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## **Design, synthesis and biological evaluation of novel, orally available tubulin depolymerizing (E) N-aryl-2-arylethanesulfonamide compounds**

### **Short Title:**

Tubulin depolymerizing sulfonamides

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*Abstract:* Many antimetabolic agents that interfere with polymerization/depolymerization of  $\alpha$  and  $\beta$ -tubulins have been successfully used for cancer treatment. These agents cause dynamic instability of microtubules resulting in cell cycle arrest in the M-phase, forming abnormal mitotic spindles. Agents such as vincristine and paclitaxel have gained wide clinical use for the treatment of various cancers, but suffer from undesired side effects, particularly neurotoxicity, and are substrates of various efflux mechanism leading to drug resistance. It is therefore desirable to discover novel antitubulin agents with fewer side effects, improved pharmacokinetic properties and better efficacy against MDR+ cancer cells.

Here, we describe a new class of small molecule antitubulin agents which appear to satisfy many of the criteria for successful development of new anticancer agents. These compounds belong to the sulfonamide family and could readily inhibit the polymerization of tubulins both in vitro and in vivo. Several of these compounds are water soluble with suitable pharmacokinetic profile and are stable and easily available by analytical methods. They exhibit potent (IC<sub>50</sub> of 5-10 nanomolar) activity against a wide spectrum of cancer cell-lines, including all drug resistant cell-lines (e.g. MES-SA, HCT116, MDA-MB, etc.) in the NCI collection and are active against solid tumor cell-lines in nude mouse xenograft assays. Preliminary drug development studies have been completed with ON 24160, which was found to be completely soluble in PBS at 62.5  $\mu$ M, and non toxic to Caco-2 and HepG2 cells after 1 and 4h of incubation, respectively. The absorptive permeability of ON24160 is high and implies intestinal absorption to be favorable. Compound ON24160 is metabolized at a moderate rate with 20% parent remaining following a 1 hour incubation with human liver S9. ON 24160 is extensively bound (>98%) to rat, dog and human plasma proteins. Preliminary toxicology studies suggest their relative safety profile and support the rationale for further development of these molecules toward clinical testing as single agents and in combination with novel mechanism directed targeted agents.

**Author Disclosure Information:** **M. Reddy**, Onconova Therapeutics Inc. Consultant/Independent Contractor, Stock Shareholder (self managed); **S.C. Cosenza**, Onconova Therapeutics Inc Consultant/Independent Contractor, Stock Shareholder (self managed); **V.R. Pallela**, Onconova Therapeutics Inc Consultant/Independent Contractor; **S.R. Natala**, Onconova Therapeutics Inc Consultant/Independent Contractor; **M.R. Mallireddigari**, Onconova Therapeutics Inc Full-time/Part-time Employee; **M. Maniar**, Onconova Therapeutics Inc Full-time/Part-time Employee, Stock Shareholder (self managed); **N.M. Iqbal**,

**A gene expression-based approach to devise combinations with gemcitabine (GEM) in pancreatic cancer (PC) identifies polo-like kinase 1 (Plk1) as a rational target**

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**Purpose:** This work is aimed at discovering targets for rational combination therapy with gemcitabine (GEM) in PC, in view of its poor single-agent efficacy. We hypothesized that upon exposure to GEM, the tumor cells would adapt by modulating key factors, especially at the gene expression level. Querying this adaptative response focusing on druggable targets would provide a window of opportunity to detect potentially vulnerable points, and identify candidates for combination. **Methods:** We selected 11 cases from a collection of freshly generated human PC xenografts with a wide range of GEM sensitivity *in vivo*. We exposed fine-needle aspiration (FNA) material to either vehicle or GEM (1 microM) for 6 hours, and compared the gene expression profile of the treated and untreated samples using a RT-PCR-based, customized low-density array with 45 genes of interest representing known drug targets. **Results:** We observed that the only dynamic gene signal that differentiated G sensitive from resistant cases was Plk1, showing > 50% down-regulation in all sensitive cases and no change in resistant cases. The variations in Plk-1 levels induced by GEM *ex vivo* were confirmed in the tumors from the 28-day GEM mice experiments, where a strong correlation between *ex vivo* and *in vivo* Plk1 dynamics existed. To assess the mechanistic implications of these observations, we used siRNA knockdown of Plk1 in a panel of six GEM-resistant PC cell lines, and tested GEM, Plk1 siRNA and the combination by MTT. Plk1 knockdown per se had a modest inhibitory effect on two cell lines, but the combination of siRNA and GEM induced synergy in three cell lines, resulting in growth arrest of up to 90%. We then treated the same panel of cell lines with GEM, ON 01910.Na or the combination of both. Whereas the effect of single-agent ON 01910.Na was marginal, the combination of ON 01910.Na and GEM induced synergy in three cell lines that were interestingly the same three found to be sensitive in the Plk1 siRNA experiment. Finally, we conducted three sets of *in vivo* experiments in three of the GEM-resistant tumors with persistent elevated levels of Plk1 with either GEM, the Plk-1 pathway inhibitor ON 01910.Na and the combination of both. Whereas GEM and ON 01910.Na alone had modest or no effect, the combined therapy resulted in a synergistic effect with significant tumor growth inhibition and tumor regressions. Globally, these results provide mechanistic specificity that targeting Plk-1 sensitizes PC to GEM. **Conclusions:** Plk1, a key mitotic regulator that modulates the transition through the G2/M checkpoints, appears to be involved in GEM resistance in PC. A rapid vulnerability assay was developed that identified Plk1 as a putative target involved in GEM resistance in PC. The novel mitotic inhibitor ON 01910.Na (which is currently in clinical trials) had synergistic activity with GEM in GEM-resistant PC xenografts.

**Short title**

Plk1 mediates gemcitabine resistance in pancreatic cancer

## **Novel anticancer agent ON 01910.Na induces phosphorylation of cdc25C-cdc2 via ATM-Chk2 activation, leading to cell cycle arrest and apoptosis of human leukemia cells**

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Previously we described the growth inhibitory effect of ON 01910.Na, a novel styryl sulfone antimotitic compound, on human leukemia cell lines U937, HL60 and K562 (Skidan et al, Proc AACR #1310:2006). This compound is currently being evaluated in multiple clinical trials. In the present study the effect of ON1910Na on the cell cycle, apoptosis and molecular targets in signal transduction were investigated. Treatment of U937, HL60 and K562 cells with ON 01910.Na (250 -1000 nM) resulted in cell cycle arrest at G2/M phase in a dose- and time-dependent manner starting at hour 4 of treatment. Cyclin B1 protein level was upregulated consistent with a block in mitotic progression. ON 01910.Na also induced an apoptotic cell death of U937 cells in a dose- and time-dependent manner. The percent of dead cells (Flow cytometry, AnnexinV/PI) increased from 5.8% to 46.6% after 24h of treatment and to 89.1% after 48h treatment with 1000 nM. Caspase-3 was activated and the apoptosis-marker PARP (poly (ADP) ribose polymerase) was cleaved after 16h treatment (1000 nM). Since ATM kinase (ataxia telangiectasia mutated kinase) is a transducer of DNA damage signals and plays a role in the coordination of cell cycle checkpoints and regulation of apoptosis, we studied the ATM kinase dependent pathway. It has been shown that the checkpoint effector kinase, known as Chk2, plays a central role in transducing DNA damage signals from ATM. Activation of Chk2 causes the phosphorylation and thereby inactivation of cdc25 family of tyrosine phosphatases. The downstream gene cdc2 (cell division cycle 2) is converted into active form by Tyr<sup>15</sup> dephosphorylation catalyzed by cdc25 tyrosine phosphatases. Consistent with these reports, our results showed that ON 01910.Na treatment leads to activation of ATM-Chk2 and then inactivation of cdc25C. Moreover, as a downstream effect, ON 01910.Na inactivates cdc2 by keeping cdc2 (Tyr<sup>15</sup>)

in an inactive form that leads to a cell cycle arrest. These data help define the novel pathway of action of ON 01910.Na in human leukemia cells.

**Phase I study of ON 01910.Na, a novel Polo-Like Kinase (Plk1) pathway modulator, administered as a continuous infusion (CI) in patients (pts) with advanced cancer.**

**Short Title:**

Phase I Study of 01910.Na.

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**Abstract:** Background: ON 01910.Na induces mitotic catastrophe and apoptosis in cancer but not "non-neoplastic" cells. Cell kill effects are exposure time-dependent in vitro. Based on these preclinical and toxicology findings, a 24hr continuous infusion (CI) study designed to determine safety and MTD of ON 01910.Na was initiated. Methods: Patients with advanced cancer received a starting dose of 250 mg/m<sup>2</sup> as a 24hr CI repeated weekly. The dose cohorts ranged from 250-2750 mg/m<sup>2</sup> based on a modified Fibonacci escalation algorithm using the accelerated titration scheme (NCI). Intra-patient dose escalation was allowed. Intensive and comprehensive plasma sampling on weeks 1 and 4 or 8 of treatment were performed to determine drug pharmacokinetics (PK). Results: 22 pts (16 females; 45-86yrs) have received ON 01910.Na. At the highest dose (2750 mg/m<sup>2</sup>), two pts have received 3 and 2 weeks of continuous therapy. The overall mean (range) number of weeks of continuous therapy delivered is 8.14 (2 to 36). One patient (850 mg/m<sup>2</sup>) discontinued therapy after week 1 for a disease-related pneumonia. Grade 2 toxicities (2-grade increase in toxicity) included fatigue (2pts) and anorexia (1pt). Fatigue (9/20 pts) was the most common side effect and there were no grade 3 or greater events noted. One pt had grade 3 weakness (week 9) at a dose of 2000 mg/m<sup>2</sup>. Overall, fatigue was transient, reversible, non-cumulative, and not dose related. Overall, fatigue was transient, reversible, non-cumulative, and not dose related. One patient with progressive rectal cancer had stable disease lasting 18 weeks. The plasma concentration time profile, of the first 21 patients, across the dose range of 250 mg/m<sup>2</sup> to 2000 mg/m<sup>2</sup>, shows that the drug reaches a steady state within 3 hours of starting infusion. The drug concentration declines exponentially after the end of infusion. The non-compartmental analysis of the data reveals that the drug has a half-life of about 2 hours with the clearance decreasing with the increase in dose. The drug exposure (AUC) increases non-linearly with the six doses studied amongst 21 patients. The steady state plasma concentration of the drug at the dose of 2000 mg/m<sup>2</sup> is 24.4 ±9.1 µg/ml. These levels far exceeds the drug concentration that have been shown to be cytotoxic in vitro in a variety of cancer cell lines, including cell-lines that were resistant to several chemotherapeutics. Conclusion: It is feasible to administer ON 01910.Na as a 24h infusion. In the dose range studied, PK profile suggests non-linear kinetics with rapid attainment of steady-state plasma concentrations in a range shown to be cytotoxic to cancer cells in vitro but with limited end-organ toxicity in vivo. Further analysis of response and toxicity as well as combination phase I studies are planned as this study continues to accrue.