Epigenetic Profiling in High-Risk MDS and AML Patients Identifies Pre-Treatment Methylation Patterns that Indicate Response to Hypomethylating Agents: A Pilot Study

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BACKGROUND

- Azacitidine is a nucleoside analog that functions as a hypomethylating agent (HMA), approved for use in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML).

- However, the complete remission (CR) rate to HMA therapy is generally <20%1,2, indicating the need to more accurately identify patients most likely to respond and benefit from therapy.

OBJECTIVES

- The primary objective of this pilot study is to assess whether the presence of epigenetic DNA methylation markers in pre-treatment bone marrow myeloblasts can reliably differentiate azacitidine responders (R) from non-responders (NR).

METHODS

- A cohort of 10 patients from Moffitt Cancer Center Total Cancer Care Database and repository.

- Bone marrow samples were collected within 3 weeks prior to their treatment with azacitidine.

- This included 5 responders (R) and 5 non-responders (NR).

- Genomic DNA Extraction and Purification
- Creation of DNA fragment libraries and Whole Genome Sequencing (WGS)
- Quantification of CpG site-specific methylation load
- Compilation of methylation scores, SNPs, INDEL
- Methylation differences at CpG sites, genes, and pathways using clustering analysis

RESULTS

- Total of ~2.04 million 'CCGG' sites were analyzed and 88% (1.81M) were present in all samples.

- Comparison of the methylation signatures of individual genes reveals potentially interesting MoA targets.

- In addition to methylation scores at single CpG sites, we have characterized methylation loads across genes, promoters and intron domains.

- Some degree of inter-individual variation is evident.

- Quantitative separation between responder and nonresponder groups. This set of CpG sites was found to be highly discriminative for clinical discrimination.

- Figure 2: Dendrogram Heatmap of CpGs: Of the 1.8M CpG sites scored in all samples, 98% had statistically different UMET levels. In this plot, the top 1,000 sites are shown in a heatmap (each row is a CpG revealing high conservation in UMET states; raw variance) across patients within a drug response group. Some degree of inter-individual variation is evident.

- The top-40 differential sites show high resolution between responder and non-responder groups. This set of CpG sites was found to be highly discriminative for clinical discrimination.

- Figure 3: Epigenetic discrimination of drug response type in azacitidine treated patients. This figure displays NFDS (non-parametric multidimensional scaling) ordinate analysis to compare CpG methylation patterns among individuals to discriminate NR vs. R epigenetic signatures that could be utilized in pre-treatment stratification applications. Bootstrap analysis estimates indicate that the probability the observed group separation existing by random chance is p < 0.0001.

- The top 40 differential sites show high resolution between responder and non-responder groups. This set of CpG sites was found to be highly discriminative for clinical discrimination.

- Figure 4: Summation of differential methylation load (ML) across broad functional classes. Summation and normalization of differential methylation levels across genes within KEGG cellular function groups. The load score is in difference of NR minus R, where positive values reflect higher methylation in NR patient profiles. Again note that these are pre-treatment profiles.

CONCLUSIONS

- These findings indicate that a targeted assay of CpG methylation sites can provide a mechanistically-based screening tool to discern potential for response azacitidine (or resistance), which warrants confirmation in a larger validation study cohort.

- A clinical screening test to guide selection of HMA therapy would be of great benefit to the ~80% of MDS/AML patients receiving this standard of care with little to no improvement in outcomes.

REFERENCES
