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The VEGF antagonist PRS-050 decreases vascular permeability in tumors and inhibits tumor growth: an analysis using dynamic contrast-enhanced magnetic resonance imaging

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INTRODUCTION

Lipocalins comprise a class of functionally diverse secretory proteins of 150 to 180 residues. Despite limited sequence homology they share a common β-barrel fold. At one end the β-barrel features four loops at the entrance of the ligand binding pocket. These loops are hypervariable in terms of conformation, length and sequence which reflects the variety of ligand binding specificities of this protein family. Human tear lipocalin (Tlc, Lcn1) shows broad ligand promiscuity indicating enhanced flexibility of its binding site. Hence, Tlc provides a promising scaffold for the engineering of lipocalins with novel specificities, so-called Anticalins.

Human tear lipocalin was used as a protein scaffold to engineer an 'Anticalin' that specifically binds and antagonizes the function of Vascular Endothelial Growth Factor (VEGF-A), a pivotal inducer of angiogenesis in physiological and pathological settings. Starting from a naïve combinatorial library where residues forming the natural ligand binding site of Tlc were randomized, followed by a few cycles of affinity maturation, the Anticalin was selected to bind to all splice forms of VEGF-A with picomolar affinity. Moreover, the Anticalin was found to cross react with the mouse and rat orthologues. The Anticalin efficiently antagonizes the interaction of VEGF with its cellular tyrosine kinase receptors in biochemical and cell-based assays,. To allow persistent systemic inhibition of VEGF, the plasma half-life of the Anticalin was extended by site-directed PEGylation. The modified Anticalin efficiently blocks the growth of tumor xenografts in *nude* mice and rats. The newly developed Anticalin may provide a novel small protein antagonist opening unique therapeutic opportunities for oncology and ophthalmology indications and is scheduled to enter clinical development in oncology in 2010. The aim of this study was to investigate the antitumor activity of PRS-050 in a preclinical model and to identify an imaging biomarker of the efficacy of this antiangiogenic therapy using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI).

MATERIAL AND METHODS

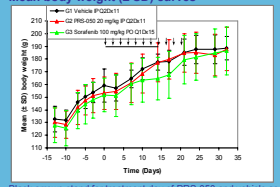
Test substance : PRS-050 prepared in PBS / **Reference substance :** Sorafenib prepared in DMSO/Tween 20/Saline
Tumor cell line : HCT-116 human colon carcinoma cell
Animals : Female *Nude* rats (Charles River, France)
Drug administration : IP route for PRS-050 and oral route (PO) for Sorafenib
Tumor induction and treatment schedule : SC inoculation of 10^7 HCT-116 cancer cells to *Nude* rats. Randomization (on tumor volume (TV) and Ktrans value) of rats at D0 in 3 groups when the mean TV reached about 400 mm^3. Treatment started at D0. One group of rats received repeated IP injections of vehicle once a day every two days (Q2Dx11) ; One group of rats received repeated IP injections of PRS-050 at 20 mg/kg/inj once a day every two days (Q2Dx11) ; One group of rats received a daily PO administration of Sorafenib at 100 mg/kg/adm for 15 consecutive days (Q1Dx15). Antitumor activity was measured as the optimal T/C% parameter. All study data, including rat weight and tumor volume measurements, clinical and mortality records, and drug treatment management were performed using Vivo Manager® software (Biosystemes, Dijon, France).
DCE-MRI protocol :
Six rats/group dedicated to MRI exams were selected on tumor volumes and Ktrans values at D0 (before start of treatment) (4 rats for the experiment and 2 in case of death).
MRI was performed at D0, D1, D3, D7 and D14.
All imaging was performed at 4.7T with an horizontal bore magnet (Pharmascan, Bruker, Germany). During the imaging protocol, the animals were maintained under anaesthesia via a constant flow of isoflurane at 2-3% delivered by a nose cone.
A T2-weighted RARE sequence (TE/TR=38/2500 ms) with a FOV=70x50 mm and a slice thickness of 1.5 mm was used for morphological description and tumor volume measurement.
DCE-MRI data was acquired during 8 minutes using a T1-weighted FLASH2D sequence (TE/TR/flip angle= 3 ms/50 ms/60°; Slice thickness=2 mm) with FOV=60x50 mm and matrix size= 108x80 at a temporal resolution of 4 s *per* image. An intravenous bolus injection of Gd-DTPA (Magnevist®, Bayer Healthcare Pharmaceuticals, Germany) at the dose of 0.1 mmol/kg was performed 30 s after acquisition start.
Tracer uptake curves derived from signal enhancement in selected regions of interest (ROI) (i.e on tumor rim and core) were fitted using a two-compartment kinetic model (Tofts et al, JMRI, 1999) for the determination of the volume transfer constant (Ktrans) using an in-house developed plugin of ImageJ®.
Ktrans distributions were computed from ROI data and normalized to voxel count to assess vascular heterogeneity and to allow for visualization of changes not shown by the mean value over a ROI.
All procedures with animals were submitted to the Animal Care and Use Committee of Pharmacy and Medicine University (Dijon, France).

CONCLUSIONS

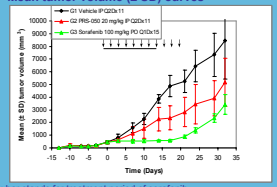
Repeated IP injections with PRS-050 at 20 mg/kg/inj following schedule Q2Dx11 were well tolerated by *Nude* rats bearing subcutaneous HCT-116 tumors while sorafenib showed evidence of toxicity at 100 mg/kg. Both treatments exhibited a marked antitumor activity.
Ktrans values measured on tumors using DCE-MRI could be a useful early biomarker of biological activity for PRS-050 treatment and a potential indicator of antitumor activity.
Ktrans values could be useful information to select responding tumors as well as to design combination treatment using PRS-050.

RESULTS

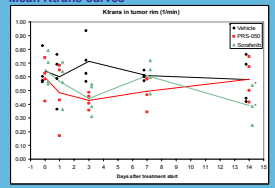
Mean body weight (± SD) curves



Mean tumor volume (± SD) curves



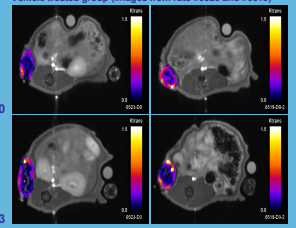
Mean Ktrans curves



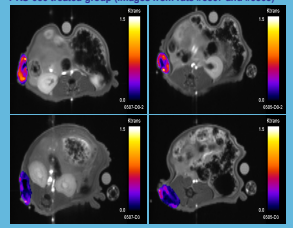
Black arrows stand for treatment day of PRS-050 and vehicle as horizontal black bar stands for treatment period of sorafenib.

Ktrans parameter maps

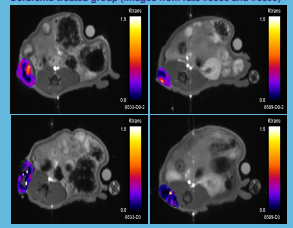
Vehicle treated group (images from rats #6523 and #6519)



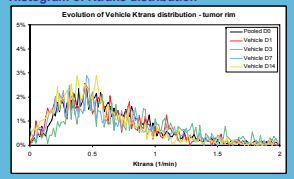
PRS-050 treated group (images from rats #6507 and #6505)

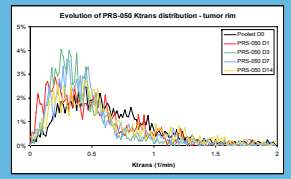


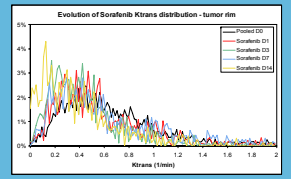
Sorafenib treated group (images from rats #6533 and #6509)



Histogram of Ktrans distribution







Summary table of antitumor activity parameters

Group	n at D0	T (Days) to V = 1.500 mm^3			T (Days) to V = 2.500 mm^3			DT (Days)			Optimal T/C (%)
		n	Mean	SD	n	Mean	SD	n	Mean	SD	
G1 vehicle IP Q2Dx11	6	6	**6.2**	2.7	6	**10.0**	0.0	6	**8.2**	1.1	-
G2 PRS-050 20 mg/kg IP Q2Dx11	6	6	**10.8**	5.2	6	**19.7**	7.9	6	**10.0**	1.7	**41** (D21)
G3 Sorafenib 100 mg/kg PO Q1Dx15	9	6	**24.0**	0.0	5	**29.0**	0.0	8	**15.0**	8.8	**13** (D17)