ABSTRACT

Background: Traditional AML prognostic markers are based on clinical characterization (e.g. age) or static biological markers present at diagnosis, such as cytogenetics and isolated molecular events (e.g. presence of FLT3 ITD mutations). No validated methods currently exist to predict the disease response to standard AML induction chemotherapy for individual patients.

Methods: Modulated SCNP using a multiparametric flow cytometry platform was performed evaluating the phosphorylation of intact and active signaling molecules in their basal states and after treatment with modulators in specific cell populations (e.g. leukemic cells). Since multiple signaling pathways may be dysregulated in AML and contribute to the likelihood of response to a given therapy, pathways that affect proliferation, apoptosis, and DNA damage were analyzed. Analyses were designed to assess assay reproducibility, identify a signaling profile associated with likelihood of response to standard induction chemotherapy (first training set, n=34), and test extrapolation of the identified profile to a fully independent set of AML samples (second training set, n=88).

Results: High assay reproducibility (Pearson correlation coefficients > 0.95) was observed. In the first training study univariate analysis revealed multiple “nodes” (modulated readouts in signaling pathways associated with disease response to conventional induction therapy (i.e. AUC of ROC > 0.86; p<0.05). Importantly, combination of some of the independently predictive nodes improved disease response stratification (AUC of ROC up to 1.0; p<0.05). Extrapolation of the assay to a second independent set of samples revealed similar findings after accounting for clinical covariates. Specifically, for patients < 60 years, the presence of intact apoptotic pathways was correlated with complete response (CR); while in samples from patients ≥ 60 years increased p-Akt and p-Erk levels in response to FLT3 stimulation correlated with non response (NR). Importantly, the predictive values of these nodes were independent from cytogenetic and FLT3/MLMT mutation status.

OBJECTIVES

Objectives: To identify nodes using Single Cell Network Profiling (SCNP) that predict for the likelihood of response to induction chemotherapy in AML (non-M3) patients.

CONCLUSIONS

- SCNP can be performed with high technical accuracy and reproducibility to quantitatively characterize the biology of AML.
- While various single node/metrics predict response to induction therapy, the combination of independently predictive node/metrics added further value.
- For patients < 60 years old, CR was associated with the presence of intact apoptotic pathways.
- In patients ≥ 60 years old, NR was associated with FLT3 ligand mediated increase in p-Akt and p-Erk.
- SCNP assays provides additional important information to current prognostic markers such as age, cytogenetics, secondary AML and molecular alterations.
- Final training study ongoing which will lead to validation of classifier for both old and young AML patients.

References


Acknowledgements

We thank all patients who have donated samples for this investigation.

Disclosures

$ Consultant to Nodality, Inc.
* Denotes Nodality, Inc. employees and stockholders

Steve M. Kornblau,15 Mark D. Minden,2 David B. Rosen,3 Santosh Putta,3 Aileen Cohen,3 Todd Covey,3 Wendy J. Fanti,3 Ute Gayko,3 and Alessandra Cesano2

1 MD Anderson Cancer Center, University of Texas , 2University Health Network, Toronto, BC, 3Nodality, Inc., South San Francisco, CA

SINGLE CELL NETWORK PROFILING (SCNP)

FIGURE 1. PATHWAYS MEASURED USING SCNP & STUDY DESIGN

Node: readout in modulated signaling pathway e.g. IL-27 → p-Stat5 (modulator→ assay readout)

Metric: analytical quantification of stimulated and basal protein activity

FIGURE 2. PATIENT AND SAMPLE CHARACTERISTICS

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>UHN</th>
<th>MDACC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Samples</td>
<td>97</td>
<td>110</td>
</tr>
<tr>
<td>Tissue</td>
<td>PBMC</td>
<td>BMMC</td>
</tr>
<tr>
<td>Sample Timepoint</td>
<td>Diagnosis / Pre-Induction</td>
<td>Diagnosis / Pre-Induction</td>
</tr>
<tr>
<td>Disease</td>
<td>Non-M3 AML</td>
<td>Non-M3 AML</td>
</tr>
<tr>
<td>Number of Nodes/Metrics</td>
<td>304</td>
<td>182</td>
</tr>
</tbody>
</table>

Samples were obtained from adult patients with non-M3 AML prior to induction chemotherapy

FIGURE 3. ASSAY REPRODUCIBILITY

2 vials for a patient cohort, each vial processed independently for assay reproducibility

Majority of node/metrics had Pearson Correlation of 0.6

Best reproducibility for node/metrics with large range of signaling (e.g. different stages in apoptosis

FIGURE 4. UNIVARIATE ANALYSIS METHODS & RESULTS

FIGURE 5. NODES ADD VALUE BEYOND AGE FOR PREDICTING INDUCTION RESPONSE

Model: CR or NR + Age (Categorical) + Node

Examples of Responder and Non-Responder in both Age Groups

CR | NR
--- | ---
IL-27 | [Total Phospho]

References


Acknowledgements

We thank all patients who have donated samples for this investigation.

Disclosures

$ Consultant to Nodality, Inc.
* Denotes Nodality, Inc. employees and stockholders

All Rights Reserved. © 2009 Nodality, Inc.