



# **VEGF-A-induced regulatory T cell proliferation, a novel mechanism of tumor immune escape in colorectal cancer: effects of anti-VEGF/VEGFR therapies.**

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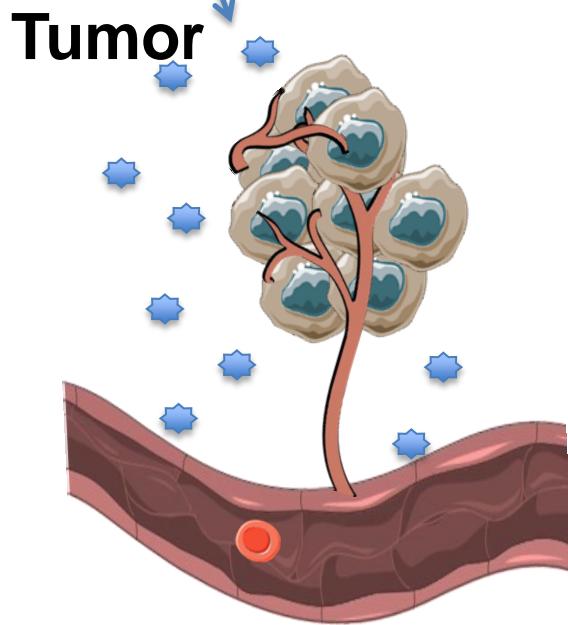
**Paris, France**

# Disclosures

Research grants from Roche and Pfizer

Anti-  
angiogenic  
molecules

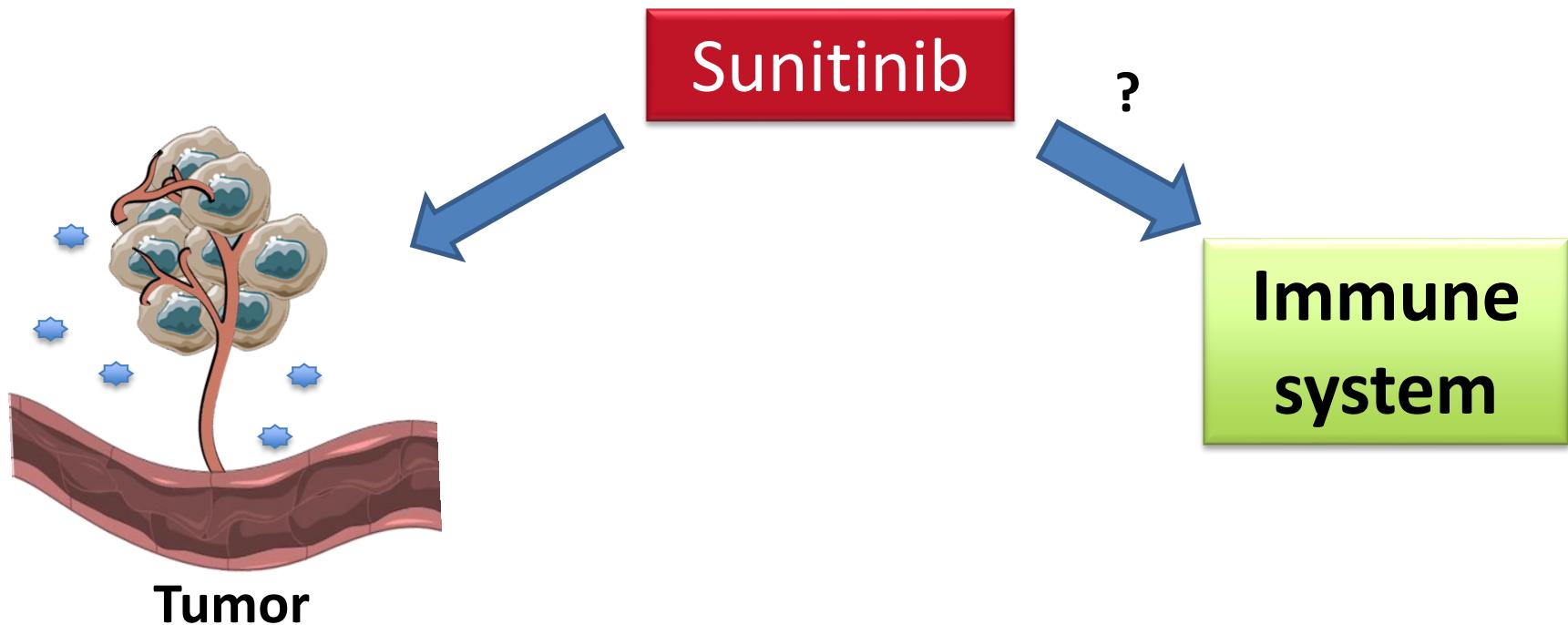
Proangiogenic  
factor (VEGF,  
PDGF ...)



Understanding  
mechanisms of action to  
define biomarkers of  
efficacy

# Anti-angiogenic molecules and immunity

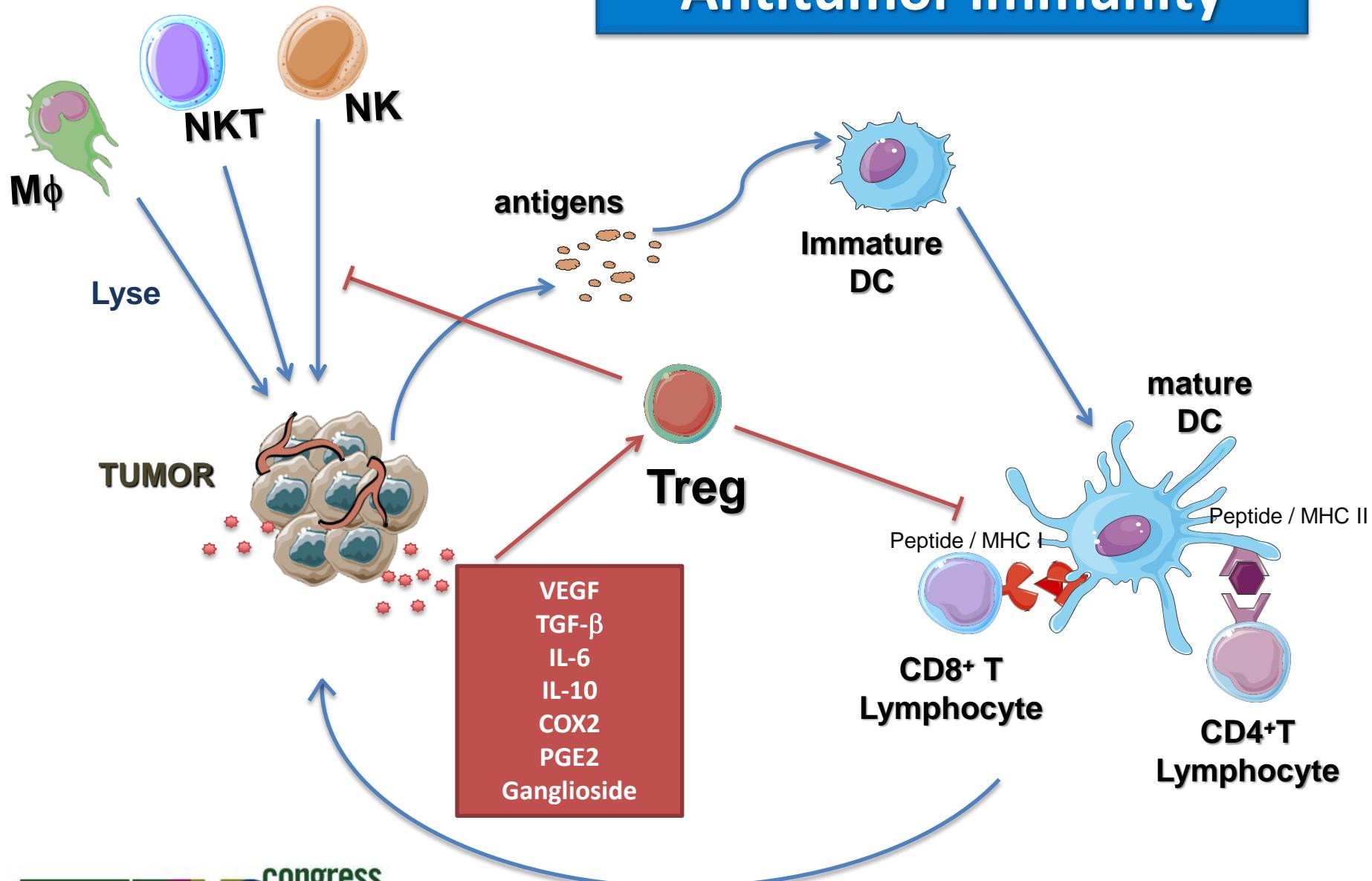
**Sunitinib = tyrosine kinase inhibitor specifically targeting VEGF-R, PDGF-R, c-kit, Flt3**



# Importance of tumor immunosurveillance

- Immunodeficient patients or patients treated by immunosuppressive molecules: enhancement of cancer incidence
  - => *Renal transplant patients: relative risk of colon cancer x 3,2 in men and x 3,9 in women (Birkeland et al., 1995 ; Sheil et al, 2001)*
- Good prognosis of T cell infiltration in cancer (colorectal cancer, ovarian cancer)
  - Galon et al., Science 2006 ; Zhang et al, NEJM 2003*

# Antitumor immunity



# Regulatory T cells (Treg)

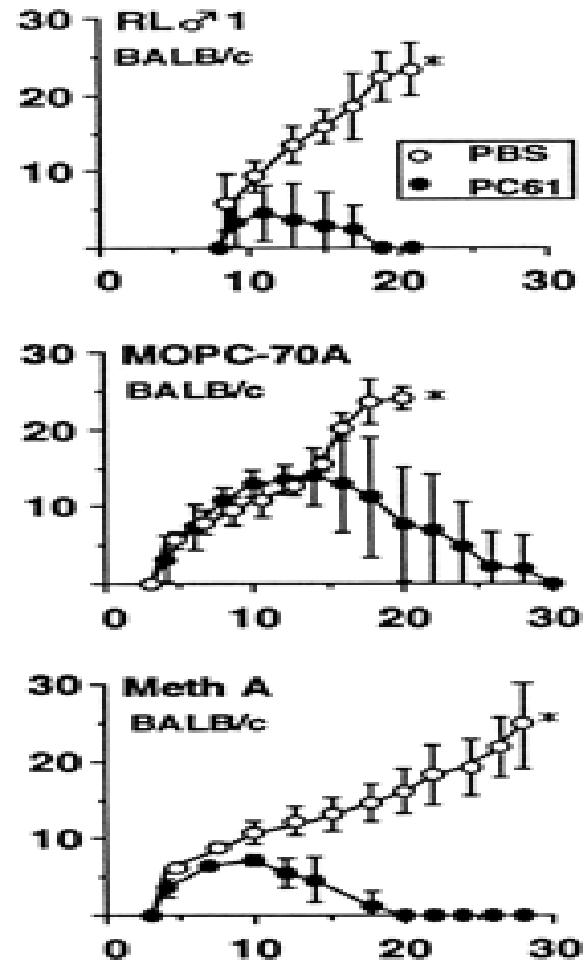
Phénotype : CD4<sup>+</sup> CD25<sup>+</sup> Foxp3<sup>+</sup>



Inhibition of anti-tumor immune response



Depletion of Treg *in vivo* induces tumor regression in different mouse tumor models



Onizuka et al. Cancer Res 1999

# Anti-angiogenic molecules and regulatory T cells

## *Sunitinib*

- inhibits the accumulation of Treg in a mouse model of renal cancer

(Xin et al., Cancer Res 2009, Ozoa-Choy et al., Cancer Res 2009).

- inhibits the accumulation of Treg in the blood of metastatic renal cancer patients

(Finke et al., Clin Cancer Res 2008 ;Ko et al., Clin Cancer Res 2009).

- Treg decrease could be a predictive marker of sunitinib response in these patients

(Adotevi et al., J Immunother 2010)

# ***Sunitinib : multi target tyrosine kinase inhibitor (VEGF-R, c-kit, PDGF-R, Flt-3)***



***Role of VEGF-A/VEGFR blockade in Treg modulation by sunitinib?***

***Direct impact of VEGF-A on Treg?***



**Mouse model of colorectal cancer (CT26)**

# Study design



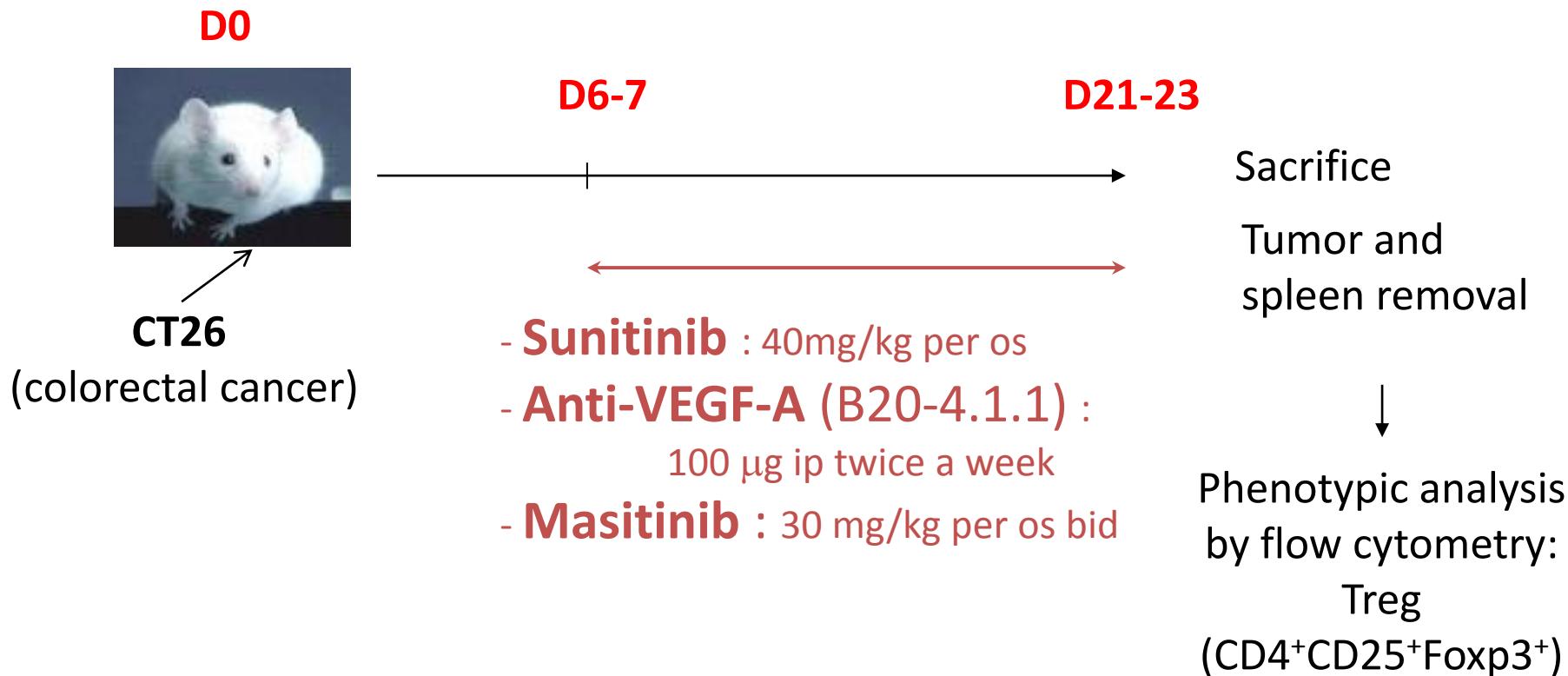
Comparing  
sunitinib  
with

anti-mouse VEGF-A antibody

(Genentech)

TKI that does not target VEGFR :  
masitinib (ABScience)  
(c-kit, PDGF-R, FAK-kinases inhibitor)

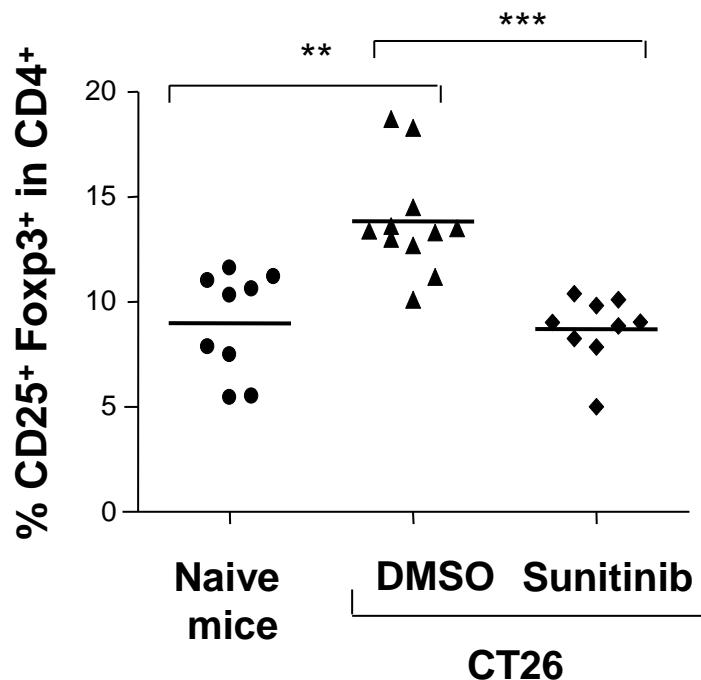
# *Experimental settings*



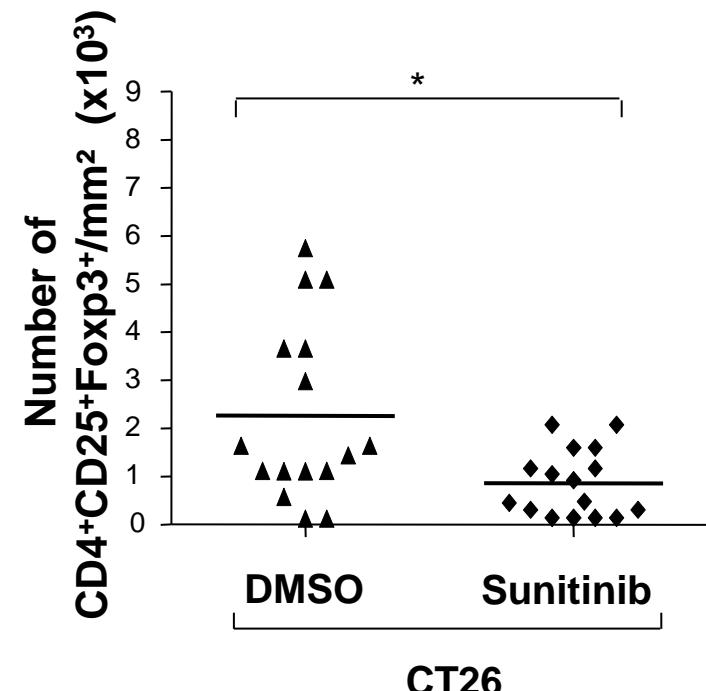
# Sunitinib decreases Treg in spleens and tumors in a mouse model of colorectal cancer



## Spleens



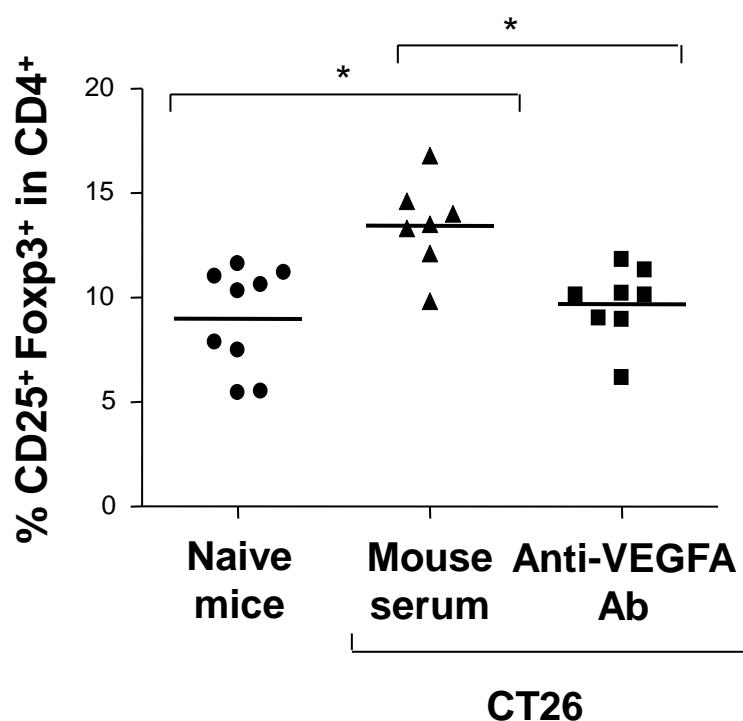
## Tumors



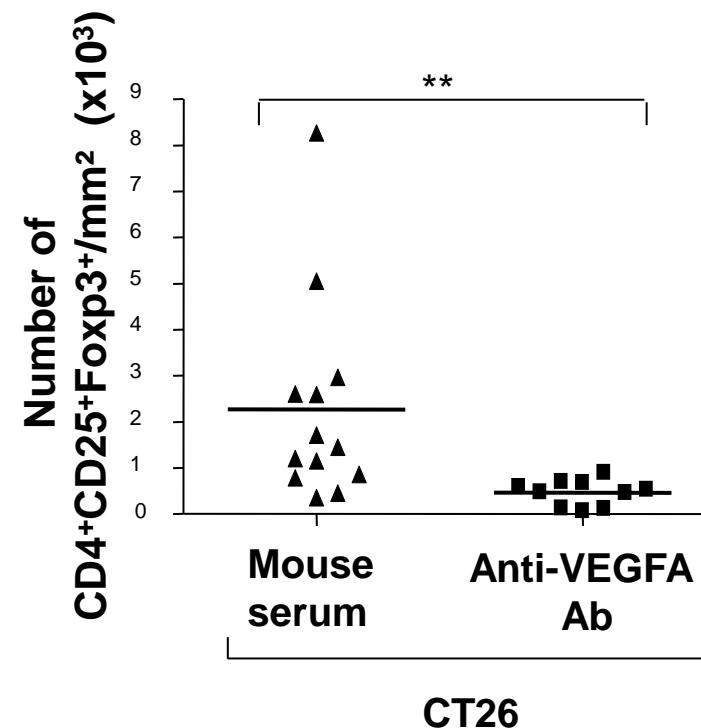
# Anti-VEGF-A antibody decreases Treg in spleens and tumors in a mouse model of colorectal cancer



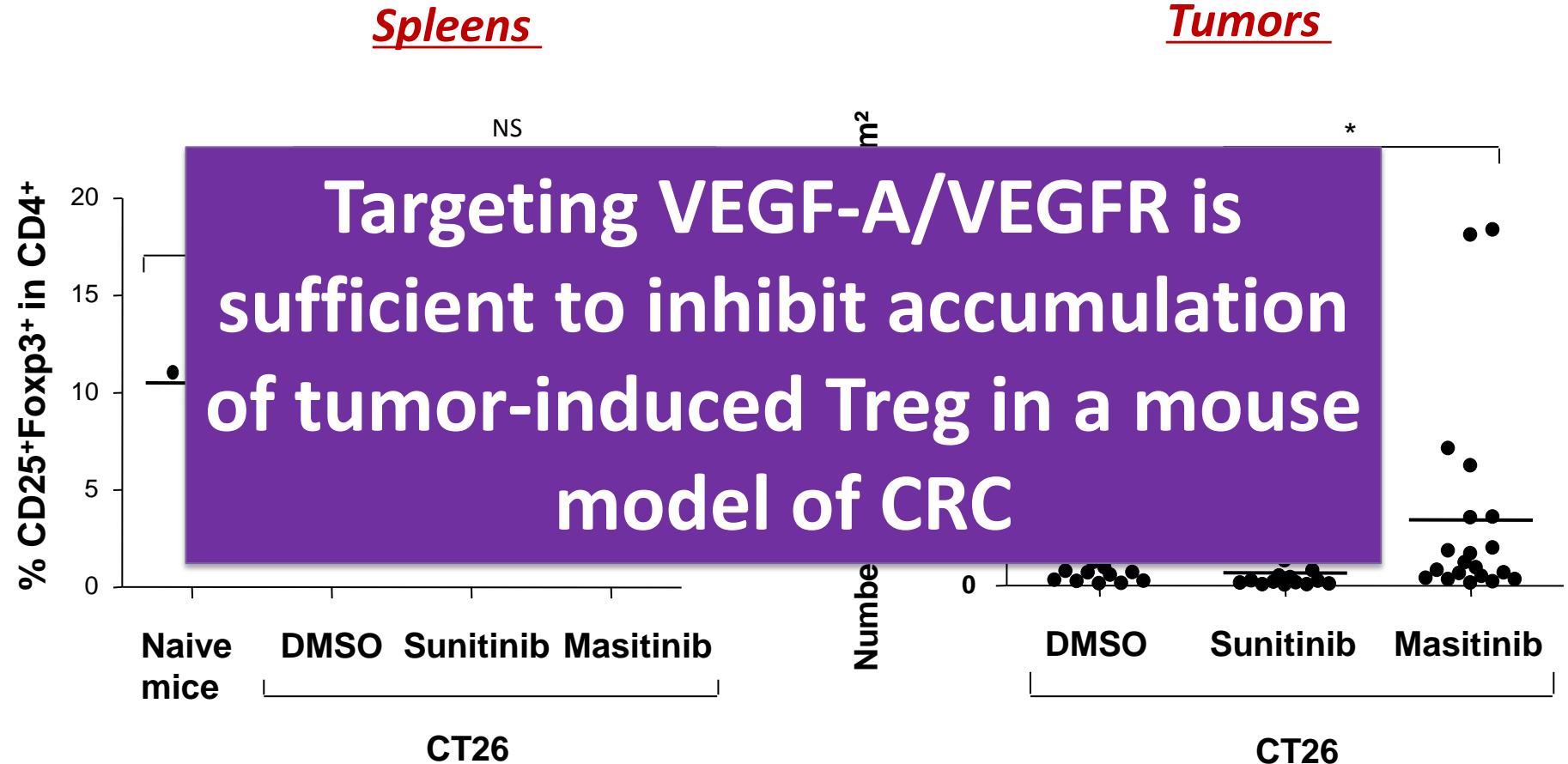
*Spleens*



*Tumors*



# Masitinib, a TKI that does not target VEGFR, does not prevent Treg accumulation in spleens and tumors in a mouse model of CRC



# *Treg modulation in metastatic colorectal cancer patients treated with anti-VEGF-A Ab (bevacizumab)?*



*Inclusion criteria:*

- metastatic colorectal cancer patients
- 1st line treatment
- No Cetuximab (anti-EGFR)

*2 groups:*

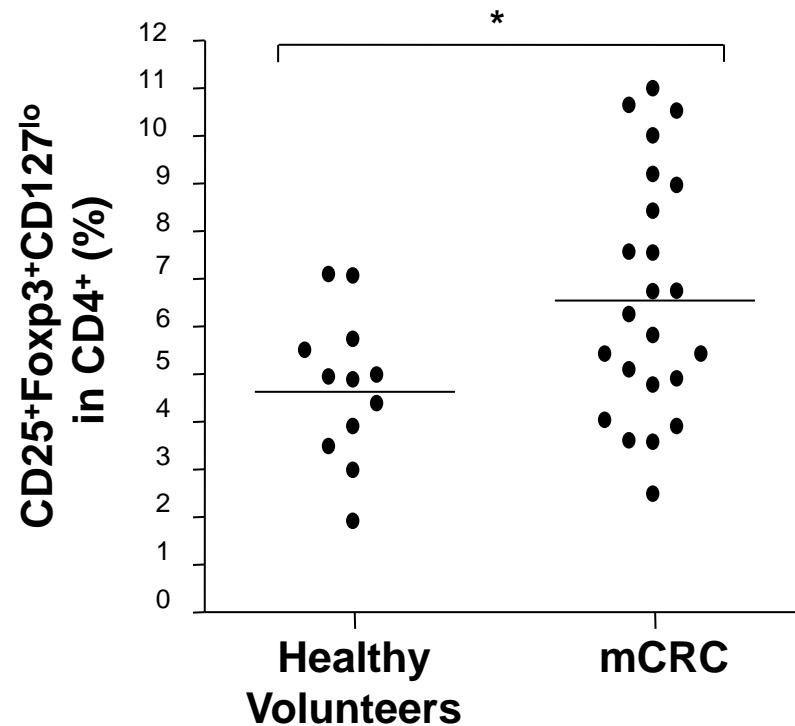
- chemotherapy alone
- chemotherapy + bevacizumab

*Blood sampling :*

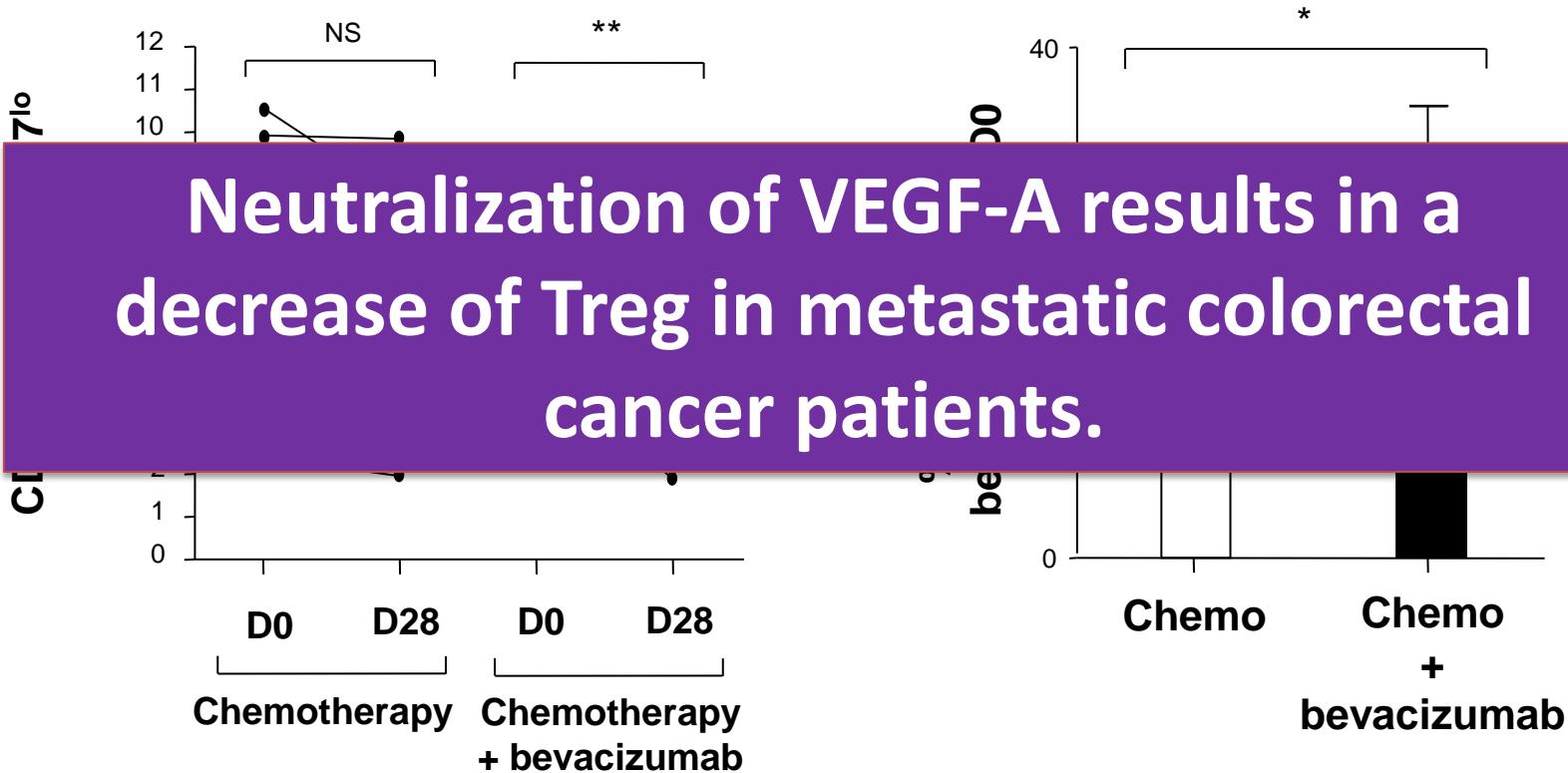
- before the first cycle of treatment (D0)
- after the second cycle of treatment (D28)

*Staining : CD4/ CD25/Foxp3/CD127*

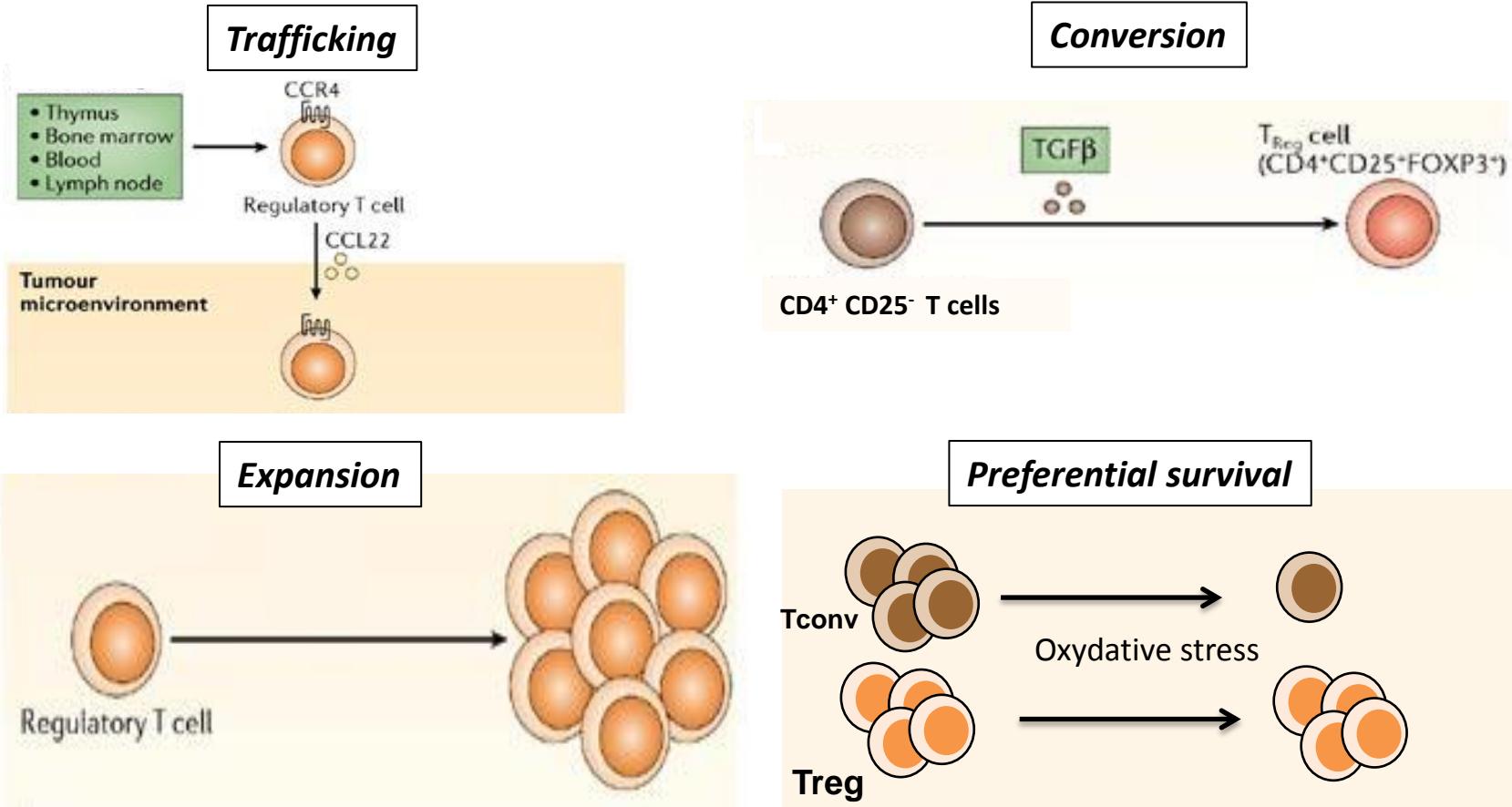
# Treg proportion is enhanced in the blood of metastatic colorectal cancer patients (mCRC) compared to healthy volunteers



# Bevacizumab associated with chemotherapy decreases Treg proportion in metastatic CRC patients unlike chemotherapy alone.



Neutralization of VEGF-A results in a decrease of Treg in metastatic colorectal cancer patients.



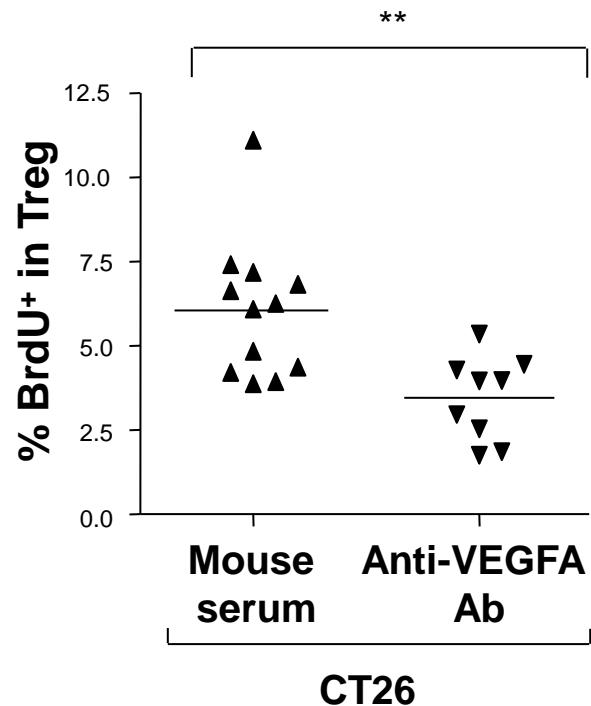
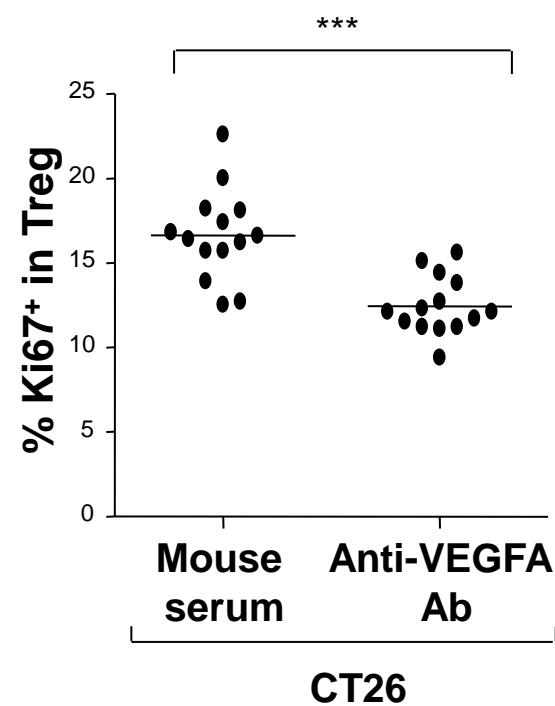
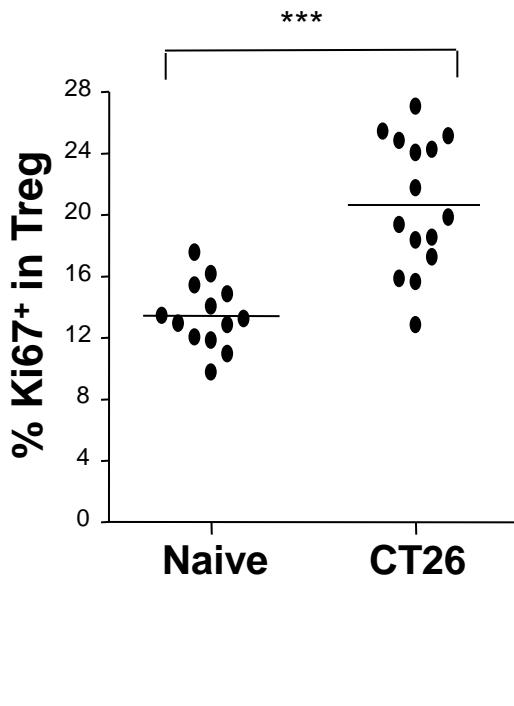
Adapted from Zou W et al., Nature Review Immunol, 2006

VEGF-A => Proliferation factor for endothelial and tumor cells  
 (Liang et al 2005 ; Shaked et al., 2005)

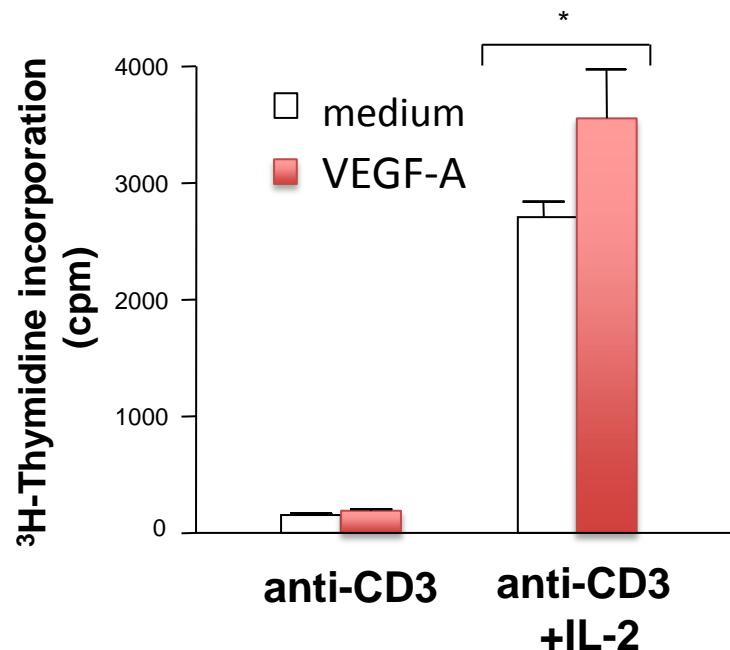
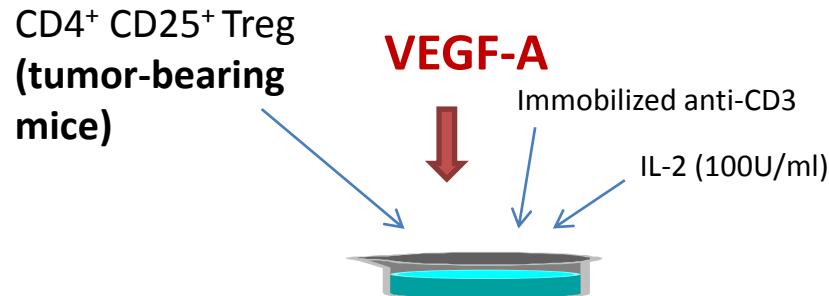
→ Does VEGF-A induce Treg proliferation?



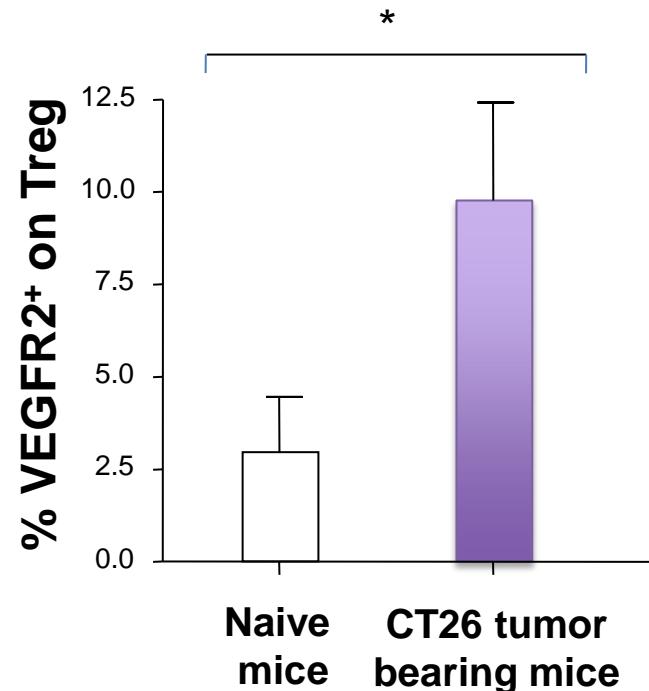
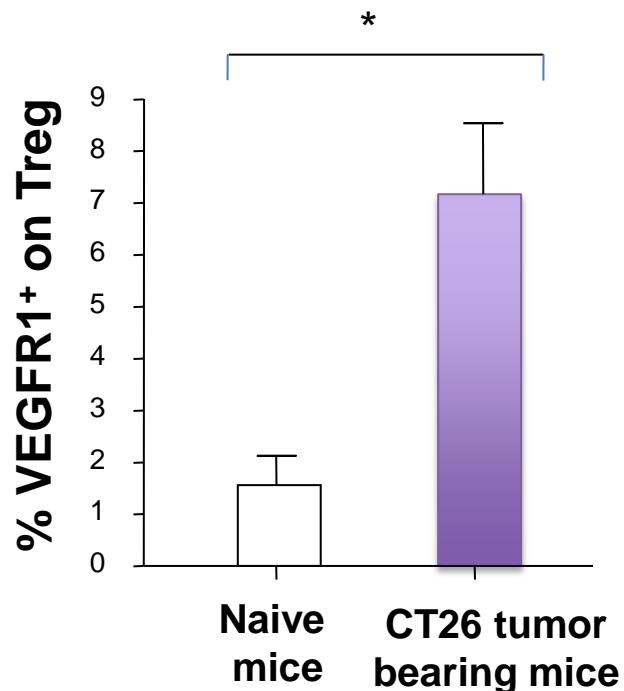
# VEGF-A induces Treg proliferation in tumor-bearing mice



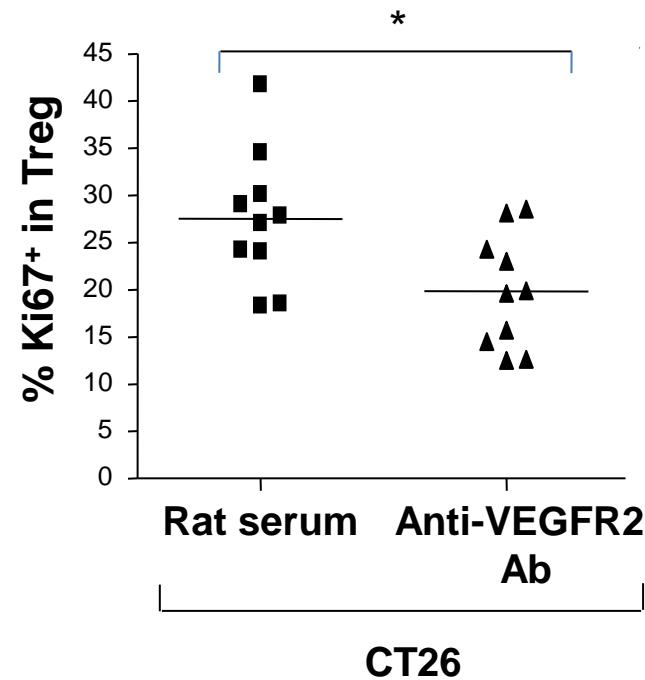
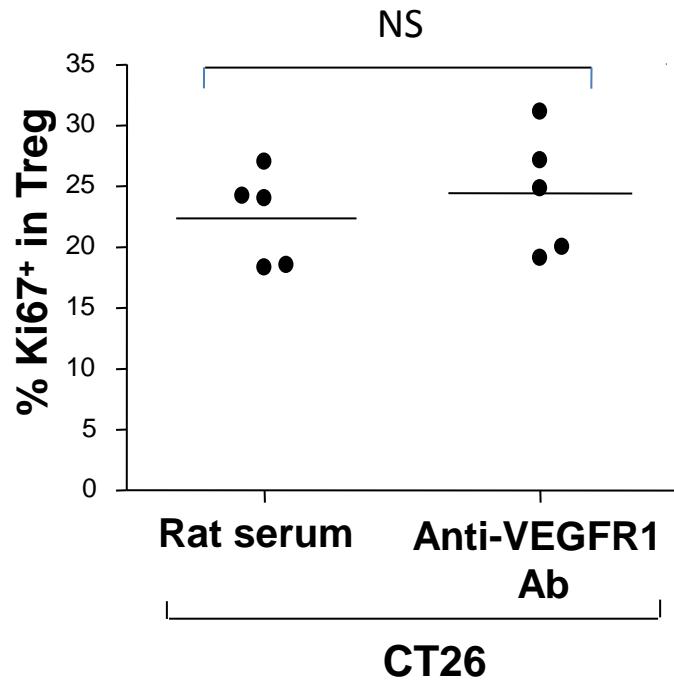
# VEGF-A directly induces Treg proliferation *in vitro*



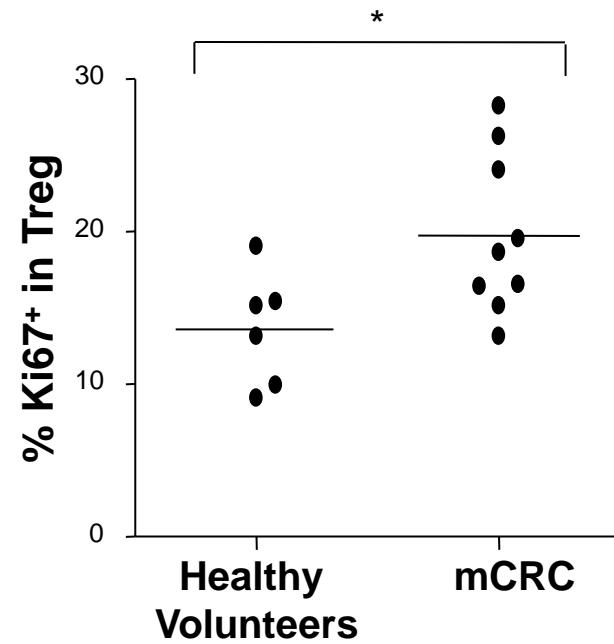
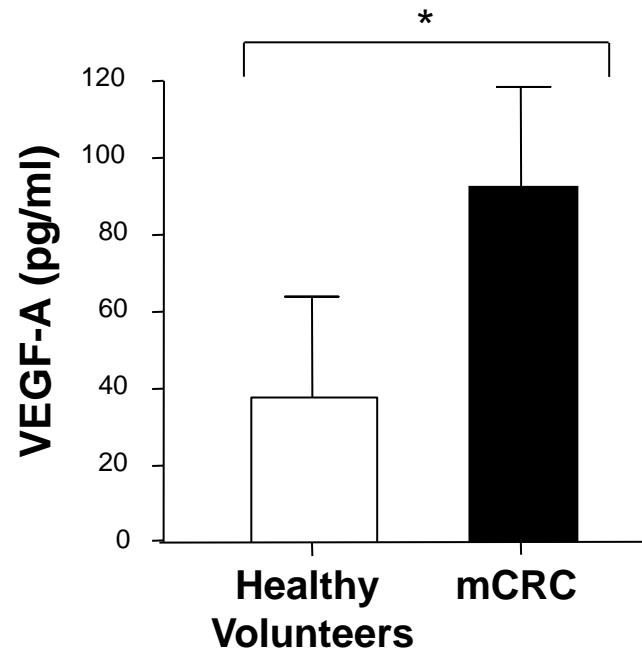
# Treg express VEGF receptors in tumor-bearing mice



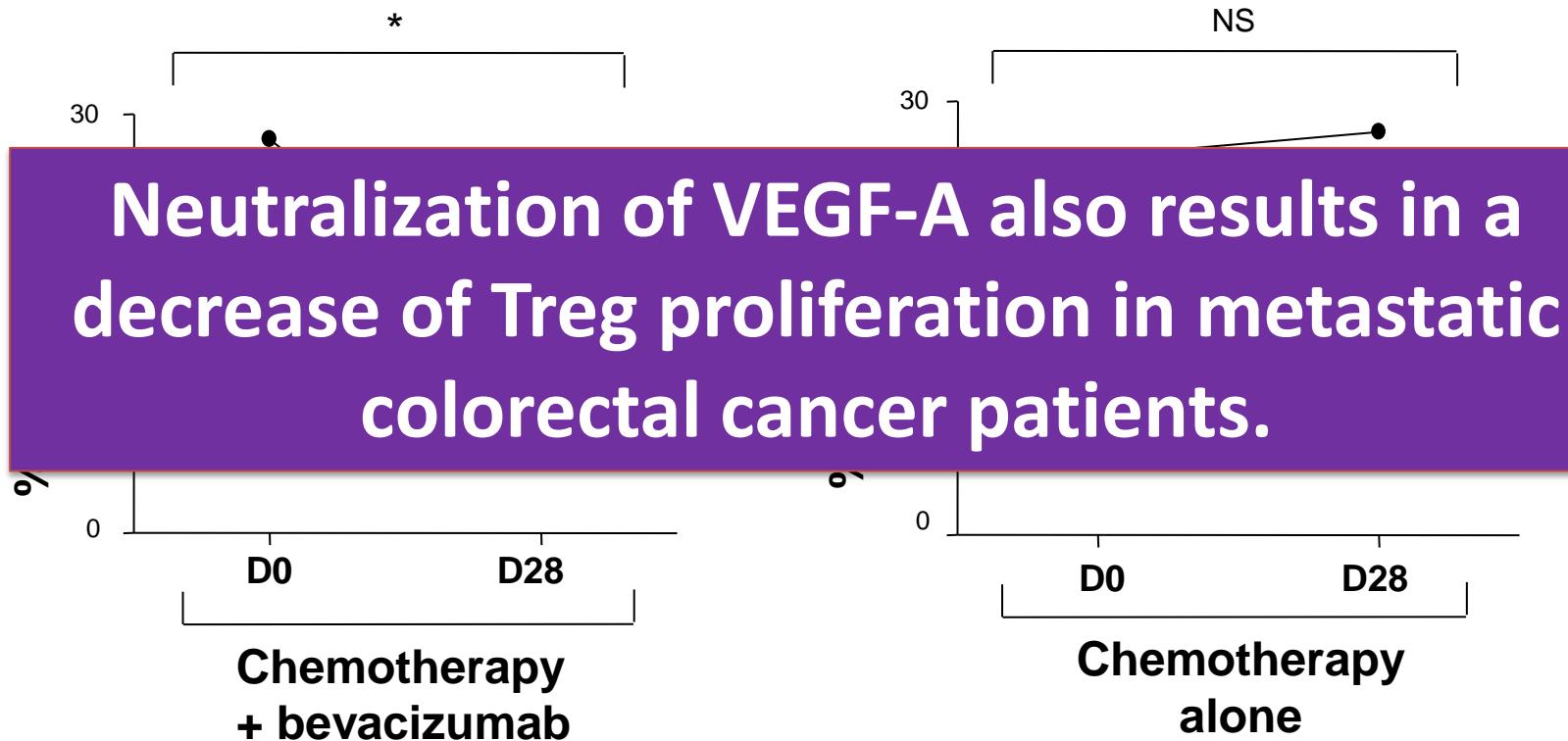
# Treg proliferation in tumor-bearing mice depends on VEGFR2



# Treg proliferation is enhanced in metastatic CRC patients compared to healthy volunteers



# Bevacizumab associated with chemotherapy but not chemotherapy alone decreases Treg proliferation in metastatic CRC patients



# CONCLUSIONS

Targeting VEGF-A/VEGFR is sufficient to inhibit Treg accumulation  
*(mouse model of colorectal cancer and metastatic CRC patients).*

VEGF-A can directly induce  
Treg proliferation

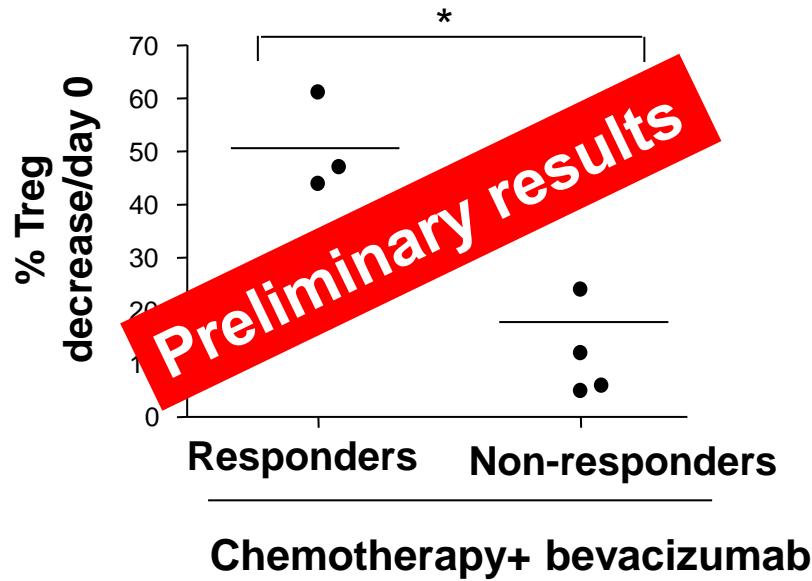


a novel tumor immune escape  
mechanism

Targeting VEGF-A/VEGFR2 decreases Treg proliferation  
*(mouse model of colorectal cancer and metastatic CRC patients).*

# Perspectives and potential therapeutic applications

- Decrease of Treg : a potential biomarker of bevacizumab response in metastatic CRC patients ?



- Role of this immunomodulation in the observed anti-tumor effect? Restauration of an efficient anti-tumoral immune response?
- Association of anti-angiogenic molecules targeting VEGF-A/VEGFR2 with immunotherapeutic strategies in CRC?

# Acknowledgments



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