

ABSTRACT

ECO-4601 is a structurally novel farnesylated diazepinone (MW 462) discovered using Ecopia's genomic platform through analysis of actinomycete loci encoding bioactive compounds. ECO-4601 is being developed as antitumor agent as it shows suitable pharmaceutical properties, including promising *in vivo* efficacy, low toxicity, rapid absorption and bioavailability in effector tissues. We have recently shown that ECO-4601 strongly inhibits proliferation of several human cancer cell lines *in vitro*, such as low and high-grade human glioma cells (IC₅₀ = 1 to 6 μM 100% inhibition). To demonstrate *in vivo* efficacy, nude mice have been inoculated with rat C6 glioma cells (5x10⁶/ml) either subcutaneously (6/group), or orthotopically in the caudate putamen (10/group). Daily treatment by i.p. route (10 - 30 mg/kg) was initiated 24 hrs following glioma cell inoculation. When tumor cells were implanted subcutaneously, treatment with ECO-4601 resulted in a 60% decrease of the tumor volume. In the orthotopic model, mice were treated daily with ECO-4601 until spontaneous death. Preliminary results suggest that ECO-4601 is distributed to the brain as we observed a seven-day increase in the median survival of treated mice as compared to the vehicle-treated group. Body weight loss observed during such chronic treatment suggest a moderate toxicity profile of the compound that has been further confirmed by acute and subchronic administration of ECO-4601 in healthy animals. Female CD-1 mice tolerated single doses ≥100 mg/kg when ECO-4601 was given by i.v. route and single or repeated doses ≥225 mg/kg when ECO-4601 was given by s.c. or p.o. routes. Preliminary pharmacokinetic data of ECO-4601 following administration by various routes suggest a similar profile to other structurally related molecules with rapid absorption and tissue distribution (<3 min). These data highlight the pharmaceutical and therapeutic potential of ECO-4601 in aggressive tumors like gliomas.

BACKGROUND

Ecopia's discovery platform

Ecopia's innovative platform, called DECIPHER® technology^(1,2), revolves around the discovery of lead compounds that have been optimized through years of natural evolution in bacteria. The DECIPHER® technology uses a combination of leading edge genomics and bioinformatics to make computer predictions of the chemical structure of potential new drugs based on gene sequence information obtained by scanning the bacterial genome. For more information please consult Ecopia's website at www.ecopiabio.com.

Scientific rationale for developing ECO-04601 in cancer

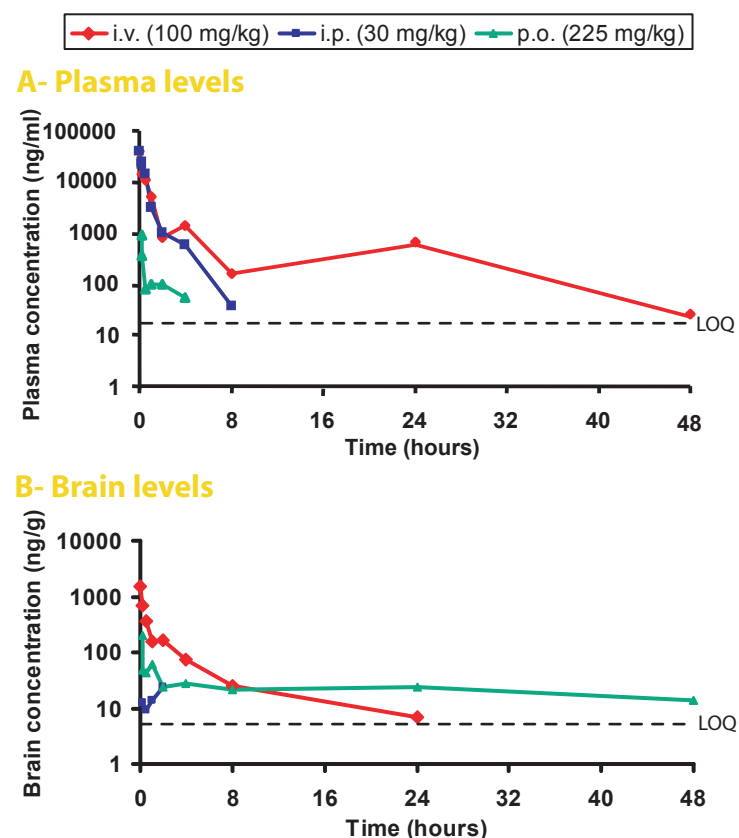
This novel molecule has shown anti-proliferative activity against several human cancer cell lines from the NCI panel. The results show that ECO-4601 at a μM range inhibits the growth of leukemia and cancer cell lines derived from lung, colon, brain, ovary, renal, prostate and breast tissues as well as melanomas.

References:

- Ahlert J, Shepard E, Lomovskaya N, Zazopoulos E, Staffa A, Bachmann BO, Huang K, Fonstein L, Czisny A, Whitwam RE, Farnet CM, Thorson JS. **The calicheamicin gene cluster and its iterative type Ienediylne PKS.** *Science* 2002 Aug 16;297(5584):1173-6.
- Zazopoulos E, Huang K, Staffa A, Liu W, Bachmann BO, Nonaka K, Ahlert J, Thorson JS, Shen B, Farnet CM. **A genomics-guided approach for discovering and expressing cryptic metabolic pathways.** *Nat Biotechnol.* 2003 Feb;21(2):187-90.

RESULTS

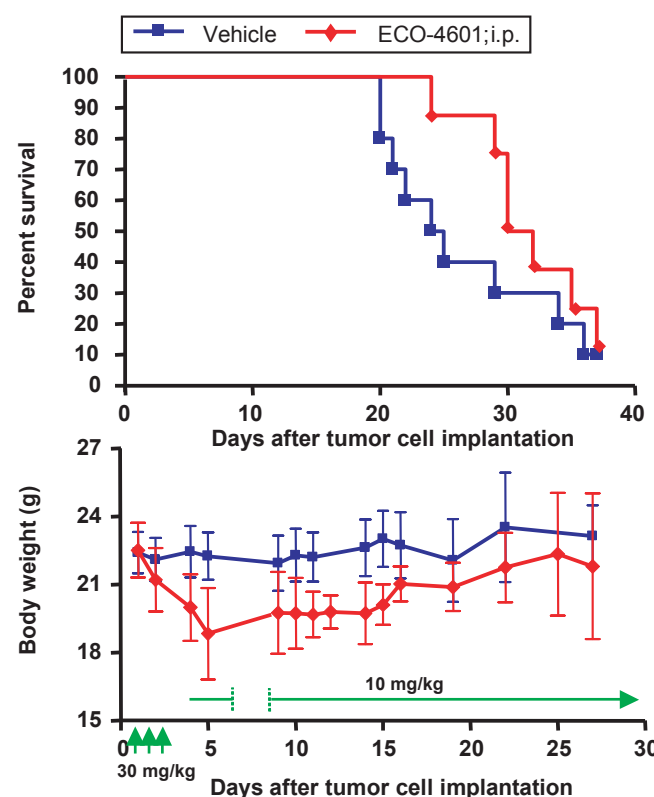
Figure 1: Plasma and brain concentrations of ECO-4601 after a single dose using different routes of administration



CD1 female mice (6 weeks of age) received a single oral (225 mg/kg), intraperitoneal (30 mg/kg) or intravenous (100 mg/kg) dose of ECO-4601. Two mice per group were sacrificed at 3 min, 10 min, 30 min, 1h, 2h, 4h, 8h, 24h and 48h. Blood was collected into EDTA containing tubes by cardiac puncture and brains were rapidly collected and immediately frozen on dry ice. Samples were analysed by LC/MS/MS. Standard curve ranged from 25 to 2000 ng/ml with limit of quantitation (LOQ) ≤ 15 ng/ml.

- Mean plasma concentrations of ECO-4601 following i.v. administration declined in a bi-phasic manner and remained above LOQ for 48h.
- PK after i.p. administration resulted in concentrations falling below LOQ after 8h, while oral administration resulted in low concentrations detectable up to 4h.
- The PK profile of ECO-4601 in brain tissue following i.v. administration was similar to that observed in the plasma (i.e. bi-phasic elimination) suggesting rapid equilibrium between both tissues.
- Lower but still detectable levels of ECO-4601 were observed in brain following i.p. and oral administrations.

Figure 2: Antitumor efficacy of ECO-4601 against orthotopic C6 glioma tumor



CD1 female nude mice (6 weeks of age) were grafted intra-cerebrally with 5x10⁴ (10 μl) C6 rat glioma cells (day 0). Treatment was initiated 24h after tumor cell implantation. Vehicle and ECO-4601 were administered i.p. 30 mg/kg qdx3 followed by 10 mg/kg qd on days 4-5 and 9-38.

- Daily treatment with ECO-4601 led to an increase survival of 7 days resulting in a 29% ILS*.
- Three consecutive doses of 30 mg/kg of ECO-4601 administered i.p. resulted in 16% decrease in body weight followed by re-gain despite continuous administration at 10 mg/kg i.p.

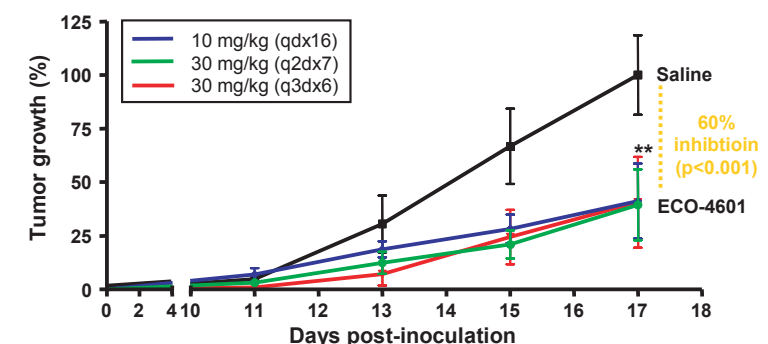
* ILS (increased life span) is expressed as mean survival time of treated animals minus that of control animals over the mean survival time of the control group. By NCI criteria, ILS% exceeding 25% indicate that the drug has significant antitumor activity.

CONCLUSIONS

- Pharmacokinetic studies with ECO-4601 following different routes of administration (oral, i.p. and i.v.) resulted in high C_{max}, short half-life and rapid distribution to tissues.
- Significant amounts of ECO-4601 were found in brain tissues following all three routes of administration strongly suggesting that the compound **crosses the blood brain barrier**.
- ECO-4601 demonstrated anti-tumor activity in both s.c. and orthotopic highly aggressive glioma tumor models with moderate and reversible toxicity.

These encouraging results support the development of ECO-4601 as a novel anticancer agent for use in humans.

Figure 3: Inhibition of newly implanted tumors by treatment with ECO-4601



CD1 female nude mice (6 weeks of age) were injected s.c. with 5x10⁵ (100 μl) C6 rat glioma cells (day 0). Treatment was initiated 24 h after tumor cell inoculation at the dose and schedule indicated in the legend. Statistical analysis of the main values was done using Anova's one-way analysis of variance (p<0.001).

- All treatments with ECO-4601 significantly decreased the growth of C6 tumors as compared to the saline-treated group.

Table 1: In vivo toxicity of ECO-4601

Administration routes	CD-1 mice	
	MTD** (mg/kg)	Observations
i.v. (bolus)	100	• Edema and necrosis at the site of injection • Can be partially prevented by avoiding vein dilatation
i.p.	30	• Intestinal occlusion; weight loss • Reversible effects • Mice tolerate 3 consecutive injections
s.c.	225	• Edema and necrosis at the site of injection • Mice tolerate several injections (by changing the site of injection)
p.o.	225	• No observed toxicity for a single administration • At 200 mg/kg, mice tolerate at least 4 consecutive gavages

** MTD = maximum tolerated dose

- PK profile, target tissue distribution and MTD evaluation suggest i.v. dosing as the preferred route of administration.