Identification of biomarkers and pathways associated with response to DOT1L inhibitor Pinometostat (EPZ-5676) in MLL-r leukemia

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Background

Pinometostat is a highly selective first in class DOT1L inhibitor currently in Phase 1 clinical trials in adult and pediatric leukemia patients (pts) with MLL rearrangements (MLL-r or MLL-PTD). Preliminary results of the adult trial have demonstrated clinical activity including complete remissions in a subset of patients (1). Investigation and identification of candidate molecular correlates of pinometostat response in both pt samples and cell lines are reported.

DOT1L Promotes MLL-r Leukemia via Aberrant H3K79 Methylation

DNA and RNA were isolated from PBMCs and/or bone marrow (BM) collected prior to treatment from 18 pts enrolled in the adult pinometostat Phase 1 study (CT.gov: NCT01684150), at the following doses 24 (n =2), 36 (n = 3), 54 (n = 6), 80 (n = 3) and 90 mg/m²/day doses (n = 4). mRNA transcript abundance was assessed using whole genome RNA-seq and DNA variants were determined using a 194 gene panel, MyAML (Genection Inc.). Correlations of transcript abundance and DNA variants detected with categorical (responder = CR or PR [n=3], or no response [n=16]) and continuous response parameters (time on study [TOS], mean = 59 days: range = 8-196 days) were performed. For cell lines, whole genome RNA-seq data was generated from 14 cell lines (MLL-r or MLL-PTD) with a range of in vitro sensitivity to pinometostat (cell proliferation IC50 2 nM to > 10 mM) and RNA transcripts identified as correlated with IC50 were submitted for pathway analysis.

Methods

RNA-seq Pathway Analysis to ID Mechanisms of Pinometostat Sensitivity

DNA-seq results generated greater accuracy and precision for MLL-variant detection
- Local FISH cytogenetics identified 10/14 MLLr cases
- NGS (MyAML) identified fusion partners in 14/14 cases
- Translocations of MLL with ENL are enriched with longer time on study (P value = 0.05)
- ELL also enriched for longer time on study
- DNAseq results show no single entity (SNP, or indel) was statistically associated with binary response criteria or time on study

DNA-seq Results

<table>
<thead>
<tr>
<th>DNA-seq Data</th>
<th>Clinical Data</th>
<th>RNA-seq Data</th>
<th>RNA-seq Data</th>
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</thead>
<tbody>
<tr>
<td>Pathway</td>
<td>Response</td>
<td>Patient</td>
<td>In vitro sensitivity</td>
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</table>

RNA-seq Pathway Analysis to ID Mechanisms of Pinometostat Sensitivity

RNA Measurements used to Infer Mechanism Strengths

- Synergy with RAS Pathway Inhibitors Observed in Cell Based Models of Acute Leukemia

Synergy with RAS Pathway Inhibitors Observed in Cell Based Models of Acute Leukemia

MOLM-13 (MLL-AF9, RAS WT)

OCI-AML4 (MLL-ENL, NRAS mut)

SKM-1 (non-MLL-r, KRAS mut)

Patient Pathway Analysis Exhibit Four Response Associated Mechanisms Identified in Cell Lines

- Inflammation – Higher in sensitive cell lines
- Stem Cell Renewal – Lower in sensitive cell lines
- PPARγ and Lipid Metabolism – Lower in sensitive cell lines
- Ras Signaling – Higher in sensitive cell lines

Patient Pathway Analysis

Patient Listing of Best Response, Time on Study and Detected DNA Variants

<table>
<thead>
<tr>
<th>Patient</th>
<th>Best Response</th>
<th>Time on Study</th>
<th>Detected DNA Variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CR</td>
<td>8</td>
<td>AML1-ETO</td>
</tr>
<tr>
<td>2</td>
<td>PR</td>
<td>196</td>
<td>AML1-ETO</td>
</tr>
<tr>
<td>3</td>
<td>CR</td>
<td>8</td>
<td>AML1-ETO</td>
</tr>
<tr>
<td>4</td>
<td>PR</td>
<td>196</td>
<td>AML1-ETO</td>
</tr>
</tbody>
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Complete Responder

B) Biological Effect (leukemiccytosis) Non-responder

Conclusions

- MLL fusion partner (e.g. t(11;19)) may influence clinical response to pinometostat
- No SNP or indel was statistically associated with binary response or time on study
- DNA-seq analysis of baseline patient samples and cell lines revealed additional candidate pathways that warrant further investigation
- Increased Ras signaling was observed in patients with longer time on study and cell lines sensitive to pinometostat
- Combination of pinometostat with RAS pathway inhibitors leads to synergistic cell killing in leukemia cell lines
- These data warrant further investigation of potential for clinical benefit from combination of pinometostat and RAS pathway inhibitors in MLLr patients.