GenPro presents azacitidine efficacy study to identify epigenetic predictors of Responder vs Non-Responder AML/MDS patients.

The work will be presented in a poster session on Sunday, December 4, 2016, 6:00 PM-8:00 PM. The project is titled, "*Epigenetic Profiling in High-Risk MDS and AML Patients Identifies Pre-Treatment Methylation Patterns That Indicate Response to Hypomethylating Agents: A Pilot Study* (Absttract #2885)." It is scheduled for Session #617 - Acute Myeloid Leukemia: Biology, Cytogenetics, and Molecular Markers in Diagnosis and Prognosis, at the San Diego Convention Center, Hall GH. The lead author on the study who will be presenting is Dr. Chetasi Talati from the Department of Malignant Hematology, Moffitt Cancer Center & Research Institute, Tampa, FL. A poster summarizing this presentation is available at: ASH 2016 Poster.

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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).
  - Responders: patients who received at least 4 cycles of therapy with azacitidine and achieved CR per IWG 2009 response criteria.
  - Non-responders: patients with inferior outcome to complete or partial remission per IWG 2009 response criteria.
- Institutional Review Board (IRB) approval was obtained.
- Overview of the process of Genomic DNA extraction and determination of methylation patters among the cohort is described in Figure 1.

RESULTS

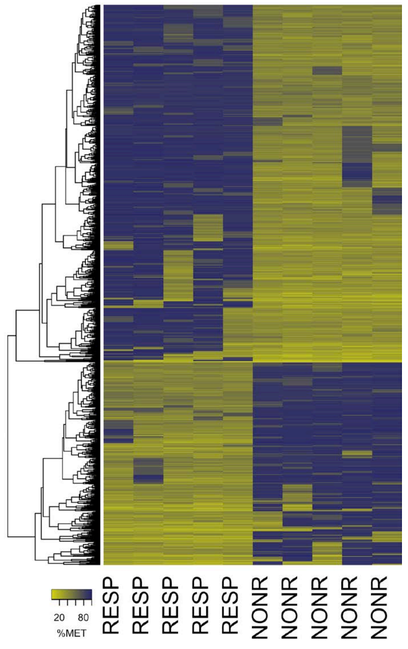

Figure 2: Dendrogram Heatmap of CpGs:

Of the 1.8M CpG sites scored in all samples, 55k had statistically different %MET levels. In this plot, the top 1,000 CpG sites are shown in a heatmap (each row is a CpG) revealing high conservation in %MET states (low 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 CpGs reveals quantitative separation between R and NR that could potentially be utilized in developing a targeted panel assay for clinical discrimination.

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

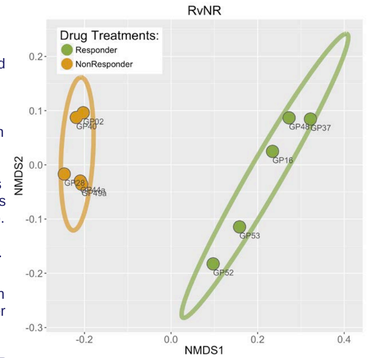

Figure 3: Epigenetic discrimination of drug response type in azacitidine treated patients.

This figure displays NMDS (non-metric 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 arising by random chance is $p < 0.0001$.

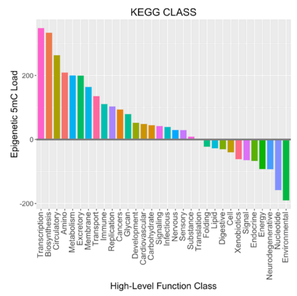

Figure 4: Summation of differential methylation load (ML) across broad functional classes

Summation and normalization of differential methylation levels across genes within KEGG cellular function groups. The load score is a difference of NR minus R, where positive values reflect higher methylation in NR. Overall, NR patient profiles are more heavily methylated than R patient profiles. Again note that these are pre-treatment profiles.

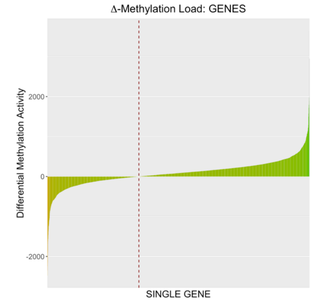

Figure 5: Summation of differential methylation load (ML) across CpG sites within genes.

Summation and normalization of differential methylation levels among all genes as presented as a rank ordered histogram (one bar = one gene; bar width too small to resolve; n=4400). The distribution is sigmoidal with sharp exponential tails at each end. The load score is a difference of NR minus R, where positive values reflect higher methylation in NR. The dotted line represents the equivalence position between NR and R. Again, the larger number of positive values are indicative of the higher methylation levels in NR patients.

Characteristics	Non-Responders (NR)	Responders (R)
Median Age (y)	71 (60-95)	68 (56-82)
Males/Females	4/1	2/3
Disease at diagnosis (n)	MDS-RAEB2 (3) Secondary AML (2)	MDS-RAEB2 (2) Secondary AML (2) De novo AML (1)
Bone marrow myeloblast (%)	17 (12-43)	19 (16-28)
Duration of Response (mo)	Not applicable	10 mo (5-19 mo)

Table 1: Patient characteristics in both non-responders (NR) and responders (R) cohort.

- Total of ~2.04 million "CCGG" sites were analyzed and 88% (1.81M) were present in all samples.
- Assessing methylation loads across genes and grouping genes within defined pathways (Kyoto Encyclopedia of Genes and Genomes or KEGG) revealed substantial hypermethylation in R, primarily in signaling pathways mediated by ErbB, TGFβ, and estrogen.
- Comparison of the methylation signatures of individual genes reveals potentially interesting MoA targets.

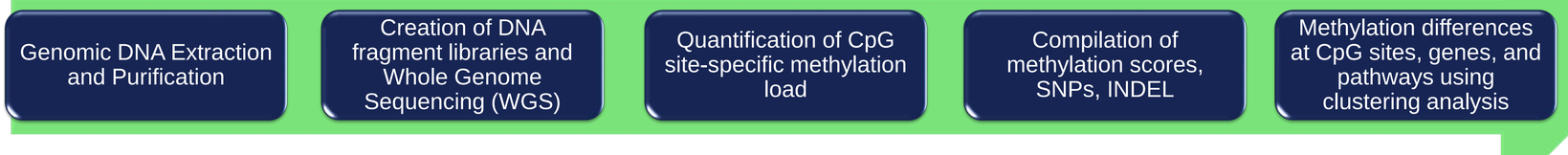

Figure 1: Process from DNA extraction from the bone marrow samples to assessment of methylation load at CpG sites via computational processing

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

1. Silverman, L. R., *et al*. Ramdomized controlled trial of azacitidine in patients with the myelodysplatic syndrome: A study of the Cancer and Leukemia Group B. *J Clin Oncol* 2002 20:2429-2440.
2. Fenaux, P., *et al.* Azacidine prolongs overall survival compared with conventional care regiemns in elderly low bone marrow blast count acute myeloid leukemia. *J clin Oncol.* 2010 Feb 1; 28(4): 562-569.