

## BACKGROUND

ECO-4601 is a structurally novel farnesylated dibenzodiazepinone (MW 462; US Patent 7,101,872) discovered using Thallion's proprietary DECIPHER® technology platform. Preclinical data suggests that ECO-4601 is a targeted anticancer drug with dual activity: selective binding to the peripheral benzodiazepine receptor (PBR) and inhibition of the Ras mitogen activated protein kinase (MAPK) signalling pathway.

The PBR was originally discovered as an alternative binding site for the benzodiazepine diazepam (Valium®), and is a critical component of the mitochondrial permeability transition pore (MPTP). This multiprotein complex is located at the contact site between inner and outer mitochondrial membranes where it regulates cholesterol transport and synthesis of steroid hormones. Upregulation of the PBR is documented in many tumor types and PBR ligands have been used as imaging tools in the diagnosis of brain tumors.

Aberrant Ras signalling has been implicated in the etiology of certain brain cancers. For example, EGFR amplifications and mutations occur in 40-50% of glioblastomas (GBM). As the PBR is overexpressed in brain tumors compared to normal brain, we examined whether ECO-4601 would preferentially target and accumulate in tumors using an intra-cerebral orthotopic tumor model in rats. Specific tumor targeting and drug accumulation may further increase the likelihood of ECO-4601 in exerting its cytotoxic effects via intracellular inhibition of Ras signalling pathways.

## METHODS

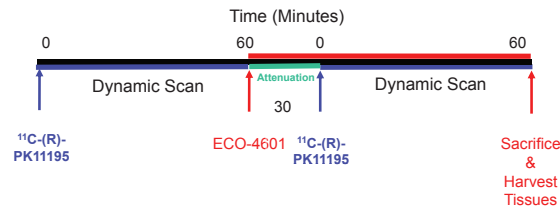
**Cell Culture and Spheroid Preparation:** Rat C6 glioma cells were purchased from the ATCC and grown in DMEM supplemented with 10% FBS, 125 U/mL penicillin G, 125 µg/mL streptomycin sulfate, and 2.2 µg/mL amphotericin B. Upon reaching confluency, spheroids were prepared using the hanging drop method described by Del Duca *et al.* (*J. Neurooncol.*, 67: 295, 2004.). Briefly, 20 µl drops of DMEM containing 15,000 rat C6 glioma cells (obtained from exponentially growing cultures) were suspended from the lids of culture dishes. After 72 hours, the resulting aggregates were transferred to culture dishes base-coated with agar and further cultured for 48 hours.

**Surgical Implantation of Spheroids:** Male Sprague-Dawley rats (250-300g) were anesthetized with 50 mg/kg ketamine and 10 mg/kg xylazine. The right cortical surface in the parietal-occipital region was exposed by craniectomy using a high-powered drill and the underlying dura and its vessels were carefully removed under a surgical microscope. A piece of the cortex was removed to expose the underlying white matter and a single spheroid was placed into the surgical defect. The craniectomy was covered with bone wax and the overlying skin sutured.

**In Vivo [<sup>11</sup>C]-(R)-PK11195 PET Imaging in Rats:** *In vivo* PET studies were performed 14 days post-tumor implantation. PET imaging studies were performed while the animal was anesthetized and placed in the supine position on the bed and at the center of the FOV of the CTI Concorde R4 microPET scanner. Each dynamic PET study lasted 60 minutes and was initiated with an IV bolus administration of [<sup>11</sup>C]-(R)-PK11195 (7.1-12.7 MBq), a specific and potent PBR ligand. Receptor occupancy studies were performed by acquisition of [<sup>11</sup>C]-(R)-PK11195 images prior to and during ECO-4601 treatment over 60 minutes. Attenuation correction factors, for each rat, were determined using a 10 minute <sup>57</sup>Co transmission scan acquired immediately prior to the dynamic scan (detailed in Figure 1). In addition, all images were scatter corrected.

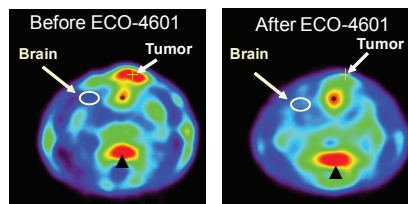
## RESULTS

Figure 1: Experimental Design



ECO-4601 was administrated by a bolus IV infusion (30 mg/kg) followed by continuous IV infusion (5 mg/h/kg) lasting through out the 60 min dynamic scan.

Figure 2: *In Vivo* PBR Occupancy by ECO-4601 in the Rat C6 Tumor Orthotopic Model



Images were reconstructed using filtered back-projection, and time-activity curves (TACs) were obtained from regions-of-interest (ROIs) in the tumor (target region), brain (reference region), and cerebellum (reference region).

The \* likely represents a necrotic area and therefore non-specific binding of the [<sup>11</sup>C]-(R)-PK11195, while the arrowhead represents non-specific soft tissue [<sup>11</sup>C]-(R)-PK11195 uptake.

Table 1: Binding Potential for Each Rat

Rat ID	BP (Baseline)	BP (ECO-4601)	% PBR occupancy
1	2.30	0	100
2	1.68	0.84	50
3	2.89	0	100
4	2.51	0	100
5	2.11	0	100
6	2.51	0	100

For all studies, the mean binding potential (BP) was determined using the simplified reference tissue method. Repeated measures t-test analysis was performed using the R statistics software.

**ECO-4601 effectively binds the PBR *in vivo* as determined by the competition assay of [<sup>11</sup>C]PK11195 using PET-Scan studies to quantify PBR occupancy.**

Table 2: Tissue/Plasma Drug Levels

	Tumor Drug Levels (µg/g)	Brain Drug Levels (µg/g)	Liver Drug Levels (µg/g)	Plasma Drug Levels (µg/mL)
Range (Average)	8.5 to 273 (176)	0.5 to 1.3 (0.807)	2.3 to 61 (24.8)	4 to 32 (16.2)

Following the completion of *in vivo* studies (see Figure 1), animals were sacrificed by anesthetic overdose and decapitated. Brain, tumour, and liver were snap-frozen in liquid nitrogen and stored at -80°C. Blood samples were collected into K<sub>2</sub>-EDTA tubes and plasma stored at -80°C. ECO-4601 was extracted with acetone and quantified by HPLC-MS/MS.

**Analysis of ECO-4601 tissue and plasma concentrations indicated that ECO-4601 preferentially accumulates in the tumor. Indeed, drug levels were >200-fold higher in the tumor compared to the normal brain. ECO-4601 accumulation in the tumor (176 µg/g) was also significant compared to liver (24.8 µg/g; 7-fold) and plasma (16.2 µg/mL; 11-fold).**

## CONCLUSIONS

- ECO-4601 crosses the blood brain barrier
- ECO-4601 binds to the PBR *in vivo*
- ECO-4601 preferentially accumulates in tumors
  - > 200-fold increase in the tumor vs normal brain
  - 11-fold increase in the tumor vs plasma

These data suggest that the presence of PBR in GBM allows for specific accumulation of ECO-4601 in the tumor and the potential for reducing normal brain toxicity.

These data, together with the inhibitory effect of ECO-4601 on activated Ras and the phase I/II clinical data showing safety and tolerability, warrant testing ECO-4601 as a treatment for GBM.