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

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

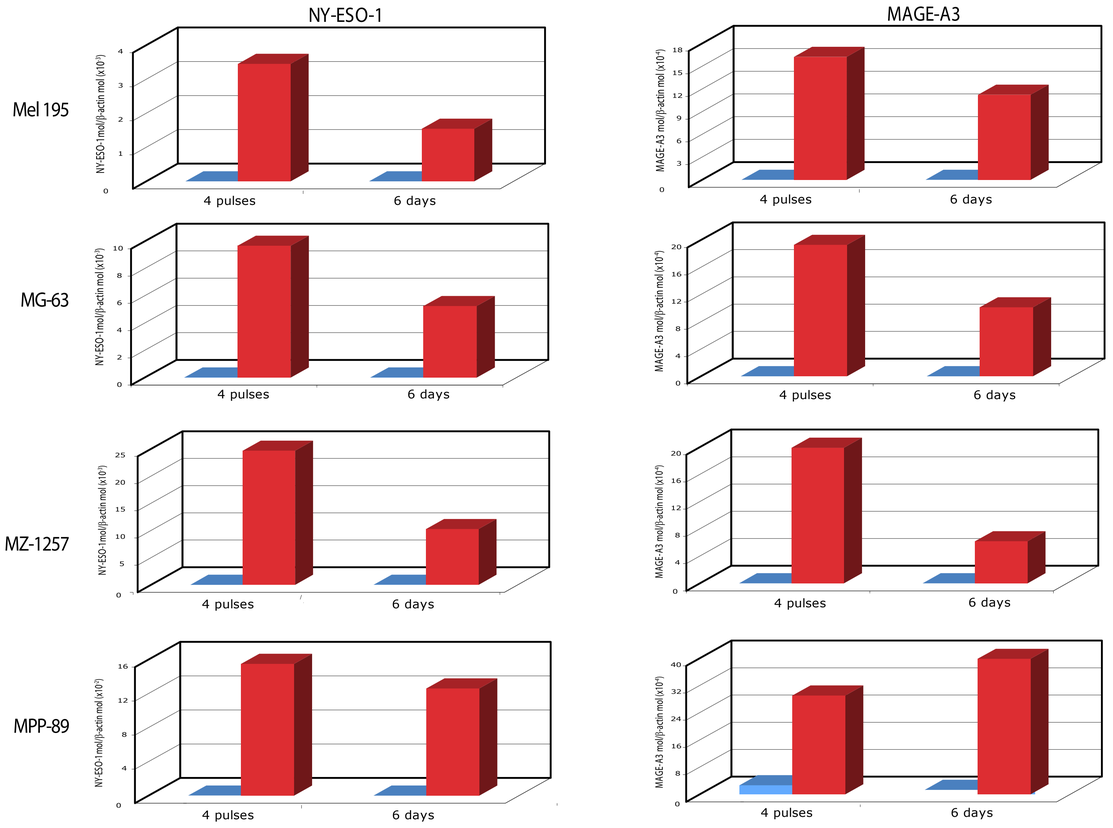
[1]Cancer Bioimmunotherapy Unit, Centro di Riferimento Oncologico, Aviano, Italy; [2]Division of Medical Oncology and Immunotherapy, University Hospital of Siena, Siena, Italy; [3]SuperGen, Inc. Dublin, California USA

ABSTRACT

Epigenetic alterations play a major role in human malignancies by affecting crucial cellular pathways in cancer initiation and progression (e.g., cell cycle control, apoptosis, invasive and metastatic potential, angiogenesis). In this context, we have demonstrated a key role of aberrant DNA hypermethylation in favoring tumor escape from host's immune recognition, through the down-regulation of different 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 findings contribute to explain at least in part the reduced clinical efficacy of immunotherapeutic approaches for cancer treatment. The present study was designed to evaluate the immunomodulatory activity of the new DNA hypomethylating agent SGI-110, a dinucleotide of 5-aza-2'-deoxycytidine and guanosine, in different solid malignancies. Five cutaneous melanoma, 2 mesothelioma, 2 renal cell carcinoma and 2 sarcoma cell lines were treated in vitro with 1 M SGI-110, added every 12 hours for 2 days (4 pulses), or treated for 6 days with addition of new drug at day 3. RT-PCR analyses showed that treatment with SGI-110 induced/up-regulated the expression of a large panel of CTA analyzed (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. Consistently, quantitative real-time RT-PCR analyses of the CTA MAGE-A3 and NY-ESO-1, which are currently utilized as therapeutic targets in clinical trials of CTA-based cancer vaccination, demonstrated that SGI-110 strongly up-regulated their constitutive levels of expression in neoplastic cells of all investigated histotypes. This latter observation was confirmed at protein level by flow cytometric analysis of the intracytoplasmic levels of CTA, in melanoma cells. Concomitantly, flow cytometric analyses revealed that treatment with SGI-110 up-regulated the expression of HLA class I antigens, HLA-A2 allospecificity and the co-stimulatory molecule ICAM-1. Altogether, these preliminary in vitro experimental 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 more effective combined chemo-immunotherapeutic approaches in patients with solid malignancies.

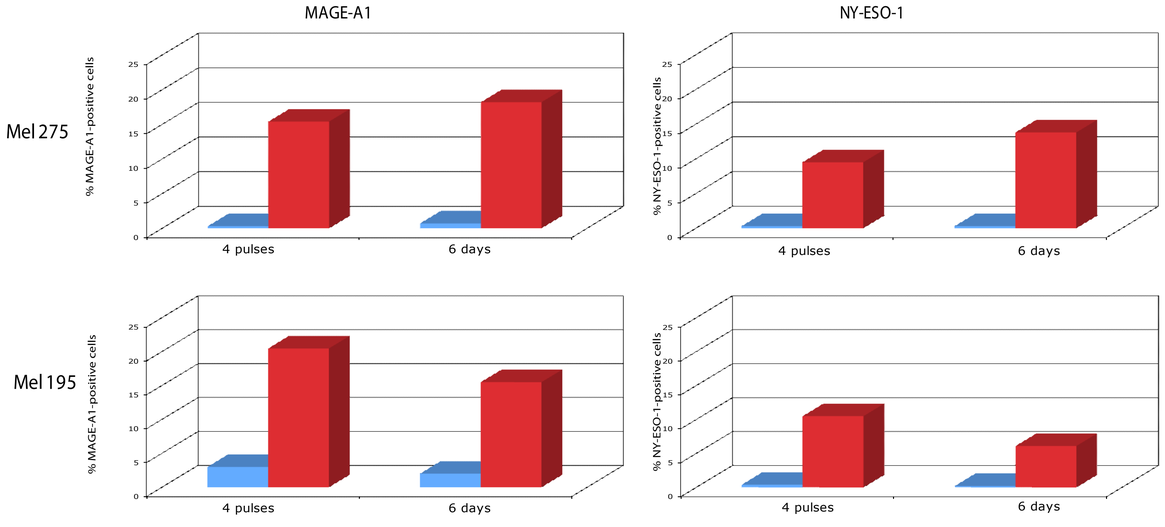
RESULTS

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 1mM 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 b-actin-specific primers. CTA expression was normalized to the expression of the housekeeping gene b-actin. Values are reported as CTA molecules/b-actin molecules. Data shown are relative to a single tumor cell line that is representative for each investigated istotype.

Indirect immunofluorescence analysis of the intracytoplasmic expression of CTA in melanoma cells exposed to SGI-110



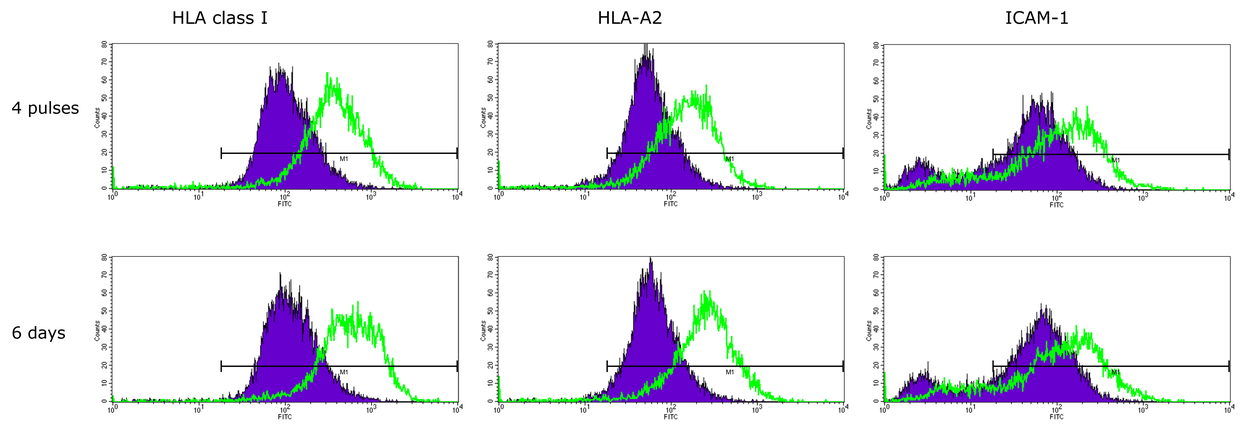
Paraformaldehyde-fixed and permeabilized human melanoma cells, either untreated (blue) or treated with 1 μM SGI-110 (red), were sequentially incubated with the anti-MAGE-A1 mAb MA454 or the anti-NY-ESO-1 mAb D8.38, and with FITC-conjugated F(ab')2 fragments of rabbit anti-mouse Ig. Cells were then analyzed by flow cytometry. Data are reported as percentage of melanoma cells positively stained by anti-CTA mAbs.

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	4 pulses	ctrl	-	++	+	-	+	-	-	-	-
			1 uM	+	++	+	++	+	+	++	++	++
			10 uM	+	++	+	++	+	+	++	++	++
		6 days	ctrl	-	++	+	-	+	-	-	-	-
			1 uM	+	++	+	++	+	+	++	++	++
			10 uM	+	++	+	++	+	-	++	++	++
	Mel 313	4 pulses	ctrl	-	-	-	-	-	-	+	-	-
			1 uM	+	+	+	+	+	+	++	++	++
			10 uM	+	+	+	+	+	+	++	++	++
		6 days	ctrl	-	-	-	-	-	-	+	-	-
			1 uM	+	+	+	+	+	+	++	++	++
			10 uM	+	+	+	+	+	+	++	++	++
	Mel 611	4 pulses	ctrl	+	++	++	-	++	+	++	+	+
			1 uM	+	++	++	+	++	+	++	++	+
			10 uM	+	++	++	+	++	+	++	++	+
		6 days	ctrl	+	+	++	-	++	+	++	+	+
			1 uM	+	++	++	+	++	+	++	++	+
			10 uM	+	++	++	+	++	+	++	++	+
	Mel 684	4 pulses	ctrl	++	++	+	-	+	-	++	-	+
			1 uM	++	++	+	+	+	+	++	+	++
			10 uM	++	++	+	+	+	+	++	+	++
		6 days	ctrl	++	+	+	-	+	-	++	-	++
			1 uM	++	++	+	+	+	++	++	++	++
			10 uM	++	++	+	+	+	++	++	++	++
	Mel 195	4 pulses	ctrl	-	-	-	-	-	-	-	-	-
			1 uM	++	++	+	+	+	+	++	++	++
			10 uM	++	++	+	+	+	+	++	++	++
		6 days	ctrl	-	-	-	-	-	-	-	-	-
			1 uM	+	++	+	+	+	+	++	++	++
			10 uM	++	++	+	++	+	+	++	++	++
Sarcoma	MG-63	4 pulses	ctrl	-	-	-	-	-	-	-	-	-
			1 uM	-	++	+	++	+	++	++	++	++
			10 uM	+	++	+	++	+	++	++	++	++
		6 days	ctrl	-	-	-	-	-	-	-	-	-
			1 uM	++	++	+	++	+	++	++	++	++
			10 uM	++	++	+	++	+	++	++	++	++
	SK-LMS-1	4 pulses	ctrl	-	-	-	-	-	-	+	-	++
			1 uM	++	++	+	++	+	++	++	++	++
			10 uM	++	++	+	++	+	++	++	++	++
		6 days	ctrl	-	-	-	-	-	-	+	-	++
			1 uM	++	++	+	++	+	++	++	++	++
			10 uM	++	++	+	+	+	++	++	++	++
Renal cell carcinoma	MZ-1257	4 pulses	ctrl	-	-	-	-	-	-	-	-	-
			1 uM	++	++	-	++	+	+	++	++	++
			10 uM	++	++	+	+	+	++	++	++	++
		6 days	ctrl	-	-	-	-	-	-	-	-	-
			1 uM	++	++	+	++	+	+	+	++	++
			10 uM	++	++	+	++	+	+	+	++	++
	LE-9104	4 pulses	ctrl	-	-	-	-	-	-	-	-	-
			1 uM	+	+	-	++	-	++	+	+	++
			10 uM	+	+	-	++	+	++	+	+	++
		6 days	ctrl	-	-	-	-	-	-	-	-	-
			1 uM	+	++	+	++	+	++	+	++	++
			10 uM	+	++	+	++	+	++	+	++	++
Mesothelioma	MES-1	4 pulses	ctrl	-	-	-	-	-	-	-	-	-
			1 uM	+	++	-	++	+	++	+	++	++
			10 uM	+	++	-	+	+	++	+	++	++
		6 days	ctrl	-	-	-	-	-	-	-	-	-
			1 uM	++	++	-	++	+	++	++	++	++
			10 uM	++	++	-	++	+	++	++	++	++
	MPP-89	4 pulses	ctrl	+	+	+	-	-	-	-	-	-
			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 (1mM and 10mM) 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 housekeeping gene b-actin. Intensity of RT-PCR products: -, not detectable; +, weak; ++, strong.

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

CONCLUSIONS

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

Specifically:

- SGI-110 induced the expression of all CTA in solid malignancies of different histotypes

- SGI-110 strongly up-regulated the constitutive levels of CTA expression in neoplastic cells of all investigated solid malignancies

- SGI-110 up-regulated the expression of HLA class I antigens, HLA-A2 allospecificity and of the co-stimulatory molecule ICAM-1

Altogether these findings demonstrate that SGI-110 is an attractive therapeutic agent to comprehensively increase immunogenicity and immune recognition of neoplastic cells from solid malignancies of different histotypes. These data provide the scientific rationale for the clinical development of SGI-110 as immunomodulating agent, to be utilized alone or in combination with CTA-based vaccines, for the treatment of cancer patients.