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

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Introduction

E-3810 is a potent VEGF1 and 2 and FGFR1 kinase inhibitor showing anti-angiogenic and anticancer activities in several in vitro and in vivo mouse models (Proceedings AACR-EDBTC-HC Meeting, Boston, November 2009; Abstracts No. 252 and 257). The aim of this study was to investigate, in a rat model, the anticancer 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 (0.5/90)

Animals: female nude (RHM/nu) rats (Harlan Laboratories, Holland)

Drug administration: Oral route (po, gavage) via a cannula for 14 days

Tumor induction and treatment schedule protocol:

SC inoculation of 10^6 Calu-6 cancer cells to male 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/rats) Adm route Treatment schedule Imaging schedule
1 21 Vehicle PO ODx14 ODx14
2 25 E-3810 10 PO ODx14 ODx14 D-1, D3, D7 and D14
3 21 Sorafenib 10 PO ODx14 ODx14 D-1, D3, D7 and D14

DCE-MRI protocol:

- Randomisation of six rats/group for DCE-MRI at D-1 based on tumor volume (35±17 mm^3) and Ktrans data (0.6±0.1 min^-1) -4 rats for the experiment and 2 in case of death.
- MR was performed at D-1, D3, D7 and D14 with a 4.7T MR scanner (Siemens, Germany). The animals were maintained under anesthesia with a continuous 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 Gad-DTPA BMA (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 (ROIs) (i.e on tumor rim and core) and characterized by:
  - M(t): the volume transfer constant was determined by fitting the curve using a two-compartment kinetic model (Tofts et al., 1997, 1999), using an in-house developed plugin of ImageJ.
  - MROI: 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 (Circulating Endothelial Cells) by FACs
- Collection of plasma to measure the circulating level of collagen IV by ELISA assay (Incold;
- Investigation of tumor vessel permeability and functionality using Hoechst fluorescent dye

Histopathology

- Observation of tumor vascular status using CD31 / SMA immunostaining (endothelial cells and pericytes)
- Investigation of tumor vessel permeability and functionality using Hoechst fluorescent dye


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.

Conclusions

Results of this study demonstrate that E-3810 and Sorafenib can be associated to induce a marked tumor regresssion and to provide a significative antivascular activity in nude mice. These activities might translate into in vivo antitumor and antiangiogenic effects of E-3810 and provide rational clinical indications for this drug candidate.