

ABSTRACT

ECO-4601 is a structurally novel farnesylated dibenzodiazepinone (MW 463) discovered using Ecopia's genomic platform for analysis of actinomycete gene loci encoding pathways leading to bioactive compounds. The compound was shown to have a broad cytotoxic activity in the low micromolar range when tested in the NCI 60 cell line panel. Since ECO-4601 was shown to cross the blood brain barrier and to inhibit the growth of several brain tumor cell lines, its *in vivo* antitumor activity was first evaluated against subcutaneous and orthotopic rat C6 glioma tumor xenografts (EORTC-NCI-AACR, September 2004 A#569). We have further evaluated the antitumor activity in human hormone-independent breast (MDA-MB-231) and prostate (PC3) cancer xenografts. The endpoint volume for tumor growth in these models was 1000 mm³ and 1500 mm³, respectively. Treatment results are presented as percent growth inhibition (%TGI), defined as the [mean excised treated-tumor volume increase (T) divided by the mean excised control-tumor volume increase (C) = T/C] x 100% subtracted from 100%.

ECO-4601 resulted in highly significant (P < 0.001) antitumor activity in the MDA-MB-231 breast tumor model (10 animals per group) when mice were dosed at 30 mg/kg (Q1D x 5 for 3 weeks) but was not effective (P = 0.34) when dosed at 50 mg/kg (Q3D x 7) using the subcutaneous (SC) bolus route. At the end of the study (D56), one mouse in the 30 mg/kg treated group (tumor volume = 126 mm³) and two mice at the 50 mg/kg treated group (tumor volumes of 0 and 726 mm³) remained. Insignificant (P = 0.5) antitumor efficacy was observed when ECO-4601 was administered by intravenous (IV) bolus injection.

In the PC3 prostate tumor model (10 animals per group), ECO-4601 resulted in significant (P < 0.001) antitumor activity with tumor stabilization and tumor regression when given by the SC bolus route at doses of 30 mg/kg (Q1D x 5 for 3 weeks) and 50 mg/kg (Q3D x 7), respectively. At the end of the study (D71), seven mice remained in the 30 mg/kg treated group with a mean tumor volume of 700 mm³ (0 to 1470 mm³) yielding one tumor-free animal (complete regression) and three partial responses. The 50 mg/kg dose resulted in five survivors with a mean tumor volume of 371 mm³ (62.5 to 726 mm³) and yielded two complete responses and three partial responses. In this tumor model, IV bolus dosing was also ineffective.

Pharmacokinetic parameters were studied following SC and IV bolus administration of ECO-4601. While IV dosing resulted in elevated C_{max} (280 μM) and short half-life (T_{1/2α} = 4.7 min and T_{1/2β} = 2.5h) leading to low drug exposure, SC and IP dosing yielded low C_{max} but sustained (μM range) drug levels for up to 8h. The antitumor efficacy of ECO-4601 thus appears to be associated with the exposure parameter AUC and/or sustained drug levels rather than C_{max}. These *in vivo* data constitute a rationale for clinical studies testing prolonged continuous administration of ECO-4601.

BACKGROUND

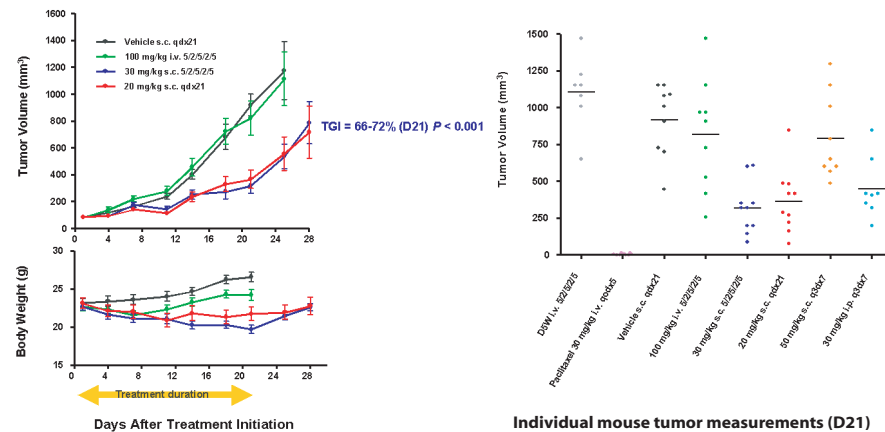
The DECIPHER[®] technology uses a combination of genomic and bioinformatic tools to make computer predictions of the chemical structure of potential new drugs based on gene sequence information obtained by scanning actinomycetes bacterial genomes.

Using this technology, 59 new chemical entities (NCEs) have been discovered, the most advanced of which is ECO-4601, a small molecule having broad *in vitro* cytotoxic activity (GI50 values in the range of 2 to 20 μM). We have previously reported that ECO-4601 has *in vivo* antitumor activity against subcutaneous and orthotopic rat C6 glioma tumor xenografts (EORTC-NCI-AACR, September 2004 A#569).

In the work presented here, the antitumor activity of ECO-4601 was further evaluated in human hormone-independent breast (MDA-MB-231) and prostate (PC3) cancer xenografts. Since antitumor activity was dependent on the route of administration, pharmacokinetic behaviour of ECO-4601 following subcutaneous (SC) or intravenous (IV) administration was assessed. Our findings show that ECO-4601 antitumor activity is dependent of total drug exposure (area under the curve, AUC) rather than on short elevated systemic drug concentrations (C_{max}).

RESULTS

Antitumor efficacy of ECO-4601 against the human breast (MDA-MB-231) tumor xenograft



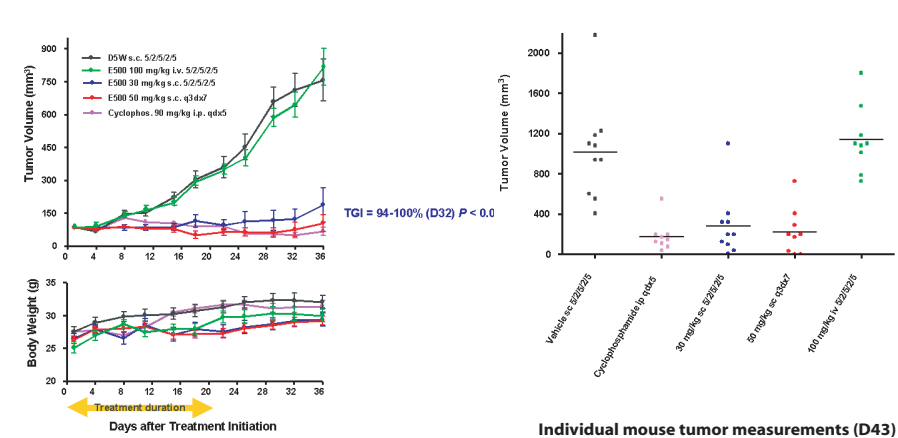
Female Hsd:ATHymic Nude-Foxn1nu mice (8-9 weeks old) were injected SC in the right flank with 5 x 10⁶ MDA-MB-231 cells (0.2 ml cell suspension). Mice were randomized into eight groups of 10 mice once tumors had reached 80-120 mm³ (day 1 of study; 8 days post tumor cell inoculation). Treatment was from day 8 to day 29 post tumor cell inoculation. Each animal was euthanized when its tumor reached the predetermined endpoint size (1,000 mm³) or at the end of the study (56 days). Tumor growth inhibition (TGI) was calculated on day 29 post tumor cell inoculation (day 21 of study), at which time the animals from the vehicle or dextrose control groups had to be sacrificed due to tumor burden.

Tumor measurements:

The length (L) and width (W) of tumor mass were measured by calliper twice a week and tumor volume (TV) was calculated as: TV = (L x W²)/2. Tumor volume at day n was expressed as relative tumor volume (RTV) according to the following formula RTV = TV_n - TV₀, where TV_n is the tumor volume at day n and TV₀ is the tumor volume at day 1. The percentage of tumor growth inhibition (%TGI) was determined by 1 - (mean RTV of treated group/ mean RTV of control group) x 100. According to the NCI standards, a %TGI of ≥ 60% is indicative of antitumor activity. Statistical analysis was calculated by the two-tailed unpaired t test and by ANOVA using the Prism software.

- ECO-4601 resulted in highly significant antitumor activity (P < 0.001) in human breast cancer xenografts when mice were dosed at 30 mg/kg (5/2/5/2/5) and 20 mg/kg (QD X 21) using the bolus SC route.
- High doses given less frequently (50mg/kg Q3DX7) were not effective.
- Insignificant antitumor activity (P = 0.5) was observed when ECO-4601 was administered by the IV bolus route.

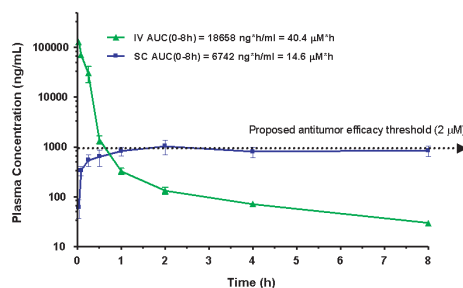
Antitumor efficacy of ECO-4601 against the human prostate (PC3) tumor xenograft



The human PC3 prostate carcinoma line utilized for these studies was maintained in athymic nude mice by serial engraftment. A tumor fragment (1 mm³) was implanted SC into the right flank of male Hsd:ATHymic Nude-Foxn1nu mice. When tumor volumes reached 80-120 mm³, mice were randomized and treatment began (day 1 of study; 19 days following tumor fragment inoculation). Each animal was euthanized when its tumor reached the predetermined endpoint size (1,500 mm³) or at the end of the study (71 days). Tumor growth inhibition was calculated 62 days after tumor implantation (day 43 of study), at which time the animals from the vehicle control group had to be sacrificed due to tumor burden.

- ECO-4601 resulted in highly significant antitumor activity (P < 0.001) with PC3 tumor stabilization and regression observed (days 8 to 15 and days 8 to 36) when given by the bolus SC route at doses of 30 mg/kg (5/2/5/2/5) and 50 mg/kg (Q3DX7), respectively.
- As with the breast tumor xenograft, bolus IV treatment was not effective.

Plasma concentration-time curves of ECO-4601 following IV and SC administration



- Bolus SC administration resulted in prolonged drug exposure at therapeutic effective (2 μM) drug levels.
- Bolus IV dosing resulted in high C_{max} (280 μM), and due to the short half-life (T_{1/2α} = 4.7 min) drug plasma concentration rapidly declined and was below quantitation after 8h (with SC dosing, plasma drug levels remain constant up to 24h).

The pharmacokinetic profile of ECO-4601 was evaluated following a bolus IV or SC at 30 mg/kg. Four (4) mice per group were sacrificed at each of 3 min, 5 min, 15 min, 30 min, 1h, 2h, 4h and 8h time points. Blood was collected into EDTA containing tubes and plasma samples were analyzed by LC/MS/MS (limit of quantitation, 25 ng/ml). The therapeutic threshold refers to the sustained plasma concentration of ECO-4601 which resulted in antitumor efficacy in mice.

CONCLUSIONS

- ECO-4601 has potent antitumor activity against human breast and prostate hormone-independent tumors.
- ECO-4601 antitumor activity is dependent on the route of administration: bolus SC administration is active while bolus IV administration is inactive in both tumor models. This is consistent with sustained plasma levels of ECO-4601 achieved by SC administration.
- Frequency of dosing depends on tumor growth rate: daily administration is required with tumor doubling time of 6 days or less.

Our findings suggest that the antitumor activity of ECO-4601 is dependent on total exposure (AUC) of drug versus elevated levels of short drug exposure (C_{max}).

These *in vivo* data constitute a rationale for clinical studies testing prolonged continuous administration of ECO-4601.