Epigenetic immunomodulation by SGI-110 combined with immune check-point blockade as a new therapeutic strategy

Abstract # 134

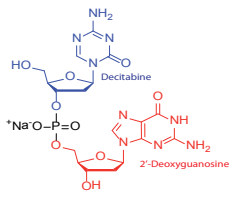
Alessia Covre[1,2], Carolina Fazio[1,2], Hugues JMG Nicolay[2], Pier Giorgio Natali[3], Pietro Taverna[4], Mohammad Azab[4], Sandra Coral[1,2] and Michele Maio[1]

[1]Medical Oncology and Immunotherapy, Department of Oncology, University Hospital of Siena, Istituto Toscano Tumori, Siena, Italy; [2]Epigen Therapeutics S.r.l., Pordenone, Italy; [3]Laboratory CINBO, University of Chieti, Italy; [4]Astex Pharmaceuticals Inc. Dublin, CA, USA

Abstract

Background: SGI-110 is a dinucleotide of decitabine (DAC) and deoxyguanosine formulated as a small volume SQ injection that extends DAC exposure compared to DAC IV. Our *in vitro* and *in vivo* evidence identified a strong immunomodulatory activity of SGI-110 on human cancer cells of different histotype and on human melanoma xenografts. We also showed a remarkable anti-tumor effect once combined with anti-CTLA-4 mAb in a syngeneic mouse model. In this study we evaluated the contribution of anti-tumor immune responses in the reduction of tumor growth achieved by this therapeutic combination.

SGI-110



SGI-110 (Astex Pharmaceuticals) is a dinucleotide of decitabine (blu) and deoxyguanosine (red) formulated as a low volume and pharmaceutically stable SQ injection allowing more extended decitabine exposure than decitabine IV injection.

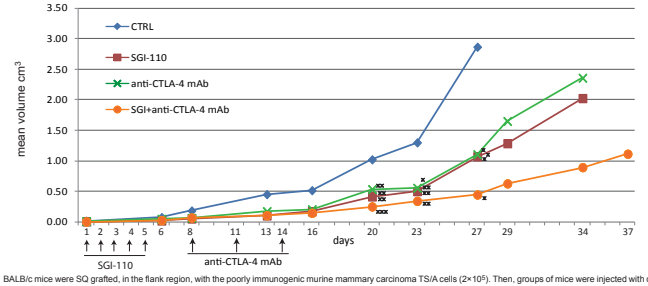
Materials and Methods: The mammary carcinoma cells TS/A (2×10^5) were implanted SQ in Balb/c mice. Animals bearing palpable tumors were treated with 3mg/kg of SGI-110 (days 1-5), alone or combined with 100µg of anti-murine CTLA-4 mAb (days 8, 11 and 14). The immunomodulatory effects of treatment were studied on tumor and normal tissues by RT-PCR and by quantitative RT-PCR analysis of murine CTA expression. Immunohistochemical evaluation of tumor infiltrating immune cells was also performed. P1A-promoter methylation was tested by quantitative Methylation-Specific PCR (qMSP) on genomic DNA from tumor tissues.

Results: The expression of P1A and Mage-a family members was induced in tumor tissues from animals treated with SGI-110, either alone or in combination with anti-CTLA-4 mAb, but not from mice treated with anti-CTLA-4 mAb alone. Levels of P1A-specific mRNA were similar in tumors from mice treated with SGI-110 alone ($3.18\text{x}10^{-04}$ P1A/β-actin molecules) or combined with anti-CTLA-4 mAb ($1.18\text{x}10^{-04}$ P1A/β-actin molecules). The DNA hypomethylating effect of SGI-110 was sustained by the reduction of P1A promoter methylation in cancer tissues from SGI-110- (16%) and combination- (7%) treated mice *vs* control. Epigenetic remodelling was restricted to tumor tissue leaving almost unaltered normal ones. The contribution of immune cells in the therapeutic effectiveness of treatment was supported by the increased frequency of tumor infiltrating CD3+ cells in the combination arm (11±1.9) vs control (3.7±1.4) or single agent, anti-CTLA-4 mAb (3±1.1) and SGI-110 (4.1±1.7), treated mice.

Conclusion: These data highlight the involvement of the immune system in the anti-tumor effect of SGI-110 combined with CTLA-4 blockade. Based on these experimental evidences, an exploratory phase I trial to evaluate safety and immunobiologic activities of the combination is being activated in advanced melanoma patients.

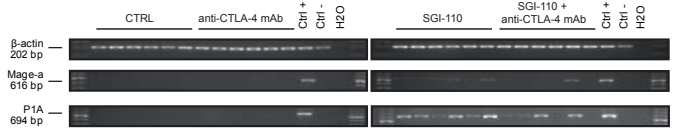
Results

Fig.1 Anti-tumor activity of SGI-110 combined with anti-CTLA-4 mAb



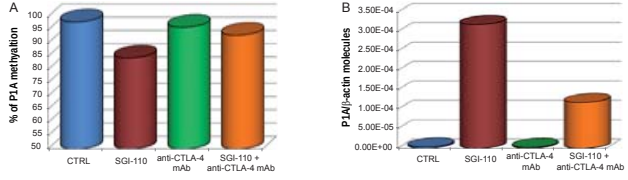
BALB/c mice were SQ grafted, in the flank region, with the poorly immunogenic murine mammary carcinoma TS/A cells (2×10^5). Then, groups of mice were injected with diluent solution for control, 3mg/kg SGI-110, 100µg anti CTLA-4 mAb or the combination of SGI-110 with anti-CTLA-4 mAb. To evaluate the effectiveness of therapies, tumor volumes (TV) from mice were measured periodically, all along the treatment, by using a caliper and calculated as follows: $TV=LD^2/2$ (in which L is the longest diameter and D the shortest one). Tumor mean values for each group are reported. Vertical arrows indicate days of different treatments. *, ps0,05 ; **, ps0,01; ***, ps0,001 *vs* TV of control group.

Fig 2. RT-PCR analysis of murine CTA expression by SGI-110 combined with anti-CTLA-4 mAb



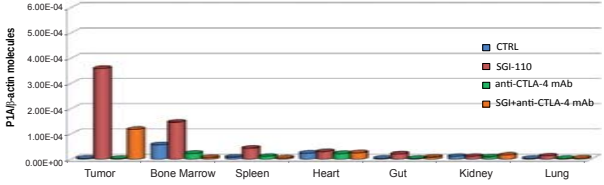
Total RNA was extracted from tumors excised from TS/A grafted mice treated with: diluent solution, as control group (CTRL), SGI-110, anti-CTLA-4 mAb, or the combination of SGI-110 with anti-CTLA-4 mAb. RT-PCR analysis was performed using P1A-, Mage-a- or β-actin-specific primer pairs. Total RNA from mouse testis and splenocytes was utilized as positive (ctrl +) or negative (ctrl -) controls respectively.

Fig 3. Regulation of P1A expression by SGI-110 combined with anti-CTLA-4 mAb



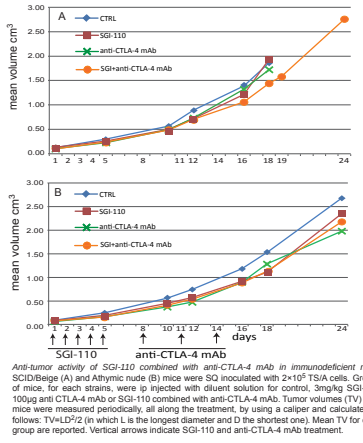
A week after the end of treatment, TS/A tumors were excised from control mice (blu) and mice treated with SGI-110 (red), anti-CTLA-4 mAb (green) or the combination of SGI-110 with anti-CTLA-4 mAb (orange) and processed to extract genomic DNA and RNA. A) Real-time qMSP analyses of P1A promoter were performed on bisulfite-modified genomic DNA using methylated- or unmethylated-specific primer pairs. Data are reported as percentage of methylation that was defined as the ratio between methylated molecules and the sum of methylated and unmethylated molecules. B) TaqMan quantitative RT-PCR reactions were performed on retrotranscribed total RNA, utilizing P1A- and mouse β-actin-specific primers. CTA expression was normalized to the expression of the β-actin gene. Values are reported as P1A molecules/β-actin molecules, on a linear scale.

Fig 4. Regulation of P1A expression by SGI-110 combined with anti-CTLA-4 mAb in tumor and normal tissues

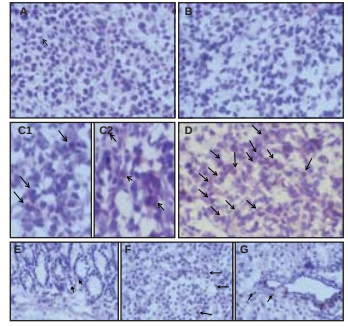


A week after the end of treatment, TS/A tumors and normal tissues were excised from control mice (blu) and mice treated with SGI-110 (red), anti-CTLA-4 mAb (green) or the combination of SGI-110 with anti-CTLA-4 mAb (orange) and processed to extract RNA. TaqMan quantitative RT-PCR reactions were performed on retrotranscribed total RNA, utilizing P1A- and mouse β-actin-specific primers. CTA expression was normalized to the expression of the β-actin gene. Values are reported as P1A molecules/β-actin molecules, on a linear scale.

Fig 5. Contribution of immune response in the anti-tumor activity of SGI-110 combined with anti-CTLA-4 mAb



Anti-tumor activity of SGI-110 combined with anti-CTLA-4 mAb in immunodeficient mice: SCID/Beige (A) and Athymic nude (B) mice were SQ inoculated with 2×10^5 TS/A cells. Groups of mice, for each strains, were ip injected with diluent solution for control, 3mg/kg SGI-110, 100µg anti CTLA-4 mAb or SGI-110 combined with anti-CTLA-4 mAb. Tumor volumes (TV) from mice were measured periodically, all along the treatment, by using a caliper and calculated as follows: $TV=LD^2/2$ (in which L is the longest diameter and D the shortest one). Mean TV for each group are reported. Vertical arrows indicate SGI-110 and anti-CTLA-4 mAb treatment.



Immunohistochemical analysis of T cell infiltrating in tumor and normal tissues: BALB/c mice were SQ inoculated with 2×10^5 TS/A cells. Groups of mice were ip injected with diluent solution, SGI-110, anti-CTLA-4 mAb and SGI-110 combined with anti CTLA-4 mAb. One week after the end of treatment, neoplastic and normal tissues were excised and processed for CD3+ infiltrating staining. Representative results from investigated mice are reported.
CD3+ staining of tumors from mice treated with diluent solution (A), SGI-110 (B), anti CTLA-4 mAb (C1, C2) and SGI-110 combined with anti-CTLA-4 mAb (D);
CD3+ staining of large intestine (E), renal cortex (F) and liver parenchyma (G) from mice treated with SGI-110 combined with anti CTLA-4 mAb.

Conclusions

-SGI-110 treatment induces a positive modulation of CTA-profile in poorly immunogenic tumor grafts, and is sustained by specific promoter demethylation;

-Modulation of CTA expression by SGI-110 is preferentially directed to tumor tissue, without significantly affecting normal tissue;

-The improved anti-tumor activity of SGI-110 combined with anti-CTLA-4 mAb is mediated by cellular immunity;

-Cellular immunity mediated by the combination regimen is preferentially directed to tumor tissue, without significantly affecting normal ones.

The immunomodulatory properties of SGI-110 make it an attractive therapeutic agent to improve the anti-tumor activity of anti-CTLA-4 mAb and to increase the partial therapeutic efficacy of immunostimulatory mAb to poorly immunogenic tumors.

A phase I-II clinical study that will first test SGI-110 epigenetic priming followed by CTLA-4 blockade in metastatic cutaneous melanoma patients is planned.

EORTC-NCI-AACR Symposium on Molecular Targets and Cancer Therapeutics. 18-21 November 2014, Barcelona

Study supported by Astex Pharmaceuticals