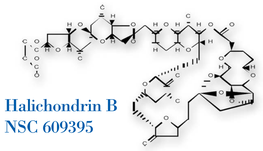
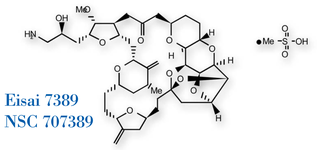
Halichondrin Analog



Halichondrin B
NSC 609395

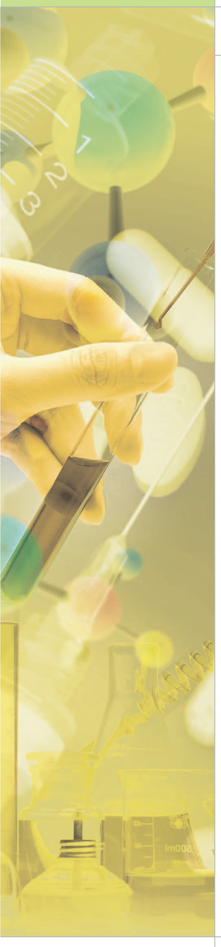


Eisai 7389
NSC 707389

SUCCESS STORY

HALICHONDRIN B (NSC 609395).....RECEIVED MAY 1986.....DN MARCH 1992

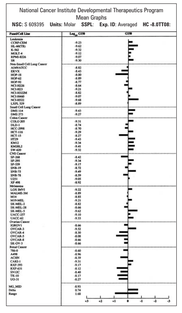
E7389 (NSC 707389).....RECEIVED AND PRESENTED TO DN SEPTEMBER 1998.....CLINICAL TRIAL APRIL 2002



Halichondrin B (NSC 609395)

Background

Halichondrin B isolated from the marine sponge *Halichondria okadai* (New Zealand) in 1985



- Highly potent cytotoxic agent.
- COMPARE analysis indicates a pattern similar to known tubulin interactive agents.
- Tubulin binding activity confirmed by Dr. E. Hamel, DTP.

Recent Testing of Halichondrin B in Human Tumor Xenografts (Sample 12)

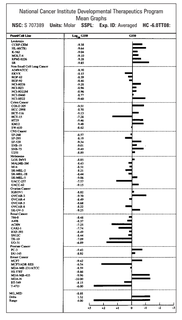
Model	Implant Site	Treatment Route	Schedule	MTD (ug/kg/dose)	Activity %T/C
LOX	IP	IP	Q4D X3	45	[153] (7/8 TF)
		IP	QD X9	>20	(8/8 TF)
LOX	ESC	IV	Q4D X3	45	2 (1/10TF)
		IV	QD X5	22	9
OVCAR-3	IP	IP	Q4D X3	>45	[170] (1/8 TF)
		IP	QD X9	>20	[144]
MDA-MB-435	ASC	IP	Q4D X3	>45	-39 (1PR&1CR)
		IP	QD X5	>20	-42 (1PR&1CR)
MDA-MB-435	ASC	IP	Q4D X3	~54	-100 (5/10 CR)
		IV	Q4D X3	~54	-100 (8/10 CR)
		IV	QD X9	13.4	-25 (2/10 CR)

- Halichondrin B accepted into DN in March of 1992 based on *in vitro* and *in vivo* data.
- Difficulty in obtaining additional material from the natural sponge stalled progress.
- Harvard licensed Dr. Y. Kishi's synthetic method to Eisai. Eisai developed modified structures and was able to identify the active pharmacophore in the molecule.

Eisai 7389 (NSC 707389)

Background

- A synthetic macrocyclic ketone derivative of the marine natural product Halichondrin B.
- Produced by chemical synthesis by the Eisai Corporation of America.
- Synthesis based on the total synthesis of Halichondrin B reported by Professor Y. Kishi, Harvard University, in 1992 (NIH-funded grant).



- Tubulin activity was indicated by COMPARE and confirmed in the laboratory by Dr. E. Hamel.
- Dr. E. Hamel's studies indicated that E7389 was a more effective inhibitor of tubulin binding than the parent Halichondrin.

In Vivo Studies

- Intermittent i.v. treatment regimens (total dosages of 2.25–9.0 mg/kg) were observed to produce 14/15 complete tumor regressions in the MDA-MB-435 breast model and 14/15 tumor-free animals bearing the NCI-H522 lung model with minimal to no toxicity.
- In the NCI-H522 lung model, 14 of the 15 animals showed tumor-free status and no signs of tumor regrowth for at least 37 days following cessation of treatment.
- E7389 clearly showed better activity than that produced by the natural Halichondrin B or Taxol® against NCI-H522 and MDA-MB-435 under conditions of the assay.

Pharmacokinetic (PK) and Toxicokinetic (TK) Studies

Single Dose PK in Rats (1.5 mg/kg given i.v.)

- Rapid tissue distribution.
- Extended terminal half-life was noted (11 hours).
- High Vdss values indicated deep tissue distribution.
- Minimal oral bioavailability of E7389 alone (2.5 mg/kg).
- Cyclosporine pretreatment enhanced oral bioavailability, which indicates that E7389 is a substrate for p-glycoprotein.

Multiple Dose PK/TK

- Pre-dose plasma levels were below level of detection, indicating the lack of drug accumulation with multiple dosing.
- Rats
  - — Toxicity was noted in male rats at high dose (0.2 mg/kg/dose) which lead to inconclusive PK.
  - — Lack of adverse clinical events in female rats administered 0.2 mg/kg/dose q4d x 3 without blood draws.
  - — Female rats exhibited similar plasma distribution and elimination profiles on days 1 and 9 at high and low (0.13 mg/kg/dose) doses.
- Dogs
  - — Similar PK profiles following administration of multiple i.v. doses.
  - — At the low dose, 0.004 mg/kg/dose (0.08mg/m^2/dose), only post-infusion levels were detectable.

Toxicology Studies

***In Vitro* Bone Marrow Assay**

Species	IC_{90} (nM)
Mouse	63.1
Dog	19.8
Human	21.7

Mouse Was Somewhat Less Sensitive—Biologically Significant?

Dog and Human Equally Sensitive

Rat Study Results

- No clinical signs of toxicity.
- Drug-related body weight loss (males only); anemia, mild leukopenia (SEG, LYM) were present in the 0.20 mg/kg/day dose group.
- Dose-related increases in AST.
- Histopathological lesions: bone marrow, thymus, skeletal muscle, and testes.
- No neurological lesions were present.
- Except for testicular lesions, toxicity was reversible.
- Maximum tolerated dose (MTD) > 0.20 mg/kg/day (1.2 mg/m^2/day).

Dog Study Results

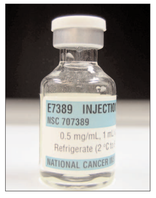
- No clinical signs of toxicity or changes in body weight.
- Dose-related leukopenia (neutropenia) in the 0.04 mg/kg/day group.
- Myelosuppression was reversible.
- No histopathological lesions were present.
- MTD > 0.04 mg/kg/day (0.8 mg/m^2/day), TDL was 0.02 mg/kg/day (0.4 mg/m^2/day).

Conclusions

- Doses of up to 0.8 mg/m^2/day in dogs and 1.2 mg/m^2/day in rats produced mild, reversible bone marrow toxicity.
- No toxicity at 0.08 mg/m^2/day in dogs or rats.
- Bone marrow toxicity occurred in both species.
- Recommended starting dose 0.12 mg/m^2/day (< 1/10 MTD in rats and ~ 1/3 TDL in dogs).

Clinical Formulation

- E7389, a highly complex, synthetic analog of Halichondrin B prepared at Eisai Research Institute via a synthetic pathway of more than 60 steps.
- Absence of any chromophore in E7389 required a selective and sensitive HPLC method for the drug substance and drug product.
- Reversed phase HPLC method with UV detection at 200 nm was developed for the bulk drug.
  - — Column: YMC J'sphere M80 ODS, 4.6 mm x 250 mm, 4-µm particles
  - — Mobile phase: CH3CN/water with 0.1% perchloric acid, pH 3.5 (38/62, v/v)
  - — Detector: UV @ 200 nm
- The expected low dosage (~ 1 mg/dose) of the solution formulation required a highly sensitive HPLC assay for the drug product.
  - — A reversed phase LC/MS/MS method was developed:
  - — Column: YMC J'sphere M80 ODS, 2 mm x 100 mm, 4-µm particles
  - — Detection: Applied Biosystems API 3000 Triple Quadruple system, ionization-electrospray (TurbolonSpray)
- Convenient solution formulation (0.5 mg/mL, 1 mL/vial) containing 5% ethanol in water for injection developed for i.v. administration.
- Clinical batch of 2,500 vials was manufactured.
- Over 1,100 vials have been distributed during the past 12 months to support the ongoing phase I and II clinical studies.



E7389 Approved for Clinical Trials in April 2002

Developmental Therapeutics Program of the National Cancer Institute

TURNING MOLECULES INTO medicines FOR THE public health