
Drug Delivery 2: Poster Presentations

Abstract #2271

ON1910Na enhances the in vivo cancericidal effect of oxaliplatin

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ON1910Na is a benzyl styryl sulfone kinase inhibitor, which induces G2/M arrest and apoptosis in cancer cells. Promising anticancer activities of ON1910Na in multiple tumor models has led it to phase I clinical trials. Cancericidal effects of ON1910Na in combination with oxaliplatin were assessed in nude mice bearing human tumor xenografts. After inoculation of 10^7 cells sc, human Bel-7402 hepatoma, DU-145 prostate cancer, DND-1 melanoma, MIA pancreatic cancer and Lovo colon cancer were treated when tumors had reached a mean volume of 200 to 630 mm³. ON1910Na (200 mg/kg, ip, q2d), oxaliplatin (5 mg/kg, ip, q2d) or the combination of both was continuously administered without recognized toxicity. The table shows tumor mass (mm³) on the day control animals were euthanized, and the day for treated animals to reach tumor size equivalent to that of controls.

These data support future investigation of combined ON1910Na and oxaliplatin in the treatment of human hepatoma, melanoma, pancreas and prostate cancers.

Enhanced anticancer activity in combination of ON1910Na with Oxaliplatin					
	Starting	Control	ON1910Na	Oxiplt	Combination
Bel-7402(DRETS ^b)	350	3000(35)	1400 ^a (60)	1100 ^a (91)	400 ^a (>120)
DND-1(DRETS)	250	3000(42)	1100(87)	1200(87)	600(>100)
DU-145(DRETS)	630	3000(28)	1600(68)	1800(58)	750(>150)
MIA(DRETS)	250	2500(18)	1000(42)	1600(29)	800(>42)
Lovo(DRETS)	200	3000(43)	1100(>84)	2000(64)	1200(77)

a) Tumor volume (mm³) at the time of euthanizing the control. b) Days to reach equivalent tumor size of the controls.

Antibodies and Immunoconjugates: Poster Presentations

Abstract #920

Mass spectrometric study of antibody-drug conjugates

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The objective is to identify one or more highly potent antibody drug conjugates for future evaluation in clinical trials. As a preclinical model, we developed the antibody conjugates which complex trastuzumab, as Herceptin® (a commercial monoclonal antibody used to treat HER2 (+) breast cancer) and two novel benzyl styryl sulfone compounds, ON 01500 and ON 013100. Both compounds are patented, highly potent and selective inhibitors which induce apoptosis in tumor cells at low nanomolar concentrations. The advantages for using compounds ON 01500 and ON 013100 are: they are highly potent against a broad spectrum of cancer cell types, have a relatively high therapeutic index, are not substrates for the multi-drug resistance (MDR) pump, and are inexpensive to manufacture on a kilogram scale.

Methods:

The formation of antibody drug conjugate with commercial Herceptin® and ON 013100 was accomplished using ON 14013100 (an N-hydroxysuccinimide activated prodrug of ON 013100) and ON 16013100 (another N-hydroxysuccinimide activated prodrug of ON 013100) at pH 8.3 and 4:1 molar ratio (antibody:drug). 10 nL aliquots of conjugates were injected directly into the ion source of a triple quadrupole mass spectrometer. Positive nanospray mass spectra were obtained and molecular masses of the intact drug-antibody conjugates were obtained from multiple charged ion patterns.

Results:

(a) Antibody-drug interactions yield mixtures simultaneously containing conjugates with a varying number of bound drug molecules, e.g., up to 8 molecules of ON 14013100 per antibody molecule; (b) The number of bound molecules for each drug was different, e.g., antibody attached 8 molecules of ON 14013100 vs. 6 molecules of ON 16013100; (c) Drug binding follows an apparently discontinuous digital progression, e.g., 2, 6, and 8 molecules of ON 14013100 bound to antibody but not 1, 3, or 7. (d) The conjugation of these molecules to monoclonal antibody could be used as stable, self-immolating linkers that by internalization could release an intracellular cytotoxic payload. (Supported by the T. J. Martell Foundation for Leukemia, Cancer and AIDS Research and by Onconova Therapeutics, Inc.)

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Mass Spectrometric Study of Drug Binding to Proteins

Short Title: Drug Binding to Proteins

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Abstract:

The objective was to characterize intact complexes of drugs with proteins, particularly human serum albumin and a variety of enzymes using mass spectrometry to calculate the number of bound drug molecules. Drugs studied: (a) Antineoplastic: tamoxifen (372 Da); doxorubicin (544 Da), methotrexate (454 Da), bleomycin (1416 Da), suramin (1429 Da) and the novel benzyl styryl sulfone analog ON 1910.Na (474 Da); (b) Other Drugs: Digoxin (791 Da); fluphenazine (438 Da), and trifluoroperazin (408 Da). Proteins studied: albumin (66 kDa) and the enzymes: papain (21 kDa), elastase (25 kDa), trypsin (24 kDa) and carbonic anhydrase (29 kDa). **Methodology:** One μL aliquots of incubates (30 min, 37°C) or plasma sample from patients on Phase I clinical trials with ON 01910.Na were injected directly into the ion source of a triple quadrupole mass spectrometer. Positive electrospray or nanospray mass spectra were obtained and molecular masses of the intact drug-protein complexes were obtained from multiple charged ion patterns. **Results:** (a) Complex formation between the drug and protein commenced immediately upon exposure. (b) Albumin-drug interactions yielded mixtures simultaneously containing complexes with a varying number of bound drug molecules, e.g., up to 21 molecules of tamoxifen per albumin molecule vs. only up to 8 molecules of digoxin per albumin; (c) The number of bound molecules for each drug was different for different enzymes, e.g., carbonic anhydrase attached 13 molecules of tamoxifen vs. 11 molecules of digoxin; (d) Drug binding followed an apparently discontinuous digital progression, e.g., 10, 11, 13, 17, 19, and 21 molecules of tamoxifen bound to albumin but not 12, 14, 15, 16, 18, or 20; (e) Similar unique phenomena occurred using several antineoplastic drugs as well as other drugs; and also using several different enzymes. We hypothesize that conformational changes caused by binding may account for the exclusion of certain binding complexes, and that conformational changes may influence protein behavior and enzymatic activity. (Supported by the T. J. Martell Foundation for Leukemia, Cancer and AIDS Research and by Onconova Therapeutics, Inc.)

Category and Subclass: CH02-05 Small molecule/protein interactions

Keywords/ Indexing: Mass spectrometry; Protein-drug binding

Kinases 1: Poster Presentations: Abstract #5391

Evaluation of ON 01910.Na, a novel modulator of polo-like kinase 1 (Plk1) pathway, and development of a cyclin-B1-based predictive assay in pancreatic cancer

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Background: Plk1 is a key mitotic regulator that modulates the transition through the G2/M checkpoint in the cell cycle. This work aimed to evaluate the activity of ON 01910.Na, a Plk1 pathway modulator, in *in vitro* and *in vivo* models of pancreatic cancer (PaCa) and to discover biomarkers predictive of efficacy. Methods: ON 01910.Na was tested in 12 PaCa cell lines. Then, mRNA and protein-based pharmacodynamic studies interrogating a panel of Plk1 pathway related markers (CDC25C, cyclin B1, cyclin D1, and Plk1, among others) were conducted to identify biomarkers correlating with activity. For validation a live collection of PaCa xenografts from fresh tumor samples obtained at the time of surgical resection was used (PancXenoBank). The *ex vivo* assays were based on fine-needle aspirate (FNA) biopsies. **Results:** ON 01910.Na showed equivalent activity to gemcitabine against PaCa cell lines. The activity of the agent correlated with suppression of two downstream mediators of PLK1, CDC25C and cyclin B1 (as measured by mRNA and protein). Detection of these markers was optimized for a FNA *ex vivo* assay. ON 01910.Na was tested in xenografts from representative pancreatic cell lines. The selected markers were evaluated in an *ex vivo* assay, using plasma and intra-tumor pharmacokinetic studies to select the dose of the assay. Cyclin B1 mRNA evaluation yielded the most optimal combination of accuracy and reproducibility. Treatment of the same cell lines with gemcitabine did not induce changes in cyclin B1. Knockdown of cyclin B1 by siRNA had no effect per se or in the response of the resistant MiaPaca2 to either of the drugs suggesting that cyclin B1 is a marker of activity and not a mediator of it. We next used the *ex vivo* assay to profile nine patient-derived cases from the PancXenoBank. Two cases were catalogued as potential responders. From each of these nine cases, a group of mice bearing at least 20 tumors was randomized to receive vehicle or ON 01910.Na for 28 days. There was a correlation between the *ex vivo* cyclin B1 assay and the sensitivity to the tested agent, as the two cases prospectively identified as sensitive met pre-specified criteria for response. Of the seven tumors predicted to be resistant, only one was found to be sensitive. In IHC testing at the end of treatment, cases showing *ex vivo* cyclin B1 down-regulation had a similar decrease in cyclin B1 protein, and there was a significant correlation between activity and IHC changes in cyclin B1. **Conclusions:** The novel anticancer agent ON 01910.Na demonstrated significant activity in a preclinical model of PaCa. A rationally designed *ex vivo* cyclin B1-based assay not only identified cases sensitive to ON 01910.Na, but also replicated the pharmacodynamic events occurring after *in vivo* exposure. Further studies expanding these findings to a broader number of xenografts and to ongoing clinical trials with ON 01910.Na are underway.