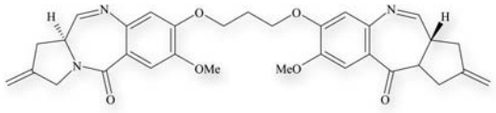
SJG-136



SUCCESS STORY

NSC 694501.....RECEIVED JANUARY 1997.....DN2A JUNE-1999.....CLINICAL TRIAL OCTOBER 2003

Government, Academic, and Foundation Collaborations

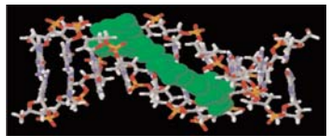
DNA Cross-Linker Grooves With Antitumor Activity
Hartley, Alley, Pepper, et al.

Excerpt From* Cancer Research, *September 15, 2004

The development of agents targeted against the molecular hallmarks of malignancy have been at the forefront of therapeutic efforts over the past 25 years. Progress has been made with the introduction of monoclonal antibodies and small molecule inhibitors directed against both oncogenes and their protein products activated in the process of carcinogenesis. Concurrently, progress has also occurred in the design of agents directed against more classical cancer drug targets. In this issue, three manuscripts from Hartley *et al.,* Alley *et al.,* and Pepper *et al.* describe the preclinical qualifications of the novel DNA interstrand cross-linking agent SJG-136. This molecule, which is based on the naturally occurring anthramycin family of antitumor antibiotics, has been designed to possess both DNA sequence-selectivity and bivalent DNA-interacting moieties, thus producing a new type of minor-groove interacting drug with marked antitumor activity in many model systems as well as in primary chronic lymphocytic leukemia cells. Importantly, for SJG-136, growth inhibition of mouse bone marrow appears to be similar to that of human bone marrow, suggesting that, in contrast to previous "minor groove binders," activity in mouse xenograft models will hopefully presage comparable activity in the clinic. Furthermore, preliminary evidence suggests that, unlike many other DNA-interactive agents, SJG-136-induced cytotoxicity is independent of p53 activation. Given that mutation and aberrant expression of the p53 tumor suppressor protein are the most frequent molecular alterations in human malignancy and contribute to genomic instability, carcinogenesis, and treatment failure, this feature of SJG-136 may prove to be one of its most interesting attributes.

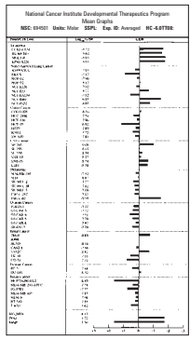
The promise of SJG-136 further illustrates the importance of government, charitable foundations, and academic researchers collaborating to bring potentially useful new treatment concepts forward as candidate molecules for early clinical trials.

A Rationally Designed Pyrrolobenzodiazepine Dimer



Illustrates the value of academic, charitable foundation, and government collaborations (David Thurston, University of London, Cancer Research Campaign, UK, and NCI).

Development Concerns



- Potent DNA minor groove cross-linking alkylating agent.
- Mechanism of action and structure similar to Bizelesin, which caused myelosuppression with human sensitivity much greater than mouse.
- Anthramycin-like compound with potential for serious cardiotoxicity.

In Vitro Data

- Potent cytotoxic with a multilog differential pattern of activity.
- COMPARE analysis shows a similarity to other DNA-binding agents but is distinct from other known agents.
- Gene expression analysis:
  – LS174T human colon tumor cells at 0.1 nM showed clear pattern of both up- and down-regulated genes.
  – Up-regulated genes include DNA repair genes RECA and XRCC1.

Colony Formation Assays (CFA) Comparing Leukemia Cells and Normal Bone Marrow Cells

Methodology	Cell Type	IC50	IC75	IC90
In Vitro Leukemia Cells CFA	HL-60 (human)	1.19	1.76	2.95
	Molt-4	0.503	1.13	1.70
Ex Vivo Bone Marrow CFA	Mouse	111	218	536
	Dog	16	41	118
	Human	21	101	167

HL-60 and Molt-4 leukemia cells are at least one order of magnitude more sensitive to SJG-136 than are bone marrow cells derived from the mouse, dog, and human.

In Vivo Studies

Hollow Fiber Assay

- Prominent growth inhibition observed both in i.p. fibers and s.c. fibers with cell kill in 5 of the 12 tumor cell types.
- Score 40 (i.p.) + 14 (s.c.) = 54.

Xenograft Models

Tumor-free responses were seen in 6 tumor models.

Xenograft Data for SJG-136

Tumor	High Dose	Toxicity	High T/C	% Growth Delay	Log Cell Kill
MDA-MB-435	0.67 mg/kg	3/6	3	41	0.20
OVCAR-3	0.67 mg/kg	0/6	7	73	0.20
UACC-62	0.67 mg/kg	0/6	22	43	0.40
OVCAR-5	0.67 mg/kg	0/6	38	32	0.00
SF-295	0.40 mg/kg	2/6	0	NA	-0.60

0.67 doses given by i.v. once every 4th day for a total of 3 doses.
0.40 dose given by i.v. daily for 5 days.

Pharmacokinetic Studies

Performed Mouse PK in the UK at the University of Bradford

- Mouse PK: High concentration of 3.8 μM obtained in plasma.

Performed at the NCI

- Drug is stable in rat plasma for 48 hours at both 4°C and 37°C.
- In human plasma, drug concentration fell to 75% of initial level after 48 hours at 4°C and to 50% of initial level after 48 hours at 37°C.

Toxicology Studies

***In Vitro* Bone Marrow Assay**

	Concentrations given in pM		
Species	IC50	IC75	IC90
Murine	83	760	2640
Dog	12	87	442
Human	16	148	826

Dog may be a more appropriate model than the rat to predict human toxicity.

Rat Studies

- Doses of 30–150 μg/m^2/day for 5 days (i.v.) produced toxicities but no deaths.
- Bone marrow suppression was prominent.
- All toxicities were reversed by day 33.

Dog Studies

- Doses of 4 and 20 μg/m^2/day for 5 days (i.v.) produced dose-dependent toxicities.
- No clinical abnormalities were observed.
- Bone marrow suppression observed at the high dose. Reversed by day 33.
- Maximum tolerated dose (MTD) determined to be 20 μg/m^2/day.
- Recommended starting dose for clinical trials was 1/6 the dog MTD: 3.33 μg/m^2/day for 5 days.

Development Conclusions

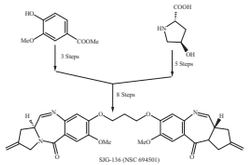
- A uniquely designed minor groove binder.
- Highly active *in vitro* and *in vivo*.
- Does not show species bone marrow disparity.
- Does not appear to be cardiotoxic.
- DLTs (bone marrow, gastrointestinal) are clinically manageable.
- MTD for dog is 2 times lower than mouse MED. However, dog bone marrow sensitivity is 2 times greater than human.
- Projected human MTD is within range.

Bulk Production and Clinical Batch Preparation

Synthesis

- Detailed studies of the highly purified SJG-136 led to the discovery of the dihydrate adduct as the actual active ingredient—subject of a collaborative patent application.

A 16-Step Synthesis, 35 gm of SJG-136 Produced by Starks Under Contract



- High reactivity of SJG-136 toward water and protic solvents poses a challenge for HPLC method development. A reversed phase HPLC that determines SJG-136 as its dihydrate adduct has been developed.
  – Column: Supelco Discovery RP Amide C-16, 4.6 mm x 150 mm, 5 μm particles.
  – Mobile phase: CH3CN/20 mM potassium phosphate buffer, pH 4.6.
  – Detection: UV at 320 nm.

Formulation

- Convenient solution formulation (0.01 mg/mL, 1 and 5 mL/vial) containing 5% dimethylacetamide in water for injection has been developed for i.v. administration.

SJG-136 Labels

SJG-136 Containers

Clinical Trials Experience

Trials in the U.K. Sponsored by Beaufour Ipsen

- Eight patients accrued.
- Starting dose 15 μg/m2.
- Current dosing scheme 60 μg/m^2 once a week for 3 weeks is well tolerated.

Trials in the U.S. Have Begun

- Starting dose 5 μg/m^2 for 3 days.

Developmental Therapeutics Program of the National Cancer Institute