No. 036

SGI-110, a novel second generation DNA hypomethylating agent, enhances sorafenib activity and alters the methylation signature of HCC cell lines

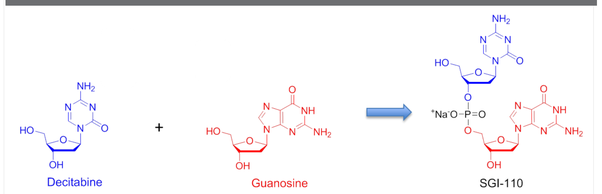
Simone Jueliger[1], John Lyons[1], Mohammad Azab[2] and Pietro Taverna[2]

Astex Pharmaceuticals, [1]436 Cambridge Science Park, Milton Road, Cambridge, CB4 0QA, UK and [2]4140 Dublin Blvd., Suite 200, Dublin, CA, 94568 USA.

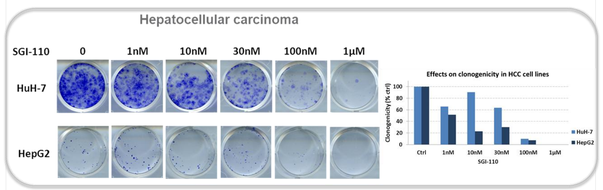
INTRODUCTION

SGI-110, is a novel second generation DNA methylation inhibitor currently in Phase I/II clinical studies for the treatment of haematological malignancies. SGI-110 is a dinucleotide of decitabine (DAC) and deoxyguanosine, designed to be more stable than decitabine to deamination by cytidine deaminase, thus offering a promising alternative to current approved hypomethylating agents. Chemosensitizing effects of SGI-110 have been seen in ovarian cancer cells to platinum but the role of epigenetic therapy in other solid tumors is still largely unknown. Since epigenetic alterations have been shown to play an important role in liver carcinogenesis, we investigated preclinically, the ability of SGI-110 to combine effectively with the kinase inhibitor sorafenib and to demethylate and induce re-expression of tumor suppressor genes in hepatocellular carcinoma (HCC) cell lines. This opens new opportunities for combination studies in solid tumors shown here in *in vitro* and *in vivo* HCC models.

SGI-110 was developed as a dinucleotide of decitabine and deoxyguanosine as a biologically more stable compound than decitabine



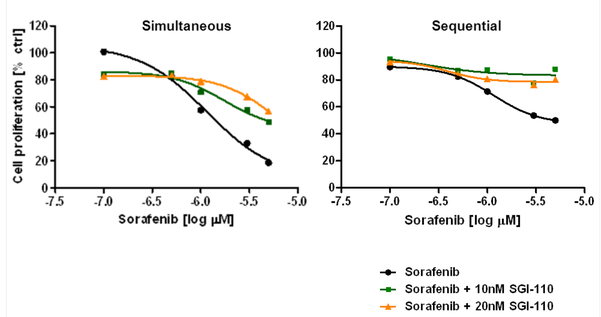
Potent reduction of clonogenic survival induced by SGI-110 in hepatocellular carcinoma cells



- SGI-110 treatment resulted in a concentration-dependent inhibition in cell proliferation in both HCC cell lines HuH-7 and HepG2 which are p53 mutant (exon 6) and wild type, respectively.
- A potent reduction in colony formation was demonstrated at low nanomolar concentrations of SGI-110 (Crystal Violet stained colonies were quantified using GelCount Counting System Oxford Optronix Ltd.).

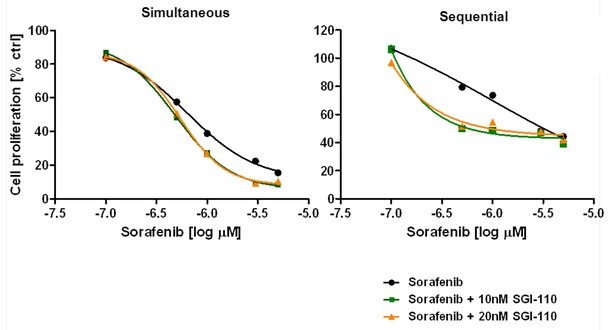
Optimization of SGI-110 treatment schedules in combination with sorafenib *in vitro*

SGI-110 in combination with sorafenib in HuH-7 cells: using simultaneous treatment or SGI-110 primed cells before sorafenib treatment



- No additive or synergistic effects were observed with either treatment schedule on HuH-7 cells.

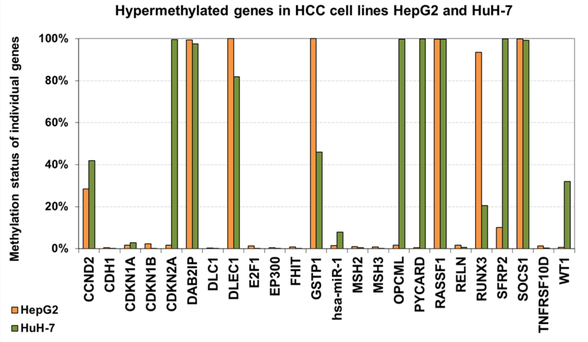
SGI-110 in combination with sorafenib in HepG2 cells: using simultaneous treatment or SGI-110 primed cells before sorafenib treatment



- Epigenetic priming with low-dose SGI-110 prior to sorafenib indicates a possible window of synergistic inhibition of cell proliferation.
- HepG2 cells treated simultaneously with SGI-110 and sorafenib showed an at least additive effect which was more evident when cells were primed with SGI-110 for 6 days.
- We next asked whether this priming effect might be due to changes in promoter methylation.

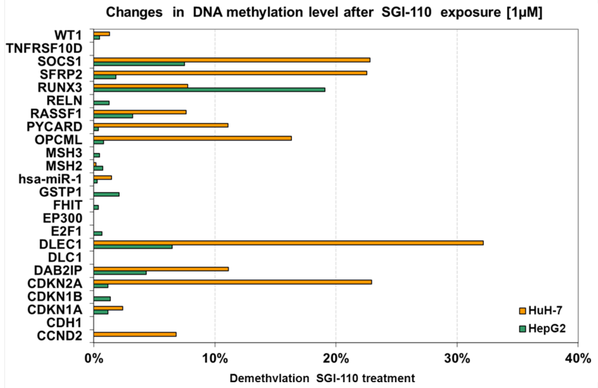
Gene-specific basal DNA hypermethylation levels and changes after SGI-110 exposure in HCC cells

Human Liver Cancer DNA Methylation Array to detect basal DNA methylation level in both HCC cell lines: HuH-7 (p53 mut(ex6) and HepG2 (p53 wt)



- A Basal DNA methylation signature was determined for 24 selected genes and showed unique methylation patterns for both HCC cell lines using EpiTect® DNA Methylation PCR Array (Qiagen).

Changes in DNA hypermethylation induced after daily SGI-110 exposure (day 1-5) in HepG2 and to an even higher extent in HuH-7 cells

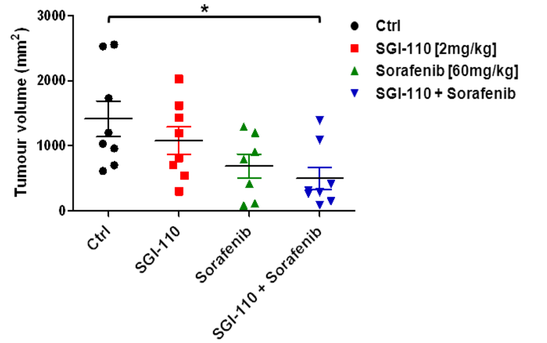


- DNA hypomethylation induced by daily SGI-110 exposure for 5 days was found to be concentration dependent.
- We have also seen changes in global LINE-1 methylation with daily SGI-110 exposure using Pyrosequencing technique and PyroMark Q24 LINE-1 Assay (Qiagen).
- The differential re-expression patterns of genes might explain the differences in sensitivity seen in these two cell lines when used in combination with sorafenib. Further experiments are undergoing to explain this phenomenon.

Establishment of *in vivo* HCC models to show the combinatory effect of SGI-110 and sorafenib

HuH-7 xenograft model in male balb/c nude mice:

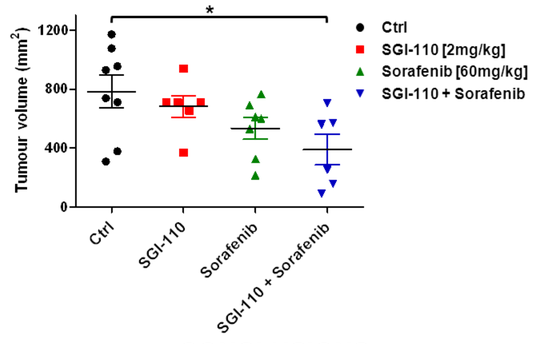
SGI-110 primed mice (s.c. day 1-3) showed an increased tumour growth reduction when combined with sorafenib (p.o. day 8-15)



- We obtained a significant tumour size reduction in both HCC in vivo models when primed with SGI-110 followed by sorafenib, *p<0.05, using 1way ANOVA with Bonferroni's Multiple Comparison Test, shown as box plot ± 95% CI).

HepG2 xenograft model in male beige scid mice:

SGI-110 primed mice (s.c. day 1-5) showed an increased tumour growth reduction when combined with sorafenib (p.o. day 8-15)



CONCLUSIONS

- Epigenetic priming with SGI-110 resulted in an at least additive effect on cell growth inhibition when combined with sorafenib.
- Distinct hypomethylation patterns were observed in two HCC cell lines characterized by differentially methylated tumour suppressor genes when exposed to SGI-110. These differences in methylation level might reflect the increased sensitivity to targeted agents in HCC as shown here in our *in vitro* and *in vivo* models.
- Priming with SGI-110 is well tolerated and may help to improve the anti-tumour activity of sorafenib.
- These pre-clinical data warrant the clinical investigation of SGI-110 in hepatocellular carcinoma.

astex™

Poster presented at EORTC-AACR-NCI Symposium on Molecular Targets and Cancer Therapeutics Nov 6-9, 2012, Dublin, Ireland.

Poster can be downloaded from www.astx.com