SGI-110, a novel 5-aza-2’-deoxycytidine containing dinucleotide, induces Cancer Germline (CG) antigen expression in Acute Myeloid Leukemia cells

Abstract

Introduction: CG antigens are genes whose normal expression is limited to the adult tests and embryonic ovary. Aberrant expression of these genes is reported in solid cancers where they are known to be immunogenic; CG targeted cancer vaccines are in clinical development for cancers with endogenous gene expression. The CG genes NY-ESO1 and MAGEA are silent in adult tissues due to CpG island hypermethylation. Treatment of non-expressing cancer cell lines in vitro with hypomethylating drugs (HMAs) like Decitabine (DAC) and Azacytidine (AZA), both FDA approved for patients with myeloid cancers, can induce CG gene expression. We hypothesized that SGI-110 (Astex Pharmaceuticals, Inc.), a second generation hypomethylating agent with superior pharmacokinetics, would induce CG antigen genes in AML cells in vitro.

Methods: HL60 and U937 cells were cultured in vitro using standard techniques and treated with phosphate buffered saline, 0.1, 0.5 μM SGI-110, 0.5μM DAC or 2μM AZA. Results are pooled from a minimum of three replicates. Viability by trypan blue exclusion and DNA, RNA and protein were obtained on day 5; DNA was bisulfite converted. Methylation (LINE-1, NY-ESO1, by pyrosequencing and MAGEA3/6 by methylation specific PCR), mRNA expression (by RT-PCR), and protein expression (by Western blot) were assessed.

Results: Treatment with SGI-110 produced dose-dependent cytotoxicity. Viability at 0.1, 1 and 5μM SGI-110 doses was 47, 11, 8% in HL60 cells and 91, 41, 22% in U937 cells. Viability at the 1μM SGI-110 dose was comparable to 0.5μM DAC (11 and 38%) and less than 2μM AZA (51 and 30% respectively) in both cell lines. Baseline LINE-1 elements were 80% methylated in HL60 cells and 68% methylated in U937 cells. In both lines 1μM SGI-110 produced optimal LINE-1 demethylation, comparable to DAC. At baseline both NY-ESO1 and MAGEA3/6 promoters were densely hypermethylated in both HL60 and U937 and neither expressed mRNA; treatment with AZA, DAC and 1μM SGI-110 hypomethylated both genes and induced mRNA expression. No mRNA expression of CG genes was detected at the 0.1μM SGI-110 dose. NY-ESO1 and MAGEA protein expression were induced following 1 and 5μM doses of SGI-110 and DAC in both cell lines; in U937 cells AZA induced MAGEA but not NY-ESO1.

Conclusions: SGI-110 induces global and gene specific hypomethylation as well as mRNA and protein expression of the CG genes NY-ESO1 and MAGEA3/6 in AML cells. This protein induction in vitro is at least as potent as that observed following AZA and DAC treatment. Induction of anti-MAGE specific T-cells has been reported in myeloid cancer patients receiving AZA + Vorinostat, suggesting that induced CG specific immunity may contribute to responses to HMAs (Goodyear et al. Blood. 2010:116:1908). These data suggest that the combination of HMAs such as SGI-110 with CG vaccination may be of value in myeloid malignancy.

Cell Viability and Baseline Global Methylation

Figure 1: A) Viability of U937 and HL60 cell lines following in vitro exposure to HMAs. At the highest dose of SGI-110 viability was low. B) Changes in LINE-1 methylation as a surrogate marker for global methylation following exposure to HMAs in vitro. At the highest dose of SGI-110 excess toxicity prevented enhanced hypomethylation.

CG Gene Specific Methylation Changes

Figure 2: A) NY-ESO1 methylation, assessed by bisulfite pyrosequencing, declines in both U937 (blue) and HL60 (green) cells following in vitro exposure to HMAs. B) MAGEA3/6 is densely hypermethylated in both U937 (above) and HL60 (below) cells at baseline by PCR, in vitro exposure to HMAs induces an unmethylated band suggesting partial hypomethylation of the promoter. For both genes, excess toxicity at the 5μM SGI-110 dose limited hypomethylation.

Induced mRNA expression of NY-ESO1 and MAGEA 3/6

Figure 3: mRNA is induced at comparable levels in both U937 (blue) and HL60 (green) cells following in vitro exposure to HMAs for both A) NY-ESO1-1 and B) MAGEA3/6. MAGEA3/6 induction in HL60 cells is markedly higher than in U937 cells by mRNA, thus data are presented on a log2 scale for clarity.

Induced protein expression of NY-ESO1 and MAGEA

Figure 4: A) U937 cells treated with decitabine or SGI-110 in vitro induce expression of NY-ESO1 protein. 1μM SGI-110 appears to be superior to both 0.5μM decitabine and 5μM SGI-110 at inducing protein expression B) HL60 cells treated in vitro induced robust protein expression of NY-ESO1. MAGEA protein expression was induced at two of the three tested doses of SGI-110 as well as with decitabine and azacitidine.

Hypomethylation and gene re-expression in HMA treated U937 tumor bearing SCID mice

Figure 5: SCID mice bearing U937 tumors were treated for five days with the HMAs described, harvested on day 7. A) LINE-1 and NY-ESO1-1 methylation changes were similar to those seen in vitro. B) MAGEA3/6 hypomethylation was induced. C) NY-ESO1-1 and MAGEA3/6 mRNA were induced. D) Both NY-ESO1-1 and MAGEA protein were induced in the tumors from treated animals with all dosing schedules.

Conclusions

- SGI-110 induces both global and gene specific hypomethylation as well as mRNA and protein expression of the CG genes NY-ESO1 and MAGEA3/6 in AML cells in vitro and in vivo in a SCID mouse model at doses comparable to those required for decitabine induction of the same genes.
- Excessive toxicity at higher doses limits hypomethylation and gene re-expression.
- HMAs such as SGI-110 may enable combination strategies with CG specific vaccination in patients with myeloid malignancy.