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Treatment of Higher Risk Myelodysplastic Syndrome Patients Unresponsive to Hypomethylating Agents with ON 01910.Na

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Abstract

In a Phase I/II clinical trial, 13 higher risk red blood cell-dependent myelodysplastic syndrome (MDS) patients unresponsive to hypomethylating therapy were treated with the multikinase inhibitor ON01910.Na. Responses occurred in all morphologic, prognostic risk and cytogenetic subgroups, including four patients with marrow complete responses among eight with stable disease, associated with good drug tolerance. In a subset of patients, a novel nanoscale immunoassay showed substantially decreased AKT2 phosphorylation in CD34+ marrow cells from patients responding to therapy but not those who progressed on therapy. These data demonstrate encouraging efficacy and drug tolerance with ON01910.Na treatment of higher risk MDS patients.

Keywords

MDS; Treatment; ON01910.Na; rigosertib; nanoimmunoassay; AKT signaling pathway

Introduction

The myelodysplastic syndromes (MDS) are a heterogenous spectrum of diseases with disparate clinical manifestations and outcomes. Based on the International Prognostic Scoring System (IPSS) risk categorization, patients are stratified into risk disease regarding their potential for survival or progression to acute myeloid leukemia (AML) with IPSS Intermediate-2 and High risk patients being at higher risk for poor clinical outcomes [1].

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Upon patients' progression to higher risk disease, therapies aimed at altering disease natural history have been used [2–4]. For patients eligible for high intensity therapy, allogeneic hematopoietic stem cell transplantation (HSCT) is considered. However, for the majority of patients lacking a suitable donor or ineligible for high intensity therapy, lower intensity treatments with hypomethylating therapy using the DNA methyltransferase inhibitors (DNMTIs) 5-azacitidine or decitabine have been used for treatment [3,4]. Although these lower intensity therapies have been beneficial for a portion of these MDS patients, patients may lack responsiveness or relapse after initial response.

For patients who have not responded to or have progressed after an initial response to DNMTIs and are not HSCT candidates, therapeutic options are generally limited to investigational therapies, in addition to supportive care. These patients have relative short survival (4.3 to 5.6 month medians) and a high risk of leukemic transformation [5, 6]. These patients, in general, are also poor candidates for other therapies due to advanced age or significant co-morbidities.

A number of compounds have been investigated in an attempt to improve treatment options for this subset of patients who have failed or are resistant to hypomethylating agent treatment. One such strategy is to therapeutically target cell cycle regulators, as altered cell cycle is a central feature of human malignancy and dysfunctional signaling in tumors ultimately affects cell cycle progression. Cell cycle progression is coordinated by cyclin/cyclin-dependent kinase (CDK) complexes and CDK inhibitors. Kinase activation generates phosphorylation cascades and mitotic spindle formation.

ON 01910.Na is a styryl sulfone mitotic and multikinase inhibitor which inhibits Polo-1 kinase (Plk1), phosphatidylinositol-3 (PI3) kinase, AKT and mitogen activated kinase (MAPK) pathways [7–9]. The drug inhibits cell cycle progression, as well as synergizing with cytotoxic drugs, selectively inducing mitotic arrest and apoptosis of cancer cells (including human lymphoma cells), while being relatively non-toxic for normal cells mediated via the PI3, mammalian target of rapamycin (mTOR) and AKT pathways [7–9]. These effects lead to tumor regression in in vivo animal models [10]. In addition, Plk1 is a critical cell cycle kinase which affects mitotic progression, spindle assembly and centrosome maturation [11]. Its inhibition leads to mitotic arrest and apoptosis [12]. Conversely, its ectopic over-expression leads to neoplastic progression [13]. Increased expression of Plk1 is noted in many human tumors, including leukemia [14].

In Phase I/II studies ON 01910.Na has shown promising therapeutic results and drug tolerance in patients with advanced solid tumors [15,16], as well as in pilot in vitro and in vivo studies of MDS pts, including those with trisomy 8 [17–19]. Correlative investigations have demonstrated that ON 01910.Na inhibited cyclin D1 accumulation and was selectively toxic to trisomy 8 cells while promoting maturation of diploid cells in CD34+ cells of trisomy 8 MDS patients treated with this drug [17–19].

With this background we designed a Phase I/II trial to evaluate the safety and potential efficacy of ON 01910.Na in higher risk MDS patients whose disease had not responded to hypomethylating agents. As ON 01910.Na is a kinase inhibitor, we measured the changes in intracellular AKT signaling as an exploratory biologic correlative adjunct for our study. The PI3K/AKT signaling pathway is essential for different physiological processes of cell growth, survival and suppression of apoptosis, and its constitutive activation has been implicated in the pathogenesis as well as the progression of a wide variety of neoplasias, including AML and MDS [20–22]. A novel and highly sensitive nano-fluidic proteomic immunoassay method (NIA) has recently been developed to quantify changes in phosphorylated protein isoforms in MDS and other tumor specimens [23,24]. We utilized

this detection method to investigate oncoprotein expression and phosphorylation in our patients' marrow samples, assessing CD34+ marrow intracellular AKT2 phosphorylation, a biomarker of apoptotic and cell cycle signaling [25], pre- and post-treatment.

Patients, Methods

The objectives of this Phase I/II study were to evaluate the efficacy and safety of ON01910.Na treatment in achieving marrow responses or hematological improvement (HI) in patients with Trisomy 8 cytogenetics or those classified as IPSS Intermediate-1, Intermediate-2 or High risk whose disease had failed to respond to at least 4 cycles of hypomethylating agents or were intolerant of these drugs. All patients had been unresponsive to a median of six cycles (range 4–13) of hypomethylating agent therapy (6 post-azacitidine, 6 post-decitabine, and 1 patient treated with both agents). In addition, at baseline, all patients were red blood cell (RBC) transfusion-dependent. All patients had reviewed and signed informed consent forms according to the guidelines of the Stanford Investigational Review Board prior to their entry into the trial.

The patients (9 males, 4 females) had IPSS Intermediate-1 (n=4), Intermediate-2 (n=2) and High (n=7) risk MDS subtypes and the cohort was comprised of patients with RAEB-1 (5 patients), RAEB-2 (4 patients) and RAEB-T (4 patients), with a median age of 75 years (range 65–86) and 1.5 year median (range 0.5–4.6) prior duration of MDS. Their cytogenetic profile included 6 patients with normal cytogenetics and 7 with abnormal cytogenetics (5 with trisomy 8 and 1 complex). This was a higher risk patient cohort, with nine of the 13 patients being IPSS Intermediate-2 or High at study entry. As the four Intermediate-1 patients were RBC transfusion dependent and had failed to respond to hypomethylating agents (after prior first line therapy), they were thus in a higher risk status than Intermediate-1 patients who would have been seen at their time of diagnosis.

Patients were required to have at least one clinically significant cytopenia and to have a baseline serum creatinine of <2 mg/dL and AST/ALT less than twice the upper limit of normal. They could not have received treatment with standard MDS therapies or investigational therapy within 4 weeks of starting ON01910.Na.

The planned total study duration was 33 weeks, which included a 2-week screening phase, a 27-week dosing phase, and a 4-week follow-up phase that began after the last dose of ON 01910.Na. Beginning at week 4, patients were assessed for response. Marrow exams were performed at baseline, and after the first, third, fifth and seventh cycles, and at other times as clinically indicated.

Prior dosing regimens for the drug in MDS have ranged from 800–1500 mg/m²/day for 2 days weekly for 3 out of 4 weeks or from 650–1700mg/m²/day for 3–6 days every 2 weeks as an continuous intravenous infusion (CIVI) [19, 26]. The initial two patients received ON 01910.Na at a dose of 800mg/m²/d CIVI x 2 days/week x 3 weeks/month cycle. However, subsequent clinical data (Onconova Investigator's Brochure, January 25, 2010) led to recommendations to alter the treatment regimen, wherein the remaining 11 patients received the drug at a dose of 1800mg/d CIVI x 3 days every 2 weeks/month cycle for the first 2 months, then monthly. These treatments were given in monthly cycles for a planned seven month course of treatment.

ON 01910.Na (rigosertib) was supplied by Onconova Therapeutics Inc, Newtown, PA, as a sterile, concentrated solution in labeled, sealed glass vials stored at 2 to 8 C. Just prior to dosing, the ON 01910.Na concentrate was diluted with aqueous infusion solution. Reconstituted ON 01910.Na was kept at room temperature and drug administration was started within 2 hours of reconstitution via an infusion set with an inline 1.2 micron filter.

Infusion bags were changed every 24 hours and a new infusion bag was administered for each of the following 24 hours until completion of the total infusion time. The therapy was provided to patients as outpatients with drug placed in a portable infusion set, with their daily return to the nursing unit for changing the infusion bags and stopping the infusion at the end of the 3 day treatment.

Statistical Methods

The response rates and the bone marrow blast and hematopoietic response (hematologic improvement, HI-E, HI, N, HI-P) rates were determined according to International Working Group (IWG) 2006 criteria [27]. Overall survival was estimated using the Kaplan-Meier method. Analysis of the primary efficacy endpoints and safety and toxicity features were performed on all enrolled patients who received at least one dose of ON 01910.Na.

Biologic Correlative Studies

Cell separation—For biologic correlative studies, aliquots of marrow samples obtained from patients pre- and post-treatment were enriched for CD34+ cells, as previously described [28]. The CD34+ marrow cell suspensions were stored frozen in heat-inactivated fetal bovine serum plus 10% dimethylsulfoxide in liquid nitrogen, were thawed at 37°C into pre-warmed phosphate buffered saline (PBS) and washed once in PBS immediately before NIA analysis.

Nano-fluidic proteomic immunoassay (NIA)—The NIA experiments were performed using a Nanopro1000 instrument (Cell Biosciences, Santa Clara, CA) [29] as previously described [23]. Total AKT2 protein expression and its various phosphorylated isoforms were detected using a rabbit polyclonal antibody that recognizes mouse and human AKT2 (Cell Signaling Technology Inc, Boston, MA, #3063). NIA is able to quantify phosphorylated and unphosphorylated isoforms of signaling proteins in a sample, using a single antibody that recognizes all isoforms of the protein. Therefore, changes in relative phosphorylation of a protein can be measured. Quantitation of AKT2 phosphorylated and unphosphorylated protein peaks was performed using Compass (version 1.8.0) analysis software, using Gaussian peaks with variable widths, as previously described [23].

Results

Clinical responses

Thirteen patients were entered into the study and all were evaluable for efficacy and toxicity (Table 1). Patients received a median of six cycles (range 2–7) of treatment, with five patients completing the planned 7 cycles. Responses according to IWG 2006 criteria were: Partial response (1), stable disease (SD) 8, including 2 with transient (2 month) hematologic improvement (HI) (2; 1 HI-E, N, 1 HI-P, N) (#111, 112), and 4 with marrow complete responses (CRs) (Tables 1 and 2). The patients with marrow CRs had survivals of 5–17+ months. Five patients had progressive disease (PD), including 4 who transformed to AML while on trial. Overall survival from time of study entry was 10 months median (range 3–17) (Figure 1); three patients remain alive, with a median time of 14 months (Table 2). Responses occurred in all FAB, IPSS and cytogenetic subgroups. Of the five patients with trisomy 8, two with stable disease (#101, 104) demonstrated a decrease in proportion of trisomy 8 marrow cells from 65 to 15% and 80 to 13% after treatment, whereas the three remaining patients with PD had no such decrements. No other cytogenetic responses were noted in the two other pts with abnormal cytogenetics.

Side effects

Grade 3/4 non-hematologic drug-related or possibly related toxicities occurred in 4 patients: 1 diarrhea, 1 dysuria, 1 fatigue, 1 epistaxis; two patients had pulmonary infections unrelated to study drug. Five patients were able to complete all 7 cycles of treatment. Per protocol guidelines, 5 patients discontinued treatment due to disease progression (4 to AML, 1 to RAEBT) (Table 1). One patient stopped therapy due to drug toxicity (severe dysuria) and two patients had treatment stopped due to infection related to their underlying neutropenias.

Biologic analyses

Using NIA, we analyzed intracellular phosphorylation of AKT2 and its isoforms in CD34+ marrow cells from five patients. The assays were performed to compare signaling at baseline and after initiating ON 01910.Na treatment (Table 3). In three responders, AKT2 phosphorylation substantially decreased by 12 to 19% following cycle 1. In contrast, in two patients who progressed on treatment, AKT 2 phosphorylation decreased by only 2% or increased by 19% post therapy. NIA is able to measure changes in different phosphorylated states of AKT2 (22,23,29); thus, we were able to further resolve changes in AKT2 phosphorylation into distinct phosphorylated states (Figure 2). Interestingly, the decrease in overall phosphorylation was predominantly due to a decrease in phosphorylated AKT2 isoform 3, by 22% to 36% in each of the three responders, whereas it either decreased only 10% or increased 22% in two non-responders whose disease progressed (Table 3). In these patients, assays performed at sequential time points up to seven cycles of treatment showed similar changes as those found following cycle 1.

Discussion

Based on our study, ON 01910.Na appears to be active in patients with higher risk MDS who have failed to respond to hypomethylating agent therapy. A high degree of stable disease was achieved in the patients during their treatment period. Transient marrow CRs occurred in four patients. The drug was tolerated relatively well with few adverse effects. As hematologic toxicity was uncommon in these previously heavily treated patients despite decreasing marrow blastic proliferation, this suggests that the drug had limited negative effects on the patients' residual hematopoietic reserve. Although responsiveness was noted frequently in either the stabilization or improvement of marrow blasts, there was little improvement in hematologic parameters. Responses occurred in all FAB, IPSS and cytogenetic subgroups. The median 10 month survival from time of study entry compares favorably with the 4.3 to 5.6 month medians previously reported in higher risk MDS patients whose disease failed to respond to hypomethylating agents [5, 6]. Effective clinical responses have been seen with this drug in other advanced human neoplasms [16,17].

Most current methods of protein detection are insensitive to detecting subtle changes in oncoprotein activation that underlie critical hematopoietic signaling processes. The requirement for large numbers of cells precludes serial cell sampling for assessing a response to therapeutics. Thus, in order to accurately measure oncoprotein expression and activation in our marrow specimens, we utilized the nano-fluidic proteomic immunoassay detection method (NIA) [23]. This method separates proteins by isoelectric focusing, followed by antibody detection of specific epitopes with chemiluminescence and quantifies total and low abundance protein isoforms. It has also previously been shown to measure changes in the expression and activation of a variety of other onco/signaling proteins with high accuracy and sensitivity in preclinical and clinical specimens of hematopoietic malignancies, before and during treatment [23,29,30].

The serine/threonine kinase AKT functions as a critical mediator of signaling downstream of PI3 kinase and is essential for different physiological processes and its constitutive activation has been implicated in the pathogenesis and the progression of a wide variety of neoplasias, including MDS and AML [20–22]. Protein and mRNA levels of the Akt2 isoform increase with the pathological grade of malignant gliomas and its down-regulation induced apoptosis in a variety of tumor cells [31]. Our NIA analysis of bone marrow cells sampled before and after ON 01910.Na treatment demonstrated substantially decreased AKT2 phosphorylation of marrow CD34+ cells in the three MDS patients assayed who responded to treatment, whereas in two patients who did not respond and whose disease progressed, there was no decrease (or even increased). Notably, NIA resolved different phosphorylated isoforms of AKT2, and uncovered a decrease in responders in phosphorylation predominantly occurring in isoform 3. These findings are of interest as AKT signaling, including that for AKT2, is a critical pathway for metabolic signaling, protection against tumor cell apoptosis, cancer cell invasion and chemoresistance in AML [25, 31–33]. Altered levels of AKT2 phosphorylation, particularly for isoform 3, may correlate with response to ON01910.Na. Extension of these preliminary studies are needed to determine whether the changes in AKT2 isoform 3 may be useful to provide a potential surrogate biologic marker for responsiveness to ON 01910.Na therapy.

In summary, the responsiveness, median survival and drug tolerance of these higher risk MDS patients to ON 01910.Na are encouraging. However, a randomized Phase III clinical trial is required to clarify the potential survival advantage and for decreasing AML progression of this therapy in such patients, particularly those whose disease failed to respond to hypomethylating agents. Such a Phase III trial is now underway, based in part on results of this study. Use of surrogate biomarker correlates such as NIA of intracellular phosphorylation of relevant signaling proteins should prove useful for determining drug mechanism of action and for patient evaluation and selection on therapeutic trials with this and other kinase inhibitors.

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Figure 1. Overall survival of patients treated with ON 01910.Na from the onset of start of study drug therapy (Kaplan-Meier curve).

Figure 2.

Treatment effects of ON 01910.Na on AKT2 phosphorylation using nanoimmunoassay (NIA) analysis: Decreased phosphorylation in CD34+ marrow cells in (a) a responding MDS patient (#101) with stable disease, (b) in a responding patient (#104) with marrow complete response, and (c) in a nonresponder (#111) whose disease progressed on treatment.

Table 1
Clinical status, treatment and responses of individual MDS patients to ON01910.Na

Patient	Age/Gender	Modified FAB	IPSS category	Treatment Cycles, Number	Marrow response	Developed AML	Survival, months
101	80M	RAEB-T	High [*]	7	SD		17
102	74M	RAEB-2	Int-2	3 ^a	SD		11
103	73M	RAEB-1	Int-1	7	SD		10
104	77M	RAEB-1	Int-2 [*]	7	SD (mCR)		8
105	66M	RAEB-1	Int-1	1 ^b	SD (mCR)		13+
106	78M	RAEB-2	High	1 ^c	PD	Yes	4
108	65F	RAEB-1	Int-1	7	SD (mCR)		17+
110	76M	RAEB-T	High	7	SD (PR)		8
111	86M	RAEB-T	High	5 ^a	SD ^f →PD		12
112	81F	RAEB-2	High [*]	5 ^d	SD ^g →PD		14+
117	75F	RAEB-2	High [*]	3 ^{c,e}	PD skin (mCR)	Yes	5
121	80M	RAEB-T	High [*]	1 ^c	PD	Yes	3
122	76F	RAEB-1	Int-1	5 ^c	SD→PD	Yes	8

Modified FAB includes RAEB-1, RAEB-2 as defined by the WHO classification. RAEB=refractory anemia with excess blasts, RAEB-T=refractory anemia with excess blasts in transformation. Int=Intermediate. Survival measured from time of study entry

^{*} Trisomy 8 cytogenetics

SD= stable disease, PD=progressive disease, mCR=marrow complete response, PR=partial response

Discontinued treatment due to:

^a Infection related to refractory neutropenia

^b Severe dysuria

^c PD (AML)

^d PD (RAEBT)

^e Leukemia cutis despite marrow CR; overt AML 1 month later

Hematologic responses:

f_T Transient hematologic improvement-erythroid, neutrophils (HI-E,N)
 g_T Transient hematologic improvement-neutrophils, platelets (HI-N,P)

Table 2

Responses to therapy with ON 01910.Na related to baseline clinical features

Modified FAB Morphologic Subgroup					
	Patients	Stable Disease	Marrow Complete response	Progressive disease	Survival, months
RAEB-1	5	4	3	1	10 [8,8,10,13+,17+]
RAEB-2	4	2	1	2	8 [4,5,11,14+]
RAEB-T	4	2 (1PR)	0	2	10 [3,8,12,17]
IPSS Category					
Intermediate-1	4	3	2	1	11.5 [8,10,13+, 17+]
Intermediate-2	2	1	1	1	9.5 [8,11]
High	7	4 (1PR)	1	3	8 [3,4,5,8,12,14+,17]
All patients	13	8	4	5	10 [3–17]

Modified FAB includes RAEB-1, RAEB-2 as defined by the WHO classification. PR= Partial response

Table 3

Nanoimmunoassay (NIA) Quantification of AKT2 Isoforms in CD34+ Bone Marrow Cells from MDS patients treated with ON 01910.Na

Patients	Clinical Response	% Change in Phospho-AKT2 levels *	% Change in Phospho-AKT2 Isoform 3 levels *
101	Stable Disease	19% decrease	22% decrease
104	Marrow CR	15% decrease	36% decrease
105	Marrow CR	12% decrease	25% decrease
111	Progressive Disease	2% decrease	10% decrease
121	Progressive Disease	18% increase	22% increase

* Percentage (%) change following cycle 1 compared to pretreatment, except for patient 111, who was evaluated post cycle 5 at time of disease progression.