

Multicenter Pharmacokinetic Evaluation of ON 01910.Na, a novel broad-spectrum anticancer agent, in Phase I Single Agent Clinical Trials in Patients with Solid Tumors

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Introduction: ON 01910.Na is an anti-cancer agent with demonstrated activity against both solid tumors and hematological cancers. The purpose of this research was to evaluate the effect of dose and administration schedule on ON 01910.Na pharmacokinetics in advanced, heavily pre-treated solid tumor patients. **Methods:** Phase I studies were performed under three clinical trial protocols conducted at Mt. Sinai and Einstein, and three sites in India covering a wide range of doses and infusion schedules: Protocol 1 (50-1375 mg/m²/day over 72 hrs); Protocol 2 (250 – 4450 mg/m²/day over 24 hr) and Protocol 3 (2400-3200 mg over 2, 4 or 8 hr);. In some subjects, ON 01910.Na pharmacokinetics were evaluated for more than 1 dosing cycle. Plasma samples were collected pre-dose, during drug administration, and up to 72 hours post-infusion. ON 01910.Na plasma levels were determined by a LC/MS/MS. **Results:** 95 data sets corresponding to 81 patients were evaluated in this study. ON 01910.Na showed biphasic elimination from the plasma, regardless of dose and administration schedule. The functional half-life of ON 01910.Na, estimated from the initial decline of plasma levels following infusion termination, was less than 2 hours. This was confirmed in data from patients receiving prolonged infusions (24 and 72 hr), as ON.01910.Na approached steady state plasma levels within several hours after dose initiation. As noted in the table below, ON.01910.Na clearance was lower at higher drug dosing rates. There were no differences in drug pharmacokinetics among dosing cycles. **Conclusion:** The pharmacokinetics of ON 01910.Na is dose dependent. A continuous IV infusion would be recommended to treat patients because of its short plasma half-life and rapid clearance. Clearance, and consequently systemic drug exposure is not affected by type of dosing (flat dosing vs. BSA adjusted). No significant differences in the pharmacokinetic profile were noted in comparing data from two US sites with data obtained at three sites in India.

Effect of Dosing Rate on ON 01910.Na Clearance

Dosing Rate (mg/m ² /hr)	N	Clearance (L/hr/m ²)
0-25	18	9.3 ± 4.7
25-50	16	10 ± 5.0
50-75	20	9.2 ± 3.1
75-100	6	5.5 ± 2.6
100-200	12	6.2 ± 3.8
200-400	8	3.3 ± 1.6
400-800	11	2.5 ± 1.8
800-1250	4	1.9 ± 0.47

ON 01910.Na, a clinical trial stage multi-kinase inhibitor, induces apoptosis in chronic lymphocytic leukemia (CLL) cells through inhibition of PI3K/AKT and activation of the JNK pathway resulting in NOXA and BIM upregulation

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Chronic lymphocytic leukemia (CLL), the most common leukemia in Western countries, is a clinically heterogeneous disease characterized by the accumulation of mature B lymphocytes, where disease progression is driven by survival and proliferation signals provided by the tumor microenvironment. ON 01910.Na (Onconova Therapeutics) belongs to the family of styryl benzyl sulfones, a novel family of non-ATP competitive kinase inhibitors. ON 01910.Na is under clinical development in hematologic and solid tumors. Here we show that ON 01910.Na induced apoptosis in CLL samples (n=28) *in vitro*, without showing significant toxicity against T-cells or normal B-cells. Twenty four CLL samples were highly sensitive to ON 01910.Na (IC₅₀ 0.89 μM) a concentration readily achieved in phase I clinical trials, three showed moderate sensitivity (IC₅₀ 4.26 μM) and one was resistant (IC₅₀ >8 μM). There were no significant differences in ON 01910.Na cytotoxicity against CLL cells expressing mutated (IC₅₀ 1.33 μM) or unmutated (IC₅₀ 1.22 μM) IgVH sequences. ON 01910.Na was similarly effective against tumor cells carrying 17p or 11q deletions. ON 01910.Na activated Bax and Bak, leading to mitochondrial depolarization. To delineate the upstream pathways underlying ON 01910.Na induced apoptosis, we performed Gene Expression Profiling (GEP) in CLL cells treated *in vitro* for 4 and 10 hours with ON 01910.Na. Gene Set Enrichment Analysis (GSEA) identified gene sets indicating BCR and PI3K inhibition (FDR<0.2). In keeping with PI3K inhibition, we found that ON 01910.Na inhibited phosphorylation of Akt and Foxo3a, and FOXO3 target genes, including Bim, were upregulated. In addition, GSEA identified induction of AP-1 gene sets (FDR<0.01) suggesting the generation of reactive oxygen species (ROS) by ON 01910.Na. Consistently, we found a rapid increase in ROS in cells treated with ON 01910.Na and ROS scavengers protected cells from its cytotoxic effect. ROS upregulated the proapoptotic BH3-only protein Noxa, and knockdown of Noxa by shRNA decreased the sensitivity to the drug by 60%. Our results identify ON 01910.Na as a promising agent in the treatment of CLL with an interesting dual mechanism of action: inhibition of the PI3K/AKT survival pathway with Foxo-mediated upregulation of BIM and the induction of oxidative stress resulting in Noxa upregulation. These data support the development of ON 01910.Na in CLL and a clinical trial at the NHLBI is currently enrolling patients

ON 01910.Na, a clinical stage anticancer mitotic inhibitor, produces prolonged hyperphosphorylation of RanGAP1•SUMO1 as a potential mechanism of G2/M arrest and apoptosis

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INTRODUCTION: The benzyl styryl sulfone ON 01910.Na (abbreviated as 1910) is a novel anticancer agent that inhibits mitotic progression and induces apoptosis in most cancer cell cultures. The compound is currently in Phase 1 and 2 trials. Available data show the drug produces three major abnormalities in tumor cell lines: (a) aberrant cell division, including irregular chromosomal segregation and cytokinesis; (b) G2/M arrest and apoptosis in many tumor cells; and (c) decreased expression of Cdc25C phosphatase. Early data suggested that 1910 was a PLK1 inhibitor. Subsequent studies did not confirm this, although 1910 was found to inhibit the PLK pathway. Precise mechanism of action continues to be investigated. **METHODS and RESULTS:** We assessed DNA damage checkpoints throughout the cell cycle and effects on signaling molecules upstream of Cdc25C following treatment of DU145 prostate cancer, Bel7404 hepatoma, U937 lymphoma or MOLT-3 ALL cells with 1910. Camptothecin (CPT) and doxorubicin (DOX) served as positive controls. Cell lysates after drug exposure for 4, 16 or 24 h were resolved on SDS-PAGE and analyzed by Western blot for Chk1, Chk2, ATM, RanGAP1•SUMO1 and their phosphorylated forms. CPT and DOX exposure resulted in activation/phosphorylation of DNA damage-responsive molecules Chk1, Chk2 and ATM by 4 h, whereas 1910 did not. However, hyperphosphorylation of RanGAP1•SUMO1 was observed within 4 h and sustained for more than 24 h following 1910 exposure. This was also seen with the anti-tubulin agent, nocodazole, but not with ON 01911, an inactive analog of 1910. Mild phosphorylation of Chk2 was observed only after 24 h exposure, suggesting that DNA damage response is not a primary effect of 1910. MOLT-3 cells, synchronized by double thymidine block, were released into medium supplemented with 1910, which resulted in peak accumulation of G2/M cells by 9-12 h. The G2/M cell fraction remained in plateau for more than 20 h. This timeframe correlated with the hyperphosphorylation of RanGAP1•SUMO1. Cleaved Lamin B, detected by 16 h and thereafter in 1910-containing release medium, confirmed an active apoptotic process during this period. MOLT-3 cells released into drug-free medium reached G2/M peak at 9-10 h and continued to cycle with synchronized transition into G1 at 15-16 h. RanGAP1•SUMO1 was not hyperphosphorylated and no cleaved Lamin B was detected. Tubulin polymerization assay revealed that 1910 and ON 01911 had little or no effect, whereas nocodazole inhibited the process. **CONCLUSION:** These findings show that 1910 is neither a direct DNA damage response inducer nor a tubulin toxin, and suggest that 1910 is an inhibitor of RanGAP1•SUMO1 phosphatase. Its mechanism of action appears to rely on prolonged hyperphosphorylation of RanGAP1•SUMO1, leading to G2/M arrest and induction of apoptosis.

Proposed Pathway of Disposition of ON 01910.Na, a Novel Clinical Trial Stage Anti-Cancer Agent: Implication of Mrp2 in Biliary Excretion in the Isolated Perfused Rat Liver System.

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Introduction. ON 01910.Na is a novel targeted anti-cancer agent under clinical investigation in Phase I and Phase II trials. Preclinical studies indicate that the compound distributes extensively to the liver but is not extensively metabolized *in vivo*. The purpose of this study was to evaluate the disposition of ON 01910.Na employing the isolated perfused rat liver (IPRL) model. The specific goals were to 1) Assess the dose-linearity of ON 01910.Na disposition; 2) Probe the role of the Mrp2 transporter on ON 01910.Na biliary excretion; and 3) Establish the effect of concurrent dosing with oxaliplatin and doxorubicin on ON 01910.Na hepatobiliary disposition. **Methods.** Perfusion experiments (n=3/group) were performed at 4 doses (0.8, 4, 8, 20 mg), targeting a range of perfusate levels between 10 and 250 ug/ml. ON 01910.Na (10 ug/ml) disposition was then studied in the presence of doxorubicin (2.5 ug/ml) and oxaliplatin (2 ug/ml). IPRL experiments were also conducted using livers from Mrp2 deficient rats. ON 01910.Na was assayed in perfusate and bile samples by HPLC. **Results.** ON 01910.Na parameter estimates are provided in the table below. The compound displayed nonlinear excretion in the IPRL, and ON 01910.Na disposition was altered in mrp2-deficient rats. The effect of co-administered chemotherapeutic agents is being investigated. **Conclusions.** ON 01910.Na showed extensive biliary excretion in the IPRL, and IPRL findings correlated with *in vivo* data in rats. ON 01910.Na biliary transport appears to be mediated in part by Mrp2.

ON 01910.Na Disposition in the IPRL

PARAMETER	ON 01910.Na (ug/ml)			
	10.0	50.0	100	250
t _{1/2} (hr)	0.460 (0.0340)	0.327 (0.0674)	0.585 (0.0810)	1.26 (0.318)
C _{max} (ug/ml)	11.3 (1.75)	56.1 (7.12)	106 (11.2)	257 (658)
AUC (0-∞) (ug*min/ml)	290 (87.1)	1480 (73.0)	4010 (963)	21100 (2580)
Cl (ml/min)	2.91 (0.753)	2.71 (0.134)	2.08 (0.557)	0.959 (0.125)
% Drug Excreted in Bile ^e	57.2 (5.04)	59.2 (7.40)	68.4 (0.870)	45.6 (2.66)

^aData presented as mean (standard deviation)