Abstract

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Chemosensitizing Effects of the Novel, Small Molecule DNA Methylation Inhibitor SGI-110 in Ovarian Cancer

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INTRODUCTION

1. The majority of women diagnosed with advanced-stage epithelial ovarian cancer (OC) experience tumor recurrence associated with the development of chemoresistance, and platinum-resistant OC is uniformly fatal.

2. Deoxycytosine methylation of CpG islands in promoter regions of tumor suppressor genes (TSGs) plays a prominent role in the development and progression of drug-resistant OC. Genes known to be silenced by methylation in OC include *MLH1, RASSF1, BRCA1, HOX* genes and others.

3. Based on preclinical studies generated by our group demonstrating that inhibition of DNA methylation reverses platinum resistance in EOC cells, we designed and recently completed a phase I/II trial using the DNA methylation inhibitor decitabine (5-aza-2'-deoxycytidine) in combination with carboplatin in patients with recurrent, platinum-resistant OC (Fang et al., *Cancer*, 2010; Matei, et al., *Cancer Research*, in press).

4. This trial demonstrated that repetitive low-dose decitabine is well tolerated when combined with carboplatin (Fang et al., *Cancer*, 2010) and has biological (i.e., DNA-hypomethylating) as well as clinical activity (Matei, et al., *Cancer Research*, in press). These results support the concept that therapies targeting epigenetic changes can be employed for clinical benefit in EOC.

5. SGI-110 (Astex Pharmaceuticals, Inc) is a DNA hypomethylating agent with demonstrated activity in restoring silenced TSG expression in cancer cells by reversal of DNA methylation.

6. As a decitabine-deoxyguanosine dinucleotide, SGI-110 has been shown to be less prone to deamination by cytidine deaminase and could have advantages over decitabine, such as better stability, less toxicity and a more convenient and less frequent SQ administration.

HYPOTHESIS AND OBJECTIVES

- Our group's long-term goal is to establish interventions targeting the epigenome as a new therapeutic strategy for ovarian cancer.
- We hypothesize that epigenetic modulators in combination with platinum will exert potent antitumor activity in preclinical models of treatment naïve and resistant, recurrent OC.
- We examined the ability of SGI-110 to resensitize cisplatin-resistant ovarian cancer cells by demethylating and derepression of drug-response genes and inhibit OC cell proliferation *in vitro* and *in vivo*.
- We conducted a 'tolerability' study to examine that SGI-110 is active in non-tumor bearing mice.
- We investigated the ability of SGI-110 to reverse aggressive ovarian cancer by targeting ovarian cancer stem cells and associated molecular pathways, including epithelial-mesenchymal transition (EMT) and transforming growth factor-beta (TGF-β).

METHODS

Figure 1. Treatment schedules.

Bi-weekly: SGI-110 and cisplatin were administrated on Day 1 and Day 4 for each cycle (7 days a cycle). Blood draws were taken on Day 1 (before treatment, baseline), Day 8, Day 15, Day 22 (first day of each cycle), Day 29 (end of treatment), and Day 46 (end of study).

QD 5: SGI-110 was administrated for 5 consecutive days from Day 1 to Day 5 and followed by cisplatin on Day 8. Blood draws were taken before Day 1 (baseline), Day 8 (before cisplatin), and Day 36 (end of study).

RESULTS

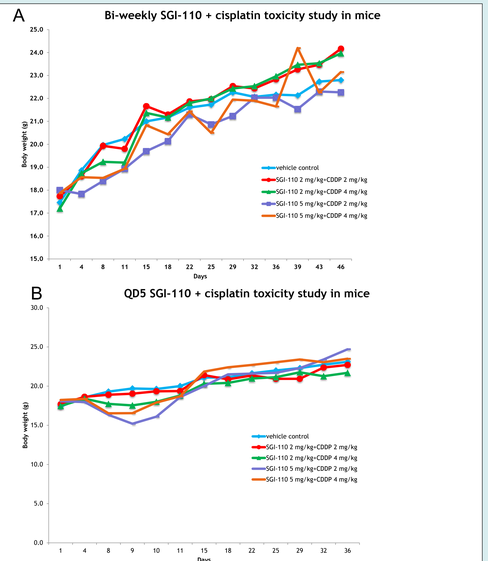

Figure 2. Body weight curves of bi-weekly or QD 5 schedule treated mice. Mice (3 per gourp) were injected with different doses of SGI-110 (2 mg/kg, 5 mg/kg, i.p.), cisplatin (2 mg/kg, 4 mg/kg, s.c.) or combination of the 2 drugs. A. combination treatment for bi-weekly schedule. B. combination treatment for QD 5 schedule. Single drug treatment did not affect the body weight gain, data not shown.

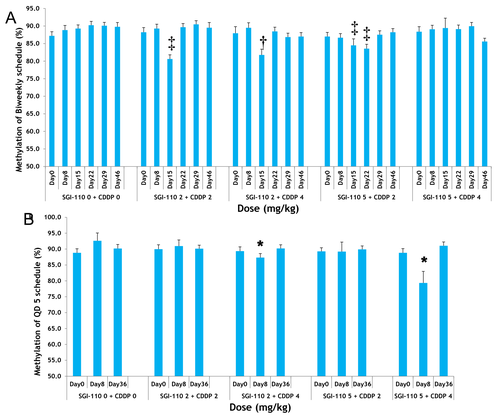

Figure 3. *LINE1* methylation changes. Blood samples were collected on different days (see Fig 1). PBMC DNA was extracted and subjected to bisulfite conversion and pyrosequencing for LINE1 methylation.
(*: $P<0.05$, †: $P<0.01$, ‡: $P<0.001$). Data for the combination treatment is shown.
A. bi-weekly, B. QD5.

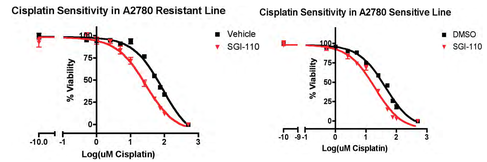

Figure 4. Resensitization of chemoresistant ovarian cancer cells by SGI -110. Ovarian cancer cell lines (A2780-sensitive, A2780-cisplatin-resistant) were treated with 5μM SGI-110, or vehicle (DMSO 1:2000) for 48 hours. SGI-110 was removed and the cells were treated with cisplatin (0-500μM) for 3 hours and then allowed to recover for 3 days. Drug-reduced cell viability was determined using standard MTT assay.

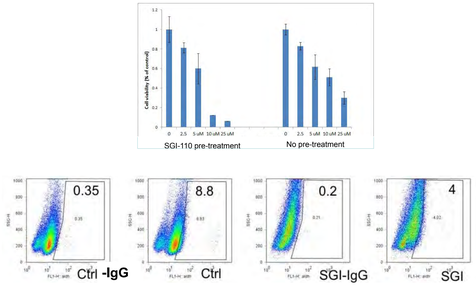

Figure 5. SGI-110 sensitizes A2780 cells to platinum and reduces the number of ALDH+ cell sub-population. A. BRDU assay measured cell proliferation 48 hours after treatment with cisplatin (2.5μM to 25μM) in A2780 cells pretreated with SGI-110 (5μM) or control for 1 week. B. Population of ALDH+ cells measured by flow cytometry in A2780 cells pretreated with SGI-110 (5μM) or control for 1 week.

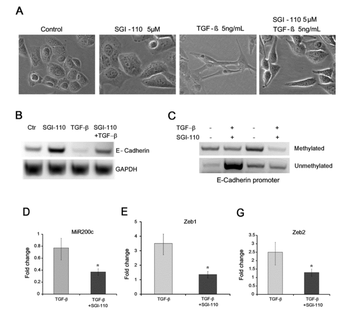

Figure 6. SGI-110 prevents TGF-β induced EMT in SKOV3 cells.
A) Morphological changes induced by TGF-**β** in SKOV3 cells. B) E-cadherin expression level (RT-PCR, top panel) and promoter methylation (MS-PCR, lower panel in SKOV3 cells treated with TGF-**β** (5ng/mL for 48 hours) in the presence of SGI-110 (5μM) or control. C) Expression level for miR200c, Zeb1, 2 (Q-RT-PCR) in SKOV3 cells treated with TGF-**β** in the presence of SGI-110 or control.

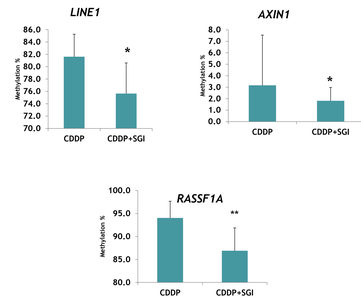

Figure 7. Pyrosequencing assessed methylation of *LINE1* and promoter methyaltion of specific genes (shown are *RASSF1A* and *AXIN1*) in A2780 orthotopic xenografts treated with cisplatin (CDDP 4mg/kg biw) for 3 weeks followed by SGI-110 (5 mg/kg/week) for 4 weeks (n=3 tumors per group).

CONCLUSIONS

1) SGI-110 is tolerable in combination with cisplatin in mice.

2) *LINE* 1 and gene specific (*AXIN 1, RASSF1*) demethylation is achieved in vivo in PBMCs and xenografts.

3)SGI-110 resensitizes ovarian cancer cells to platinum.

4) SGI-110 reduces the number of ALDH+ ovarian cancer cell population.

5) SGI-110 prevents TGF-β induced EMT by direct effects on E-cadherin expression and indirectly by affecting expression of EMT regulators Zeb1-2 and miR-200.

6) SGI-110 combined with platinum warrants further study in clinical and preclinical models of ovarian cancer.

ACKNOWLEDGEMENTS:

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