cisplatin resistance mechanism (epigenetic silencing of MLH1) of A2780 cells by conferring MLH1 promoter demethylation and re-expression.
- SGI110 sensitises OAW28 and Ovcar8 cells to cisplatin. These models have distinct mechanisms of cisplatin resistance to the A2780 cells.
- We identify epigenetic silencing of ZIC1 as a potential cisplatin resistance mechanism in Ovcar8 and OAW28 cells and demonstrate that SGI110 restores ZIC1 expression.
- We demonstrate that SGI110 reverses the cisplatin resistance of OC models by restoring expression of epigenetically silenced genes. This supports our ongoing Phase II trial evaluating SGI110 in combination with carboplatin, in platinum-resistant recurrent ovarian cancer patients (NCT01696032).

References
1.Lum et al., 2013. Gynecologic Oncology. 130.369-376
2.Huang et al., 2013. Epigenetics. 8;6.624-634

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