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The VEGF receptor inhibitor KRN951 decreases vascular permeability in tumors and inhibits tumor growth: an analysis using dynamic contrast-enhanced magnetic resonance imaging

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INTRODUCTION

- There is evidence that vascular endothelial growth factor (VEGF) has a key role in angiogenesis and may also contribute to tumor progression by increasing vascular permeability in tumors. Two high affinity receptors for VEGF are found on endothelial cells, the fms-like tyrosine kinase (TK) receptor Flt-1 and the kinase insert domain-containing receptor KDR. VEGF is a dimeric glycoprotein that binds to the endothelial receptors Flt-1 (VEGFR-1) and KDR (VEGFR-2), which possess intrinsic tyrosine kinase activity. The suppression of tumor angiogenesis has become a promising strategy in cancer treatment and a wide variety of therapies directed at interfering with this process are under development.
- KRN951 is a potent VEGFR-2 tyrosine kinase inhibitor. We have previsouly shown that repeated *PO* administrations of KRN951 at 0.2 and 1.0 mg/kg/adm were well tolerated by *Nude* rats bearing a panel of human SC tumors. Moreover, we have shown that KRN951 displayed a strong antitumor activity in a panel of 14 human tumor models xenografted in *Nude* rats. The DCE-MRI was used to identify surrogate markers of antitumor activity of new compounds in order to transfer them for clinical development.
- The aim of the study was to investigate the antiangiogenic and antitumor activity of KRN951 in a model of Calu-6 human lung tumor xenografted in *Nude* rats using DCE-MRI technology.

EXPERIMENTAL METHODOLOGY

- ✓ **Test substance : KRN951 prepared in 0.5% methylcellulose**
- ✓ **Tumor cell line : Calu-6 human lung carcinoma cell**
- ✓ **Animals : Rowett Nude rats (Hralan SD.Inc.Indianapolis)**
- ✓ **Drug administration : Oral route (PO) via a canula**

- **Tumor induction and treatment schedule**
  - SC inoculation of 10^7 Calu-6 cancer cells to Nude rats
  - Randomization of rats at D0 in 3 groups when the range of tumor volume reached 274-287 mm^3
  - Treatment start at D1 for 14 consecutive days (D1-D14)
  - One group of rats received a daily PO administration of vehicle for 14 consecutive days (D1-D14)
  - One group of rats received a daily PO administration of KRN951 at 0.2 mg/kg/adm
  - One group of rats received a daily PO administration of KRN951 at 1.0 mg/kg/adm
  - Antitumor activity measured as the Tumor Growth Inhibition (TGI%) defined as followed:

$$TGI\%\ at\ DX = \frac{(Mean\ RTV\ of\ vehicle\ group\ at\ DX - RTV\ of\ treated\ group\ at\ DX)}{(Mean\ RTV\ of\ vehicle\ group\ at\ DX - 1)} \times 100$$

The effective criteria for TGI% is >50% (RTV: Relative Tumor Volume).

- **DCE-MRI protocol**
  - Seven rats/group dedicated to DCE-MRI analysis were selected with tumor volumes between 245 and 310 mm^3 at D0 (4 rats for the experiment and 3 in case of death)
  - DCE-MRI acquisition on 4 rats/group at D0, D3, D14 and D22 (at D3 and D14, MRI analysis was performed 6 hours after the PO treatment)
  - MRI experiments were carried out on a Siemens 1.5 T Magnetom Vision. A flexible surface coil of dimensions 16x34 cm was used. During MRI, the rat was anaesthetized with a ketamine/xylazine mixture administered by intramuscular injection. The rat tail vein had been cannulated for contrast agent bolus administration before placing the animal in the magnet. Rats were positioned in the supine position within the coil. Volume measurement and anatomical description of the tumors were carried out using a multi-slice T2-weighted sequence (TR 4500 ms/TE 54 ms/NEX 2/Slice 2 mm) with an inplane resolution of 400 µm. A FLASH2D gradient recalled echo imaging sequence (TR 200 ms/TE 6 ms/NEX 1/Slice 3 mm) with an inplane resolution of 600 µm was used to evaluate the tumor blood vessel permeability.

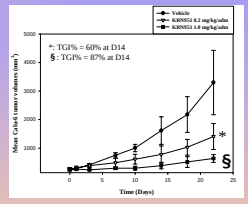
    The contrast agent Gd-DTPA (Magnevist®, Schering AG, Germany) was injected at 0.15 mmol/kg

    A bi-compartmental and bi-directional kinetic model for the measurement of vascular permeability (Ktrans) have been previously described by Tofts *et al.*(JMRI, 1997). The tracer uptake curves derived from the signal enhancement in the selected regions of interest (ROI) (eg. on the periphery and center of the tumor) were analysed using a program developed under PV-Wave (VNI, Boulder, Colorado, US).
- **Histological study of vascularity and perfusion of tumors**
  - Sacrifice of 3 rats at D0, D3, D14 and D22 for permeability and functionality of vessels studies (Hoechst/Dextran-FITC dye injections, 1min and 30sec before sacrifice respectively)
  - SC tumor collection for further histology analysis

Summary tables of TGI% values at D14 (or D13 for BxPC-3 model)

Treatments Human tumor model in Nude rats	TGI%	
	KRN951 at 0.2 mg/kg/adm (D0-D13)	KRN951 at 1.0 mg/kg/adm (D0-D13)
BxPC-3 (pancreas)	61	94
Caki-1 (renal)	27	>100
CGL-9 (glioma)	46	96
DU 145 (prostate)	90	>100
LoVo (colon)	60	>100
LS 174T (colon)	86	100
MDA-MB-231 (breast)	77	>100
NCI-H460 (lung)	69	86
NIH:OVCAR-3 (ovarian)	25	99
PC-3 (prostate)	74	96
SK-HEP-1 (liver)	60	90
SK-OV-3 (ovarian)	93	99
ZR-75-1 (breast)	77	94

Antitumor activity of KRN951 against Calu-6 human tumor xenografted in Nude rats

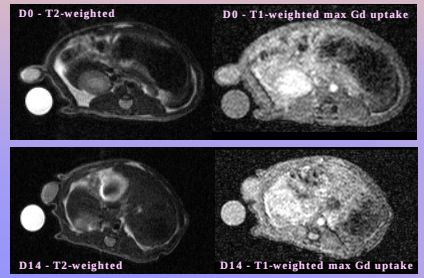


✓ **KRN951 displayed a significant antitumor activity in the Calu-6 tumor bearing Nude rat model**

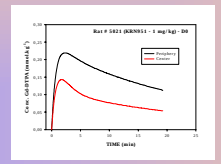
CONCLUSIONS

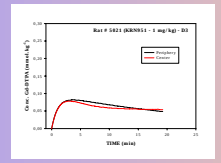
- KRN951 administered PO was well tolerated by *Nude* rats bearing human SC Calu-6 tumors and displayed a marked antitumor activity in the Calu-6 human lung tumor model,
- DCE-MRI allowed to detect early (D3) and significant antitumor activity of KRN951 and a decrease of capillary permeability and tumor washout in KRN951-treated tumors,
- These results are consistent with a rapid inhibition of the VEGF signalling pathway and were confirmed by functional histology, indicating that antitumor effects caused by KRN951 were associated with a significant decrease of the functional tumor blood vessels number in KRN951-treated tumor between D3-D22,
- These findings suggest that DCE-MRI analysis on tumor-bearing rats is well-adapted for the early characterization of tumor response to KRN951 treatment.
- K^{Trans} represents a suitable surrogate marker based on the mechanism of action of KRN951 as a VEGFR-2 inhibitor and provides useful and supporting results in favor of further clinical development of KRN951

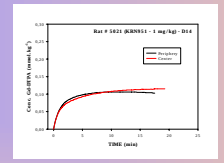
T2 and T1-weighted MR images of Calu-6 tumors before and after 14 days of KRN951 treatment

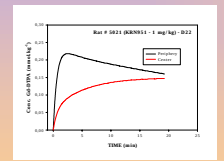


Results of MRI analysis for tumor vascular hyperpermeability quantification

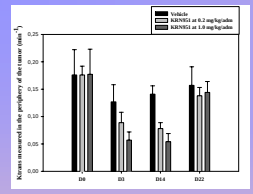




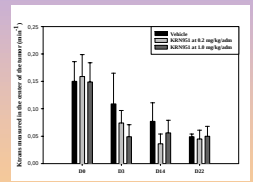




K_{trans} (tumor periphery)

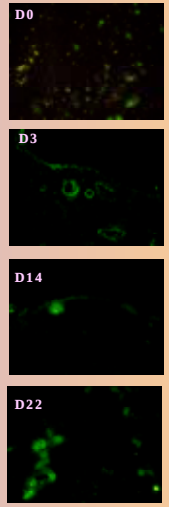


K_{trans} (tumor center)



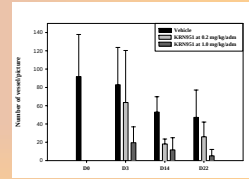
✓ **KRN951 induced a significant decrease of vascular permeability (Ktrans) in the periphery and center of the Calu-6 tumors**

Dextran-FITC tumor perfusion (KRN951-treated rat - 1 mg/kg)



Hoechst tumor perfusion (KRN951-treated rat - 1 mg/kg)

Number of vessels



✓ **KRN951 decreased significantly the number of functional vessels at D3, D14 and D22 in Calu-6 tumors**