The dual selective VEGFR-FGFR kinases inhibitor E-3810 decreases tumor perfusion and inhibits tumor growth: an analysis using dynamic contrast-enhanced magnetic resonance imaging

 # 1367

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Introduction

E-3810 is a potent VEGFR1, 2 and 3 and FGFR 1 kinases inhibitor showing anti-angiogenic and antitumor activities in several *in vitro* and *in vivo* mouse models (Proceedings AACR-EORTC-NCI Meeting, Boston, November 2009; Abstracts No. 252 and 257). The aim of this study was to investigate, in a rat model, the antitumor activity of E-3810 and its anti-angiogenic effects by using DCE-MRI and to correlate the response with a panel of biological markers.

Material and Methods

- Test substances : - E-3810 prepared in 0,5% Methocel
  - Sorafenib prepared in DMSO/Tween20/saline (5/5/90)
- Tumor cell line : human Calu-6 squamous cell lung carcinoma
- Animals : female Hsd : RH-*Foxn1*^{rnu} rats (Harlan Laboratories, Holland)
- Drug administration : Oral route (po, gavage) via a canula for 14 days
- Tumor induction and treatment schedule protocol :
  - SC inoculation of 10^7 Calu-6 cancer cells to *Nude* rats
  - Randomization of rats at D-1 (13 days after the injection of cells) in 3 groups.

Groups	Number of rats	Treatment	Dose (mg/kg/adm)	Adm route	Treatment schedule	Imaging Schedule
1	21	Vehicle	-	PO	Q1Dx14	D-1, D1, D3, D7 and D14
2	25	E-3810	10	PO	Q1Dx14	
3	21	Sorafenib	100	PO	Q1Dx14	

DCE-MRI protocol :

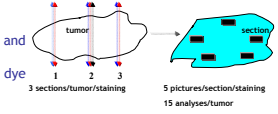
- Randomization of six rats/group for DCE-MRI at D-1 based on tumor volum (385±117 mm³) and Ktrans data (0.68±0.10 min^{-1} (4 rats for the experiment and 2 in case of death).
- MRI was performed at D-1, D1, D3, D7 and D14 on a 4.7T Pharmascan horizontal bore (Bruker, Germany). The animals were maintained under anaesthesia via a constant flow of isoflurane at 2-3% delivered by a nose cone.
- Morphological description and tumor volume were assessed with a T2w RARE sequence (TE/TR=38/2500 ms, FOV=70x50 mm, slice thickness=1.5 mm)
- DCE-MRI : follow up of contrast agent uptake in the tumor during 8 minutes after an intravenous *bolus* injection of Gd-DTPA (Magnevist®, 0.1 mmol/kg) using a T1w FLASH sequence (TE/TR/flip angle= 3 ms/50 ms/60°, slice thickness=2 mm) at a temporal resolution of 4 s per image.
- Tracer uptake curves were derived from signal enhancement in selected regions of interest (ROI) (i.e on tumor rim and core) and characterized by :
  - K^{trans} : the volume transfer constant was determined by fitting the curve using a two-compartment kinetic model (Tofts et al, JMRI, 1999), using an in-house developed plugin of ImageJ.
  - **IAUC** : the initial area under the curve was computed by integration between injection time and 60 sec after injection time.

Biomarkers evaluation

- Sacrifice of 4 satellite tumor bearing *Nude* rats/group at D1, D3, D7 and D14
  - Collection of blood to determine the absolute count number of circulating endothelial cells (CD45-CD31+) by FACS,
  - Collection of plasma to measure the circulating level of collagen IV by ELISA assay (Exocel),
  - Collection of SC tumors for assessment of number, maturity and functionality of vessels

Histopathology

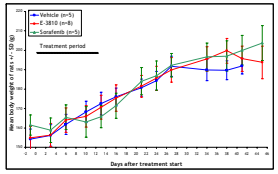
- Histological study of vascularity and perfusion of tumors
  - Investigation of tumor vascular status using CD31 / SMA immunostaining (endothelial cells and pericytes),
  - Investigation of tumor vessel permeability and functionality using Hoechst fluorescent dye (injected 1min before sacrifice).



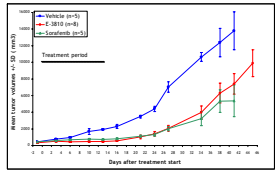
All procedures with animals were submitted to the Animal Care and Use Committee of Pharmacy and Medicine University (Dijon, France).
Principe d'éthique de l'expérimentation animale. Directive n°86/609 CEE du 24 Nov. 1986, Décrêt n°87/848 du 19 Oct. 1987, Arrêté d'Application du 19 Avril 1988.
WORKMAN P. et al., UKCCCR guideline. Br. J. Cancer, 77: 1-10, 1998.

Results

No body weight loss of tumor bearing *Nude* rats treated with E-3810 and sorafenib was observed during the course of the study.



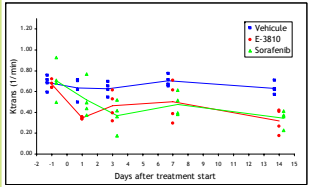
E-3810 and Sorafenib displayed a significant antitumor activity in the Calu-6 tumor bearing *Nude* rat model.



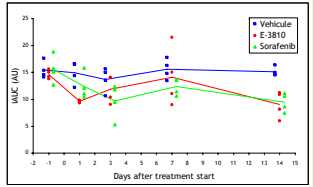
E-3810 and sorafenib induced a significant growth delay of Calu-6 tumors (increase of tumor doubling time and time to reach a defined tumor volume of 2000 mm³).

	Time to reach V (~ 2000 mm³) ± SD (days)	Tumor doubling time ± SD (days)	Optimal T/C%
Vehicle	14.2 ± 1.6	7.5 ± 0.1	NA
E-3810	28.9 ± 9.2 (p=0.0052)	11.6 ± 3.1 (p=0.0090)	22 (D13)
Sorafenib	31.5 ± 11.1 (p=0.0100)	16.9 ± 3.1 (p=0.0002)	26 (D27)

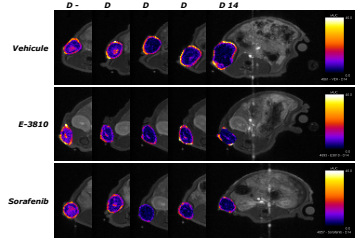
Ktrans in Calu-6 tumor rim was decreased after treatment with E-3810 and Sorafenib.



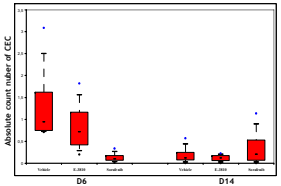
Mean iAUC in Calu-6 tumor rim was decreased after treatment with E-3810 and Sorafenib.



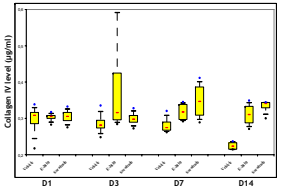
Longitudinal, whole tumor iAUC parametric mapping on one representative rat from each group showed a decrease contrast agent uptake in tumors treated with E-3810 and sorafenib.



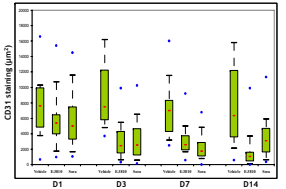
The absolute count of circulating endothelial cells in tumor bearing *Nude* rats was not changed vs controls after treatment with E-3810 and sorafenib.



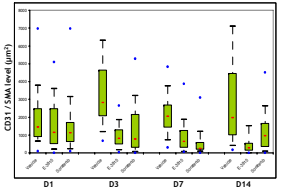
The circulating collagen IV level in tumor bearing *Nude* rats was different after treatment with E-3810 and sorafenib only at D14.

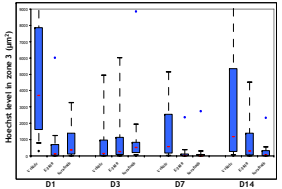


The CD31 stained area in Calu-6 tumors was lower at D1, D3, D7 and D14 for rats treated with E-3810 and sorafenib compared to vehicle-treated rats.



The CD31 / SMA co-stained area in Calu-6 tumors was lower at D3, D7 and D14 for rats treated with E-3810 and sorafenib compared to vehicle-treated rats.





The Hoechst stained area in Calu-6 tumors was lower at D1, D7 and D14 for rats treated with E-3810 and Sorafenib compared to vehicle treated rats.

Conclusions

- Repeated *PO* administrations of E-3810 or Sorafenib were well tolerated by Calu-6 tumor-bearing *Nude* rats,
- E-3810 and Sorafenib displayed a marked antitumor activity in the model of Calu-6 human lung tumor bearing *Nude* rats,
- The antitumor effect of E-3810 and Sorafenib is coupled with a strong antiangiogenic activity against tumors as indicated by a significant decrease of tumor permeability/perfusion and decrease of microvessels density,
- The change of tumor vascular status was measured using DCE-MRI as early as a few days after first treatments with E-3810 and Sorafenib at doses inducing tumor stabilisation,
- These results support the use of non-invasive DCE-MRI technology to follow the changes in tumor vasculature after E-3810 treatment as early biomarker during clinical trials

The remarkable antitumor and antiangiogenic activity of E-3810 make this compound an interesting candidate for clinical development.