

**Combination of sorafenib, vorinostat and bortezomib for the treatment of poor-risk AML: report of two consecutive clinical trials**

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## Introduction

Older age, unfavorable cytogenetics, and FLT3-ITD mutation are adverse prognostic indicators in acute myeloid leukemia (AML) placing the disease under poor-risk category [1-5]. Complex biologic factors in poor-risk AML confer resistance to chemotherapy [6-9]. Incorporation of novel non-chemotherapeutic targeted agents into the treatment of poor-risk AML is therefore on demand. Subject of such approaches may include epigenetic pathways that affect tumor suppressor genes. Apoptosis in leukemic blasts can be promoted through upregulation of tumor suppressors DAPK1, RUNX3, and p16INK4A/CDKN2A [10,11]. We previously identified an oncogenic pathway in AML repressing promoter of DAPK1 gene through an interaction of p52NF- $\kappa$ B and histone deacetylases [12]. Furthermore, leukemia stem cell promotion occurs in epigenetically repressed FLT3-ITD positive (+ve) or complex cytogenetics disease [13]. Indeed, a cause for suboptimal response to single-agent FLT3 inhibitors may be an epigenetic collaboration in the pathogenesis of FLT3-ITD-mediated AML [14,15].

We hypothesized that epigenetic de-repression of specific tumor suppressor genes in addition to inhibition of kinase-dependent proliferation systems can promote clinical anti-leukemic effect in AML. We therefore designed sequential clinical trials to explore the safety and efficacy of epigenetic modification along with targeting selected cell proliferation pathways in poor-risk AML. The first trial combined FLT3/RAF inhibitor sorafenib and histone deacetylase inhibitor vorinostat in a phase 1 setting. The second trial tested addition of bortezomib to the previous combination for further tumor suppressor de-repression through inhibition of p52NF- $\kappa$ B. Our findings on the molecular attributes of these studies have been reported separately [16]. This report describes clinical aspects of the two consecutive trials.

## Patient selection

Both studies were approved by Indiana University School of Medicine Institutional Review Board. Sor/vor study included adult patients with a diagnosis of relapsed and/or refractory AML or older than 70 with previously untreated disease. Any cytogenetic or molecular profile was acceptable. The sor/vor/bor study enrolled only relapsed and/or refractory AML with FLT3-ITD+ve profile and/or poor-risk cytogenetics including -5, -7, or complex

karyotype. Such selection was due to observation of response in patients with poor-risk cytogenetics or FLT3-ITD+ve disease in sor/vor trial.

### **Trial Design and Treatment**

The sor/vor trial followed a 3+3 dose-escalation schedule in a phase I setting. Sorafenib was given orally at a dose of 400 mg twice daily, and oral vorinostat was given with dose escalation in successive cohorts. Each cycle of 21 days consisted of treatment for 14 days followed by 7 days of break. An interrupted schedule was chosen to avoid excessive myelosuppression. Patients who achieved at least 50% reduction of bone marrow blasts with the first cycle, were eligible to receive the second cycle. At least 10% reduction of bone marrow blasts, but not a complete remission with the second cycle, would make patients eligible for the third cycle. A maximum of 3 treatment cycles was planned. No further treatment would be given beyond a complete remission (CR).

Sor/vor/bor was a phase I/II study with a 3+3 dose-escalation schedule in the phase I part. Vorinostat was given orally at 200 mg twice daily, initially in an interrupted fashion only on days 1-4 and 8-12, and later continuously. Oral sorafenib and intravenous bortezomib were given with dose escalation in successive cohorts. Each cycle consisted of 14 days of treatment followed by 7 days of break, 21 days total. Patients with continued response would continue on study. Up to 2 more cycles could be given beyond a CR. The phase II part of the study followed the same schedule of phase I, given at safe dose determined by the phase I part.

Response was assessed by the guidelines reported by Cheson [17], and dose-limiting toxicity (DLT) was defined as any grade 3 or 4 non-hematologic toxicity.

### **Results**

#### **First trial (sor/vor study)**

**Toxicity and optimal dose.** Patient characteristics are summarized in table 1. A total of 15 patients were enrolled, and 13 completed at least one cycle of therapy. Median age was 61 years (37-74), with 8 males and 5 females. Two patients were in their 3<sup>rd</sup> relapse, one had

relapsed after allo-transplant, one older patient had previously untreated disease, and rest of patients were refractory to chemotherapy. One patient had grade 1 hand-foot syndrome, and another grade 2 nausea. One/six patient in the third (last) cohort developed grade 4 diarrhea in the setting of neutropenic fever. The safe dose was determined as sorafenib 400 mg and vorinostat 200 mg both twice daily for 14 days, followed by 7 days of rest. To further assess the safety and response, three more patients were enrolled into the study at the established optimal dose.

**Response.** With the first cycle of treatment, seven/sixteen (44%) patients demonstrated a partial remission (PR) and 1/16 (6%) achieved a complete remission (CR) and remained disease-free for 5 months. Four partial responders received 1-2 more cycles with initial response but experienced disease progression. Three partial responders to the first cycle did not receive a second cycle, one per personal preference and two due to increasing circulating blasts. The patient with CR carried a complex cytogenetics including monosomies 5 and 7.

### **Second trial (sor/vor/bor study)**

**Toxicity and optimal dose.** Seventeen patients were enrolled in the phase I part (table 2). Fifteen patients completed at least one cycle of treatment. All patients had relapsed and/or refractory disease, and had received at least two lines of chemotherapy. Median age was 51 years (24-73), with 10 males and 7 females. Most common toxicities were gastrointestinal disturbances (64.2%) including diarrhea, nausea, or vomiting reaching grade 2 in 14.2% of patients with no grade 3 or 4. Others included fatigue (31.3%), rash (21.4%), and neutropenic fever (7.1%); none greater than grade 2. The safe dose was sorafenib 400 mg and vorinostat 200 mg twice daily for 14 days, and bortezomib 1.3 mg/m<sup>2</sup> IV on days 1, 4, 8, 11, every 21 days.

**Response.** In the phase I study 5 patients achieved CR with incomplete platelet recovery (CRi). Responses occurred across different cohorts. A total of 20 patients were enrolled into the phase II study. Majority of patients were heavily pretreated including stem cell transplantation in 5. Six patients were deemed unevaluable for response. Of 14 evaluable patients, 7 (50%) carried FLT3-ITD mutation and 7 (50%) had either complex cytogenetics or monosomy 7. One patient (7%) achieved CR, 1 (7%) CRi, and 2 patients (14%)

experienced a PR. Overall responses with all 37 patients in both phases, including unevaluable patients, were 1 CR (3%), 6 CRi (16%), and 2 PR (5%). All responders in both phases of the study carried FLT3-ITD mutation. Responders all experienced relapse within 2 cycles of consolidation, and none was able to undergo transplant due to variety of reasons.

## Discussion

The two consecutive studies were based on a hypothesis that epigenetic de-repression of tumor suppressor genes combined with inhibition of kinase-dependent proliferation systems can introduce a collaborative anti-leukemic effect. The first study demonstrated safety of combined kinase inhibitor sorafenib and epigenetic modulator vorinostat. In this study a clinical response, including 1 CR and 6 PR were observed in poor-risk AML. Analysis of bone marrow blasts in this study demonstrated up-regulation of tumor suppressors DAPK1, RUNX3, and CDKN2A along with reduction in FLT3-induced Id1 [16].

The second trial established safety and assessed efficacy of sorafenib, vorinostat, and bortezomib combination. Bortezomib was added to further enhance de-repression of tumor suppressors through inhibition of p52NF- $\kappa$ B. In this study 1 CR (3), 6 CRi (16%), and 2 PR (5%) were observed. In this study also an upregulation of tumor suppressors of interest was noted in responders [16]. Maximum response in both studies occurred within the first 2 cycles of treatment. The depth of response, as is evidenced by number of CR and CRi, was greater in the second study, reflecting potential role of proteasome inhibition in this combination. Responses in both studies were distributed among different dose levels, suggesting sensitivity of leukemic cells to the combination regardless of clinical dosing.

Single-agent sorafenib has shown efficacy in AML when administered continuously, with median time to best response of 27 to 46 days [18,19]. Majority of responders in both of our studies achieved best response within the first 14 days of therapy. Indeed, intracellular molecular changes predictive of response were observed in the AML blasts obtained from patient marrows as early as 3-4 days following initiation of treatment [16]. The expedited response observed in our studies is logically attributable to addition of vorinostat and possibly bortezomib.

Patients were not kept on treatment extensively due to limitations in drug availability, but all relapses in sor/vor/bor occurred during consolidations, indicating long-term treatment would not be beneficial. The short duration of response to sorafenib in FLT3-ITD+ve disease is attributed to emergence of D835 tyrosine kinase domain mutation [18]. Additional epigenetic modulation in our studies did not improve such response duration.

The two studies demonstrated safety of combination of sorafenib, vorinostat, and bortezomib at full approved doses in AML. These studies also showed potential for response to novel targeted-only therapies in poor-risk AML. These studies offer a platform towards further development of such rational combinations for the treatment of AML.

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### **Conflict of interest**

Hamid Sayar received research funding from Bayer/ Onyx. Other authors declare no conflict of interests.

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Table 1. Sor/vor study patient characteristics

Cohort	Patient	Age/Sex	AML status	Cytogenetics	FLT3-ITD	Response
1	1	71/M	3 <sup>rd</sup> rel	Normal	-	No
	2	56/M	Rel, post transplant	Normal	-	No
	3	65/M	Primary ref	-7	-	PR
2	4	72/M	3 <sup>rd</sup> rel	t(1;6)	-	No
	5	72/M	Rel/ref	t(18;21)	+	PR
	6	37/F	Rel/ref	Normal	-	No
3	7	58/M	Rel/ref	Normal	+	PR
	8	74/M	Untreated	-5, -7	-	CR
	9	46/F	Rel/ref	Complex	-	No
	10	74/M	Primary ref	Complex	-	PR
	11	60/F	Primary ref	Normal	-	No
	12	70/F	Untreated	Normal	-	PR
	13	42/F	Primary ref	Normal	+	PR
	14	53/M	2 <sup>nd</sup> rel	Trisomy 8	-	No
	15	67/M	Rel/ref	Normal	-	No
	16	70/F	Rel/ref	Normal	-	PR

Rel: Relapsed; Ref: Refractory; PR: Partial remission; CR: Complete remission

Dosing: Sorafenib 400 mg twice daily in all cohorts. Vorinostat 100 mg twice daily in cohort 1; 200 mg in AM and 100 mg in PM in cohort 2; 200 mg twice daily in cohort 3. Each cycle consisted of 14 days of treatment and 7 days of rest.

Table 2. Sor/vor/bor study patient characteristics

Phase 1	Patient	Age/Sex	AML status	Cytogenetics	FLT3-ITD	Response
Cohort 1	1	33/M	Rel post transplant	(-7)	-	Unevaluable
	2	67/M	Rel/Ref post transplant	(-7q)	+	Unevaluable
	3	64/F	Ref	Complex	-	No
	4	54/F	Rel/ref	Complex	-	No
Cohort 2	5	55/F	Rel/ref	Normal	+	CRi
	6	67/M	Rel/ref	Normal	+	CRi
	7	64/M	Ref	Normal	+	Initial response
Cohort 3	8	35/M	Ref	Unavailable	+	Unevaluable
	9	73/M	Ref	Complex	-	No
	10	51/M	Rel	Complex	-	No
Cohort 4	11	63/M	Ref	Complex	-	No
	12	46/F	Ref	46,XX,t(2;8)	+	Initial response
	13	50/F	Ref	Normal	+	CRi
Cohort 5	14	37/M	Rel post transplant	Unavailable	+	CRi
	15	40/F	Ref	Unavailable	+	No
	16	46/M	Ref	(-7)/Complex	-	No
	17	24/F	Rel post transplant	46,XX,t(12;15)	+	CRi
Phase 2	7 patients	2 Females 5 Males Age 41-72	4 Rel/ref 1 Ref 2 Rel post transplant	Normal	+	1 CR 1 CRi 2 PR
	7 patients	3 Females 4 Males Age 50-71	5 Rel/ref 2 Rel post transplant	Complex (-5) or (-7)	-	No
	6 patients	4 Females 2 Males Age 54-73	3 Rel/ref 3 Ref	2 Normal 3 Complex 1 (-5)	2 + 4 -	Unevaluable 3 discontinued due to patient preference 2 died due to neutropenic fever 1 lost to followup

Rel: Relapsed; Ref: Refractory; CRi: Complete remission with incomplete platelet recovery

Dosing: Sorafenib 200 mg twice daily in cohort 1 and 400 mg twice daily in next cohorts. Vorinostat 200 mg twice daily on days 1-4 and 8-12 in cohorts 1-4, and continuously in cohort 5. Bortezomib 1.0 mg/m<sup>2</sup> on days 1,8 in cohorts 1 and 2; 1.3 mg/m<sup>2</sup> on days 1,8 in cohort 3; and 1.3 mg/m<sup>2</sup> on days 1,4,8,11 in cohorts 4 and 5. Each cycle consisted of 14 days of treatment and 7 days of rest.