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

D. Russell, L. deGraffenried, J. Freeman, J. Silva, W. Friedrichs, M. Beeram. *University of Texas Health Science Center at San Antonio, Medicine/Medical Oncology, San Antonio, TX, USA*

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

P.W. Howard[1], A. Tiberghien[1], S.J. Gregson[1], G.-D. Kang[1], M. Stephenson[1], K. Kiakos[2], S. Klee[1], J.M. Hartley[2], J.A. Hartley[1,2], D.E. Thurston[1,3]. [1]*Spirogen Ltd, London, United Kingdom;* [2]*University College London, Department of Oncology, London, United Kingdom;* [3]*The School of Pharmacy, Dept of Pharmaceutical & Biological Chemistry, London, United Kingdom*

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

I. Puzanov[1], W. Lee[1], J.D. Berlin[1], M.W. Calcutt[1], D.L. Hachey[1], W. Vermeulen[1], J. Low[2], M.L. Rothenberg[1]. [1]*Vanderbilt-Ingram Cancer Center, Division of Hematology-Oncology, Nashville, TN, USA;* [2]*National Cancer Institute, CTEP, Bethesda, MD, USA*

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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