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Lobaplatin – A third-generation platinum agent with high in vitro efficacy across a wide range of cancer cells

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Platinum-based chemotherapy plays an important role in the management of solid tumors since more than 30 years. Thousands of new platinum analogues have been synthesized since the discovery of cisplatin to overcome adverse effects, induction of resistance and limitations in clinical activity. But only three compounds (cisplatin, carboplatin, oxaliplatin) have a world wide approval for the treatment of cancers. Platinum drugs are used as single compounds or in combination with other chemotherapeutic agents in many standard therapeutic regimens.
The present study was designed to explore the *in vitro* activity of lobaplatin against a wide range of human tumor cell lines including resistant subtypes. Results from proliferation experiments under various conditions and the effects on the cell cycle of tumor cells are presented together with data from apoptosis assays.
In comparison to other platinum derivatives which are in clinical use or in development (cisplatin, oxaliplatin, nedaplatin, satraplatin, and carboplatin) we have found that lobaplatin expresses a high activity on tumor cell lines of different origin. In the XTT proliferation assay lobaplatin exhibits IC_{50} values from 0.3 μM to 61 μM (mean IC_{50} 19.4 μM) against 24 tumor cell lines, together with a maximum of growth inhibition between 78% and 100% (mean 85.2%). The efficacy of lobaplatin is much higher than the activity of cisplatin, nedaplatin, and carboplatin. No cross-resistance to cisplatin could be observed on cisplatin resistant cell line A2780cis. In addition lobaplatin retains activity against multi-drug resistant cell lines with P-gp1 and MRP1 overexpression in comparison to their sensitive wild types.
Flow cytometric analysis demonstrates that treatment of KB/HeLa cells with lobaplatin affects the cells in a time and concentration dependent manner. Treatment with high doses of lobaplatin (10 μM) leads to a considerable enrichment of cells in S-phase after 24h and up to 120h. Cells treated with low doses of lobaplatin (3.16 and 1 μM) showed a delayed G2/M arrest after 48h with an increase of apoptotic cell population for at least 120h. The results are underlined by cell count experiments designed to distinguish effects on cell viability and on cell proliferation.
The new results corroborate that lobaplatin has excellent *in vitro* activity against various cancer cell lines, and support the further clinical development of the candidate in phase II trials. Lobaplatin is already on the market in China.

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D-501036, a novel selenophene-base triheterocycle derivative, exhibits potent in vitro and in vivo anti-tumoral activity, which involves DNA-damage and ATM activation

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Background: D-501036, 2,5-bis(5-hydroxymethyl-2-selenienyl)-3-hydroxy-methyl-N-methylpyrrole, a novel selenophene derivative, was identified in our laboratory as a novel antineoplastic agent. In this study, we examined the *in vitro* and *in vivo* anticancer efficacy, and explored the mechanism of action of this compound in human cancer cells.
Materials and Methods: The *in vitro* and *in vivo* anticancer effect of D-501036 was examined by the MTS assay and human xenograft model. DNA gel eletrophoresis, flow cytometry, ICP-MS, and Western blotting were used to reveal molecular events in this study.
Results: D-501036 exhibited a broad spectrum of antitumor activity against many human cancer cells with GI_{50} in nanomolar range. However, very low toxicity was found to the primary culture of kidney, lung and fibroblast. No cross-resistance with D-501036 was observed in KB-Taxol, vincristine, or CPT-resistant derivative cell lines. Significant S phase arrest followed by sub-G_1 population accumulation after cancer cells exposed to D-501036. DNA fragmentation and caspase-3 activation further indicated that D-501036 induced cell death through an apoptotic pathway. Furthermore, rapid activation of p53, p21 and ATM signaling pathway and *en masse* DNA damage were found in D-501036-treated cancer cells. However, our results demonstrated that D-501036 did not intercalate into chromosome nor inhibit the topoisomerase I/II enzyme activity, indicates that D-501036-induced DNA-damage is unlikely through the change of DNA topology. Significant amount of ROS production was found shortly after cancer cells exposed to D-501036, but partial reverse D-501036 cytotoxicity by pretreated with antioxidant. Furthermore, large amount of DNA-adduct in the D-501036-treated tumor cells by ICP-mass were found.
Conclusions: Taken together, these results suggested that the tumor growth inhibition function of D-501036 might contribute by the induction of ROS and DNA-adduct formation. Intraperitoneally administrated of D-501036 to Ncr nude/nude mice resulted a complete abrogate the growth of xenografted human renal carcinoma cells. Based on the striking antitumor efficacy of this substance, we believed the potential for this polyselenophene compound becomes to an efficacious anticancer drug is remarkably promising.

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Phase I trial of the histone deacetylase inhibitor valproic acid with the topoisomerase I inhibitor, karenitecin in advanced melanoma

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Background: Metastatic melanoma is a devastating disease currently lacking effective treatment. We have previously shown that the novel topo I inhibitor, karenitecin, can produce clinical benefit in 34% of patients with advanced melanoma. In melanoma cell lines, we have shown that pretreatment with the histone deacetylase inhibitor (HDACi), valproic acid (VPA), can increase binding of topo I poisons to DNA, potentiate apoptosis and produce synergistic cytotoxicity. Similar results were obtained in the A375 melanoma xenograft model with synergistic suppression of tumor growth. Based on this data we hypothesized that sequential VPA followed by karenitecin could produce enhanced clinical response without augmenting bone marrow suppression, which is the major toxicity of karenitecin.
Methods and Study Design: The *primary objective* was to determine the toxicity and maximum tolerated dose (MTD) of VPA given orally twice daily for 5 days sequentially with karenitecin given I.V. daily for 5 days every 3 weeks. The *secondary objectives* were to determine the pharmacokinetic parameters of VPA and karenitecin in combination. Patient had adequate organ function, ECOG PS 0–1 and progressive disease on at least 1 systemic therapy.
Results: Twenty-five patients have been enrolled to date. VPA doses of 30 mg/kg/day, 45 mg/kg/day and 60 mg/kg/day for five days were well tolerated when combined with karenitecin at 0.8 mg/m^2/day for five days. Karenitecin dose was therefore escalated to 1 mg/m^2/day for five days and this could be combined with VPA at 60 mg/kg/day for five days. No DLT was observed. Doses were escalated to VPA 90 mg/kg/day and at this level 2/3 patients experienced neuron-vestibular DLT. Subsequently, VPA was de-escalated to 75 mg/kg/day and this proved tolerable. PK for valproic acid and karenitecin are described in this combination. Histone acetylation in PBMC was examined and shows a linear increase with dose.
Conclusion: This phase I trial demonstrates that the HDACi VPA can be combined at an effective dose with full doses of the topo I inhibitor karenitecin. Encouraging clinical responses (prolonged stable disease and minor responses) have been seen in this heavily pretreated patient population at all VPA dose levels. A Phase II trial ith this combination in melanoma is currently ongoing.

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A novel HPLC/MS assay to measure DNA interstrand cross-linking efficacy in oligonucleotides of varying sequence

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Until now the main method of evaluating the DNA interstrand cross-linking ability of cancer chemotherapeutic agents (in terms of both DNA binding affinity and sequence selectivity) has involved the electrophoresis of radiolabelled oligonucleotides on denaturing gels after incubation with an agent. To avoid the use of radioactivity we have developed a method based on ion-pair RPLC/mass spectrometry which allows characterization and quantitation of drug–DNA interstrand cross-links formed within short oligonucleotides. The other advantage of this assay is that all species separated by the chromatographic process can be identified by mass spectrometry. Using this methodology we have investigated the rate and sequence-selectivity of the DNA interaction of SJG-136 (SG2000), a pyrrolobenzodiazepine (PBD) dimer currently being evaluated in Phase I clinical trials in the UK and USA. The interaction of SJG-136 was

studied with four sequences of dodecanucleotides: d(TATAGATCTATA)$_2$ [*Seq-1*], d(TATAGTACTATA)$_2$ [*Seq-2*], d(ATATGATCATAT)$_2$ [*Seq-3*] and d(TATA**I**ATCTATA)$_2$ [*Seq-4*] (where I = Inosine), specifically designed to highlight aspects of the mechanism of action of SJG-136. The method was found to be sensitive and selective enough to allow separation of the unbound and drug-bound oligonucleotide species by HPLC, and to allow identification of the individual species by mass spectrometry. The method also allowed kinetic information to be obtained, which established that SJG-136 cross-links these oligonucleotides with an order of preference of *Seq-1* > *Seq-2* > *Seq-3* > *Seq-4*, a result that could be predicted from the known mechanism of action of the agent and previous molecular modeling and gel electrophoresis studies, thus validating the assay.
In conclusion, we have successfully developed a method to measure the rate and sequence selectivity of DNA interstrand cross-linking which is sensitive, rapid and does not require radiolabelled DNA. In addition, the method could be automated for high-throughput analysis.

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Histone deacetylase inhibition modulates estrogen receptor expression at multiple levels in breast cancer cells

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Tamoxifen (TAM) is one of the most effective current therapies for breast cancer. However, only 60% of estrogen receptor expressing (ER+) breast cancers respond to TAM (40% de-novo resistance). Continuous treatment with TAM beyond 5-years leads to emergence of acquired resistance in these initially-responsive cancers. The development of alternative approaches for the treatment of patients with tamoxifen resistance is therefore of significant clinical importance. One approach currently being studied clinically for the treatment of metastatic breast cancer is the use of histone deacetylase (HDAC) inhibitors. These compounds are thought to impact tumor progression both at the level of gene transcription and protein stability. In this current study, we developed an *in vitro* model of tamoxifen resistance to test the efficacy of a hydroxamic acid derivative, Trichostatin A (TSA) at modulating breast cancer cell growth both in tamoxifen responsive as well as tamoxifen resistant cells. Forty-eight (48) MCF-7 breast cancer single-cell colonies were isolated from a heterogeneous population, with 16 of the 48 (33%) surviving 4 months of continual growth in charcoal-stripped media with 10^{-7} M tamoxifen. ER expression was lost in 9 of the 16 (56%) resistant clones. The efficacy of TSA at suppressing growth was studied in both the ER+ and ER− cells, both with and without cotreatment with tamoxifen. TSA was more effective in the ER+ cells both as a single agent, and in combination with tamoxifen. The molecular mechanisms mediating this enhanced response were studied and focused on the effects of TSA on ER activity and expression. Both luciferase reporter assays using the ER core promoter as well as QPCR results indicate that TSA suppresses ER gene transcription in a dose-dependent fashion. Additionally, assessment of protein stability indicates that TSA targets ER for protein degradation. Further tests are ongoing to determine if suppression of the ER is a significant mechanism by which TSA inhibits breast cancer cell growth. These data have important clinical ramifications for the clinical development of HDAC inhibitors for the treatment of breast cancer patients.

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Design, synthesis and evaluation of extended heterocycle linked C8/C8' pyrrolo[2,1-c][1,4]benzodiazepine DNA cross-linking agents

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We have previously reported the design and synthesis of several pyrrolobenzodiazepine (PBD) dimers. One example SG2000 (SJG-136) is currently undergoing phase I clinical trials in the UK and U.S.A. SG2000 recognises and cross-links at an embedded puGATCpy sequence, with recognition arising from the formation of an aminal linkage to guanine and an N10 hydrogen bond to adenine. In order to extend the base pair coverage, and increase the sequence selectivity of the molecules, we have explored the replacement of the 1,3-propyldioxy linker found in SG2000 with a heterocyclic polyamide chain with the potential to recognise DNA sequences itself.
A homologous series of symmetrical dimers, comprising 2–8 heterocycles was synthesised using classical peptide coupling chemistry. Molecular modelling showed that the longest member of the series could span up to 19 base pairs and, significantly, calculations suggested that a molecule of this length would have the potential to recognise a single specific gene within the entire human genome. A series of unsymmetrical dimers spanning approximately 11 base pairs and containing both pyrrole and imidazole heterocycles has also been prepared.
These extended PBD dimers are potent cytotoxic agents with IC_{50} values ranging from 182 to 12 nM (K562 human leukaemia cell line), whereas control molecules lacking the PBD units are not cytotoxic. The symmetrical dimers are efficient cross-linking agents in plasmid and cellular DNA, with the bisulphite adduct of the octapyrrole dimer producing detectable DNA cross-linking in cells at a 10 nM concentration measured using the single cell gel electrophoresis (comet) assay. DNase I footprinting studies on the unsymmetrical dimer AT235 containing three pyrroles showed binding at an AT-rich GAWWWTC (W = A or T) sequence.
These studies demonstrate that heterocycle-linked PBD dimers are able to bind to DNA in a sequence-selective manner and are highly cytotoxic. Further studies are underway to elucidate more precisely the sequence selectivity of these agents.

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Phase I and pharmacokinetic trial of SJG-136 administered on a daily × 5 schedule

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Background: SJG-136 is a pyrrolobenzodiazepine dimer that forms DNA crosslinks via covalent binding of guanine residues in the minor groove in a sequence-specific manner. *In vitro* testing of SJG-136 in the NCI's 60-human cell line screen demonstrated a broad pattern of antitumor activity in sub-nmol concentrations.
Methods: Patients with refractory solid tumors received SJG-136 over 20 min IV daily × 5 days, every 21 days. The starting dose was 6 μ/m^2/day. Design 3B of the accelerated doset itration schedule (Simon et al, JNCI, 1997) was used. Blood and urine samples were obtained for pharmacokinetic (PK) assessment on Days 1 and 5 of Cycle 1. A sensitive HPLC/MS/MS bioassay of SJG-136, with a lower limit of quantitation of <50 pg/ml was developed.
Results: 7 patients (5M, 2F) have been enrolled. Median age: 47 (range: 24–75), median ECOG PS: 1 (range: 0–1), median # of prior regimens: 3 (range: 1–5). Dose levels tested (μg/m^2/day) and # of pts treated at each dose level: 6 (1 pt), 12 (1 pt), 24 (3 pts), 48 (2 pts). Cycle 1 DLT in the form of Grade 3 soft tissue edema, dyspnea, and fatigue was observed in 1 pt at the 48 μg/m^2/day dose level. Similar toxicities occurred during Cycle 2 in two additional pts treated at the 24 μg/m^2/day dose level. These episodes were accompanied by a precipitous drop in serum albumin (mean: 37%) without proteinuria. Symptoms resolved over 2–3 weeks following discontinuation of the study drug and aggressive diuresis with spironolactone. Other non-dose-limiting toxicities include delayed increases in transaminases, alk. phosphatase, myalgias and weight gain. No significant myelosuppression has been observed. SJG-136 was reproducibly detected and the PK analysis revealed dose-dependent increases in AUC and Cmax (Table 1). In some patients, repeated SJG-136 dosing appeared to induce substantial changes in volume of distribution (V_{ss}). No objective responses have been observed; 1 pt with melanoma and 2 with colorectal cancer have achieved stable disease by radiographic criteria for up to 3 months, accompanied by 25 – 30% decreases in CEA for both pts with colorectal cancer.

Dose level	# pts	Cmax (ng/mL)		AUC (ng min/mL)		Vss (mL)	
(μg/m^2/day)		day 1	day 5	day 1	day 5	day 1	day 5
6	1	1.02	0.98	47	52	24,751	23,230
12	1	3.72	4.83	163	193	10,614	10,413
24	3	9.14 ±1.57	6.77 ±2.57	470 ±123	8735 ±2218	14,660 ±6502	
48	2	7.64	7.54	333	340	20,254	23,369

Data are mean±SD.

Conclusions: The DLT of SJG-136 administered on a daily ×5 basis is soft tissue edema, fatigue and hepatotoxicity. The MTD of daily ×5 schedule is 12 μg/m^2/day. Day 5 increase in V_{ss} may correlate with subsequent development of significant soft tissue edema. Therefore, additional patients are being enrolled on shortened, daily ×3 schedule with steroid premedication and aggressive diuresis support to assess amelioration of toxicities, starting at 20 μg/m^2/day.
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