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Determinants of Demethylation and Clinical Response In AML Patients Treated With SGI-110, a Novel Subcutaneous (SQ) Hypomethylating Agent (HMA) In a Phase 1 Study

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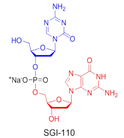
Introduction



SGI-110 is a dinucleotide of decitabine and deoxyguanosine and is a potent inhibitor of DNA methylation in-vitro and in-vivo. A Phase 1 study established the biologically effective dose of SGI-110 in MDS/AML patients as 60 mg/m^2 SQ daily x 5 and demonstrated clinical responses that correlated with hypomethylation induction. Here, we analyze AML patients treated at pharmacologically effective doses of SGI-110 looking for determinants of hypomethylation and response.

Methods

We studied patients with relapsed/refractory AML who were treated at a therapeutic dose range of SGI-110 (36 mg/m^2-125 mg/m^2).

DNA methylation pre/post treatment (for pharmacodynamics or PD) was estimated by bisulfite-pyrosequencing for the LINE-1 repetitive element as well as the INS6 CpG island gene promoter which is highly methylated in all somatic tissues. Gene expression at baseline and after treatment was measured by qPCR. For detecting mutations, we performed exome sequencing.

Results

We analyzed samples from 27 patients with AML. Median age was 64, (range, 29–86), 18 were Males (67%), 13 (48%) had poor cytogenetics at study entry and 59% had prior exposure to a hypomethylating agent. **(Table 1)** Overall, peak LINE-1 demethylation generally occurred on Day 8 and correlated strongly with INSL6 demethylation (R=0.91, p<0.0001). In individual patients, peak LINE-1 demethylation ranged from +1% to -39%. **(Fig. 1)**

We next examined expression of a panel of genes (CDA, P15, P21, DNMT3B, DNMT3A, DNMT1, CTCF) at baseline. High expression of DNMT3b (but not DNMT1) was associated with a trend for reduced demethylation (R=-0.20, p>0.05). Unsupervised classification grouped the patients into four clusters: A (N=2), B (N=6), C (N=10), and D (N=9). Cluster D is characterized by high DNMT3b expression, low P15 expression, low CDA expression and reduced demethylation (demethylation average -10.9% in cluster D compared to -22.7% in clusters B and C, p=0.06). **(Fig. 2)**

Next, we examined SGI-110 mediated induction of gene expression for 4 genes (P15, P21, DNMT3B and CTCF). **(Fig. 3)** P15 was the only significantly induced gene (p=0.03), and this was only observed in patients who were treated on the daily x 5 regimen (compared to weekly x 3). In this group of 15 patients with induced P15, peak P15 induction was on Day 8 and averaged 2.4 fold. P15 induction was associated with a trend for increased demethylation on Day 8 (R=0.28) and on Day 29 (R=0.37), p>0.05 for both. Of the 27 patients, 5 showed major clinical responses (2 CR, 3 CRi/CRp). LINE-1 and INSL6 demethylation averaged -21.1% and -16.4% in responders compared to -13.14% and -11.35% in non-responders. **(Table 2)** A three gene classifier score (low CDA, low P15 and high DNMT3B) was associated with low LINE-1 demethylation (R=0.43, p=0.025) as well as resistance to SGI-110 (mean score 6.2 in non-responders compared to 0.5 in responders, p=0.047). Finally, peak induction of P15 was similar in responders and non-responders, but sustained induction (at Day 29) was higher in responders (3.1 fold) than in non-responders (1.0 fold). **(Fig 5)**

A genetic signature of response could not be identified among those 8 genes that were examined by sequencing. IDH1 or IDH2 mutations were identified in 5 patients of whom one achieved CR and was positive for substitution R132H of IDH1, which has been described to induce epigenetic alterations and may predict poor clinical outcome in AML. The TP53 polymorphism NM_000546:c.C215G:p.P72R, which has been associated with differential response to chemotherapy in AML was identified in 9 subjects, 3 of whom responded to SGI-110. **(Table 1)**

Fig.1 Peak LINE-1 demethylation generally occurred on Day 8 and correlated strongly with INSL6 demethylation (R=0.91, p<0.0001)

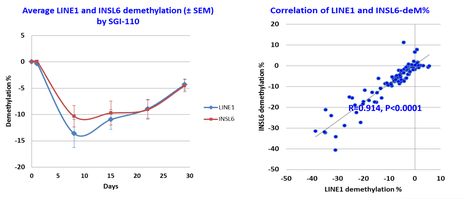
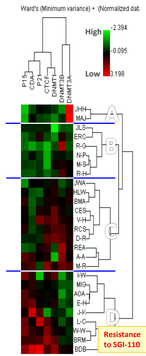


Fig.2 Base-line gene expression clustering



Cohorts	SUBJID	DNMT1	P21	CDA	CTCF	P15	DNMT3A	DNMT3B	LINE1 deM %	INSL6 deM %	Responder
4W	JHH	-1.37	0.50	-1.89	-1.06	1.03	5.76	-1.06	-4.19		
4D	MAJ	-2.06	-0.16	-0.23	-1.52	1.59	6.29	0.65	-5.72	-4.63	
A	Average	-1.72	0.17	-1.06	-1.29	1.31	6.03	-0.21	-4.40	-4.41	
	SD	0.49	0.47	1.18	0.33	0.40	0.37	1.21	1.87	0.31	
7D	JLS	-3.16	0.14	-1.04	-1.16	5.21	-2.14	-0.37	-24.32	-15.55	
4W	ERC	-2.11	1.17	-1.46	-1.51	3.49	-1.59	2.25	-22.66	-22.53	CR
5D	R-G	-1.49	-0.78	-4.40	-2.50	3.40	-2.04	5.35	-34.67	-21.23	CR
6D	N-P	-1.04	-0.20	-0.36	-2.39	4.41	-1.33	1.05	-34.76	-32.27	
7D	M-S		-0.03	-1.76	-2.42	4.84		2.06	-14.11	-6.16	
5W	R-H	-1.31	-0.02	-2.42	-1.80	4.08	-0.56	3.10	-5.92	-4.42	
B	Average	-1.82	0.05	-1.91	-1.96	4.24	-1.53	2.27	-22.74	-17.03	
	SD	0.85	0.64	1.40	0.56	0.72	0.64	1.88	11.37	10.58	
5D	JWA	-0.50	0.64	0.00	-1.03	2.43	-1.06	-0.13	-23.28	-18.91	CRi
4D	HLW	-0.39	-0.58	-2.16	-0.83	6.08	0.09	0.59	-10.35	-9.40	
4D	BMA	-0.54	0.12	-2.55	-0.62	7.99	0.19	1.33	-13.34	-10.83	CRi
7D	CES	0.44	1.06	-2.38	-0.68	6.68	-0.89	1.73	-32.49	-24.02	
6W	V-H	0.50	2.60	-1.59	0.05	6.48	-0.22	2.11	-7.49	-7.61	
6W	RCS	0.22	0.23	-1.88	-0.40	7.46	0.13	3.10	-11.52	-8.65	CRp
5W	D-R	0.61	0.03	-0.85	0.21	5.32	0.34	3.50	-5.65	-5.42	
5D	REA	-1.52	0.96	-2.01	-0.25	3.76	0.10	5.22	-38.61	-40.64	
5W	A-A	-2.55	0.63	1.59	-0.53	9.69	-0.34	4.78	-5.97	-4.45	
5W	M-R	-0.43	-0.28	-2.70	0.79	8.05	1.55	3.95	-9.27	-10.16	
C	Average	-0.42	0.54	-0.91	-0.33	6.39	-0.01	2.62	-15.81	-14.01	
	SD	0.99	0.89	1.80	0.55	2.14	0.72	1.78	11.64	11.13	
7D	I-W	0.25	-1.84	1.38	-0.40	10.66	-1.43	-1.47	-10.81	-7.70	
6D	MIG	-1.01	0.31	1.67	-0.86	6.72	-2.08	-0.14	-14.70	-12.99	
4W	AOA	-0.72	0.79	0.96	-0.95	9.44	-1.68	-1.29	-1.99	-3.20	
6D	E-H	-0.38	0.53	1.78	-0.93	9.33	-1.46	-0.26	-19.42	-11.74	
5W	J-K	-1.11	1.79	7.69	-2.23	7.05	-2.98	0.65	-4.75	-3.43	
4W	L-C		3.91	0.14	0.14	10.23	-0.90	-0.07	1.39	0.14	
7D	W-W	-0.57	0.97	1.58	0.69	10.81	-1.31	1.48	-35.05	-15.08	
4W	BRM	0.33	1.90	2.11	1.01	8.53	-1.11	0.59	-6.31	-2.28	
5D	BDB	0.99	4.70	7.75	0.02	11.35	-0.16	1.92	-7.13	-4.81	
D	Average	-0.28	1.45	3.01	-0.39	9.32	-1.46	0.16	-10.98	-6.79	
	SD	0.73	1.95	2.84	0.99	1.64	0.78	1.14	11.03	5.35	

Fig 3. SGI-110 mediated induction of gene expression for 4 genes (P15, P21, DNMT3B and CTCF).

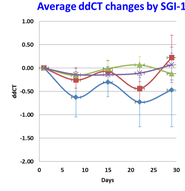
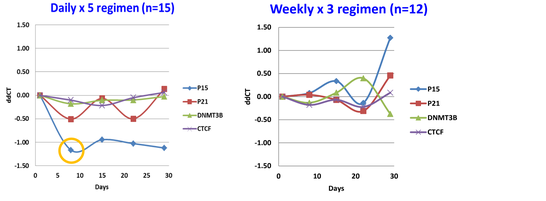


Table 2. AML Patients with DNA demethylation (±SEM) by SGI-110 treatment

	Responders (n=5)	Non-Responders (n=22)
Average MAX. LINE1 deM%	– 21.10 ± 4.14	– 13.51 ± 2.54
Average MAX. INSL6 deM %	– 16.43 ± 2.81	– 11.35 ± 2.40

Fig 4. ddCT changes of the genes in the Daily (n=15) and Weekly (n=12) Cohorts



P15 was the only significantly induced gene (p=0.03), and this was only observed in patients who were treated on the daily x 5 regimen (compared to weekly x 3). In this group of 15 patients with induced P15, peak P15 induction was on Day 8 and averaged 2.4 fold.

Table 1. Representative Pathogenic Characteristics in SGI-110 treated patients (n=27)

Cohorts	SUBJID	Age	Gender	Cytogenetic Risk	PB blasts at Screening (%)	BM blasts at Screening (%)	Platelet Count(K/uL) at Screening	WBC (K/uL) at Screening	FLT3-ITD/NPM1	IDH1/2 mutation	TP53 polymorphism	Responder
4W	JHH	64	M	poor	3	20	22	0.8	neg/neg			
4D	MAJ	59	M	intermediate	2	14	32	1.6	neg/neg			
	Average											
7D	JLS	60	M	poor	17.4	9	46	0.9	neg/neg			
4W	ERC	64	M	poor	3	8	20	1.9	neg/neg		TP53 P72R	CR
5D	R-G	70	M	intermediate	0	16.2	58	3.3	neg/neg		TP53 P72R	CR
6D	N-P	71	F	intermediate	11	17.6	52	2.5	neg/neg			
7D	M-S	71	F	intermediate	12	64.4	55	1.6	neg/neg			
5W	R-H	41	M	poor	1	7	120	2.5	neg/neg		TP53 P72R	
	Average				7.4	20.4	58.5	2.1				
5D	JWA	79	M	poor	26	35	13	2.3	neg/neg			Cri
4D	HLW	66	M	poor	2	81	31	1.2	neg/neg			
4D	BMA	70	F	intermediate	0	35	342	7.3	neg/pos	IDH1 R132H		Cri
7D	CES	86	F	poor	14	23	26	5.3	neg/neg		TP53 P72R	
6W	V-H	83	F	intermediate	12	16	149	12.8	neg/neg			
6W	RCS	37	F	intermediate	1	22	16	3.4	neg/neg		TP53 P72R	CRp
5W	D-R	78	M	intermediate	22	18	73	2.9	neg/neg	IDH1 V178I, IDH2 R140Q	TP53 P72R	
5D	REA	46	M	poor	N/A	46	17	3.4	neg/neg			
5W	A-A	53	M	poor	49	31	15	7.7	pos/neg			
5W	M-R	49	M	unevaluable	25	71.2	12	0.4	neg/neg	IDH1 R132C		
	Average				16.9	37.8	69.4	4.7				
7D	I-W	53	F	poor	95	93	12	8	neg/neg			
6D	MIG	78	F	poor	77	76.5	55	25	neg/neg			
4W	AOA	29	M	poor	87	89	5	11.2	neg/neg			
6D	E-H	78	M	intermediate	93	71	38	95.2	neg/neg			
5W	J-K	59	M	intermediate	90	54	5	3.7	neg/neg			
4W	L-C	73	M	poor	65	47	12	7.4	pos/pos		TP53 P72R	
7D	W-W	64	M	intermediate	63	80	75	30.3	pos/neg	IDH2 R140Q	TP53 P72R	
4W	BRM	44	M	unevaluable	25	60	198	26.5	neg/neg		TP53 P72R	
5D	BDB	46	F	intermediate	90	75	106	21.8	neg/pos	IDH1 R132H + V178I		
	Average				75.7	71.7	56.2	25.5				

Fig 5. Induction of P15 in responders and non-responders

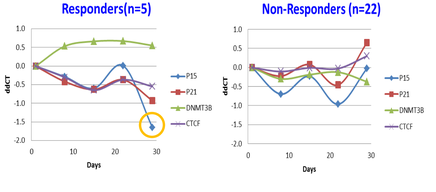


Table 3. A three gene classifier score (low CDA, low P15 and high DNMT3B) was associated with low LINE-1 demethylation (R=0.425, p=0.027) as well as resistance to SGI-110 (mean Z-score 0.40 in non-responders compared to -1.75 in responders, p=0.047).

SUBJID	CDA Z-score	P15 Z-score	DNMT3B (Z-score)	3 Z-score sum	LINE1 deM.%	Resistance to SGI-110	Responder
ERC	-0.56	-1.04	-0.38	-1.98	-22.66	No	Yes
R-G	-1.58	-1.07	-2.00	-4.64	-34.67	No	Yes
RCS	-0.71	0.32	-0.83	-1.21	-11.52	No	Yes
JWA	-0.06	-1.40	0.85	-0.60	-23.28	No	Yes
BMA	-0.94	0.50	0.09	-0.34	-13.34	No	Yes
CES	-0.88	0.06	-0.11	-0.93	-32.49	No	No
M-S	-0.66	-0.57	-0.28	-1.52	-14.11	No	No
REA	-0.75	-0.94	-1.93	-3.62	-38.61	No	No
JLS	-0.42	-0.45	0.87	0.01	-24.32	No	No
HLW	-0.80	-0.15	0.48	-0.47	-10.35	No	No
N-P	-0.18	-0.72	0.24	-0.66	-34.76	No	No
W-W	0.49	1.47	0.02	1.98	-25.42	No	No
I-W	0.42	1.42	1.55	3.39	-10.66	No	No
E-H	1.25	0.89	0.92	3.06	-19.42	No	No
MIG	0.52	0.07	0.86	1.45	-14.70	No	No
V-H	-0.60	-0.01	-0.31	-0.93	-7.49	Yes	No
R-H	-0.89	-0.83	-0.83	-2.55	-5.92	Yes	No
D-R	-0.35	-0.41	-1.03	-1.79	-5.65	Yes	No
JHH	-0.71	-1.88	1.34	-1.25	-3.07	Yes	No
MAJ	-0.13	-1.69	0.45	-1.37	-5.72	Yes	No
BDB	2.62	1.65	-0.21	4.06	-3.39	Yes	No
M-R	0.88	0.53	-1.27	0.13	-9.37	Yes	No
A-A	0.49	1.09	-1.70	-0.12	-5.97	Yes	No
AOA	0.28	1.00	1.46	2.73	-1.99	Yes	No
L-C	-0.01	1.27	0.82	2.09	1.39	Yes	No
BRM	0.67	0.69	0.48	1.84	-6.31	Yes	No
J-K	2.60	0.18	0.45	3.23	-4.75	Yes	No

Conclusions

At a therapeutic dose of SGI-110, we identified a gene expression signature **(high DNMT3B, low P15, low CDA)** associated with reduced demethylation and resistance to SGI-110 and we find trends for associations between demethylation and response, as well as sustained P15 induction and response. These associations will be further investigated in the ongoing Phase 2 study of SGI-110 in AML.

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