DNA Methylome Alterations in Platinum Resistant Ovarian Cancer Tumors

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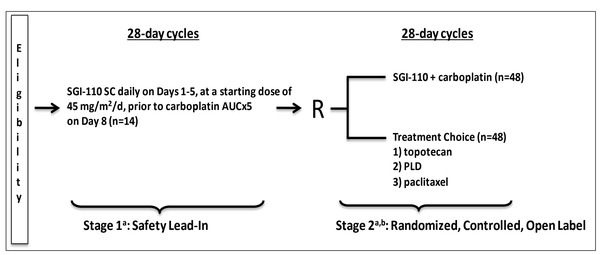
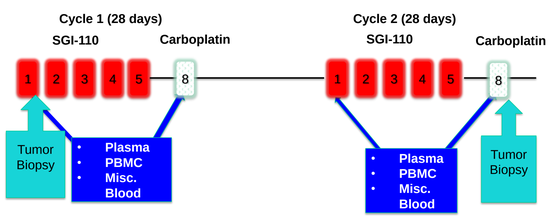
ABSTRACT

Epigenetic changes, particularly DNA methylation aberrations have been implicated in acquired resistance to platinum in ovarian cancer. An ongoing phase I/II multi-institutional clinical trial uses the novel DNA methyl transferase (DNMT) inhibitor SGI-110 to re-sensitize recurrent platinum resistant ovarian cancer to carboplatin. Tumor biopsies or malignant ascites were collected at baseline and after two cycles of SGI-110 administered daily for 5 days in low dose (30mg/m^2). The goal of the current study was to analyze global DNA methylation profiles of platinum resistant tumors and compare them to the methylome of untreated, platinum-sensitive ovarian tumors. LINE1 methylation and promoter methylation of selected genes (*MAGE-A2, MAGE-A3, MAGE-A11, NY-ESO, RASSF1, MLH1, and HOXA11*) were quantified by pyrosequencing before and after SGI-110 treatment (n=12 paired samples). Epigenetic profiling using the Infinium HumanMethylation450 BeadChip (HM450) revealed extensive methylation changes when comparing recurrent platinum resistant ovarian tumors (n=42) to primary, untreated ovarian cancer specimens analyzed as part of the TCGA project (n=10). Six hundred and four promoters were significantly differentially methylated (adjusted p<0.05, absolute methylation changes β>0.2), among which, 498 and 106 were hypermethylated or hypomethylated respectively in recurrent platinum resistant ovarian tumors. *DNMT1, 3A, and 3B mRNA* levels in the tumors were highly variable (n=19). Analysis of a limited number of paired samples (n=7) revealed no significant changes in global methylation or in *DNMT* expression levels induced by treatment with SGI-110 (adjusted p>0.05). However, the DNMT inhibitor induced significant methylome alterations in selected patients. Significant hypomethylation of *MAGE-A3* and *MAGE–A11* promoters (p<0.05) was detected. Correlations between methylation changes and clinical outcomes are being explored.

PHASE I/II CLINICAL TRIAL

SGI-110 is a novel dinucleotide resistant to cytidine deaminase and a potent inhibitor of DNA methyl transferase.



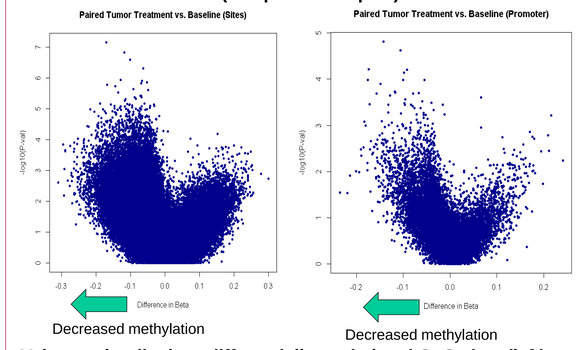


MATERIALS AND METHODS

- DNA Methylation: Infinium HumanMethylation450 array (485,577 methylation sites).
- DNA Methylation measured by pyrosequencing.
- *mRNA* expression levels *m*easured by real-time RT-PCR.
- DNMT protein expression measured by IHC.

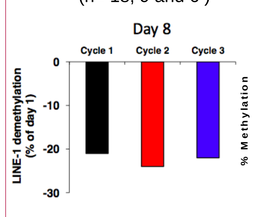
RESULTS

SGI-110-Induced Changes in DNA Methylation (n=7 paired samples)

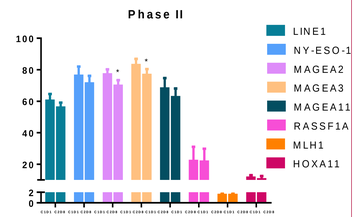


Volcano plot displays differentially methylated CpG sites (left) or promoters (right) in paired tumor biopsies pre and post treatment

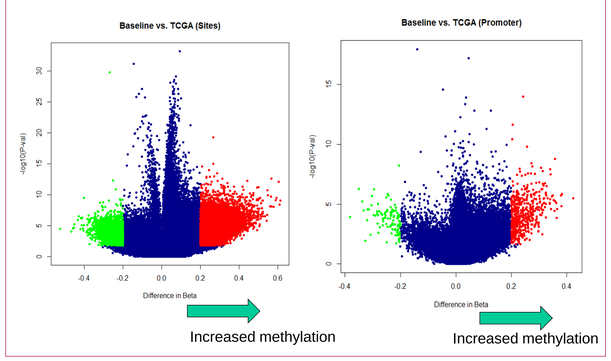
LINE 1 Methylation (pyrosequencing) PBMCs (n= 18, 9 and 6)



Gene Specific Methylation (n=12)



Comparison of DNA Methylation in baseline tumors (platinum resistant, n=42) with TCGA database (platinum sensitive, n=10)



Comparison of DNA Methylation with TCGA database

		Sites	Promoter
Total number		124,524	29,165
Hypermethylation (FDR<0.05)	Δ β > 0	83,627	10,363
	Δ β > 0.2	**17,815**	**498**
Hypomethylation (FDR<0.05)	Δ β < 0	40,897	3,514
	Δ β< -0.2	**3,391**	**106**

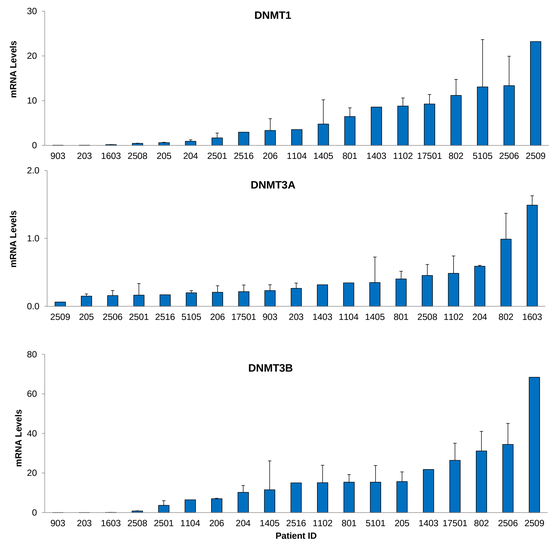
Top Canonical Pathways

Name	p-value	Ratio
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	1.04E-03	4/287 (0.014)
Dendritic Cell Maturation	2.38E-03	3/169 (0.018)
Superpathway of Inositol Phosphate Compounds	3.03E-03	3/184 (0.016)
Role of Osteoblasts, Osteoclasts and Chondrocytes in Rheumatoid Arthritis	4.62E-03	3/214 (0.014)
Ascorbate Recycling (Cytosolic)	4.75E-03	1/3 (0.333)

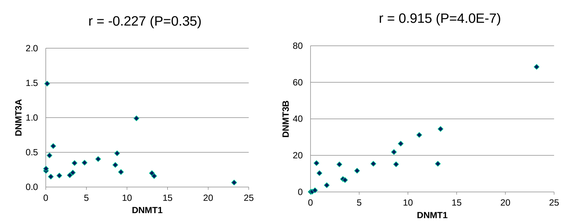
Top Upstream Regulators

Upstream Regulator	p-value of overlap	Predicted Activation State
IFNL1	1.74E-04	
miR-325-5p (and other miRNAs w/seed CUAGUAG)	6.67E-04	
miR-4680-5p (miRNAs w/seed GAACUCU)	1.43E-03	
PIK3R6	1.47E-03	
PIK3R5	1.47E-03	

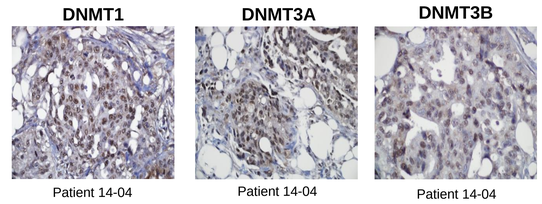
DNMT *mRNA* Baseline Levels in OC Tumors (n=19)

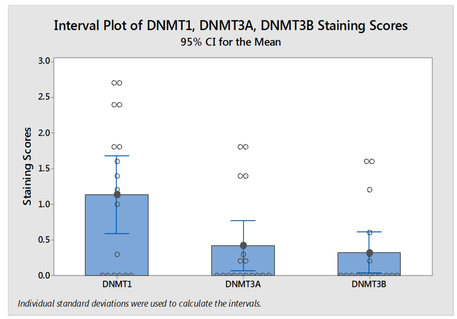


Correlations Between DNMT mRNAs Baseline Levels

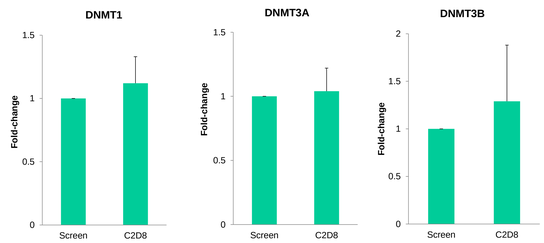


DNMT Expression by Immunohistochemistry (n=16)





Effects of SGI-110 on DNMT *mRNA* Levels (n=6)



CONCLUSIONS

1. SGI-110 induced significant hypomethylation in ovarian cancer tumors and PBMCs (global, LINE1 and gene specific).
2. There are extensive differences in DNA methylation between this set of platinum resistant tumors and tumors analyzed by the TCGA project.
3. Baseline DNMT *mRNAs* and protein expression levels were highly variable among patients.
4. SGI-110 did not alter DNMT mRNA expression levels.

ACKNOWLEDGMENTS

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