

Safety Profile in Primates of the Anticancer Agent ECO-4601, a Novel PBR Ligand and Ras Signalling Inhibitor

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ABSTRACT

Background: ECO-4601 is a structurally novel farnesylated dibenzodiazepinone (MW 462) discovered through Ecopia's proprietary Decipher® technology drug discovery platform. An initial *in vitro* assessment indicated cytotoxic activity against a wide panel of tumor cell lines, including several brain tumor cell lines. As the compound was identified through *in vitro* cytotoxic assays, its molecular target(s) were unknown at the time of discovery. The compound binds selectively, with nM affinity, the peripheral benzodiazepine receptor (PBR), preferentially expressed in tumors. Pharmacokinetic studies and antitumor evaluation indicated that efficacy is dependent on sustained plasma concentrations, rather than high C_{max} levels followed by rapid elimination. **Methods:** A study was conducted to assess the toxicity and toxicokinetic profile of ECO-4601 when administered to cynomolgus monkeys for 14 consecutive days by continuous intravenous infusion (CIV). This route and schedule of administration was chosen to achieve sustained plasma concentrations and was similar to that currently used in the clinical Phase I trial. ECO-4601 was administered to cynomolgus monkeys by CIV at doses of 5, 15 and 30 mg/kg/day, during a 14-day period, followed by a 14-day recovery period. **Results:** The highest dose level (30 mg/kg/day) was very well tolerated. This dose resulted in sustained drug plasma concentrations of 10-20 µM, which is well above target and expected therapeutic drug concentrations to be achieved in humans (2-5 µM). Furthermore, when treatment was stopped, drug plasma concentrations declined rapidly and there was no persistence in tissues. There were no effects on body weight, blood pressure and electrocardiographic activity, and no treatment-related ocular or neurologic abnormalities. Treatment-related changes observed were limited to: 1) occasional inappetence; 2) a modest degree of regenerative anemia (reversible) with no other hematologic abnormalities noted; 3) elevations in serum cholesterol and triglycerides, and a decrease in serum albumin (all reversible); and 4) diffuse vacuolization of hepatocytes and accumulation of foamy histiocytes in the spleen, which appeared to reflect clearance of the vehicle. **Conclusions:** An adequate margin of safety for ECO-4601 was established under clinically relevant dosing conditions in monkeys, which supports advancement into clinical trials.

BACKGROUND

The DECIPHER® technology uses a combination of genomic and bioinformatics to make computer predictions of the chemical structure of potential new drugs based on gene sequence information obtained by scanning the actinomycetes bacterial genome. Using this technology, we have identified ECO-4601, a small molecular weight farnesylated dibenzodiazepinone. This natural product was shown to inhibit cellular proliferation of different types of tumor cell lines and to have potent *in vivo* antitumor activity against rat glioma (AACR-NCI-EORTC 2004, # 569), human breast and prostate xenografts (AACR-NCI-EORTC 2005, #A28).

Mechanistic studies indicated that ECO-4601 binds the PBR (AACR 2005, #5896), resulting in apoptosis, and inhibits the Ras-mitogen-activated protein kinase pathway (AACR-NCI-EORTC 2006), involved in cell proliferation and migration. This dual activity makes ECO-4601 unique among the novel targeted anticancer therapies.

The encouraging pre-clinical results led Ecopia to further develop ECO-4601 and initiate a Phase I clinical trial against solid tumors. In order to support human clinical trials and identify a starting dose, GLP toxicology studies were performed in cynomolgus monkeys for 14 consecutive days by (CIV). This route and schedule of administration was chosen to achieve sustained plasma concentrations and is similar to that used in the current clinical Phase I trial.

RESULTS

STUDY DESIGN

Group Numbers	Group Designation	Dose Level (mg/kg/day)	Dose Concentration (mg/mL)	Main		Recovery	
				Male	Female	Male	Female
1	Saline Control	0	0	2	2	0	0
2	Vehicle Control*	0	0	3	3	2	2
3	Low Dose	5	0.104	3	3	0	0
4	Mid Dose	15	0.312	3	3	0	0
5	High Dose	30	0.625	3	3	2	2

* The individual concentrations of each component of the vehicle control dose formulation was equivalent to the individual concentrations of the same components in the dose formulation of the High Dose (30 mg/kg/day) group. The test and vehicle control articles were infused intravenously (24 hours/day) into the femoral vein at a dose rate of 2 mL/kg/hour for 14 consecutive days.

PARAMETERS EVALUATED

- **Clinical Signs, Body Weight, Food Consumption**
- **Clinical Pathology**
 - Hematology (including coagulation), clinical chemistry and urinalysis
- **Macroscopic and Microscopic Pathology**
- **Safety Pharmacology**
 - Neurological examinations
 - Ophthalmoscopy
 - Blood Pressure
 - Electrocardiography (ECG)
- **Toxicokinetics**
 - Blood samples were taken on Days 1, 2, 6, 10, and 15 of the treatment period. On Day 1, samples were collected at 2, 6, and 12 hours after initiation of treatment. Additional samples were collected at 30 hours after the start of infusion (Day 2) and on Days 6 and 10 (at approximately 6 hours after the bag changes). At the end of the 14 days of infusion, on Day 15, samples were collected at 1 hour prior to cessation of treatment, and at 5min, 30min, 1h, 2h, 4h, 8h, and 24h post treatment.
 - Brain, liver, and kidneys were harvested 24h after the end of treatment.

RESULTS

- **Mortality and Clinical Signs:**
 - One mid-dose female was euthanized on Day 10 due to complications associated with presence of catheter and unrelated to ECO-4601.
 - Minor clinical signs were observed at ≥ 15 mg/kg/day, including decreased appetite.

- **Body Weight and Food Consumption:** No effects
- **Blood Pressure, ECGs, Ocular and Neuro Exams:** No effects
- **Clinical Pathology:**

- Moderate reduction in RBC counts and related parameters at 30 mg/kg/day, with an increase in reticulocyte counts.
- Changes in RBC morphology consistent with erythrocyte regeneration; no clear effect on bone marrow cytology.
- Elevation in serum cholesterol and triglycerides and reduction in serum albumin at 30 mg/kg/day
- No changes in urinalysis parameters.

All clinical pathology changes were completely reversible

- **Excretion and Tissue Levels:**

- Low levels of ECO-4601 in urine samples and high levels in fecal samples, suggesting excretion via the bile.
- Low concentrations of ECO-4601 in kidney, brain, liver in mid and high-dose groups 24h following the end of infusion, suggesting no accumulation in tissues.

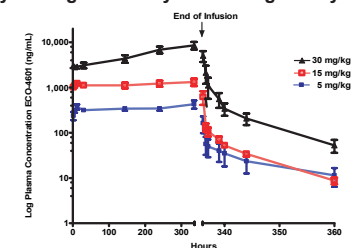
- **Organ Weights:** No clear effects

- **Necropsy Findings:** No effects

- **Histopathology:**

- No effects of ECO-4601 (no infusion site lesions).
- Findings attributable to the vehicle consisted of minimal, diffuse vacuolization of hepatocytes and accumulation of foamy histiocytes in the spleen of 2/6 animals each from the vehicle control and ECO-4601-treated groups, believed to reflect a clearance process for the vehicle.

Pharmacokinetic Profile of ECO-4601 in Cynomolgus Monkeys Following 14-day CIV



ECO-4601 Drug Levels in Selected Tissues 24 hours Following End of Infusion

Groups	Liver	Kidneys	Brain
5 mg/kg/day	BLQ	BLQ	BLQ
15 mg/kg/day	19-91 ng/g; 3/6 animals	22 and 27 ng/g; 2/6 animals	51 ng/g; 1/6 animals
30 mg/kg/day	20 to 74 ng/g; 5/6 animals	BLQ	19-92 ng/g; 4/6 animals

BLQ – Below detection limit (5 ng/g)

Summary of Pharmacokinetic Results in Monkeys from a 14-day CIV Infusion Study

Dose (mg/kg)	C _{ss} (ng/mL)*	AUC _α (ng/mL*h)	CL (L/h/kg)	V _{ss} (L/kg)	V _z /F (L/kg)	T _{1/2Z} (h)	T _{last} (h)
5	358 ± 85** (~0.8 µM)	119,018 ± 26,690	0.61 ± 0.14	7.1 ± 3.9	10 ± 3	12 ± 3	355 ± 8
15	1,173 ± 340 (~2.5 µM)	400,116 ± 126,140	0.56 ± 0.13	3.6 ± 2.0	6.8 ± 2.9	8.3 ± 2.2	360 ± 0
30	6,283 ± 3,650 (~15 µM)	1,874,950 ± 945,067	0.27 ± 0.11	10.7 ± 6.2	3.2 ± 1.7	8.1 ± 1.0	360 ± 0

*Average of plasma concentration between 30h and 14 days.
**Values are Mean ± SD.

Steady-state ECO-4601 plasma concentrations were observed throughout the 14-day CIV infusion in the 5 mg/kg/day and 15 mg/kg/day groups.

For the high dose group of 30 mg/kg/day, ECO-4601 plasma concentration increased throughout the 14-day infusion period from ~6 µM at Day 1 to ~9.4 µM at Day 6, to ~15 µM by Day 10, and to ~18.5 µM by day 15.

Plasma concentrations in the 15 mg/kg/day and the 30 mg/kg/day groups exceeded drug levels observed in the *in vivo* antitumor activity experiments.

When infusion of ECO-4601 in the different groups was terminated, rapid elimination of ECO-4601 from plasma was observed in all groups. The half-life of ECO-4601 varied from 8-12h.

CONCLUSIONS

ECO-4601 is a unique and new targeted therapeutic anticancer drug candidate with dual activity: selective binding to the PBR, resulting in apoptosis, and inhibition of the Ras-MAPK pathway, which is involved in cell proliferation and migration. Encouraging pre-clinical results allowed for the initiation of a Phase I clinical trial against solid tumors.

Toxicokinetics:

- ECO-4601 plasma concentrations in monkeys were significantly above the plasma drug levels observed in antitumor activity studies (2 µM).
- When dosing of ECO-4601 was terminated after 14 days, the drug was rapidly eliminated from plasma and tissues, indicating no drug accumulation.

Toxicity (Observations attributable to ECO-4601 at 30 mg/kg):

- Decreased appetite
- Reversible hematology and clinical chemistry changes
- Diffuse vacuolization of hepatocytes and accumulation of foamy histiocytes in the spleen (clearly vehicle-related)
- No treatment-related effects on body weight, blood pressure and electrocardiographic activity
- No treatment-related ocular or neurologic abnormalities

- **The no-adverse-effect level (NOAEL) corresponded to 15 mg/kg/day (180 mg/m²), based primarily on the anemia that was seen in the 30-mg/kg/day animals, although this was fully reversible.**

- **These data led us to define our starting dose for the Phase I clinical trial at 30mg/m²/day.**