

Synergistic Effects of a Novel Water-soluble Small Molecule, ON 013105, and Rituximab on Mantle Cell Lymphoma *In vitro* and *In vivo*

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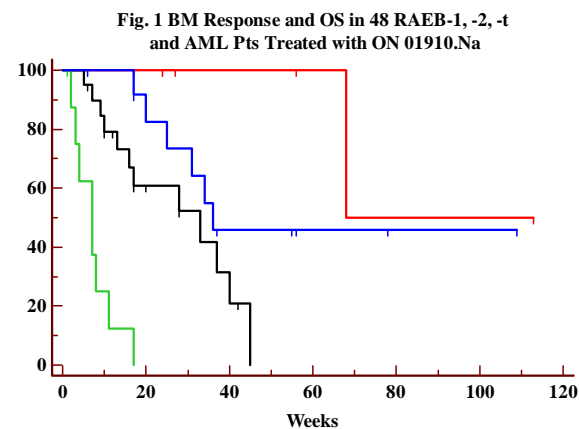
Mantle cell lymphoma (MCL) is a well-defined subtype of B-cell non-Hodgkin's lymphoma characterized by a t(11;14)(q13;q32) chromosomal translocation, and associated with constitutive over-expression of cyclin D1. MCL generally has poor clinical outcome marked by relapse. There is considerable need for novel and more effective agents against MCL. ON 013105 belongs to the styryl benzylsulfones, a novel family of non-ATP competitive kinase inhibitors with potent antitumor activity. Here, we report that ON 013105 induced cell death in a dose-dependent manner in two well-characterized MCL cell lines, Granta 519 and Z138C. *In vitro* cell death was preceded by the activation of caspases 3 and 9 and cleavage of PARP, indicating induction of apoptosis. In addition, ON 013105-treated cells exhibited reduced expression of cyclin D1 and c-myc. These effects on expression and apoptosis were not evident in cells treated with ON 013101, an inactive (non-cytotoxic) isomer of ON 013105. Since it is common clinical practice to combine Rituximab (RTX) with chemotherapy regimens in treating CD20+ B cell-lymphoma, we studied ON 013105 combined with rituximab, and found ON 013105-induced apoptosis more efficiently than when employed as a single agent. The combination effect on cell death was synergistic in nature. To further study this activity, we focused on Mcl-1, a member of the anti-apoptotic Bcl-2 family known to inhibit apoptosis induced by cytotoxic stimuli through antagonizing pro-apoptotic Bcl-2 family members. We observed a dramatic decrease in Mcl-1 expression in cells treated with ON 013105 (but not with ON 013101) in combination with RTX, compared to ON 013105 alone. We also evaluated the effects of ON 013105 in combination with Doxorubicin or Vincristine and found that both these compounds also significantly enhanced the cytotoxic effects of ON 013105. *In vivo* pharmacokinetics studies in a mouse model system revealed that plasma concentrations up to 50 μ M could be safely achieved by administering ON 013105 at 100 mg/kg via i.v or i.p routes. Significant levels of ON 013100 (30-40% of the peak levels of ON 013105), an active metabolite, were also detected in the circulation, presumably due to the *in vivo* dephosphorylation of ON 013105 by phosphatase action. ON 013105 was well tolerated in mice, both as a single agent and when used in combination with rituximab, and there were no systemic toxic effects to the host and no loss in body weight. *In vivo* efficacy studies in mouse xenograft models employing transplanted MCL cells demonstrated that ON 013105 effectively inhibited tumor growth in a dose-dependent manner. ON 013105 at 25 mg/kg (Q2D) and 75mg/kg (Q7D) induced 46% and 80 % reduction of tumor volume, respectively, compared to controls, over 4 weeks of treatment. Moreover, ON 013105 at 25 mg/kg (Q2D) in a combination regimen with RTX (2.5 mg/kg, Q3D) induced over 85% reduction of tumor volume. Though *in vivo* efficacy studies of ON013105 (25 mg/kg, Q2D) in combination with Doxorubicin (3.5mg/kg, Q7D) or Vincristine (0.3mg/kg, Q2D) showed drastic decrease in tumor growth in mouse models, this effect was accompanied by severe side effects to the host, including mortality. In sum, ON 013105, alone and in combination with RTX may be a potent therapeutic agent against MCL. A Phase I dose escalation trial of ON 013105 as a single agent is underway in patients with relapsed/refractory lymphoma including MCL.

Overall Survival in Patients with a Myelodysplastic Syndrome or Acute Myeloid Leukemia Treated With ON 01910.Na Correlates with Bone Marrow Blast Response

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We analyzed bone marrow (BM) response and overall survival (OS) in 48 patients (pts) with a myelodysplastic syndrome (MDS) and WHO/FAB subtypes of refractory anemia with excess blasts (RAEB) -1, -2 or -t and acute myeloid leukemia (AML), enrolled in 4 independent ongoing clinical trials of the novel small molecule ON 01910.Na. Pts received ON 01910.Na administered as a continuous intravenous infusion (CIV) from 2 to 6 days weekly or every other week with BM response initially assessed per protocol by week 4 or 8. A BM complete response (CR) ($\geq 50\%$ decrease from baseline BM blast and decrease below 5% for at least 4 weeks, per MDS IWG 2006 criteria) or an initial 50% decrease of BM blasts by week 4 to 8 was documented in 19/48 (40%) treated pts and was associated with a significant increase in overall survival (OS) ($p = 0.0001$) by the method of Kaplan-Meier (Figure 1 and Table 1). This relationship was still significant when excluding AML patients ($p = 0.008$). Six pts (3 RAEB-1, 3 RAEB-2) had complete BM response. Five of these six pts previously failed to respond or relapsed after azacitidine/decitabine and five out of six are alive (17 to 113 weeks follow-up; one death at 68 weeks). Eleven pts (5 AML, 3 RAEB-t, 2 RAEB-2 and 1 RAEB-1) could not be assessed at 4-8 weeks with follow-up BM evaluation; their median OS was 7.5 weeks; 9 of these 11 pts previously failed to respond or relapsed after azacitidine/decitabine. Among 10 pts with trisomy 8 cytogenetics (4 had an initial BM response), median survival was 25 weeks. FAB/WHO classification of all 48 pts was also significantly correlated with survival (Table 2, $p=0.003$). Overall, ON 01910.Na infusions were well tolerated. Eight pts had hematological improvements at various time points after starting therapy. Median OS was 33 weeks in the subset of 29 RAEB-1,-2,-t pts refractory or relapsing after azacitidine/decitabine, and a significant association ($p = 0.056$) between BM response and OS was also found in these pts (Table 1). The median survival of MDS pts who had failed to respond to prior treatment with decitabine has been reported to be approximately 17 weeks (Jabbour et al, Cancer 2010, in press). These results suggest a strong correlation between BM blast response and OS and the predictive value of BM response to ON 01910.Na for estimating overall survival of higher risk MDS or AML pts.



4-8 Week BM Blast Reduction (%)	BM CR	$\geq 50\%$ Initial Response	$< 50\%$ Initial Response	Not Assessed	P value Logrank test
Figure Legend	Red	Blue	Black	Green	
RAEB-1, RAEB-2, RAEB-t, & AML pts, N = 48	6	13	18	11	
Median Survival (weeks)	Not reached	36	28	7.5	P = 0.0001
RAEB-1, RAEB-2, RAEB-t pts relapsed/refractory to azacitidine/decitabine, N = 29	5	6	12	6	
Median Survival (weeks)	68	Not reached	33	3.5	P = 0.056

FAB/WHO Classification	RAEB-1	RAEB-2	RAEB-t	AML ($>30\%$ BM blasts)	P value Logrank test
N pts	11	18	10	9	
Median Survival (weeks)	Not reached	28	20	11	P = 0.003

Evaluation of ON01910.Na In Patients with a Myelodysplastic Syndrome(MDS) or Acute Myeloid Leukemia (AML) Relapsed or Refractory to Hypomethylating Agents: A Phase I Study

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Background: ON 01910.Na a novel benzyl styryl sulfone derivative is under clinical development in hematologic malignancies. It is a multi-kinase/PI3 kinase inhibitor that promotes G2/M arrest and selectively induces apoptosis in cancer cells. Leukemic cells exhibit significantly higher levels of sensitivity to ON 01910.Na compared to normal marrow progenitors and increasing cytotoxicity upon prolonged and repetitive exposure (Skidan Proc AACR 2006; Chen Proc AACR 2008). Azacitidine (AzaC) is first line therapy for patients (pts) with higher-risk MDS and produces a response rate of 50%. Pts relapsed or refractory to hypomethylating based therapies have a poor prognosis and there are no accepted effective second line treatments, thus a need for new agents.

Methods: A phase I/II study of ON 01910.Na is being conducted in pts with hematological malignancies. In the phase I component pts are entered in cohorts of escalating doses in a classic 3+3 design in doses ranging from 650 up to 1700 mg/m²/d continuous IV infusion (CIV) for durations from 72 hours up to 144 hours every 2 weeks (1 cycle) for 4 cycles of treatment during the induction phase. Subsequent treatments are administered every 3 to 4 weeks. A CBC is performed weekly and a bone marrow (BM) is performed at baseline and week 4, 8, and then q3 months thereafter. Pts with higher-risk disease had to have failed a hypomethylating agent.

Results: Ten pts with MDS or AML relapsed/refractory to a hypomethylating agent have been treated with ON 01910.Na thus far (table 1). The study cohort comprised pts with a diagnosis (Dx) RAEB-2 (4 pts), RAEB-T (1 pt), and AML (5 pts) (median age of 75 years). Their cytogenetic profile included 1 pt with normal, 2 with intermediate (+8), and 7 pts with poor risk cytogenetics (monosomy 7 and/or complex). Patients were treated between 5 and 70 weeks. Responses according to IWG 2006 criteria were observed in the BM and peripheral blood: Marrow CR (3), hematologic improvement (HI-P) (2); erythroid (1) platelet (1). An additional 2 pts had a >50% BM blast decrease from baseline but not to < 5%. Thus, 5/10 (50%) demonstrate a bone marrow response. Survival of these pts was 7.3, 15.7, and 16.4 months; one patient remains on study 5+ months. Four of the five responders had MDS at the initiation of treatment: RAEB-2 (3), CMMoL (1), AML (1). Responders had monosomy 7 (2), trisomy 8 (1) and complex cytogenetics (2). One pt had an elimination of the MDS clone and the others had persistence of the abnormal karyotype throughout their treatment course. Five pts had SD without HI at 4 weeks, 2 pts progressed to AML. All 5 non-responders had AML; 4 with a proliferative course. These latter received only 2 (2) or 3 (3) cycles before succumbing to disease related infectious complications. Survival for these patients ranged from 1.3 - 2 months with a median duration on study of 42 days. The most frequent side effects grade 2 for all pts included fatigue, anorexia, nausea, and dysuria in patients receiving extended duration infusions. One pt had a grade 3 urinary frequency. No hematologic toxicities occurred and no bone marrow toxicity or hypoplasia was noted. Pharmacokinetic studies are ongoing, data to date demonstrate no evidence of drug accumulation in patients who are treated repeatedly.

Conclusion: ON 01910.Na appears to be safe and well tolerated in patients with refractory or relapsed MDS and AML. ON 01910.Na has biologic activity with reduction in BM blasts, eradication of the MDS clone and improvement in the peripheral blood counts in some pts. These effects are associated with increased survival albeit in limited numbers of pts treated thus far. Further study of ON 01910.Na is warranted to better define biologic activity, appropriate target populations and to define mechanism of action.

Table 1.

Pt ID	Initial Dx/ OnStudy Dx	Prior Therapy	On Study % BM Blasts	Max BM Response (IWG 2006 criteria)	Dosing Cohorts (mg/m ² /d) CIV	# of cycles	Duration On Study (mo)	OS (mo)
001	High/AML	AzaC	80	PR	650	10	7	7.3
002	Int-1 MF/high	AzaC	11	CR	1050	14	15.7	15.7
003	AML/AML	AzaC+saha	45	NR	1050	2	1.6	2
004	High/high	AzaC	17	CR	1050	19	16.4	16.4
005	Int-2/AML	AzaC	91	NR	1375	2	1.2	1.5
006	CMMoL/CMMoL	Decitabine	22	PR	1375	4	1.6	4.7
007	Int-2/AML	AzaC	66	NR	1375	3	1.4	1.7
008	Int-1/AML	AzaC	44	NR	1700	3	1.4	1.7
009	MDS-MF- AML/AML	Decitabine	51	NR	1700	3	1.2	1.3
010	Int-2/high	AzaC & decitabine	15	CR	1375	7	5+	5+

(Leuk Res. 2012 Jan;36(1):98-103. Epub 2011 Sep 14)

American Society of Hematology, 52nd Annual Meeting, 4-7 December 2010, Orlando, FL

Session: Myelodysplastic Syndromes: Poster III; Monday, December 6, 2010, 6:00 - 8:00 PM; Board # III-789

Abstract #4010

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

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Patients with IPSS intermediate or high risk myelodysplastic syndrome (MDS) have few treatment options once their disease fails to respond to hypomethylating agent therapy. The styryl sulfone mitotic inhibitor ON 01910.Na, inhibits Polo-1 kinase, PI3-kinase and AKT pathways, and the drug has shown promising results in patients (pts) with advanced solid tumors (Jimeno et al, J Clin Onc 26:5504, 2008) and in Phase I/II studies of MDS pts, including those with trisomy 8 (Sloand et al, Proc ASH 2007, #822, Proc ASH 2008, #1651). In an ongoing Phase II clinical trial, we have treated 10 MDS pts unresponsive to at least 4 cycles (range 4-12 cycles) of hypomethylating agent therapy (5 post-azacytidine, 4 post-decitabine, and 1 pt treated with both agents) with ON 01910.Na. The pts had IPSS Intermediate-1 (n=3), Intermediate-2 (n=4) and High (n=3) risk MDS. The study cohort comprised pts with RAEB-1 (4 pts), RAEB-2 (3 pts) and RAEB-T (3 pts), with a median age of 80 years (range 65-86) and 2.3 year median (range 0.4-5.4) prior duration of MDS. Their cytogenetic profile included 5 pts with Good, 4 with Intermediate [t(8,10), t(14,18), +8, and (8+, 19+)], 1 with Poor risk cytogenetics (+8, 5q-, 1p-). At baseline, all pts were red blood cell transfusion-dependent. After the initial 2 pts were treated with 800mg/m²/day x 2day continuous IV infusion (CIVI)/week x 3weeks/month, the subsequent 8 patients received 1800mg/day x 3day CIVI q2weeks/month x 2 months, then monthly. Patients underwent bone marrow sampling and evaluation after every other cycle of treatment. To date, 7 pts have completed at least 2 cycles of therapy, with 2 pts having completed the full treatment course of 7 monthly cycles (median 4.7 for all pts). Responses according to IWG 2006 criteria were: Marrow CR (mCR) (2), Partial response (1), stable disease (SD) with hematologic improvement (HI) (2; 1 HI-E, N, 1 HI-P, N) = 5/10 overall responses (50%). Four pts had SD without HI, 1 pt progressed to AML. mCRs occurred in those with <10% marrow blasts. The 3 patients with trisomy 8 alone or with additional cytogenetic abnormalities achieved 1 mCR, 2 SD, associated with decrements in proportions of +8 karyotypes from 80% and 65% to 15% in the 2 pts with additional abnormalities. Grade 3/4 non-hematologic drug-related or possibly related toxicities occurred in 4 pts: 1 GI, 1 dysuria, 1 fatigue, 1 epistaxis; 2 pts had pulmonary infections unrelated to study drug. Two pts discontinued treatment early due to drug toxicity (1) or AML progression (1). No hematologic toxicities occurred. Nine of 10 pts remain alive (5.4 month median, range 2.5-13+) after study entry. To determine if a biologic response to ON 01910.Na treatment could be quantified, a novel nanoscale immunoassay (NIA) was used to measure changes in AKT kinase family activation in CD34+ cells isolated from the bone marrow specimens collected before and during treatment. In the subset of specimens analyzed to date, phosphorylation of AKT2 decreased by 36% in pts who had a response to therapy, suggesting that ON 01910.Na treatment altered oncogenic signaling in the MDS pts. Completion of NIA analysis will demonstrate whether changes in individual AKT isoforms (implicated in cell cycle arrest and apoptosis) occur uniquely in pts who have a clinical response to treatment. These preliminary data demonstrate encouraging efficacy and drug tolerance with ON 01910.Na in a substantial portion of higher risk MDS patients whose disease had been unresponsive to hypomethylating agent therapy. Accrual and continuing therapy for trial completion are ongoing.