

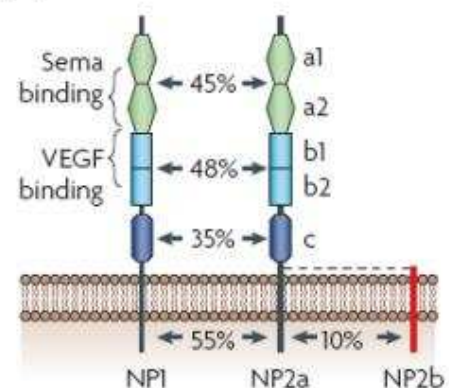
Oncogenic properties of Neuropilin-2

Camille Grandclement, Romain Bedel, Bernadette Kantelip, John Wijdenes, Maud Gounand, Adeline Bouard, Jérémy Balland, Virginie Mougey, Jean-Paul Rémy-Martin, Michael Klagsbrun, Christophe Ferrand, Xavier Pivot, Pierre Tiberghien, Christophe Borg.

- **Neuropilins (Nrp1 and Nrp2)** : single-pass transmembrane non tyrosine-kinase glycoproteins
- **Ligands** : VEGF, semaphorins, growth factors
- **Ellis, J. Natl Cancer Inst, 2008** : inhibition of NRP-2 expression in colorectal carcinoma inhibited cancer development (VEGF/PI3K/AKT)



NRP-2 : target for anti-tumoral therapy



Neuropilins in tumor biology

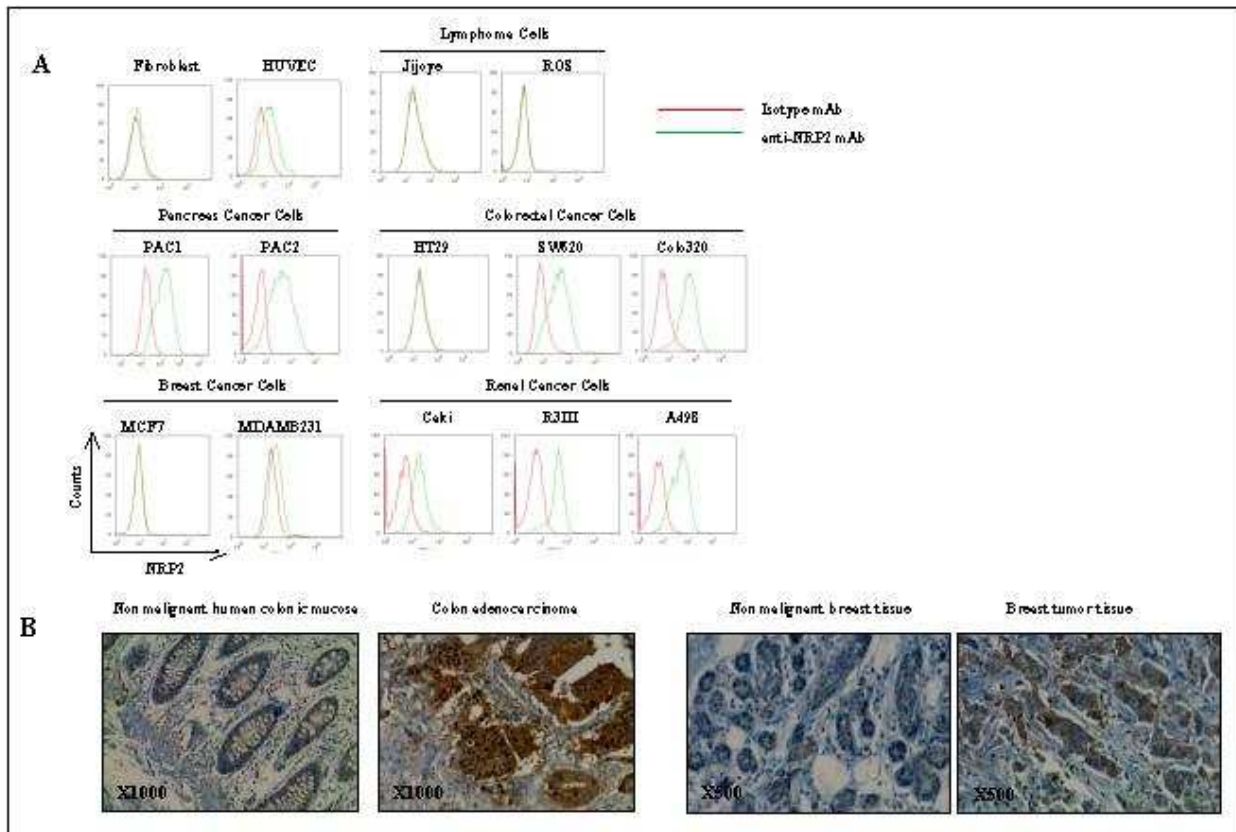


Fig. 1 Expression of NRP2 in human cancer cells

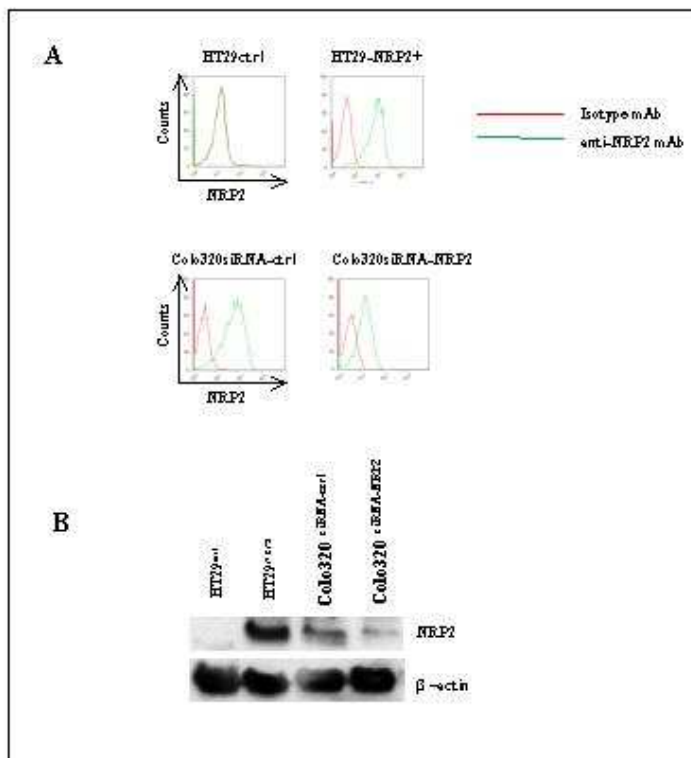


Fig. 2 Generation of colon cancer cell lines expressing or not NRP2

Fig. 3
NRP2
promotes
tumor
proliferation

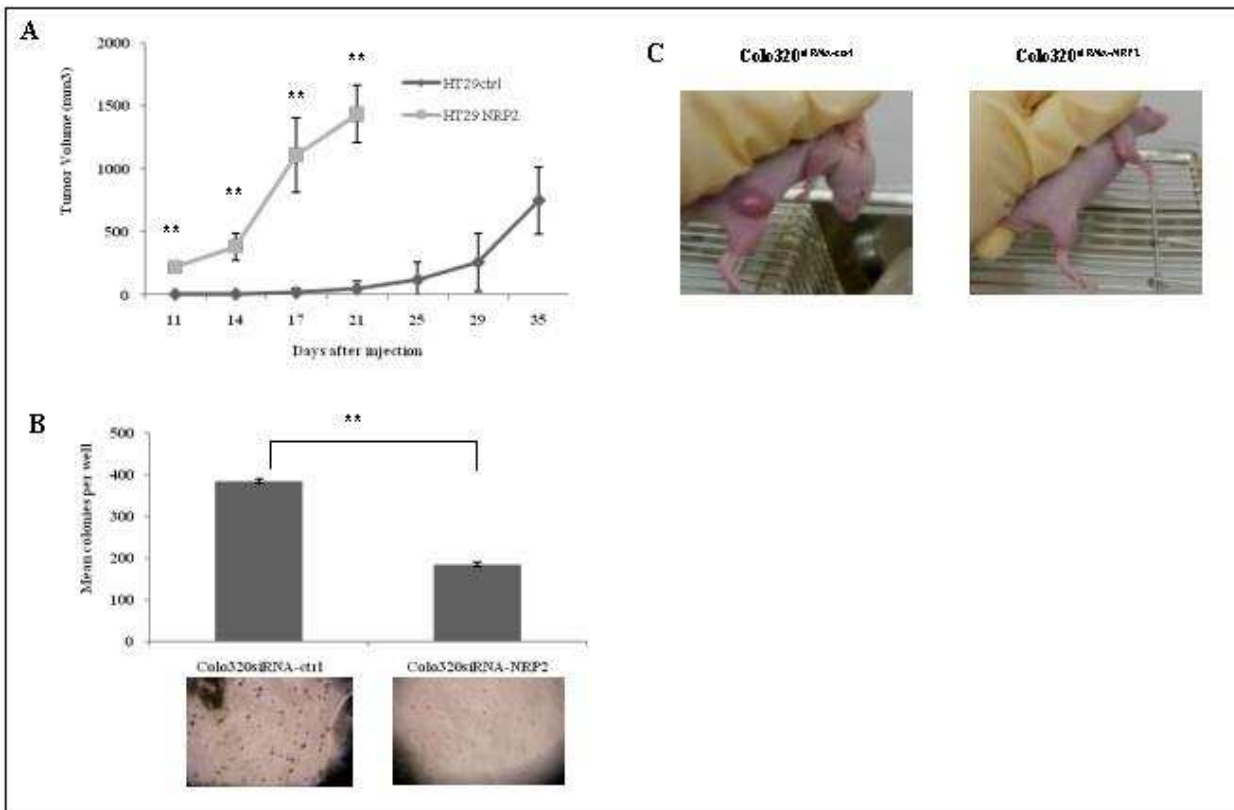
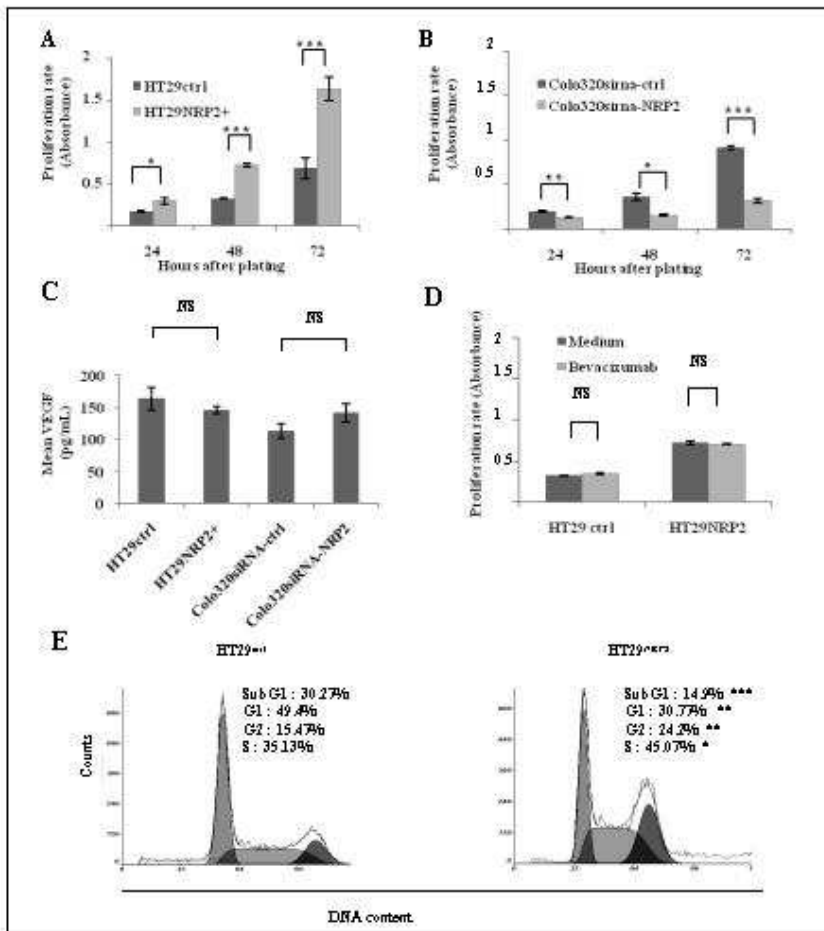


Fig. 4 NRP2 ablation using siRNA blocks xenograft progression.

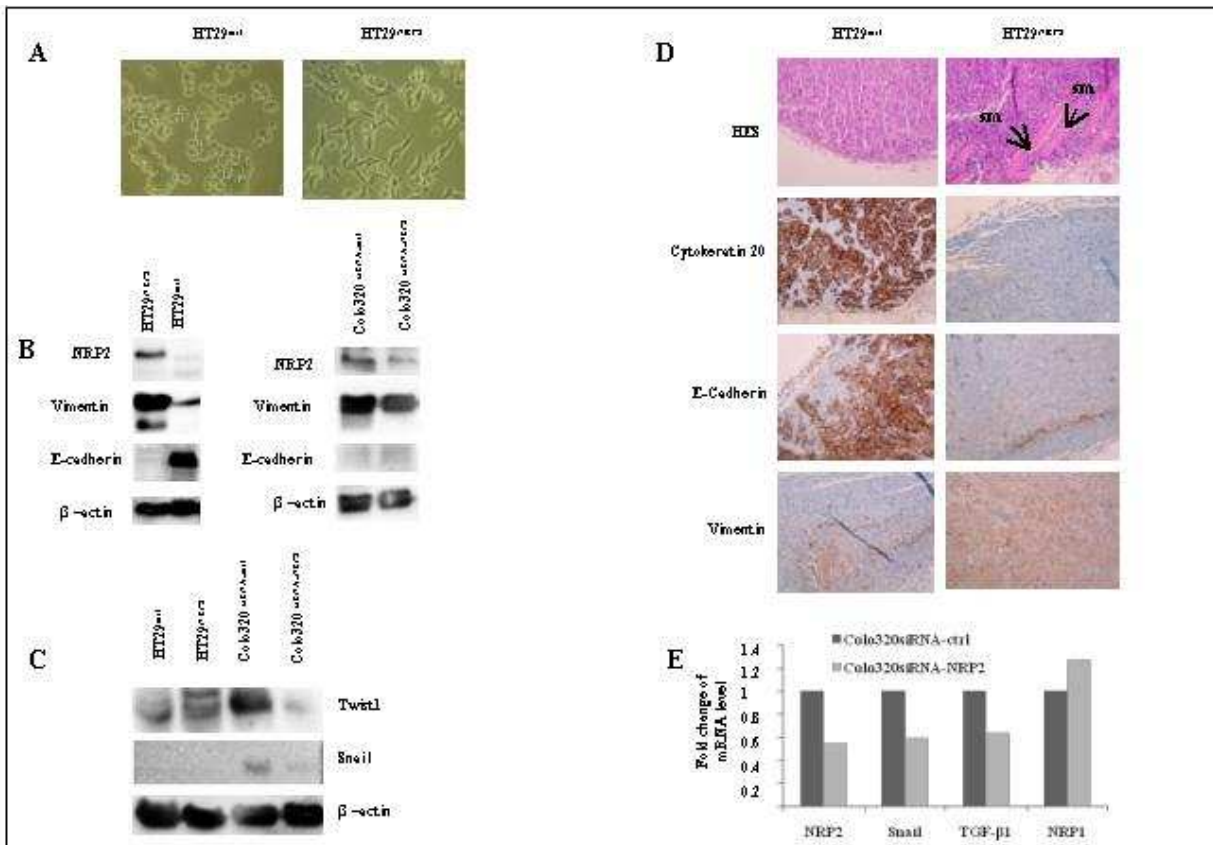
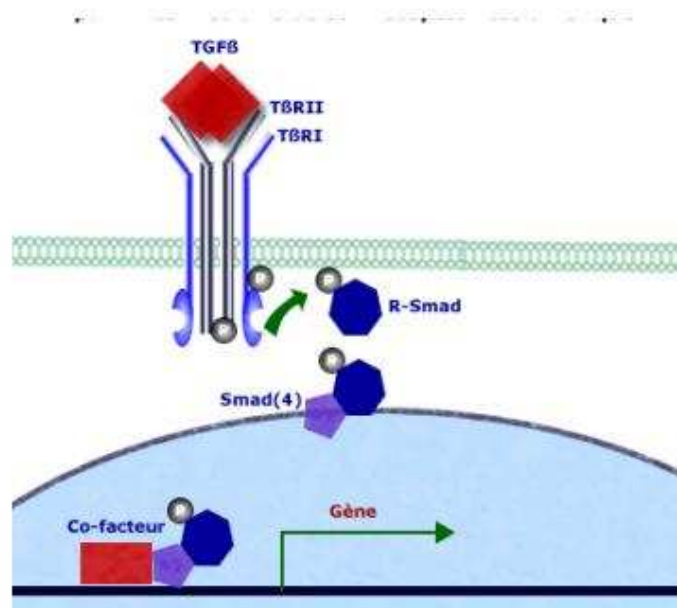


Fig 5. NRP2 induces epithelial to mesenchymal transition.

- Prud'homme G. J, Glinka Y, J. Leuk. bio, 2008 : *NRP1 is a receptor for transforming growth factor B-1, activates its latent form and promotes T regulatory T cell activity*

- TGF- β 1 signaling pathway**



NRP-2 promotes TGF- β 1 pathway (1)

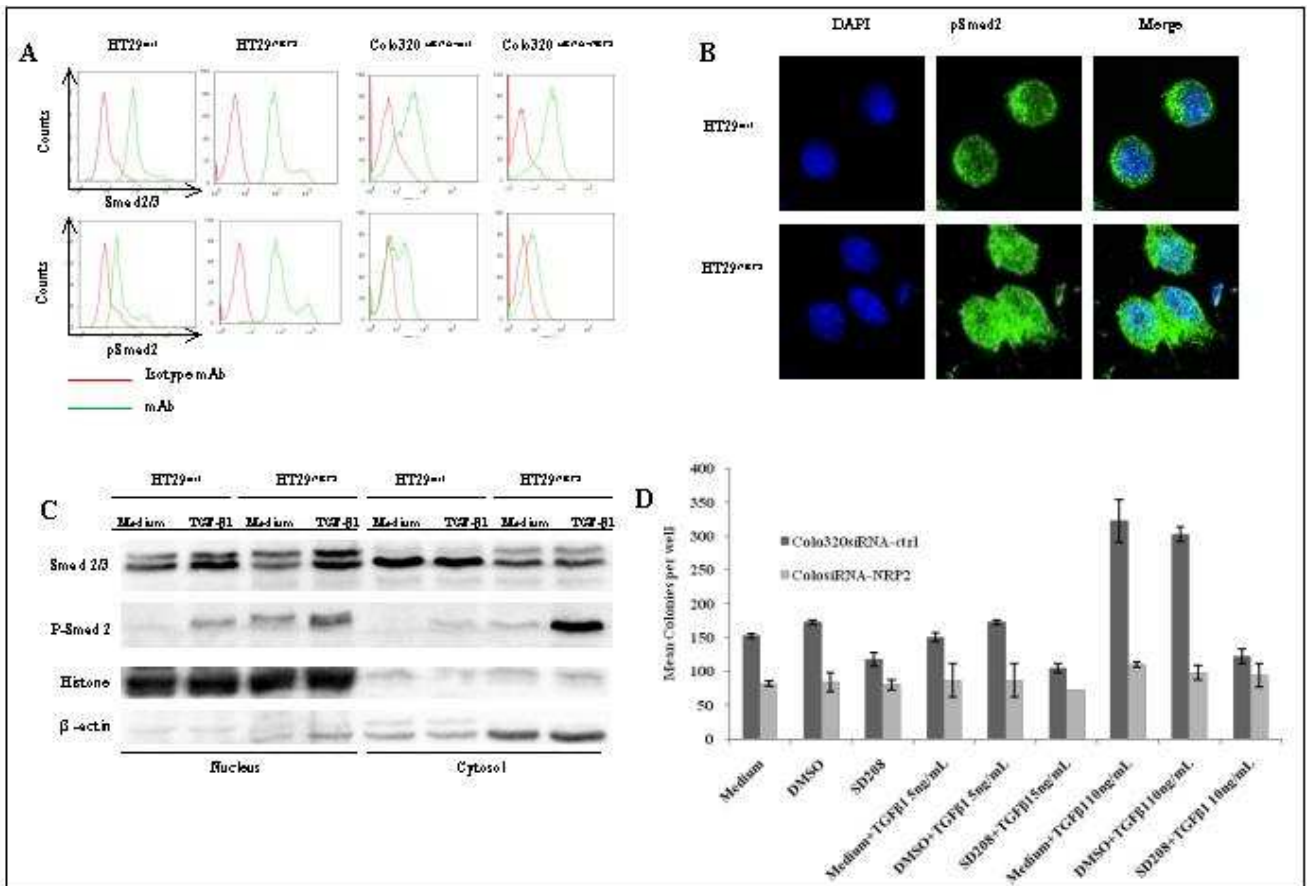


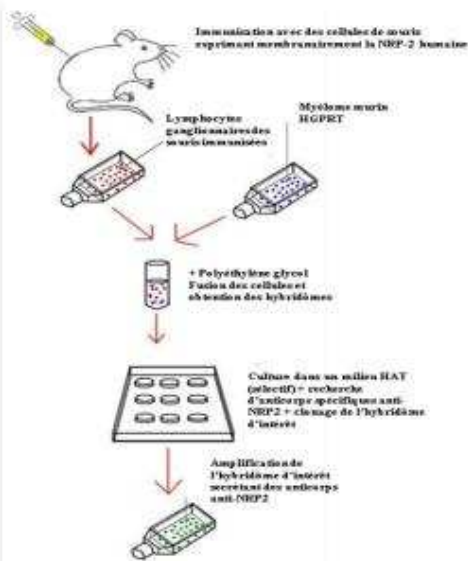
Fig. 6 NRP2 promotes TGF-β1 pathway.

**« Innovative immune Targeting of Cancer » platform
Besançon, France**



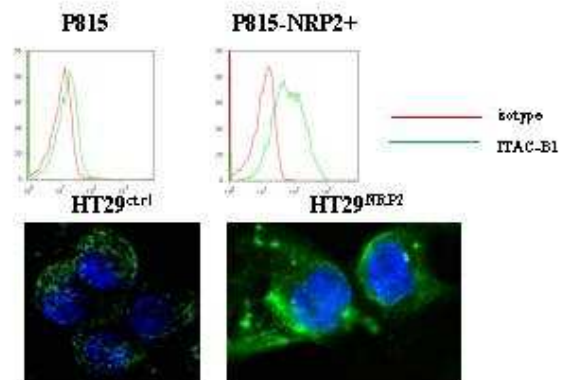
Production of mouse monoclonal antibody targeting hNRP-2 : ITAC-B1

- Immunization of Balb/c mice



• (Köhler and Milstein protocol)

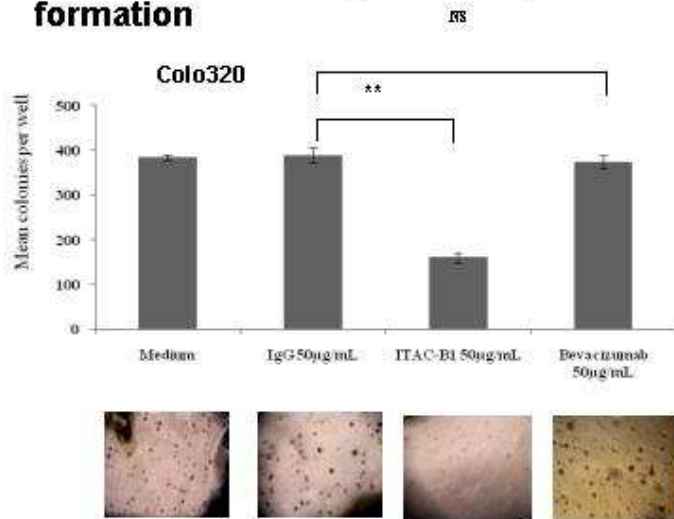
- Screening and identification of a secretant hybridoma : ITAC-B1



ITAC-B1 : specific anti-NRP2

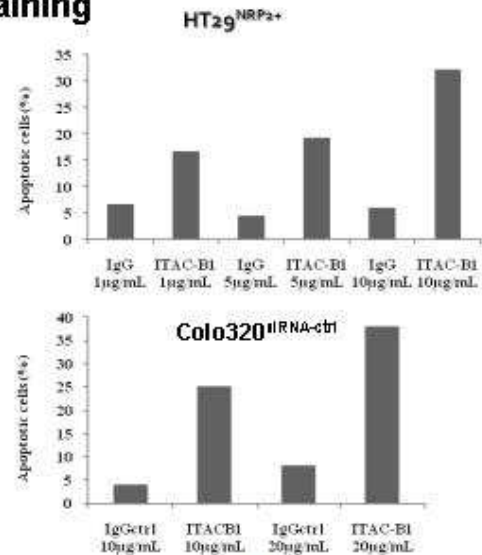
Targeting hNRP-2 : Biological effects

- Co-culture in soft agar : colony formation



ITAC-B1 inhibits colony formation

- Cell apoptosis test : AnnexinV staining

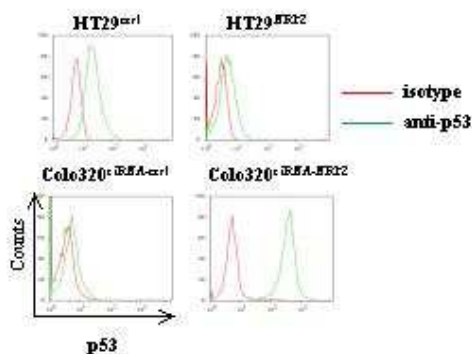


ITAC-B1 induces cell apoptosis in a dose dependant manner

Neutralizing NRP-2 to induce p53 expression

- Cao et al., Cancer Research, 2008 : *Neuropilin-1 Upholds Dedifferentiation and Propagation Phenotypes of Renal Cell Carcinoma Cells by Activating Akt and Sonic Hedgehog Axes*

- Intracellular staining

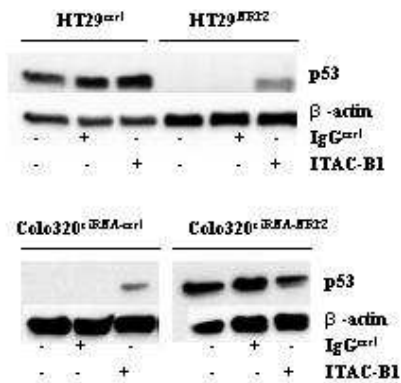


- Xenografts : IHC

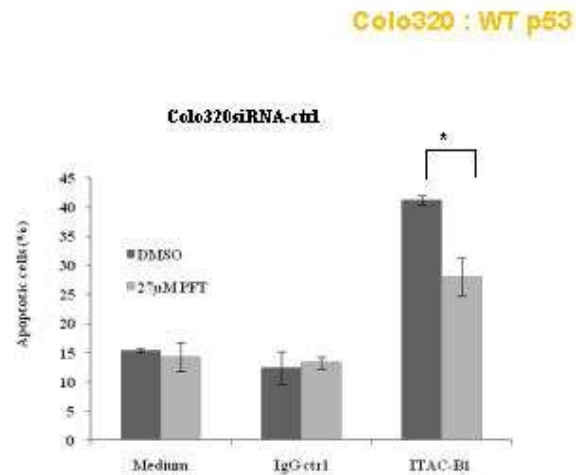


Neutralizing NRP-2 to induce p53 expression

- Western-blot :
ITAC-B1, 36h : induction of p53



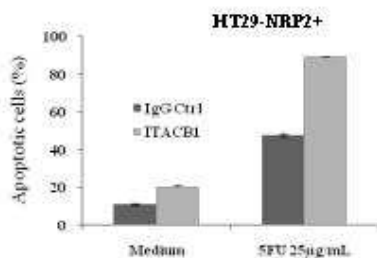
- Pretreatment with Pifithrin-α attenuates ITAC-B1 induced apoptosis



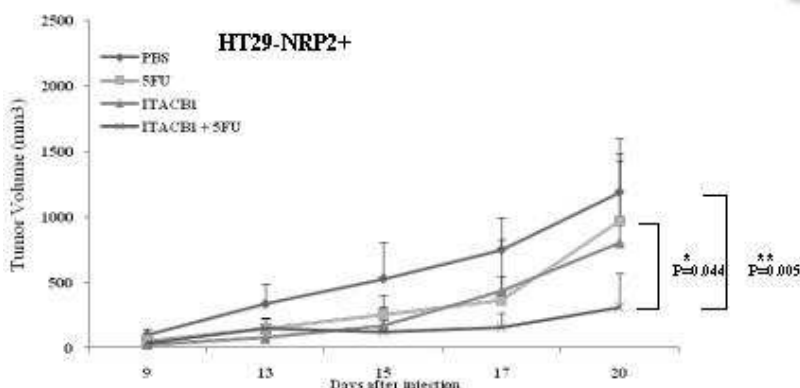
ITAC-B1 induces in part p53-dependant apoptosis

Preclinical model : Neutralizing NRP-2 to increase the cytotoxicity of classic chemotherapy

- Combination of ITAC-B1 and 5FU



Group	Dose and route	Schedule
Control	Vehicle ip	
5FU	20mg/kg ip	Daily x5
	100mg/kg ip	Days 15, 19
ITAC-B1	30mg/kg ip	Day 0
	20mg/kg ip	Every two days
5FU+	20mg/kg ip	Daily x5
	100mg/kg ip	Days 15, 19
ITAC-B1	30mg/kg ip	Day 0
	20mg/kg ip	Every two days



ITAC-B1 combined to 5FU treatment significantly reduces tumor progression

Acknowledgements



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