

IN VIVO IMMUNOMODULATORY ACTIVITY OF SGI-110, A SECOND GENERATION HYPOMETHYLATING AGENT, IN HEMATOLOGIC MALIGNANCIES

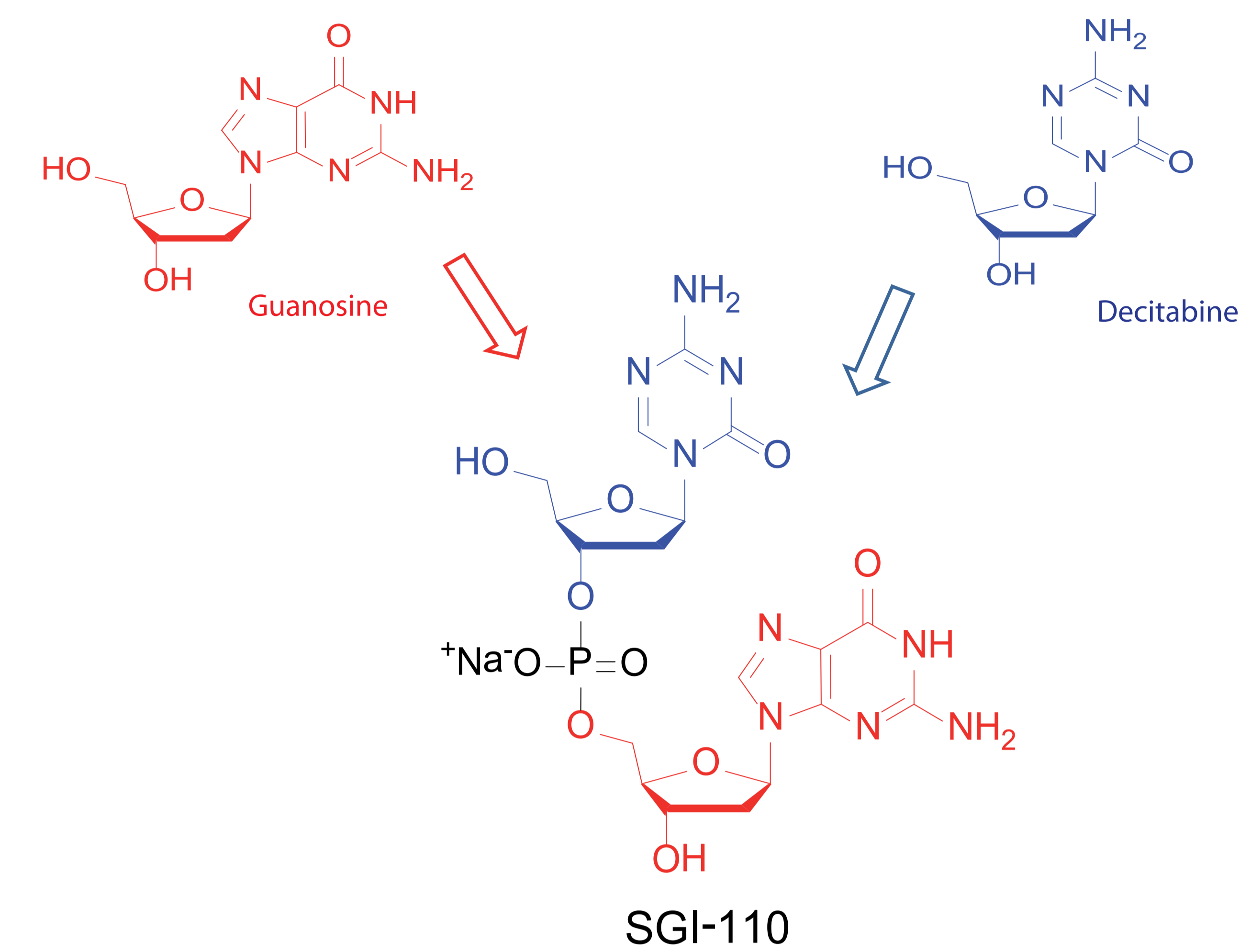
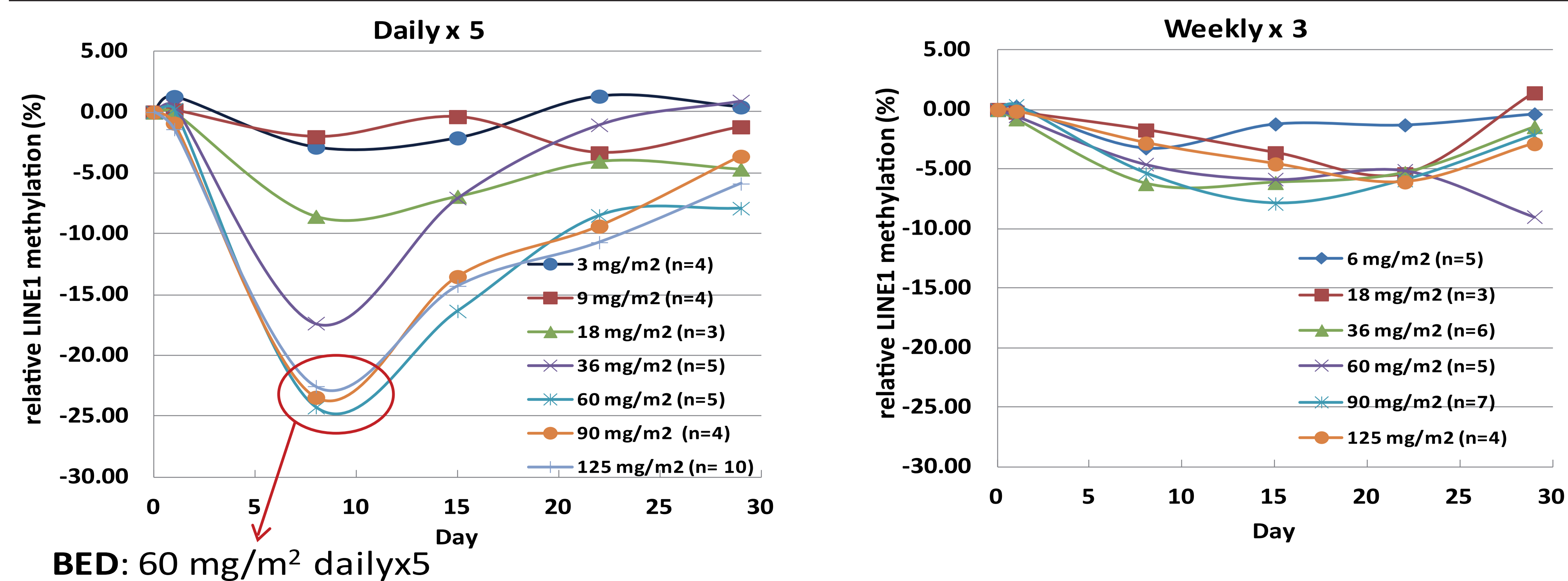
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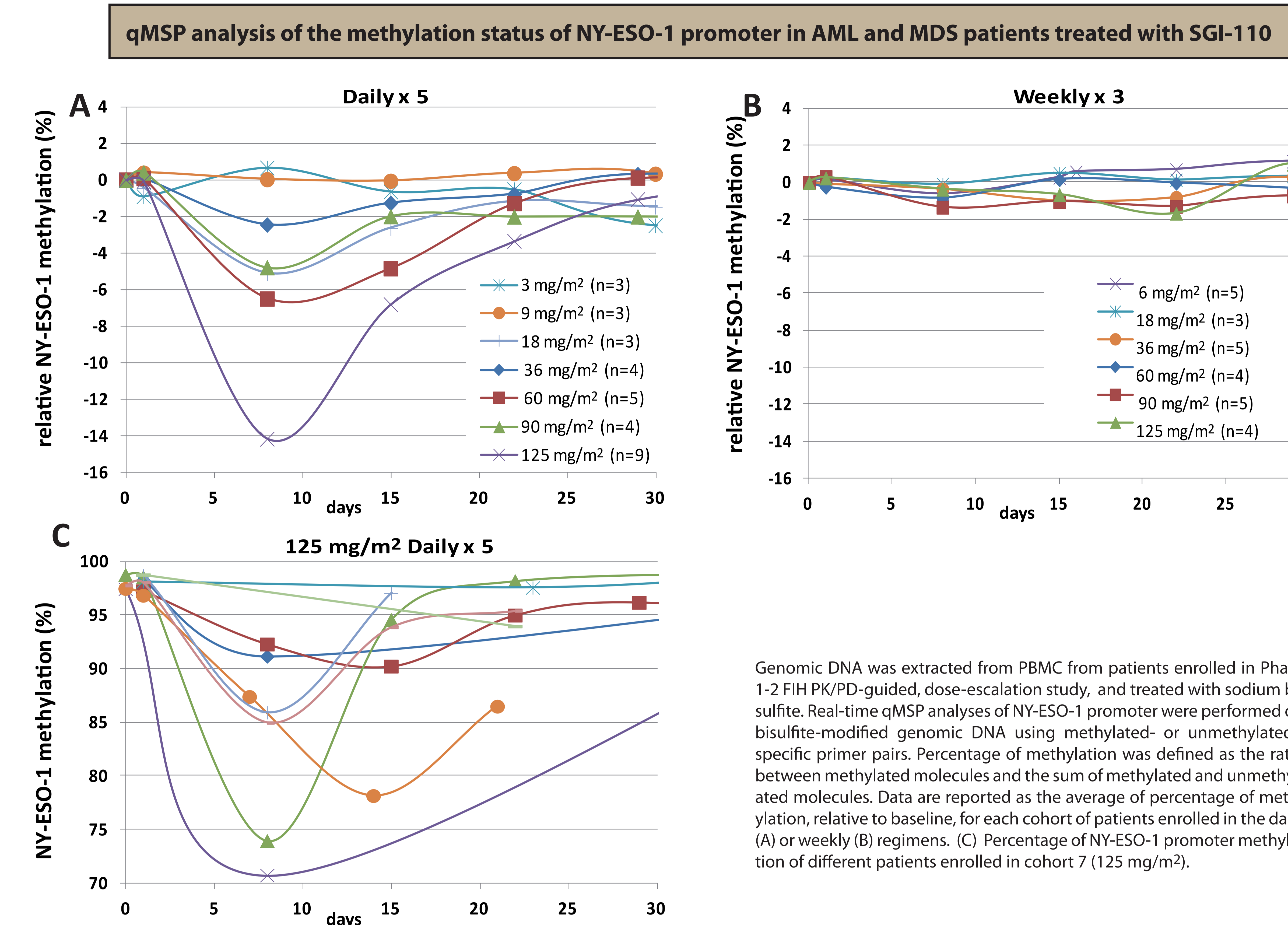
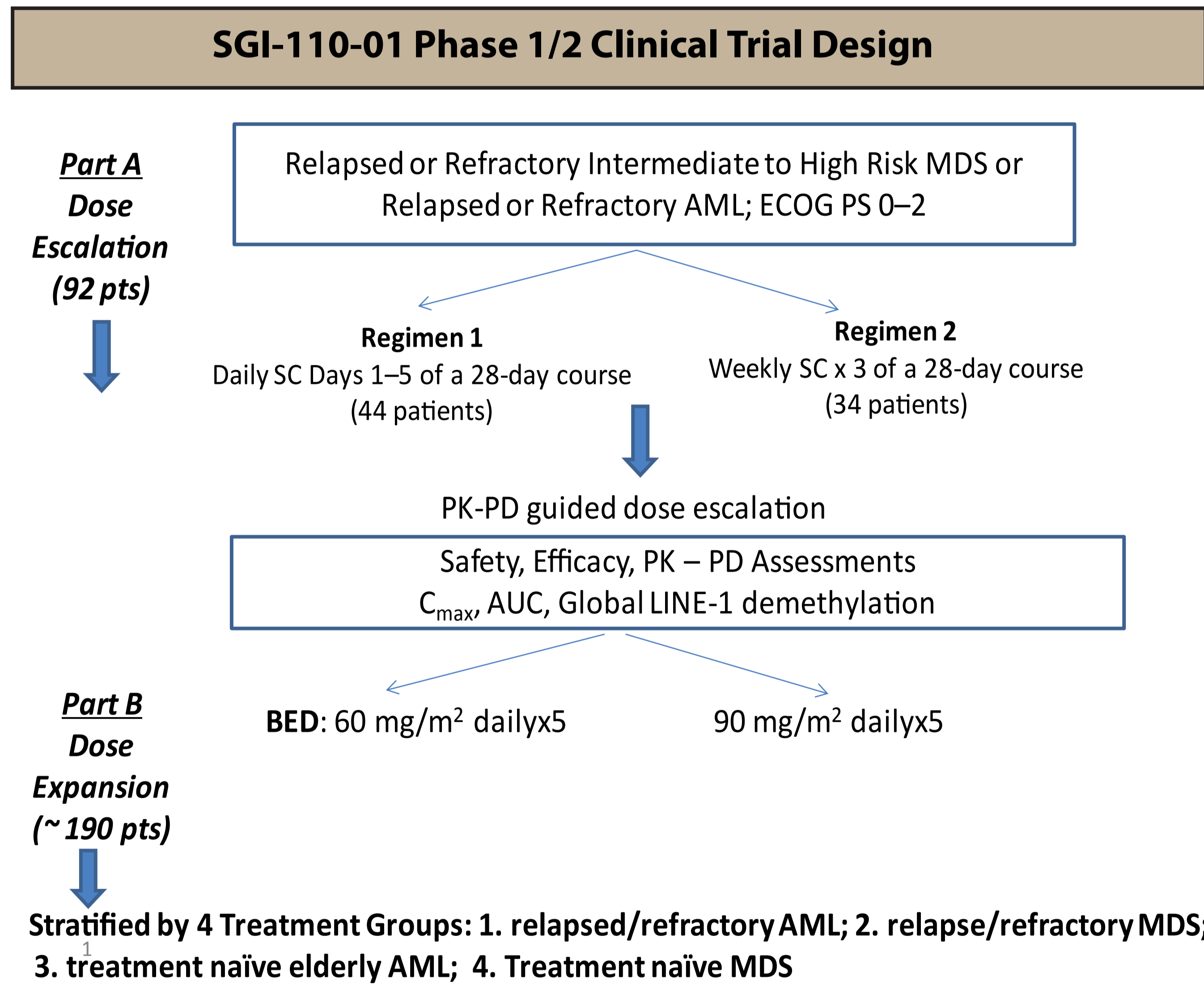
Abstract

Second generation DNA hypomethylating agents own pleiotropic properties supporting their clinical application in cancer therapy. Among these, SGI-110 is a dinucleotide synthesized as a deoxyguanosine combined with decitabine to protect the latter from the cytidine deaminase inactivation, thus increasing its in vivo exposure and efficacy. A randomized Phase 1-2 FIH PK/PD-guided, dose-escalation study is being conducted on MDS or AML patients, to determine safety and tolerability of SGI-110 and to establish maximum tolerated dose and biologically effective dose (BED). Subjects were randomized to one of two SQ regimens (daily x 5 or once weekly x 3, both administered in 28-day courses). The PK profile showed efficient conversion of SGI-110 to decitabine, longer apparent half-life and lower C_{max} than predicted equivalent decitabine IV doses. A Dose-related LINE-1 hypomethylation was observed in patients treated with the daily regimen; a plateau in maximum average hypomethylation was evident at higher daily doses (60-125 mg/m²) and therefore the BED for the dailyx5 schedule was established at 60 mg/m². Here, we investigated the immunomodulatory activity of in vivo administration of SGI-110 in enrolled AML and MDS patients. The hypomethylating properties of in vivo SGI-110 on Cancer Testis Antigen (CTA)-specific promoters and the resulting effects on the levels of CTA expression were analyzed in PBMC from patients. Quantitative methylation-specific PCR analyses showed that the daily regimen reduced the constitutive methylation levels in promoters of investigated CTA (i.e., NY-ESO-1 and MAGE-A1) in a dose-dependent manner. The major hypomethylation was observed in patients treated with the highest dose of SGI-110 (Cohort 7, 125 mg/m²), showing an average percentage of hypomethylation of 14.2 for NY-ESO-1-promoter (range 5.0 to 26.7) and 16.3 for MAGE-A1-promoter (range 3.7 to 35.6). In patients treated with the highest doses (Cohorts 4-6, 60 mg/m² – 125 mg/m²) of the weekly regimen, it was 4.4 (range 0.4 to 6.2) and 2.5 (range 0.1 to 9.9) for NY-ESO-1- and MAGE-A1-promoter, respectively. The effects of the hypomethylating activity of in vivo SGI-110 on the constitutive CTA expression were evaluated by quantitative real-time RT-PCR. The induction/up-regulation of NY-ESO-1, MAGE-A1, MAGE-A3 expression were transient and observed in 11/18 (NY-ESO-1/ β -actin molecules range 10⁻⁵ to 1.4*10⁻³), 5/18 (MAGE-A1/ β -actin molecules range 10⁻⁵ to 8.2*10⁻⁵) and 6/18 (MAGE-A3/ β -actin molecules range 10⁻⁵ to 2.4*10⁻³) patients treated with the highest doses of daily regimen (Cohorts 4-7). In weekly regimen the induction/up-regulation were detected in 10/14 (NY-ESO-1/ β -actin molecules range 10⁻⁵ to 1.8*10⁻³), 1/14 (1.3*10⁻⁵ MAGE-A1/ β -actin molecules) and 11/14 (MAGE-A3/ β -actin molecules range 10⁻⁵ to 5.9*10⁻³) patients treated with the highest doses (cohorts 4-6). Considering the biologically effective doses (Cohorts 5-7), the induction/up-regulation of NY-ESO-1, MAGE-A1, MAGE-A3 expression was observed in 9/15, 4/15 and 5/15 patients enrolled in daily regimens, respectively. These newly identified immunomodulatory properties of SGI-110, combined with its favorable pharmacologic and pharmacokinetic features, define SGI-110 as a potentially useful agent to implement new and more effective combined chemo-immunotherapeutic approaches in patients with hematologic malignancies.

LINE1 Methylation Analysis in blood DNA from AML and MDS Patients enrolled in study SGI-110-01
(data from Issa JP and Chung W, Temple Univ. and presented in Kantarjian HM et al, Abstracts of the 54th ASH Annual Meeting, Blood 120 (21), 2012 abstract 414)

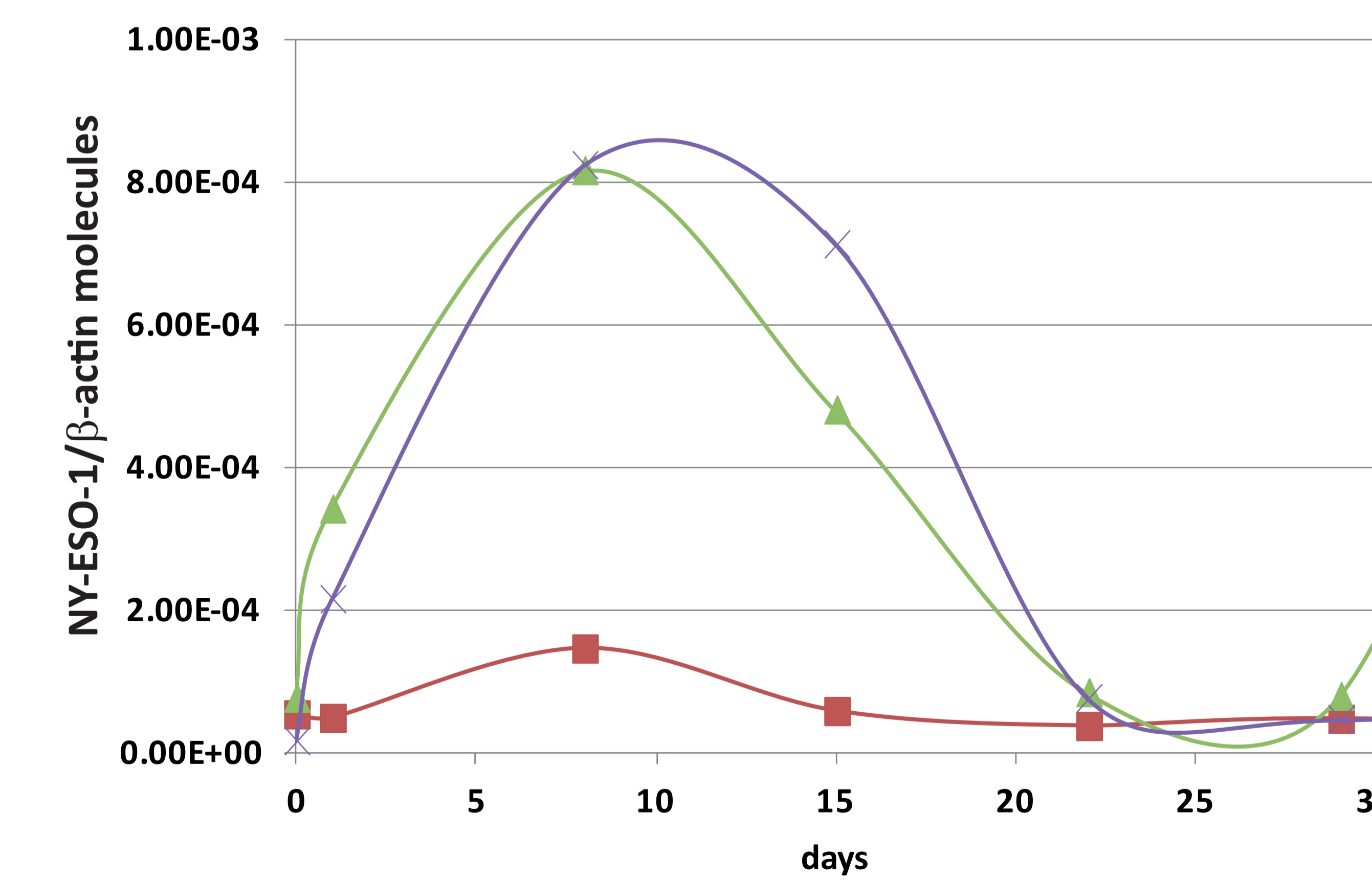


SGI-110 (Astex Pharmaceuticals) is a dinucleotide of decitabine (blu) and deoxyguanosine (red) formulated as a low volume and pharmaceutically stable SQ injection allowing more extended decitabine exposure than decitabine IV injection.



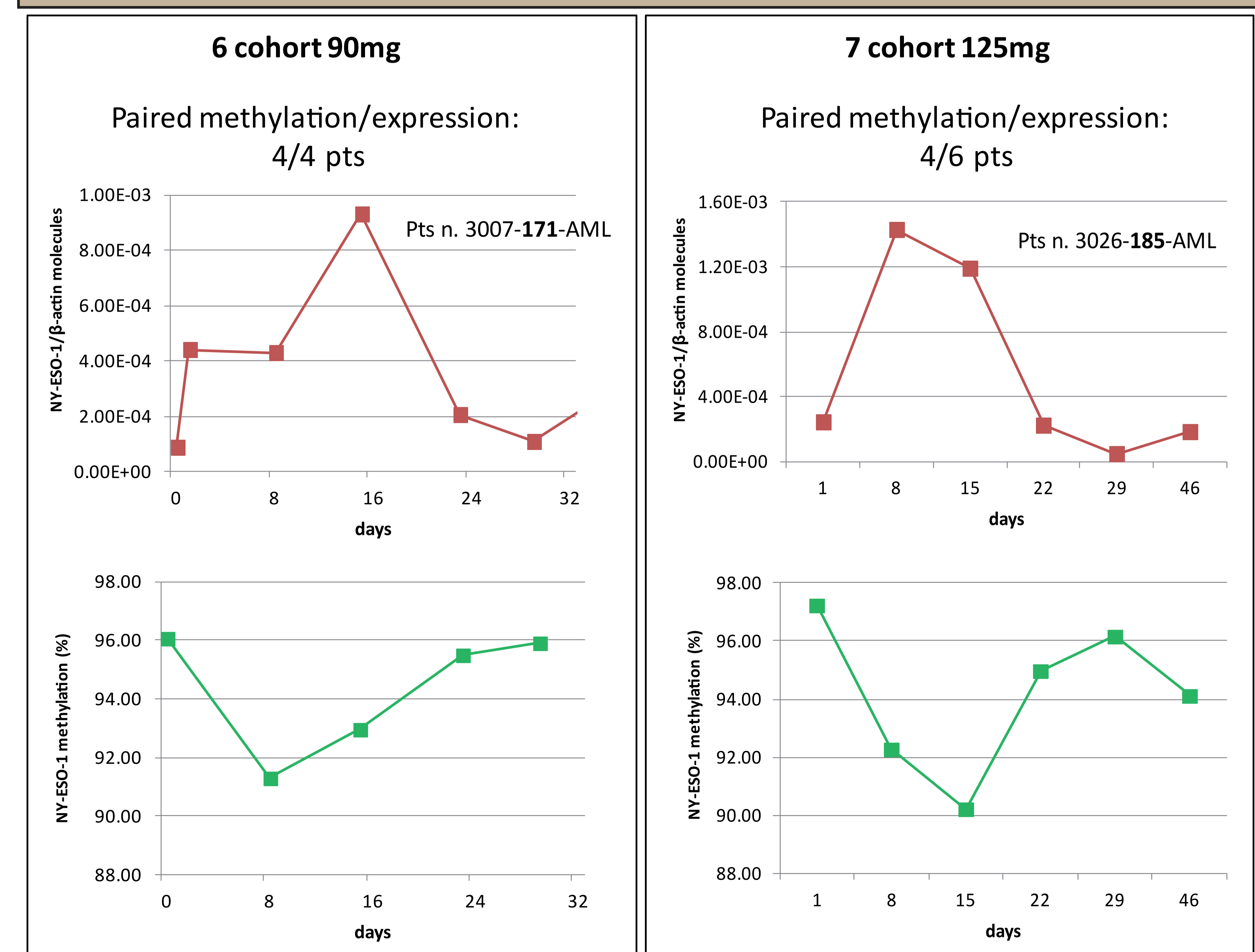
Genomic DNA was extracted from PBMC from patients enrolled in Phase 1-2 FIH PK/PD-guided, dose-escalation study, and treated with sodium bisulfite. Real-time qMSP analyses of NY-ESO-1 promoter were performed on bisulfite-modified genomic DNA using methylated- or unmethylated-specific primer pairs. Percentage of methylation was defined as the ratio between methylated molecules and the sum of methylated and unmethylated molecules. Data are reported as the average of percentage of methylation, relative to baseline, for each cohort of patients enrolled in the daily (A) or weekly (B) regimens. (C) Percentage of NY-ESO-1 promoter methylation of different patients enrolled in cohort 7 (125 mg/m²).

Quantitative RT-PCR analysis of NY-ESO-1 expression in PBMC from AML and MDS patients treated with SGI-110.

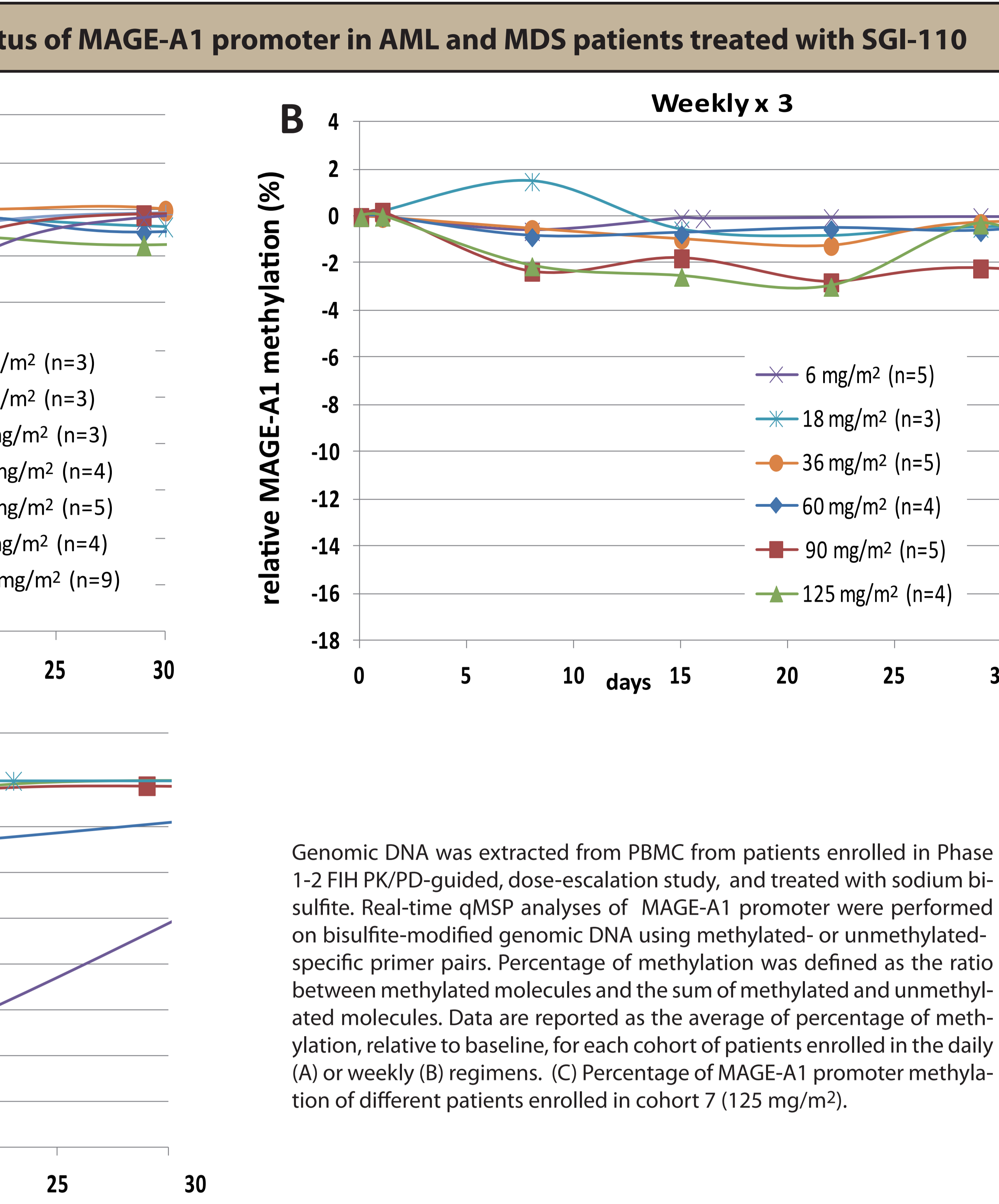


Total RNA was extracted from PBMC from patients enrolled in cohorts 5 (red), 6 (green) and 7 (violet) of Phase 1-2 FIH PK/PD-guided, dose-escalation study. TaqMan quantitative RT-PCR reactions were performed on retrotranscribed total RNA, utilizing NY-ESO-1- and β -actin-specific primers. CTA expression was normalized to the expression of the β -actin gene and data shown are reported as the average of NY-ESO-1 expression.

Comparison of quantitative RT-PCR and qMSP analyses of NY-ESO-1 in AML and MDS patients treated with SGI-110



Available paired RNA and bisulfite modified DNA samples were analyzed by quantitative real-time analyses for NY-ESO-1 expression (red line) and NY-ESO-1 promoter methylation (green line), at different time points. In 4 out of 4 patients from cohort 6 (90 mg/m²) and 4 out of 6 patients from cohort 7 (125 mg/m²) of daily regimens, NY-ESO-1 expression levels were directly associated to NY-ESO-1 promoter hypomethylation. Data shown are relative to one representative patient of each cohort.



Genomic DNA was extracted from PBMC from patients enrolled in Phase 1-2 FIH PK/PD-guided, dose-escalation study, and treated with sodium bisulfite. Real-time qMSP analyses of MAGE-A1 promoter were performed on bisulfite-modified genomic DNA using methylated- or unmethylated-specific primer pairs. Percentage of methylation was defined as the ratio between methylated molecules and the sum of methylated and unmethylated molecules. Data are reported as the average of percentage of methylation, relative to baseline, for each cohort of patients enrolled in the daily (A) or weekly (B) regimens. (C) Percentage of MAGE-A1 promoter methylation of different patients enrolled in cohort 7 (125 mg/m²).

Conclusions:

This work has identified a novel property of SGI-110 to re-express immune-biologically relevant antigens in AML and MDS patients;

SGI-110 induces global genomic DNA demethylation in PBMC from AML and MDS patients, in a dose-dependent manner;

SGI-110 reduces the constitutive methylation levels of NY-ESO-1 and MAGE-A1 promoters in PBMC from AML and MDS patients, in a dose-dependent manner;

SGI-110 induces NY-ESO-1, MAGE-A1 and MAGE-A3 expression in PBMC from AML and MDS patients;
The daily x5 treatment schedule was more effective as compared to the weekly x3 treatment schedule.

Altogether these findings identify novel immunomodulatory properties of SGI-110, providing the scientific rationale for its clinical development to implement new combined chemo-immunotherapeutic approaches for the treatment of cancer patients.

Study supported by Astex Pharmaceutical

