Identification of novel biomarker candidates for the treatment outcome prediction of SGI-110, a novel hypomethylating agent, in AML patients using Differential Methylation Hybridization (DMH) Technology

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Introduction

SGI-110 is a dinucleotide of decitabine (DAC) and deoxyguanosine designed to be more stable than decitabine to deamination by cytidine deaminase, thus offering a promising alternative to current approved HMAs. In our preclinical experiments, SGI-110 affected the clonogenic survival of malignant cells in various cancers, induced gene-specific as well as global LINE-1 (Long Interspersed Nucleotide Element 1) DNA hypomethylation in cell lines and xenograft models.

Here, we have identified novel predictive DNA-methylation biomarker candidates using an approach based on DMH profiling data of the NCI-60 cell line (Fassbender A et al, Methods Mol Biol 2010). Cell line stratification was based on EC_{50} values from Colony Forming Assays and LINE-1Methylation measurements.

Both data sets were used to classify the cell lines into SGI-110 sensitive and resistant groups and then used to generate three marker candidate sets with 249 genomic marker candidate sites in total that may be used for further assessment and classification, and might serve as a first step towards a predictive test.

Differential Methylation Hybridization (DMH) Technology as a tool to identify novel biomarker candidates

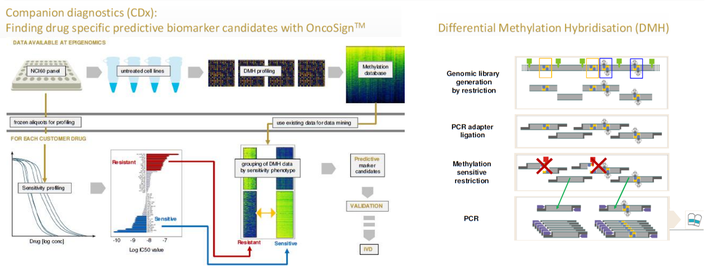
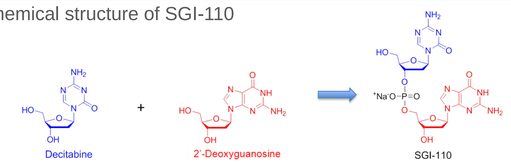


Figure 1: DMH is a discovery technology for unbiased profiling of methylated loci throughout the genome. Epigenomics' proprietary DMH 2nd generation microarray was used to generate the Oncosign™ data set which covers about 50,000 genomic loci. This DNA-methylation dataset includes the genome-wide profiles from untreated NCI-60 cell lines and was used to search for marker candidates based on cell line sensitivity to SGI-110.

SGI-110 is a dinucleotide of decitabine and deoxy-guanosine that protects decitabine from deamination

Figure 2: Chemical structure of SGI-110



Cancer Cell Line Profiling: Colony Formation Assays and LINE-1 DNA Methylation Analysis to determine sensitivity to SGI-110

- Cells were exposed to either vehicle control only or SGI-110 treated daily for 3 days with concentrations of 1nM, 10nM, 20nM, 30nM, 100nM and 1µM SGI-110.
- On day 4 cells were transferred into MethoCult H4434 classic (StemCell Technologies) for colony cultures for an additional 12-14 days. Colony growth was analysed by a GelCount Counting System and EC_{50} values for colony growth inhibition after SGI-110 exposure was calculated from each sigmoidal dose response curve (GraphPad Prism).
- Additionally, DNA extracts were prepared to measure DNA methylation level for LINE-1 using the PyroMark CpG LINE-1 Assay on a PyroMarkQ24 system.

Cell line stratification to group SGI-110 sensitive and resistant cell lines used for DMH analysis

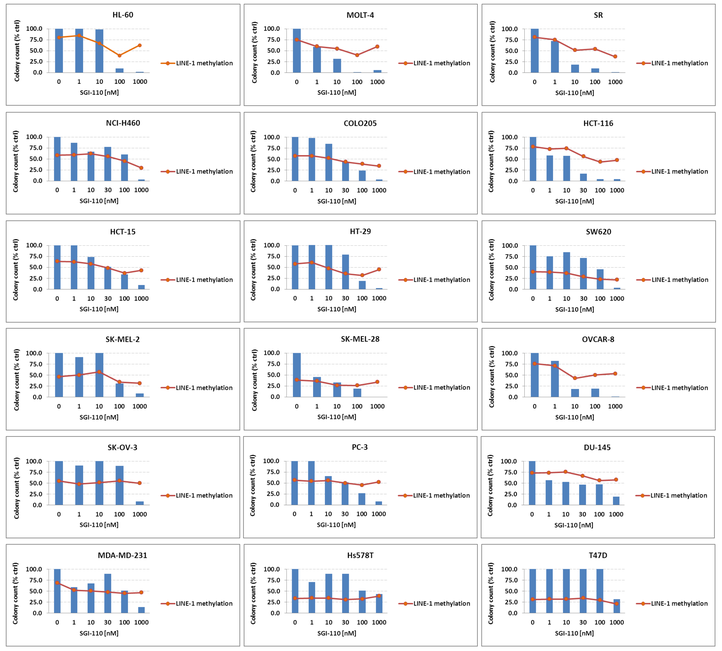


Figure 3: Clonogenic assay and LINE-1 methylation analysis of 18 cell lines from seven different cancers from the NCI-60 cell line panel. Cells were analysed for their ability to inhibit colony formation (shown in blue bars for increasing concentrations of SGI-110). As a second parameter all cell lines were also profiled for their sensitivity to LINE-1 demethylation (as shown in orange lines for each SGI-110 concentration).

Stratification of Cell Lines based on EC_{50}

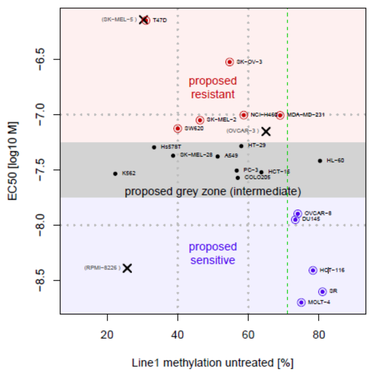


Figure 4: Cell lines plotted by EC_{50} as derived from colony forming assays of cell lines treated at different concentrations (y-axis) against LINE-1 methylation of untreated cell lines (x-axis). Cell lines excluded from the analysis were marked with crosses.

The background of the plot defines proposed EC_{50} intervals for the cell line stratification:

- light red for resistant cell lines
- light blue for sensitive cell lines
- grey for unclassified cell lines

The green vertical dotted line at 71% LINE-1 shows that all cell lines classified as sensitive have a LINE-1 methylation >73% and all classified as resistant < 70%.

Hence the classification by EC_{50} is dependent on the global DNA-Methylation.

Profiles of DHM probe sensitivities and resistance to colony formation, tumor suppressor genes and LINE-1 EC_{50} values

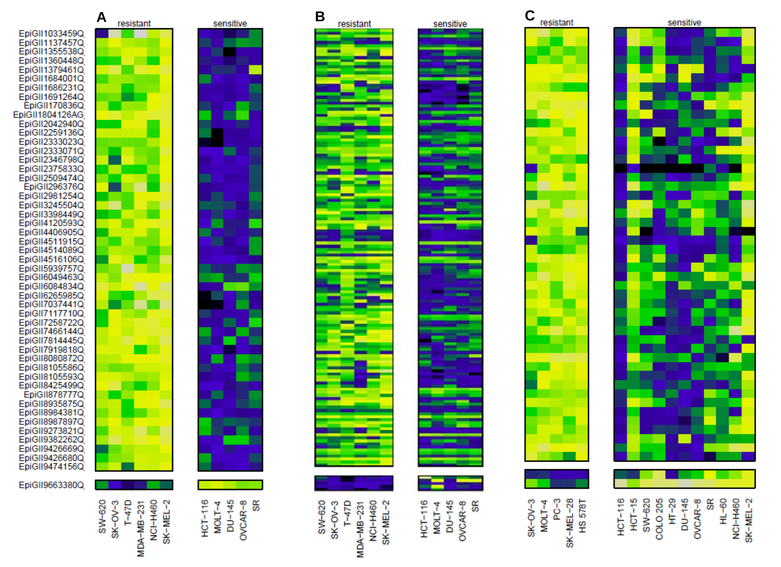


Figure 5: Coloured methylation plots for marker candidates grouped by A) SGI-110 EC_{50} colony formation data (best 50 candidates), B) Tumor Suppressor Genes from the colony formation data only (best 149 candidates) and C) LINE-1 demethylation (best 50 candidates). Colour codes methylation for fragments (rows) measured for samples (columns) from 0% over 50% and 100% to > 100% methylation in relation to a diploid genome. Y axis: DMH fragments. X axis: Cell lines grouped as named on the bottom.

Conclusions

- Cell line specific colony forming capability pre and post SGI-110 treatment was observed in the different cell lines over a wide range representing the difference in SGI-110 sensitivity
- There is a correlation between high LINE-1 methylation level with high sensitivity to SGI-110. Five cell lines classified as sensitive using EC_{50} values were among the 6 cell lines with the highest methylation >73%, whereas cell lines classified as resistant were methylated less than 70%
- The LINE-1/Oncosign comparison showed that LINE-1 methylation levels clearly indicate a good correlation to DNA-Methylation data measured with DMH from over 50k sites supporting the role of LINE-1 as useful indicator for SGI-110 sensitivity.
- As a next step 55 fragment candidates identified from the best 249 candidate markers from this analysis will be compared in 18 cancer cell lines and DNA samples derived from whole blood from AML patients that were classified into responders and non-responders after treatment with SGI-110 in our phase 1-2 study.
- These biomarker candidates may help to characterise the responsiveness of cancer patients to HMA and help to identify patient populations that preferentially respond to SGI-110.

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Poster available to download from Astex's website at www.astx.com