

BACKGROUND

ECO-4601 (U.S. Patent 7,101,872) is a structurally novel farnesylated dibenzodiazepinone with broad μM *in vitro* cytotoxic activity and *in vivo* antitumor efficacy in rat glioma, human hormone-independent prostate and breast tumor xenografts. Preclinical data suggests that ECO-4601 is a targeted anticancer compound with dual activity: (i) selective binding to the peripheral benzodiazepine receptor (PBR), resulting in targeting and accumulation of the drug in the tumor, and, (ii) inhibition of the Ras-MAPK pathway, which is involved in cellular proliferation and migration. Antitumor activity is dependent upon continuous exposure, and a target plasma ECO-4601 efficacy concentration of $2\mu\text{M}$ was determined. The pharmacokinetic profile of ECO-4601 was tested in a Phase I/II clinical trial in patients with advanced solid tumors.

METHODS

ECO-4601 was administered as a 2-week continuous i.v. infusion (CIV) on an out-patient basis using an ambulatory pump, followed by 1 week off in repeated 21 day cycles. The trial included dose-escalation and dose-extension portions, with comprehensive pharmacokinetics (PK) during the first and second cycles. A typical treatment cycle is shown below (Fig. 1). PK samples were obtained on days 1, 2, 8, 15, 16 at various timepoints. ECO-4601 doses of 30, 60, 120, 180, 270, 360, and 480 mg/m²/day were evaluated in 14 patients in the escalation portion and in 12 additional patients treated at 480 mg/m²/day. Patients were enrolled in a sequential manner starting with the dose escalation portion in which 1 to 6 patients per dose level were included until the appearance of dose limiting toxicity. ECO-4601 plasma concentrations were determined using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with EDTA-K2 as anti-coagulant agent. The lower limit of quantitation (LLOQ) was established at 1 ng/mL. Pharmacokinetic parameters were calculated using the software program WinNonlin version 5.2 (Pharsight Corp.).

RESULTS

The first six (6) patients dosed at 30 mg/m²/day had a mean ECO-4601 plasmatic concentration of 0.50 μM , which was in agreement with the predicted value calculated from PK studies in animal species (mouse, rat, and monkey). Apparent volume of distribution (V_z) was 108 ± 21 L and clearance (CL) was 9.7 ± 3.8 L/h. Inter-patient PK variability was moderate (39% CV for CL) (Table 1). The mean terminal half-life ($t_{1/2, z}$) was 8.4 ± 2.6 hours and the post-infusion half-life ($t_{1/2, \alpha}$) was less than 30 min. After the infusion ceased, ECO-4601 was rapidly eliminated from the bloodstream within 24 h at all dose levels.

Table 1: PK Parameters and Values

Patient* No.	Dose (mg/m ² /day)	Steady-state levels** (μM)	C _{DAY15} (ng/mL)	C _{SS} *** (ng/mL)	T _{max} (h)	AUC _{inf} ($\mu\text{g/mL}\cdot\text{h}$)	CL (L/h)	t _{1/2,z} (h)	V _z (L)
1 to 6	30	0.51	221 ± 76	236 ± 83	150.8 ± 100	76 ± 32	9.7 ± 3.8	8.35 ± 2.63	108 ± 21
7	60	0.62	288.5	284.7	332.6	93.3	14.9	8.90	191.6
8	120	1.39	494.3	640.8	24.0	230.2	12.5	9.40	169.6
9	180	1.53	670.0	706.8	165.8	239.1	16.3	8.87	208.6
10	270	2.52	1113.9	1163.8	24.0	382.7	14.8	6.95	148.7
12	360	3.53	1596.5	1628.9	24.0	511.9	14.6	7.66	161.1
14 to 25	480	5.56	2886 ± 543	2571 ± 557	209 ± 133	836 ± 210	16.7 ± 5.1	15.4 ± 3.1	213 ± 75

* Patients #11 (270 mg/m²/day) and #13 (360 mg/m²/day) did not complete Cycle 1.
**The steady-state concentrations calculated from C_{SS}
*** C_{SS} calculated with C_{24h}, C_{DAY8}, C_{DAY15}

Dose escalation (Phase I):

ECO-4601 exposure parameters (C_{SS} and AUC) increased proportionally with increasing dose levels. Steady-state was achieved by 24 hours and remained constant at day 8 and prior to the end of infusion on day 15. Figure 3 shows that steady-state concentrations increased linearly as a function of increasing dose levels. As presented in Table 1, doses of 270, 360 and 480 mg/m²/day resulted in ECO-4601 steady-state plasma concentrations of 2.52, 3.53 and 5.56 μM , respectively. These plasma concentrations are above the estimated therapeutic threshold (2 μM ~ 1000 ng/mL) previously determined in animal efficacy studies. An additional twelve (12) patients were dosed in the dose extension portion at the selected dose of 480 mg/m²/day, reaching plasma concentrations 2-fold higher than the estimated therapeutic threshold.

Dose extension portion (Phase II):

As shown in Table 1, PK data were evaluable for 12 patients treated at the recommended phase II dose of 480 mg/m²/day. At this dose level, mean (standard deviation) values for steady-state concentration (C_{SS}) and AUC from time zero to infinity (AUC_{inf}) were 2.57 $\mu\text{g/mL}$ (0.557) and 836 $\mu\text{g/mL}\cdot\text{hr}$ (210), respectively. Steady-state plasma concentrations varied between 4.5 and 7 μM (Fig. 4a). Interpatient variation in exposure was minimal, varying 2.1- to 2.3 fold from the minimum to maximum value at the 480 mg/m²/day dose level, with a corresponding coefficient of variation (CV%) of 22-25%. As featured in Figure 4b, systemic clearance (CL), half-life (t_{1/2, z}), and volume of distribution (V_z) during the terminal phase were independent of dose level; the overall mean (standard deviation) values for all dose levels were 14.6 L/h (4.95), 8.6 h (1.7), and 179 L (70.0), respectively.

Multiple treatment cycles:

Blood samples were collected during cycles 1 and 2. Steady-state concentrations in human plasma were comparable between both cycles, indicating that no drug accumulation had occurred. The mean terminal half-life (t_{1/2, z}) of ECO-4601 was similar in both cycles.

Fig 4: PK Profile of ECO-4601 in Human Plasma

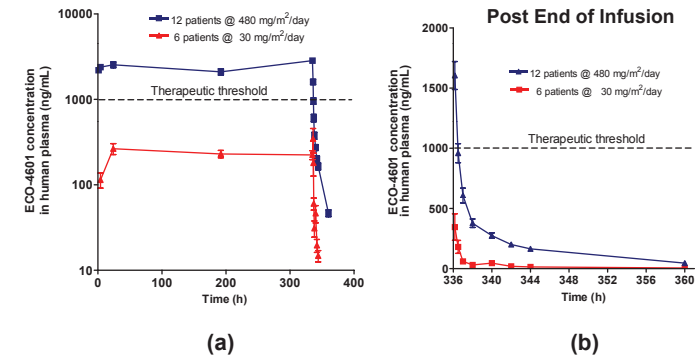


Fig 3 : Dose Proportionality

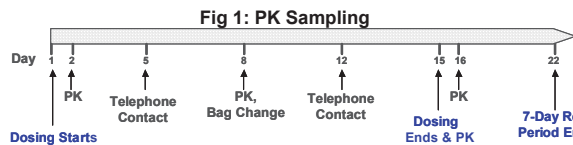
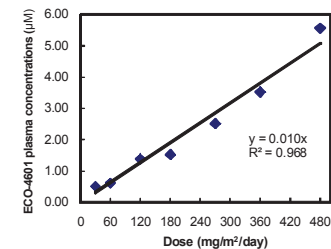
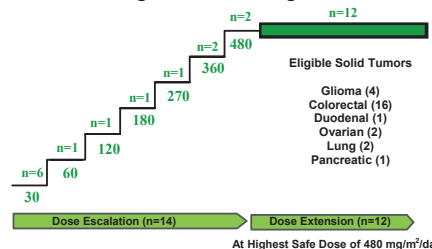


Fig 2: Clinical Design



A schematic of the Phase I clinical design and the number of patients enrolled for each advanced solid tumor type are provided (Fig. 2). Eligible solid tumors included high grade glioma, colorectal, prostate, pancreatic, lung, ovarian and breast cancers, for which standard therapy had failed or was unavailable.

CONCLUSIONS

- Estimated therapeutic ECO-4601 plasma concentrations are reached at doses of 270 mg/m²/day or greater.
- At 480 mg/m²/day, ECO-4601 plasma levels are more than 2-fold higher than the estimated therapeutic level.
- Dose proportionality is observed with increasing dose levels.
- ECO-4601 is rapidly eliminated.
- ECO-4601 is well tolerated.
- A dose of 480 mg/m²/day is the selected dose for upcoming Phase II clinical trials.

The data presented here, together with the preliminary evidence of efficacy seen in this trial support further development of ECO-4601 in a larger Phase II trial.