Immunomodulatory activity of SGI-110, a 5-aza-2'-deoxycytidine-containing demethylating dinucleotide

Sandra Coral[1], Luca Sigalotti[1], Giulia Parisi[1,2], Francesca Colizzi[1], Alessia Covre[1,2], Elisabetta Fratta[1], Ester Fonsatti[2], Hugues JM Nicolay[1,2], Pietro Taverna[3] and Michele Maio[1,2]

[1]Cancer Bioimmunotherapy Unit, Centro di Riferimento Oncologico, Aviano, Italy; [2]Division of Medical Oncology and Immunotherapy, University Hospital of Siena, Siena, Italy; [3]SuperGen, Inc. Dublin, California USA

ABSTRACT

Background: We have recently reported that aberrant DNA hypermethylation down-regulates the expression of components of the "tumor recognition complex" (i.e., HLA class I antigens, tumor-associated antigens belonging to the cancer/testis antigens (CTA) class and accessory/co-stimulatory molecules) in neoplastic cells of different histotypes. These evidences strongly suggest that the extent of DNA methylation of cancer cells might favour tumor-escape from host's immune recognition, contributing to the reduced clinical efficacy of immunotherapeutic approaches for cancer treatment. In this scenario, the present study was designed to evaluate the immunomodulatory potential of new DNA hypomethylating agents (DHA) on neoplastic cells from solid tumors, aiming to identify novel strategies to improve the clinical response to cancer immunotherapies.

Materials and methods: Cutaneous melanoma, mesothelioma, renal cell carcinoma and sarcoma cell lines were treated in vitro with the new DHA SGI-110, a dinucleotide of 5-aza-2'-deoxycytidine and guanosine. RT-PCR, quantitative RT-PCR and flow cytometric analyses were performed to investigate changes induced by treatment with SGI-110 in the constitutive immune profile of investigated cancer cells. CTA promoter methylation was evaluated by bisulfite sequencing.

Results: Treatment with SGI-110 induced/up-regulated the mRNA expression of a large panel of CTA (i.e., MAGE-A1, -A2, -A3, -A4, -A10, GAGE 1-2, GAGE 1-6, NY-ESO-1, SSX 1-5) in all investigated cell lines. Accordingly, exposure to SGI-110 up-regulated the constitutive expression of MAGE-A and NY-ESO-1 proteins, currently utilized as therapeutic targets in clinical trials of CTA-based cancer vaccination. Treatment with SGI-110 also up-regulated the expression of HLA class I antigens, HLA-A2 allospecificity and of the co-stimulatory molecule ICAM-1, in all investigated histotypes. Bisulfite sequencing analysis revealed a demethylation of MAGE-A3 promoter (-113/+130) following SGI-110 treatment of neoplastic cells, demonstrating a direct role of DNA methylation in the induction of this CTA.

Conclusions: These evidences strongly suggest that SGI-110 may represent an attractive therapeutic agent to comprehensively increase immunogenicity and immune recognition of neoplastic cells from solid tumors, and provide the scientific rationale for its clinical development to design new and possibly more effective chemo-immunotherapeutic approaches in patients with solid malignancies.

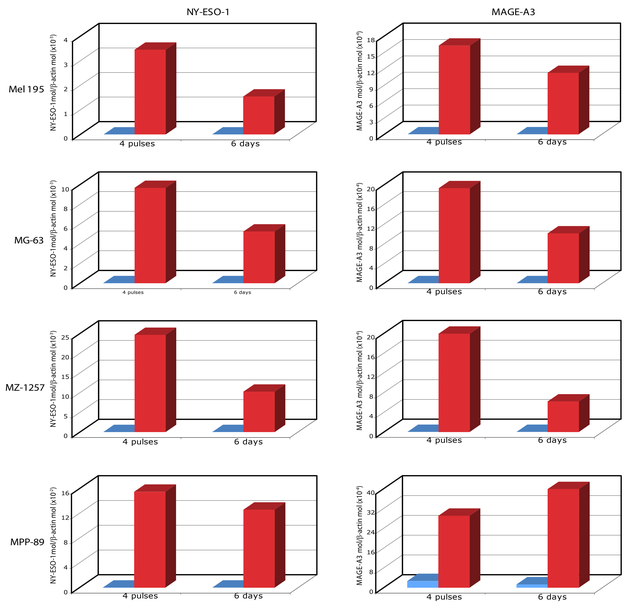
RESULTS

RT-PCR analysis of CTA expression in cell lines from different solid malignancies treated with SGI-110[a]

Histotype	Cells	Treatment	SGI-110	MAGE-A1	MAGE-A2	MAGE-A3	MAGE-A4	MAGE-A10	GAGE 1-2	GAGE1-6	NY-ESO-1	SSX 1-5
Melanoma	Mel 275		ctrl	-	++	+	-	+	-	-	-	-
		4 pulses	1 uM	+	++	+	++	+	+	++	++	++
			10 uM	+	++	+	++	+	+	++	++	++
		6 days	ctrl	-	++	+	-	+	-	-	-	-
			1 uM	+	++	+	++	+	+	++	++	++
			10 uM	+	++	+	++	+	-	++	++	++
	Mel 313		ctrl	-	-	-	-	-	-	+	-	-
		4 pulses	1 uM	+	+	+	+	+	+	++	++	++
			10 uM	+	+	+	+	+	+	++	++	++
		6 days	ctrl	-	-	-	-	-	-	+	-	-
			1 uM	+	+	+	+	+	+	++	++	++
			10 uM	+	+	+	+	+	+	++	++	++
	Mel 611		ctrl	+	++	++	-	++	+	++	+	+
		4 pulses	1 uM	+	++	++	+	++	+	++	++	+
			10 uM	+	++	++	+	++	+	++	++	+
		6 days	ctrl	+	+	++	-	++	+	++	+	+
			1 uM	+	++	++	+	++	+	++	++	+
			10 uM	+	++	++	+	++	+	++	++	+
	Mel 684		ctrl	++	++	+	-	+	-	++	-	+
		4 pulses	1 uM	++	++	+	+	+	+	++	+	++
			10 uM	++	++	+	+	+	+	++	+	++
		6 days	ctrl	++	+	+	-	+	-	++	-	++
			1 uM	++	++	+	+	+	++	++	++	++
			10 uM	++	++	+	+	+	++	++	++	++
	Mel 195		ctrl	-	-	-	-	-	-	-	-	-
		4 pulses	1 uM	++	++	+	+	+	+	++	++	++
			10 uM	++	++	+	+	+	+	++	++	++
		6 days	ctrl	-	-	-	-	-	-	-	-	-
			1 uM	+	++	+	+	+	+	++	++	++
			10 uM	++	++	+	++	+	+	++	++	++
Sarcoma	MG-63		ctrl	-	-	-	-	-	-	-	-	-
		4 pulses	1 uM	-	++	+	++	+	++	++	++	++
			10 uM	+	++	+	++	+	++	++	++	++
		6 days	ctrl	-	-	-	-	-	-	-	-	-
			1 uM	++	++	+	++	+	++	++	++	++
			10 uM	++	++	+	++	+	++	++	++	++
	SK-LMS-1		ctrl	-	-	-	-	-	-	+	-	++
		4 pulses	1 uM	++	++	+	++	+	++	++	++	++
			10 uM	++	++	+	++	+	++	++	++	++
		6 days	ctrl	-	-	-	-	-	-	+	-	++
			1 uM	++	++	+	++	+	++	++	++	++
			10 uM	++	++	+	+	+	++	++	++	++
Renal cell carcinoma	MZ-1257		ctrl	-	-	-	-	-	-	-	-	-
		4 pulses	1 uM	++	++	-	++	+	+	++	++	++
			10 uM	++	++	+	+	+	++	++	++	++
		6 days	ctrl	-	-	-	-	-	-	-	-	-
			1 uM	++	++	+	++	+	+	+	++	++
			10 uM	++	++	+	++	+	++	+	++	++
	LE-9104		ctrl	-	-	-	-	-	-	-	-	-
		4 pulses	1 uM	+	+	-	++	-	++	+	+	++
			10 uM	+	+	-	++	+	++	+	+	++
		6 days	ctrl	-	-	-	-	-	-	-	-	-
			1 uM	+	++	+	++	+	++	+	++	++
			10 uM	+	++	+	++	+	++	+	++	++
Mesothelioma	MES-1		ctrl	-	-	-	-	-	-	-	-	-
		4 pulses	1 uM	+	++	-	++	+	++	+	++	++
			10 uM	+	++	-	+	+	++	+	++	++
		6 days	ctrl	-	-	-	-	-	-	-	-	-
			1 uM	++	++	-	++	+	++	++	++	++
			10 uM	++	++	-	++	+	++	++	++	++
	MPP-89		ctrl	+	+	+	-	-	-	-	-	-
		4 pulses	1 uM	++	++	++	++	++	+	++	++	++
			10 uM	++	++	++	++	++	+	++	++	++
		6 days	ctrl	+	+	+	-	-	-	-	-	-
			1 uM	++	++	++	++	++	++	++	++	++
			10 uM	++	++	++	++	++	++	++	++	++

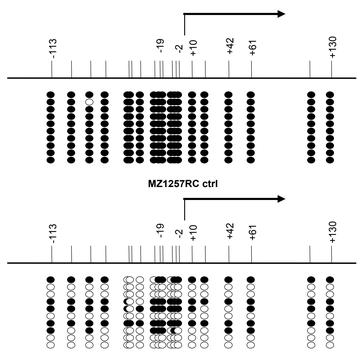
[a]Total RNA was extracted from human melanoma, sarcoma, renal cell carcinoma and mesothelioma cell lines, either untreated (ctrl) or treated with SGI-110 (1μM and 10μM) every 12 hours for 2 days (4 pulses), or treated for 6 days with addition of new drug at day 3. RT-PCR reactions were performed using gene-specific primers. RNA integrity and cDNA quality were confirmed by amplification of the house-keeping gene β-actin. Intensity of RT-PCR products: -, not detectable; +, weak; ++, strong.

Real-time RT-PCR analysis of CTA expression in cells from different solid malignancies treated with SGI-110



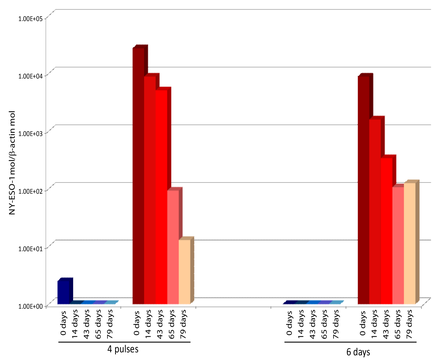
Total RNA was extracted from human melanoma, sarcoma, renal cell carcinoma and mesothelioma cell lines, either untreated (blue) or treated (red) with 1μM SGI-110 every 12 hours for 2 days (4 pulses), or treated for 6 days with addition of new drug at day 3. SYBR Green quantitative RT-PCR reactions were performed on retrotranscribed total RNA from untreated or SGI-110-treated cells, utilizing MAGE-A3-, NY-ESO-1- and β-actin-specific primers. CTA expression was normalized to the expression of the housekeeping gene β-actin. Values are reported as CTA molecules/β-actin molecules. Data shown are relative to a single tumor cell line that is representative for each investigated istotype.

Bisulfite sequencing analysis of the methylation status of MAGE-A3 promoter in solid malignancies treated with SGI-110



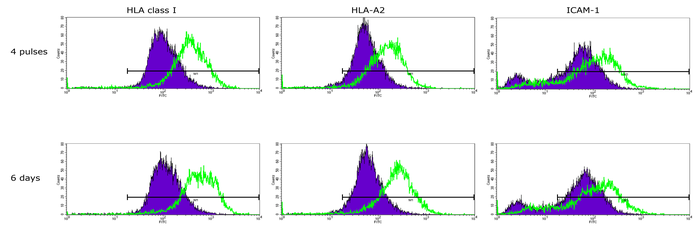
Genomic DNA was extracted from MZ-1257RC cells, untreated (ctrl) or treated with 1 μM SGI-110 for 6 days with addition of new drug at day 3, and subjected to sodium bisulfite modification followed by sequence analysis of PCR amplified MAGE-A3 promoter. Each circle represents a CpG site, with empty circles representing unmethylated cytosines and black circles representing methylated cytosines. The position of the CpG dinucleotides is reported with respect to the transcription site (+1).

Time-dependency of CTA expression induced in solid malignancies by SGI-110



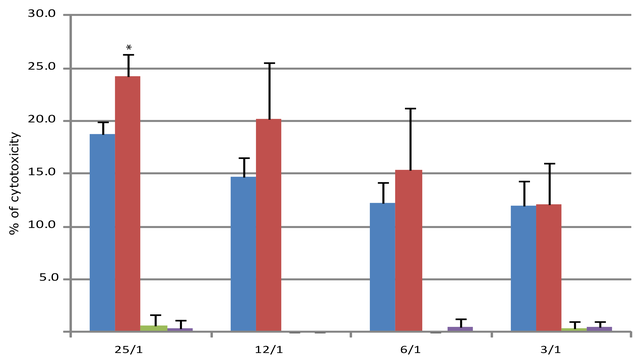
Total RNA was extracted from Mel 275 melanoma cells untreated (blue) or treated (red) with 1μM SGI-110 every 12 hours for 2 days (4 pulses), or treated for 6 days with addition of new drug at day 3, and in vitro cultured for the indicated days after the end of treatment. SYBR Green quantitative RT-PCR reactions were performed on retrotranscribed total RNA, utilizing NY-ESO-1- and β-actin-specific primers. CTA expression was normalized to the expression of the housekeeping gene β-actin. Values are reported as NY-ESO-1 molecules/β-actin molecules multiplied by 10^6 and visualized on logarithmic scale.

Indirect immunofluorescence analysis of the expression of selected immune molecules in melanoma cells treated with SGI-110



Human melanoma cells either untreated (solid purple), or treated with 1 μM SGI-110 (empty green), were sequentially incubated with the anti-HLA class I antigens mAb W6/32, the anti-HLA-A2 mAb BB7.2 or the anti-ICAM-1 mAb 84H10, and with FITC-conjugated F(ab')2 fragments of rabbit anti-mouse Ig. Cells were then analyzed by flow cytometry

Susceptibility of SGI-110-treated melanoma cells to lysis by gp100-specific CTL



Cytolytic activity of HLA-A2-restricted gp100-specific CTL was tested by LDH-release assay against Mel 275 melanoma cells, untreated (blue) or treated (red) with 1μM SGI-110 every 12 hours for 2 days, at effector/target (E/T) ratios of 25:1, 12:1, 6:1 and 3:1. Mel 40 melanoma cells (green) and K562 leukemia cells (violet) were used as control target cells. *, $p<0.05$

CONCLUSIONS

This study has identified novel immuno-biological activities of SGI-110

Specifically:

- SGI-110 persistently induced the expression of CTA in solid malignancies of different histotypes, through the DNA hypomethylation of their promoter region
- SGI-110 strongly up-regulated the constitutive levels of CTA expression in solid malignancies of different histotypes
- SGI-110 up-regulated the expression of HLA class I antigens, HLA-A2 allospecificity and of the co-stimulatory molecule ICAM-1 on neoplastic cells
- Phenotypic changes induced by SGI-110 on neoplastic cells significantly ($p<0.05$) increased their lysis by tumor antigen-specific CTL

Altogether these evidences demonstrate that SGI-110 represents an attractive therapeutic agent to comprehensively increase immunogenicity and immune recognition of neoplastic cells from solid malignancies of different histotypes, and provide the scientific rationale for its clinical development to design new and possibly more effective chemo-immunotherapeutic approaches in patients with solid malignancies.