

DOWN-REGULATION OF ANGIOGENIC GROWTH FACTOR EXPRESSION AFFECTS VASCULARIZATION WITHOUT IMPAIRING TUMOR RESPONSE TO TAXANES

M. Passiu¹, D. Coltrini², A. Garofalo¹, R. Dossi¹, M. Zucchetti³, A. Riva⁴, M. Presta², R. Giavazzi¹

¹Laboratory of Biology and Treatment of Metastasis and ³Laboratory of Cancer Pharmacology, Mario Negri Institute for Pharmacological Research, Bergamo and Milan, Italy; ²Dept. Biomed. Sci. Biotech., University of Brescia, Italy; ⁴Indena S.p.A., Milan, Italy.

E-mail: giavazzi@marionegri.it



www.marionegri.it

BACKGROUND

- ◆Vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2) are two key regulators of tumor angiogenesis.
- ◆Down regulation of FGF-2 and/or VEGF leads to an altered vasculature, and delays tumor growth (Giavazzi *et al.*, Am. J. Pathol. 2003).

AIM OF THE STUDY

The present study investigated whether down-regulation of FGF-2 and/or VEGF affected tumor response to paclitaxel and IDN 5109, an oral bioavailable taxane analogue.

METHODOLOGY

- ◆Cell cultures
Tet-FGF-2 was originated by transfection of the human endometrial adenocarcinoma (HEC-1-B) with human FGF-2 cDNA under the control of the tetracycline promoter (Tet-off system) (Giavazzi *et al.*, Cancer Research 2001). AS-VEGF/Tet-FGF-2 was derived by transfection of Tet-FGF-2 cells with VEGF₁₂₁ anti-sense cDNA (Giavazzi *et al.*, Am. J. Pathol. 2003). FGF-2 expression was down regulated by adding doxycycline (2 ng/mL) in the culture medium.

- ◆Proliferation assay
IDN 5109 (Nicoletti *et al.*, Cancer Research 1999) and paclitaxel (PTX) were dissolved in DMSO. Tumor cells were pre-cultured for 3 days in presence or absence of doxycycline before plating. After 24 h IDN 5109 or PTX were added and incubated for additional 72 h. At the end of the incubation, cells were fixed and stained with crystal violet, and proliferation was calculated from absorbance at 540 nm.

- ◆In vivo tumor growth
Nude mice (Animal production NCI, Frederick, MD) were randomized to receive tetracycline (2 mg/mL) or not in the drinking water, before the subcutaneous transplant of Tet-FGF-2 or AS-VEGF/Tet-FGF-2 cells. Drug treatment started when tumors reached approximately 150 mg. Drug response was evaluated as T/C % (median RTW of treated tumors over median RTW of control tumors)x100 and growth delay (T-C) was calculated as difference in days for treated vs control tumors to reach 800 mg.

- ◆Drug administration
IDN 5109 was prepared as a 30 mg/mL stock solution in 50% Tween 80 and 50% dehydrated alcohol and further diluted with 0.9% saline immediately before administration. IDN 5109 was given p.o. daily at 20 mg/kg and every 5 days at 80 mg/kg. PTX was prepared as a stock solution of 12.5 mg/mL in 50% Cremophor EL and 50% ethanol, diluted in 0.9% saline and administered i.v. at 20 mg/kg every 4 days. Control animals received the correspondent vehicle.

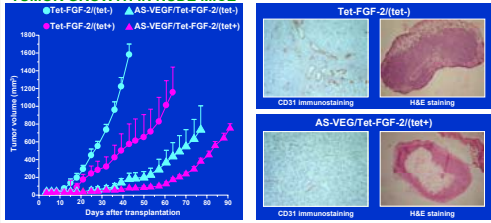
- ◆Pharmacokinetics
IDN 5109 was measured in the biological specimens (blood, normal and tumoral tissues) by high-performance liquid chromatography (HPLC) assay, as described previously (Nicoletti *et al.*, Cancer Research 1999).

THE TUMOR MODEL

Tumor line	Growth factor expression		Intratumor vessels	Tumor necrosis (%)
	Tetracycline	FGF-2 VEGF		
Tet-FGF-2 (Tet-)	-	↑ ↓	58 ± 15	10 ± 1.0
Tet-FGF-2 (Tet+)	+	↓ ↑	30 ± 8.5	11 ± 1.0
AS-VEGF/Tet-FGF-2 (Tet-)	-	↑ ↓	26 ± 7.5	43 ± 6.8
AS-VEGF/Tet-FGF-2 (Tet+)	+	↓ ↑	21 ± 10	56 ± 5.6

Tet-FGF-2 = FGF2 under the control of tetracycline promoter (Tet-off)
AS-VEGF/Tet-FGF-2 = transfected with VEGF₁₂₁ antisense

TUMOR GROWTH IN NUDE MICE



IN VITRO, EFFECT OF IDN 5109 AND PTX ON TUMOR CELL PROLIFERATION

Tumor line	Doubling time (h)	IC ₅₀ (nM) ¹	
		IDN 5109	PTX
Tet-FGF-2 (Tet-)	84 ± 1.5	0.8 ± 0.1	7.0 ± 1.6
Tet-FGF-2 (Tet+)	80 ± 2.0	0.9 ± 0.1	7.0 ± 1.7
AS-VEGF/Tet-FGF-2 (Tet-)	91 ± 1.8	1.0 ± 0.1	6.0 ± 1.5
AS-VEGF/Tet-FGF-2 (Tet+)	89 ± 2.4	1.0 ± 0.7	6.0 ± 0.1

- ¹IC₅₀ values are the mean of 4 replicates (±SD) for growth inhibition in a 72 h proliferation assay.
- ◆Tumor cell growth was comparable, independently of FGF-2 and/or VEGF expression.
- ◆In vitro the modulation of FGF-2 and/or VEGF did not influence the response of tumor cells to chemotherapeutic agent.

IN VIVO, TUMOR RESPONSE TO IDN 5109 AND PTX

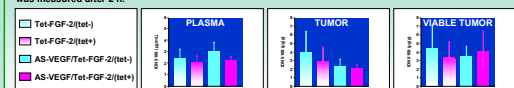
Tumor line	T/C % (day)	
	IDN 5109	PTX
Tet-FGF-2 (Tet-)	20 (+48)	23 (+41)
Tet-FGF-2 (Tet+)	28 (+66)	26 (+50)
AS-VEGF/Tet-FGF-2 (Tet-)	23 (+69)	33 (+64)

IDN 5109 80 mg/kg p.o.; PTX 20 mg/kg i.v.

- ◆IDN 5109 and PTX inhibited the growth of Tet-FGF-2 and AS-VEGF/Tet-FGF-2 xenograft at similar extent when compared to the corresponding vehicle.

UP-TAKE OF IDN 5109 IN THE Tet-FGF-2 TUMOR VARIANTS

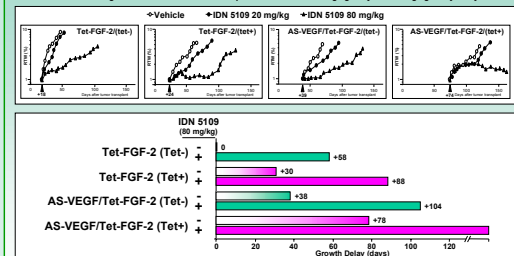
Mice bearing Tet-FGF-2 and AS-VEGF/Tet-FGF-2 were given IDN 5109 (80 mg/kg, p.o.) and the drug up-take was measured after 2 h.



- ◆Plasma pharmacokinetic profiles were similar in the four groups of mice.
- ◆The concentration of IDN 5109 in the tumors with down regulation of VEGF (AS-VEGF) was lower than the other groups; however drug level corrected for the area of viable tumor tissue was similar.

DOWN REGULATION OF FGF-2 AND/OR VEGF AND TREATMENT WITH IDN 5109

Nude mice were randomized to receive tetracycline (Tet+) or not (Tet-) in the drinking water. Treatment started when tumors reached 150 mg. IDN 5109 was administered p.o. at the dose of 20 mg/kg daily and 80 mg/kg every 5 days.



- ◆The addition of chemotherapy increased the growth delay in all the groups.
- ◆Down-regulation of FGF-2 and/or VEGF delayed tumor growth, but it did not influence the relative effect of chemotherapy in tumor xenografts.
- ◆The best therapeutic response was obtained in tumors with both the growth factors down regulated simultaneously and treatment with IDN 5109.

CONCLUSIONS

- ◆The modulation of VEGF and/or FGF-2 affected tumor growth and vascularization, *in vivo*.
- ◆The modulation of FGF-2 and/or VEGF did not affect tumor cell proliferation and response to chemotherapy, *in vitro*.
- ◆Tumor xenografts in which FGF-2 and/or VEGF were modulated responded to PTX and IDN 5109.
- ◆The best therapeutic activity on tumor xenografts was observed when both the growth factors were down-regulated, thus suggesting the "antiangiogenic" effect of the growth factor modulation potentiates the activity of chemotherapy.

AACR 97th Annual Meeting 2006 - April 1-5, 2006, Washington, DC



www.indena.com