Results of a phase I/II trial of belinostat in combination with idarubicin in AML – favorable impact on mainly intermediate cytogenetic risk AML can be predicted by gene expression profiling

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Introduction

Belinostat (PKD101), a histone deacetylase inhibitor, has demonstrated effective cell killing in leukemia cells. Showing also a synergistic effect in combination with anthracyclines in vitro, a favorable impact on the dismal clinical course of acute myeloid leukemia (AML) was suggested (Schlenk et al. ASH 2008). Recently, an open-label, multi-center, dose-escalation Phase 1/2 study to evaluate safety, explore efficacy, pharmacodynamics, and pharmacokinetics of belinostat and idarubicin combination in patients with acute myeloid leukemia (AML) has demonstrated anti- leukemic effect both of belinostat alone and in combination with idarubicin (PKD101-CLN-15, ClinicalTrials.gov ID: NCT00878722). The investigations reported here includes correlation of response with gene expression (n=13) and molecular markers (n=41) in patients with AML. Molecular markers are studied in detail for 25 patients.

Study design

Phase I/II, open label, dose-escalation (accelerated titration design or standard 3+3 schedules)

- **Schedule A**: Belinostat 1000 mg/m² 30-minute IV infusion daily Day 1-5 plus idarubicin from 5 mg/m² on Day 5 escalated to 10 mg/m² on Days 4 and 5.
- **Schedule B**: CIV dose escalation 25-800 mg/m² monotherapy. Belinostat CIV 1000 mg/m² for 48 hours plus idarubicin from 5 mg/m² after 24 hours 7.5 mg/m² after 24 and 48 hours.

**Primary objectives**: Explore efficacy (response rate), determine safety and tolerance

**Secondary objectives**: Time to response, duration of response overall survival, relapse free survival, event free survival and remission duration, examine PK, examine PD

**Pharmacodynamic investigations**

Cyto genetic changes were studied by conventional chromosome banding analysis as previously described (Schlenk et al. 2008).

Molecular genetics analyses for gene mutations affecting Fli3, T3 (evaluation of the presence of internal tandem duplications = ITC, and mutations of the tyrosine kinase domain = TDK) and NPM1, and gene fusions such as RUNX1/AML1, CBFB/MYH11, and MLL/AF4 were performed according to standard procedures (Fohring et al. Blood 2002, Döhner et al. Blood 2005, Schlenk et al. N Engl J Med 2008).

**Gene expression profiling** analysis was performed using Affymetrix U133plus2.0 microarrays. Data analysis was performed using BRB Array Tools (available at http://llnis.nch.nih.gov/BRB-ArrayTools.html). Data was normalized using the RMA (Robust Multi-array Average) algorithm and filtered based on present calls (p<0.01).

Results

**Patient characteristics**

<table>
<thead>
<tr>
<th>Gene Expression Profiling Results</th>
<th>All</th>
<th>Non Responders</th>
<th>Responders</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Efficacy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.047</td>
</tr>
<tr>
<td>PR</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0.047</td>
</tr>
<tr>
<td>Overall response rate</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0.047</td>
</tr>
</tbody>
</table>

**Prediction of Response**

A blinded response prediction based on in vitro data indicated that patient selection based on gene expression analysis could potentially increase the response rate to study treatment (Figure 2).

** Gene expression**

The gene expression analysis of 19564 genes comparing responders (n=4) versus non-responders (n=9) revealed a significant (p<0.05 level), univariate test gene expression pattern associated with the response to belinostat comprising 1905 genes. Table 3 shows genes of special interest.

**GO (gene ontology)** class comparison analysis shows a significant enrichment of gene ontologies for responders (CR+PR) including categories associated with epigenetic regulation such as the GO category “histone methyltransferase activity” (HMA) (comprising e.g. MLL, ASHNL, MEF1, and SUZ12) and “histone deacetylation activity (HDAC)” (comprising e.g. HDAC7, HDAC2, SIRT5, and HDAC6). LS permutation p-value (HMA 0.00388 / HDAC 0.01309). KS permutation p-value (HMA 0.0833 / HDAC0.00189).

**Cytogenetic Karyotype and Response**

AML cases with Intermediate Risk Cyt aberrations tend to respond better than cases with High Risk cytogenetics (p<0.14).

Conclusions

- Belinostat in combination with idarubicin demonstrated anti-leukemic effect. The objective response rate was 17% (3/18) in regimens with 30 min IV belinostat and 31% in belinostat CIV regimens (5/16).
- Gene expression profiling based on 13 patients (4 responders and 9 non-responders) revealed a strong gene expression pattern associated with the response to belinostat. The respective gene expression pattern harbored predictive information as based on an in vitro cell line derived predictor a blinded belinostat response prediction was feasible.
- Gene Ontology categories “histone methyltransferase activity” and “histone deacetylation activity” were significantly enriched in the class of responders.
- Karyotype analysis suggests that AML cases with intermediate risk cytogenetics tend to respond better to a belinostat than patients with high risk cytogenetics (p<0.05; n=41). 5 CR and 2 PR were observed in 25 (28%) AML cases with low/intermediate risk cytogenetic aberrations, whereas no CR and 2 PR were seen in 16 (13%) high risk AML cases.
- Further studies are warranted to explore the potential association of belinostat response and AML intermediate risk cytogenetics, high risk cases might nevertheless profit from an epigenetic treatment approach with a histone deacetylation inhibitor.