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 (PXD101), a histone deacetylase inhibitor, has demonstrated effective cell killing in leukemic 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 (PXD101-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 design), multi-center study for examination of two schedules.

Schedule A: Belinostat 1000 mg/m² 30-minute IV infusion daily Day 1-5 plus idarubicin from 5 mg/m²×1 on Day 5 escalated to 10 mg/m² on Days 4 and 5.

Schedule B: Belinostat CIV dose escalation 25-800 mg/m² monotherapy , Belinostat CIV 1000 mg/m²/d 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

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

Molecular genetics analyses for gene mutations affecting *FLT3* (evaluation of the presence of internal tandem duplications = ITD, and mutations of the tyrosine kinase domain = TKD) and *NPM1*, and gene fusions such as *RUNX1/CBFA2T1*, *CBFB/MYH11*, and *MLL/AF9* were performed according to standard procedures (Fröhling 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://linus.nci.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 1

Patient Characteristics and Demographics	Schedule A 30 min iv inf	Schedule B 24 or 48 hours CIV	All
Age Range (years)	56-80	28-83	28-83
Gender (n) female (F); male (M)	4 (F);14 (M)	12(F);11(M)	16 (F); 25 (M)
No. of Prior Anti Leukemic Treatments	0-3	0-5	0-5
Cancer Type (n)			
De novo AML	11	10	21
AML after MDS	7	9	16
Treatment related AML	0	2	2
De novo MDS	0	2	2
WHO Classification (n)			
I (recurrent cytogenetic translocations)	2	0	2
II (multilineage dysplasia)	5	10	15
III (therapy required)	0	2	2
IV (not categorized)	11	11	22
Duration of AML, range (years)	0-8.8	0-2.7	0-8.8
ECOG Score 0;1;2 (n)	7;5;6	7;11;5	14;16;11

Efficacy Overview

Belinostat in combination with idarubicin demonstrated anti-leukemic effect both in the conventional belinostat 5-day regimen (30-minute IV, Day 1-5 every 3 weeks) and in the 48-hour CIV regimen (every 2 weeks), both based on a daily dose of belinostat of 1000 mg/m².

Table 2

Schedule			Best Overall Response n (%)			Number of Patients
			CR+PR	SD	PD	
A: 1000 mg/m² 30 min IV belinostat schedules			3 (17%)	2 (11%)	13 (72%)	18
B: 1000 mg/m² CIV belinostat schedules			5 (31%)	4 (25%)	7 (43%)	16
B: 25-800 mg/m² CIV belinostat schedules			1(14%)	1(14%)	5(71%)	7
Schedule	**Belinostat (mg/m²/day)**	**Idarubicin (mg/m²/day)**	**Best Overall Response**	**Response Duration (Weeks)**	**Event Free Survival (Weeks)**	**AML Type**
A:30 min IV 3 weeks cycle	1000, D1-D5	10, D5	CR	26.3	28.7	De novo
	1000, D1-D5	7.5, D4, D5	CR	20.6	28.3	After MDS
	1000, D1-D5	10, D4, D5	CRi	28.1	28.1 (2.7)*	After MDS
B:CIV 48 h 2 weeks cycle	800, D1-D2	None	PR	5.4	11.4	De novo MDS
	1000, D1-D2	5 after 48 h	CR	30.3	32.1	After MDS
	1000, D1-D2	5 after 24h + 48h	PR	19.6	26	After MDS
	1000, D1-D2	5 after 24h + 48h	1 CR 2 PR	Range 3-6.1	Range 3.9-8.1	De novo MDS(CR) After MDS (PR), Treatment related AML(PR)

*Went of study in Cycle 1, but continued observation without treatment and relapsed after 28.1 weeks.

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).

Figure 1.
Gene expression profiling.
Gene expression profiling: comparison of 4 responders and 9 non-responders: strong gene expression pattern associated with belinostat response; top 50 candidates include genes involved in epigenetic deregulation (such as *MLL*) as well as *TP53*, whose transcriptional activity was shown modulated by histone deacetylases. An additional interesting candidate was *CCT5*, chaperonin containing TCP1, subunit 5 (epsilon), a known interactor of HDAC3 and HDAC5 that encodes for a molecular chaperone that is a member of the chaperonin containing TCP1 complex (CCT).

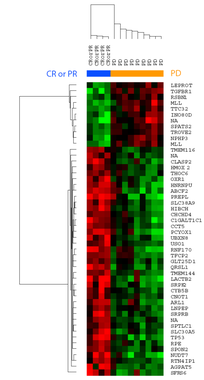
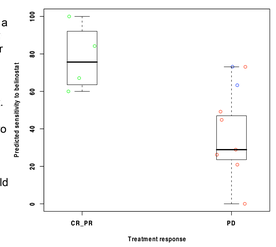


Figure 2.
Prediction of response Blind prediction of sensitivity to belinostat with a response predictor developed previously based on *in vitro* data from the NCI. After unblinding, the predicted sensitivity of responders (CR+PR) versus non-responders (PD) was distributed as shown. Predicted sensitivity to belinostat. A t-test between responders and non-responders yields a p-value of 0.02. If two non-responders that did not receive an effective dose (blue) are omitted, the p-value drops to 0.009. Selecting patients with a predicted sensitivity above 50 could increase the response rate from 4/13 (31%) to 4/7 (57%) or 4/5 (80%).



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`s) including categories associated with epigenetic regulation such as the GO category “histone methyltransferase activity (HMA)” (comprising e.g. *MLL, ASH2L*, *MEN1*, and *SUZ12*,) and “histone deacetylase activity (HDAC)” (comprising e.g. *HDAC7, HDAC2, SIRT5,* and *HDAC2)* . LS permutation p-value (HMA 0.00038 / HDAC 0.01309) KS permutation p-value (HMA 0.0833/ HDAC0.00198) Efron-Tibshirani's GSA test p-value (HMA 0.005 (+)/HDAC 0.06 (-)).

Table 3

Gene symbol	Fold-change	Parametric p-value (Rank)	Description
MLL	0.408	7.69E-05 (5)	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila), Crucial role in leukemogenesis via epigenic deregulation
CCT5	2.256	0.000135 (10)	Chaperonin containing TCP1, subunit 5 (epsilon), known interactor of HDAC 3 and HDAC 5
TP53	3.974	0.0001817 (11)	Tumor protein p53, HDAC`s modulate p transscriptional activity via regulation of p53-DNA binding

Cytogentic Karyotype and Response

AML cases with Intermediate Risk Cyto aberrations tend to respond better than cases with High Risk cytogenitics (p=0.14).

Table 4

Disease Classification/Karyotype	Number of Patients	CR	PR	SD
Low Risk: t(8;21) (n=1)	1	-	1	-
Intermediate Risk: CN-AML (n=16); t(9;11) (n=2) Cytogenetic Abnormalities not Classified as Favorable or Adverse (n=6)	24	5	1	3
High Risk: Complex Karyotype (n=10); -7/del(7q) (n=2); t(6;9) (n=1); inv(3)/t(3;3) (n=3)	16	-	2	2

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 Othology categories “histone methyltransferase activity ” and “histone deacetylase activity” were significantly enriched in the class of responders.
- Karyotype analysis suggests that AML cases with intermediate risk cytogenetics tend to responded better to a belinostat than patients with high risk cytogenetics (p=0.14; 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 deacetylase inhibitor.

Disclosures: Knoblauch and Tjørnelund: *Topotarget:* Employment.