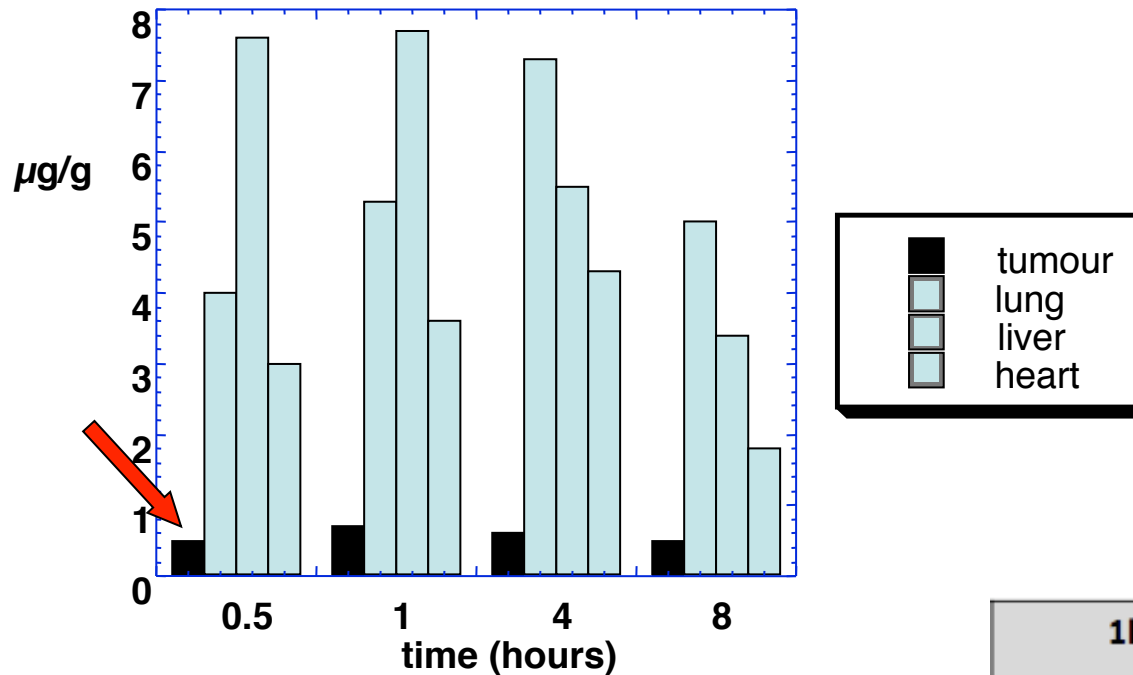


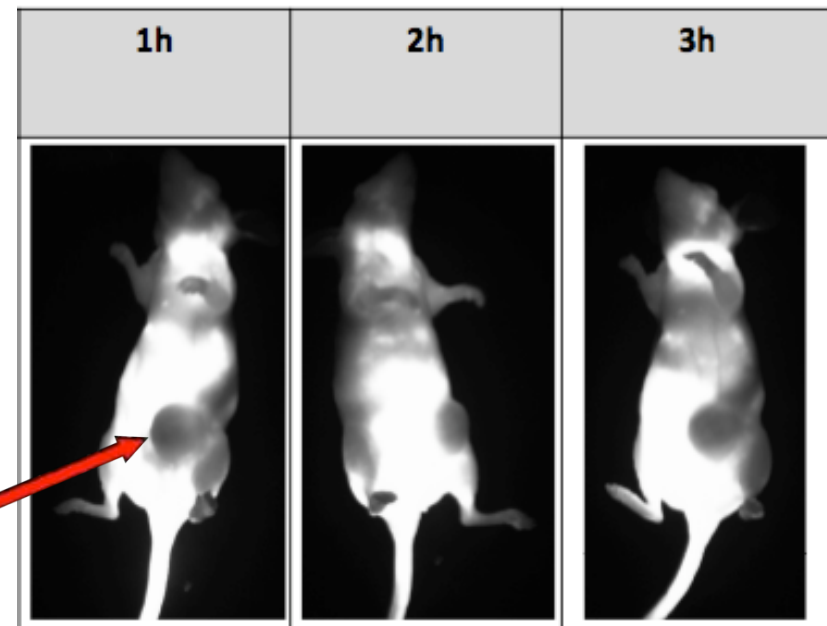
No targeting without specific binding



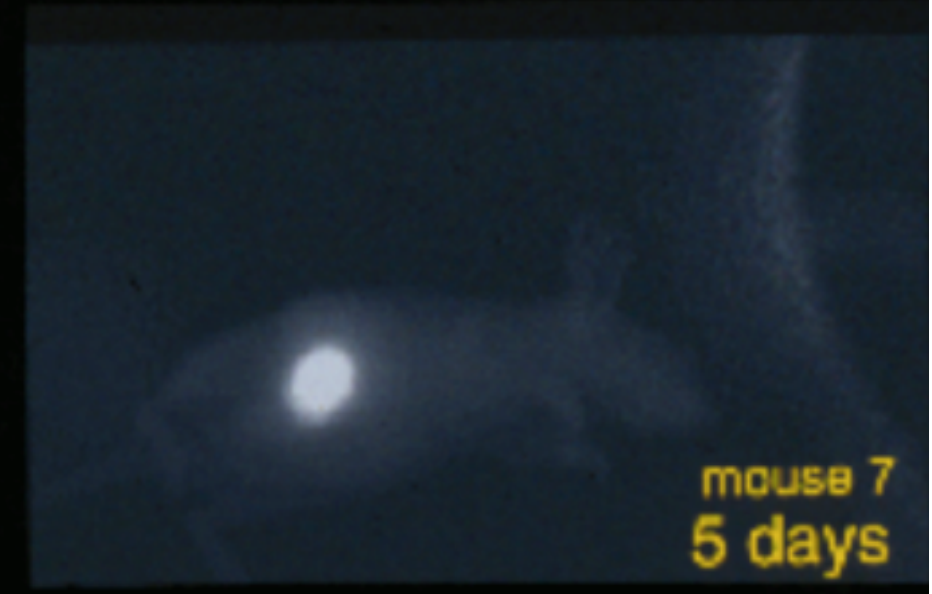
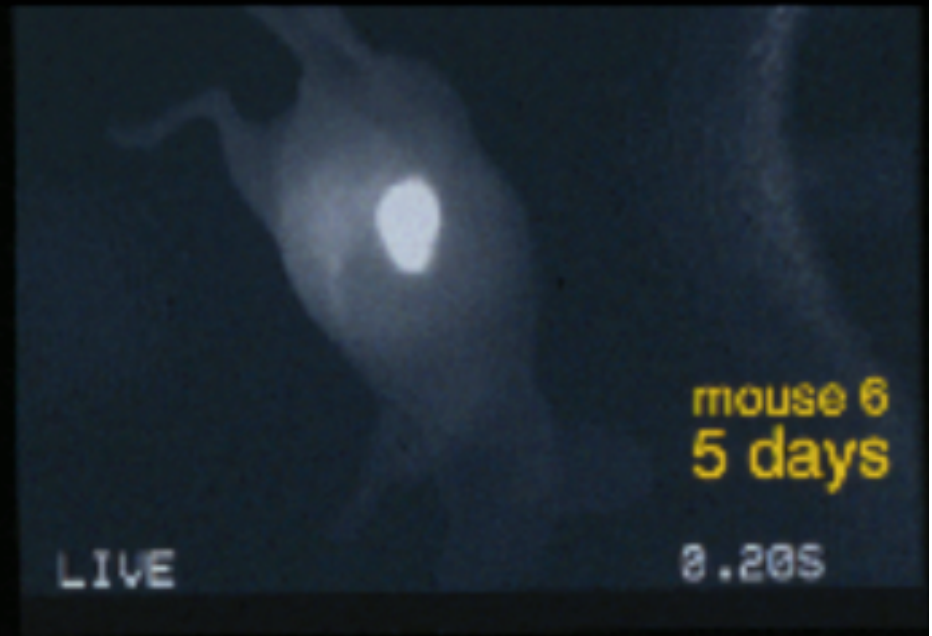
Tissue distribution of doxorubicin

Bosslet et al. (1998) *Cancer Res.* 58, 1195-1201

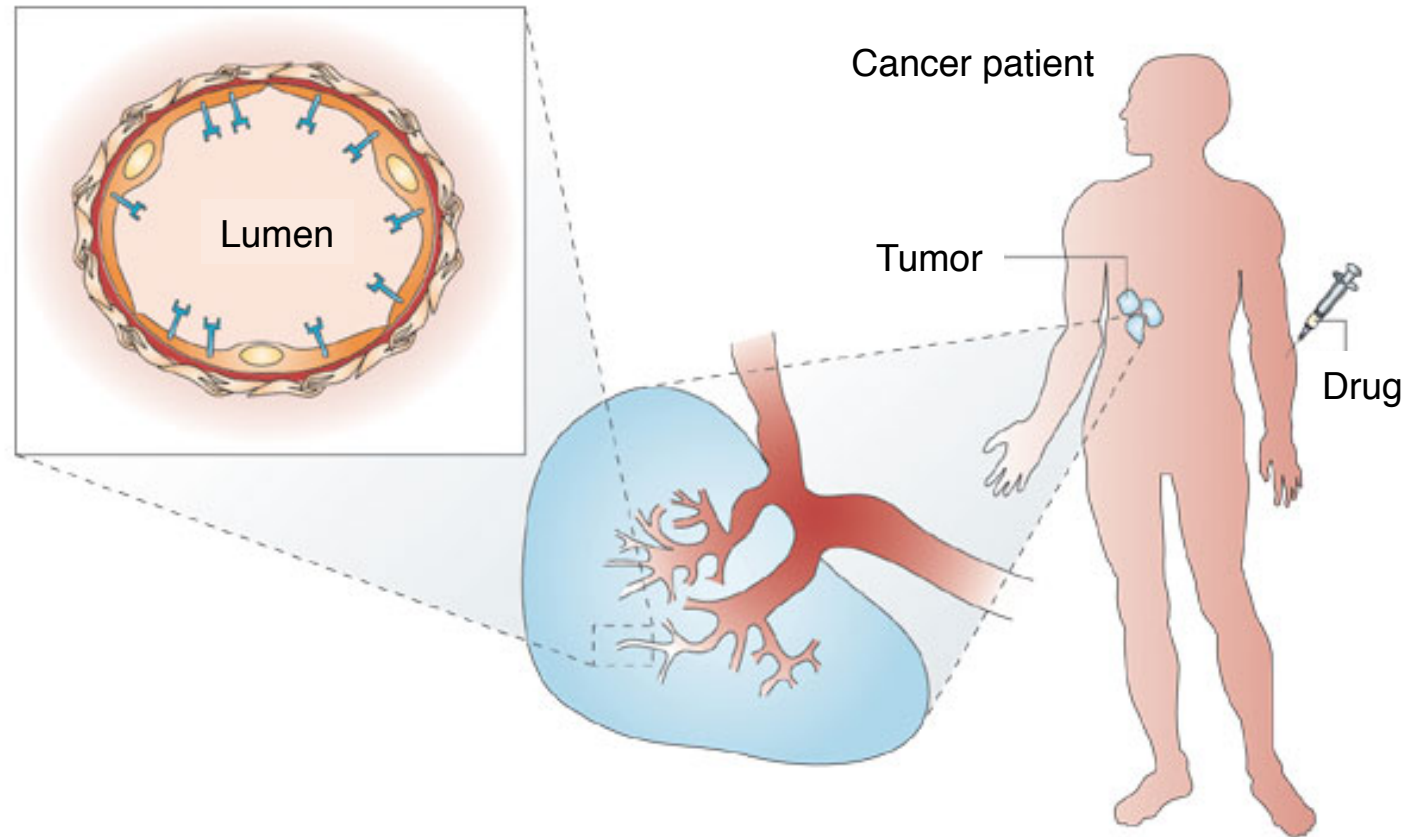
A fluorescent drug which goes everywhere in the body, except to the tumor (black)



Antibodies are able to selectively localize on tumors



Ligand-based targeting of the tumor neo-vasculature



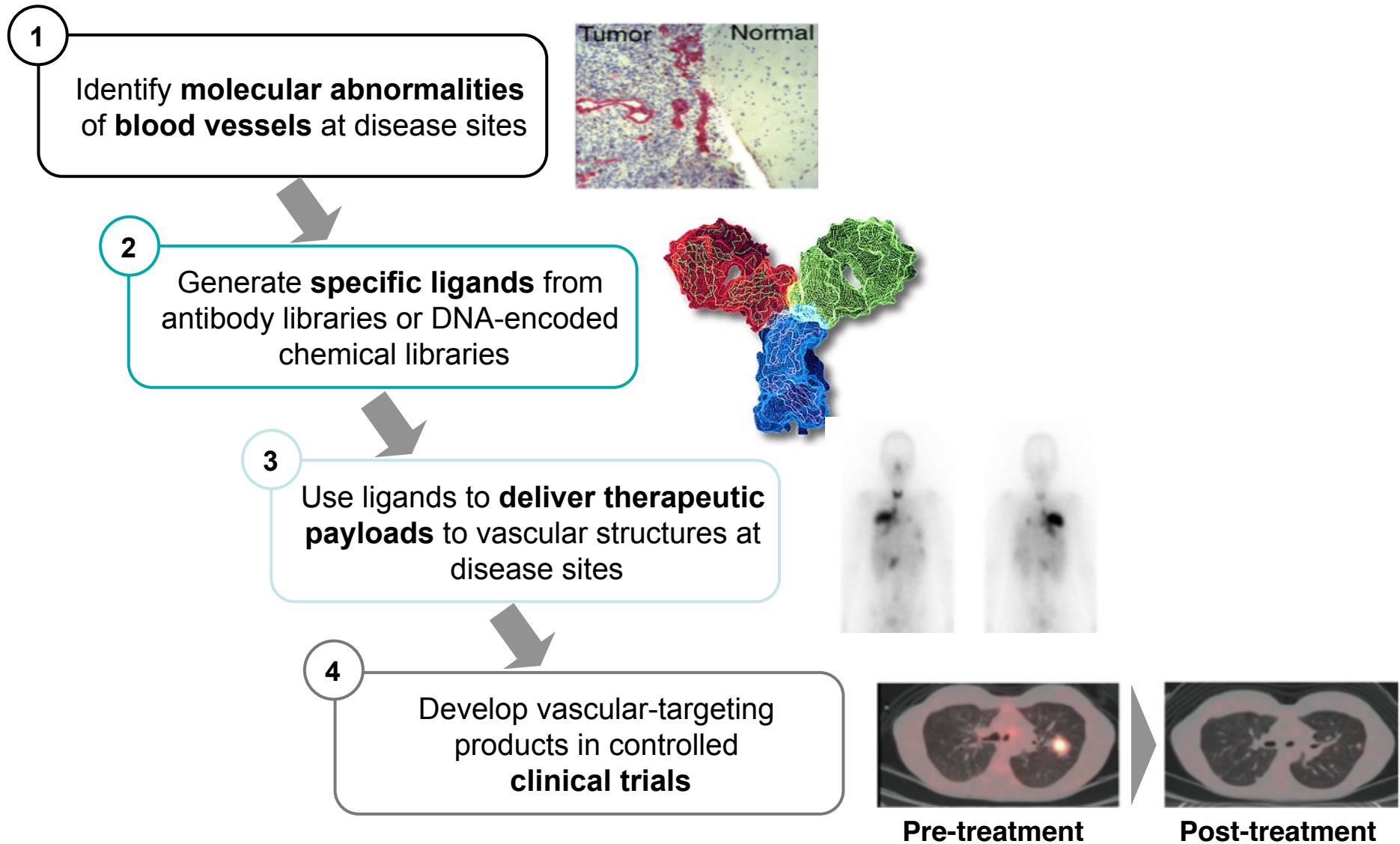
Nature Reviews | **Cancer**

Neri, , Winter (1997) *Nature Biotech.*, 15, 1271

Neri & Bicknell (2005) *Nature Rev. Cancer*, 5, 436

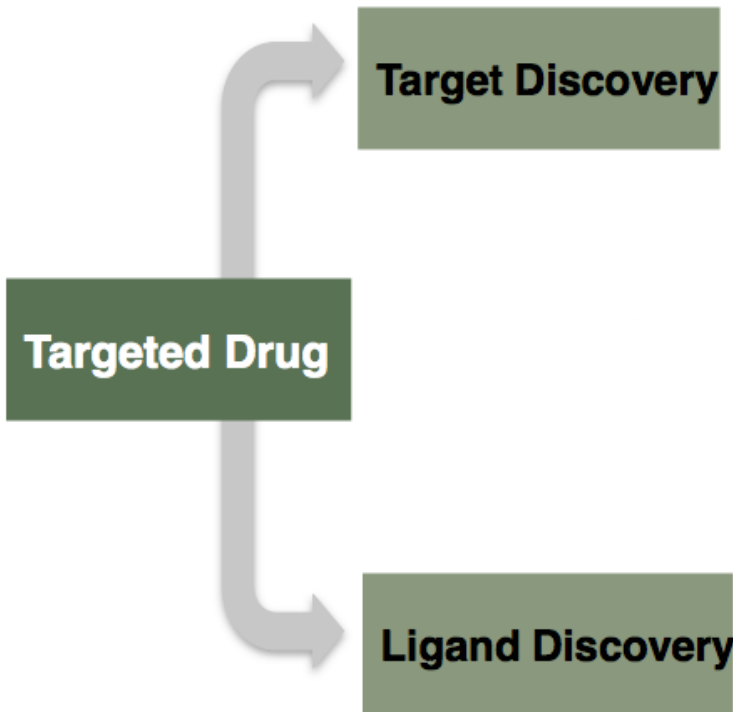
Neri & Supuran (2011) *Nature Rev. Drug Discov.*, 10, 767

Vascular Targeting strategy

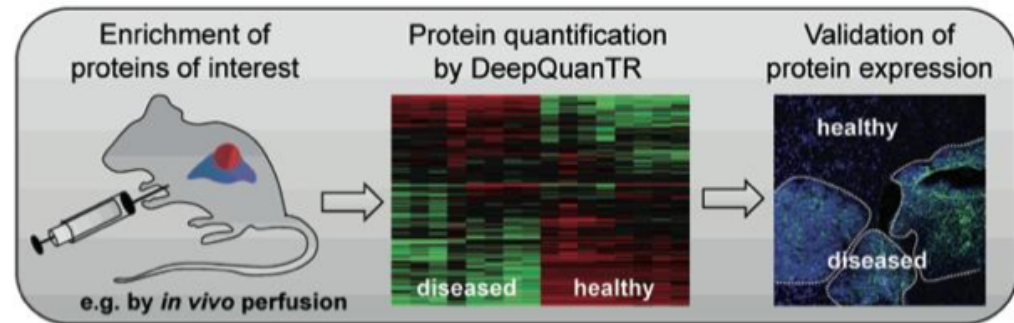


Enabling technologies

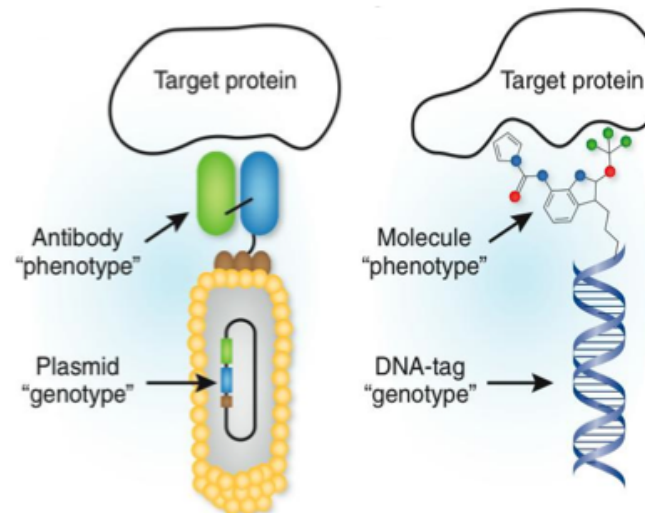
Complementary technologies combined into a vascular targeting strategy



Mass spectrometry-based discovery of markers of angiogenesis

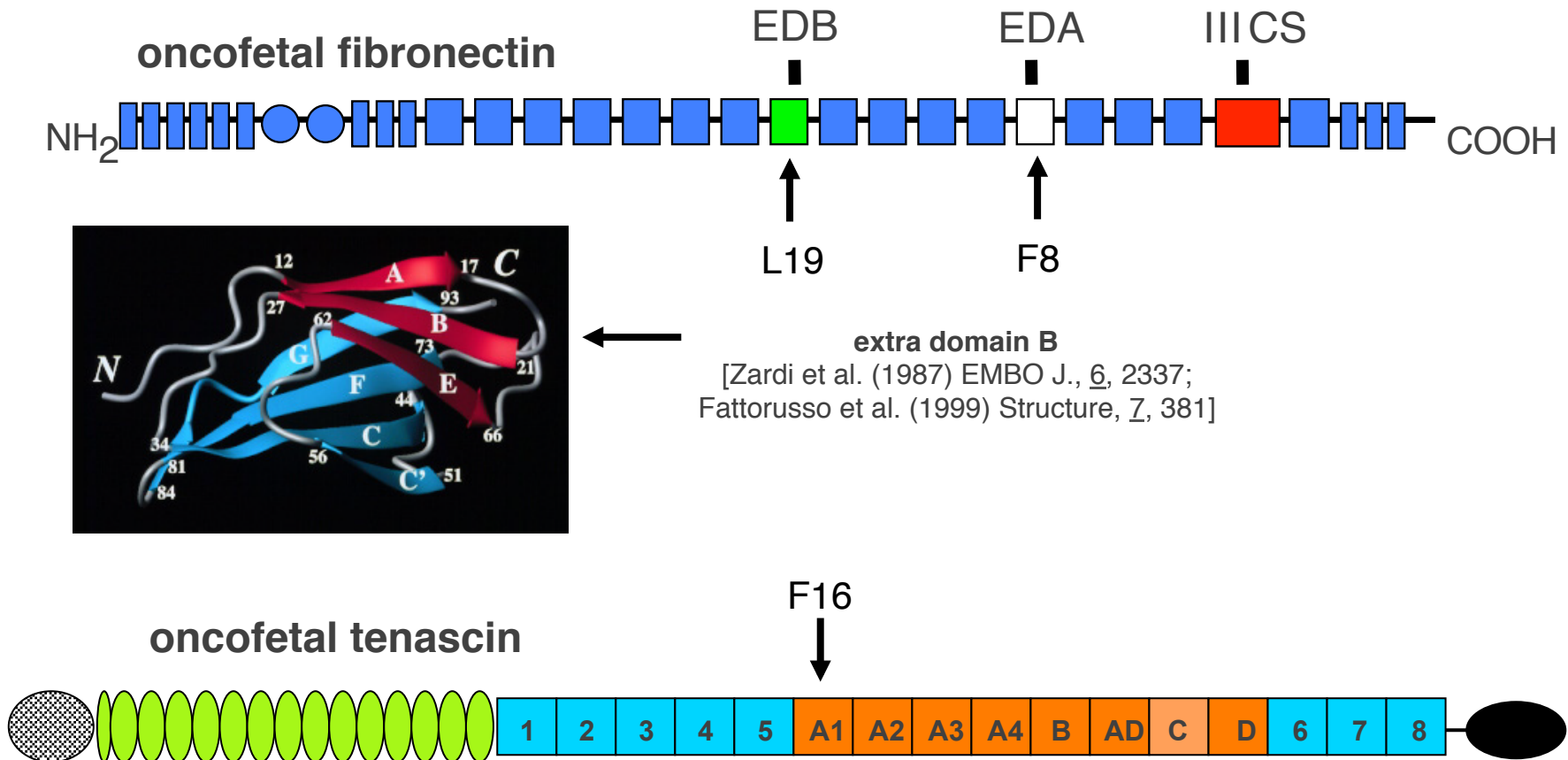


Antibody libraries and DNA-encoded chemical libraries

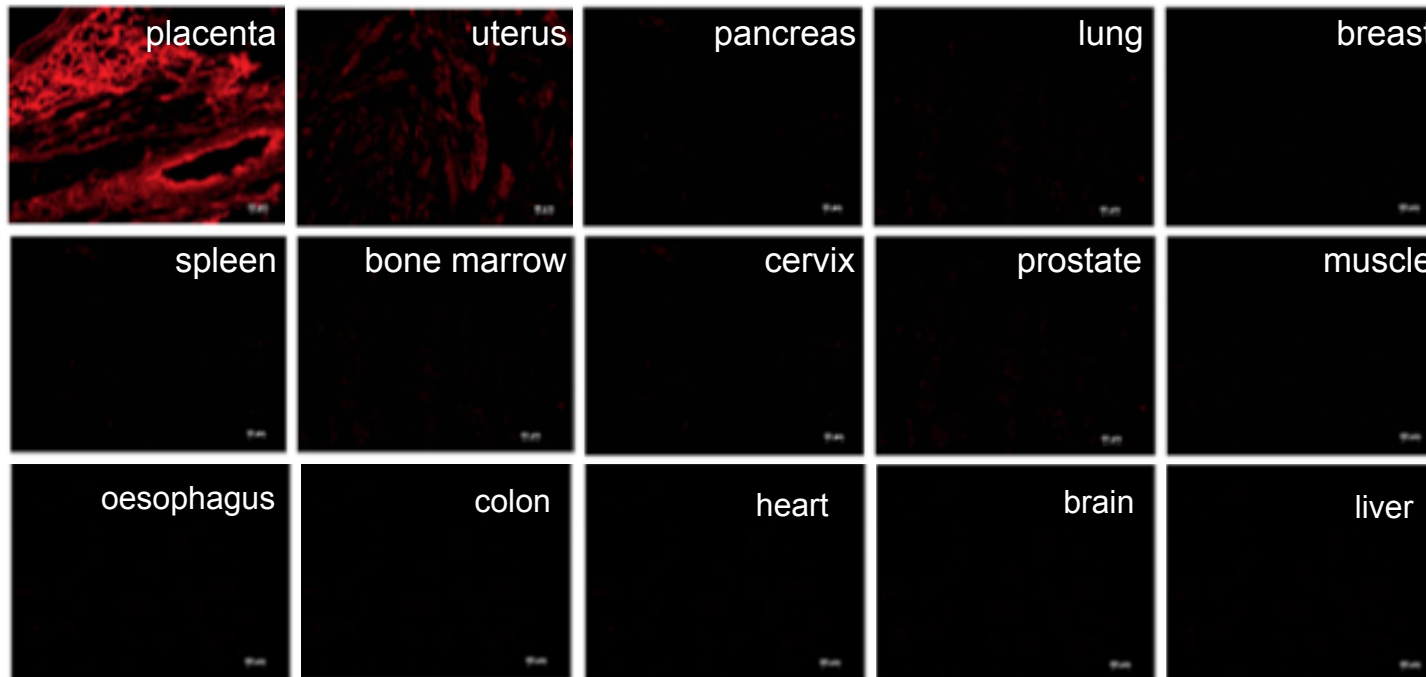


Splice Isoforms of Fibronectin and Tenascin-C as Targets

- Splice isoforms of fibronectin and tenascin represent two excellent classes of markers of angiogenesis. Human antibodies in industrial development include L19, F8 and F16.



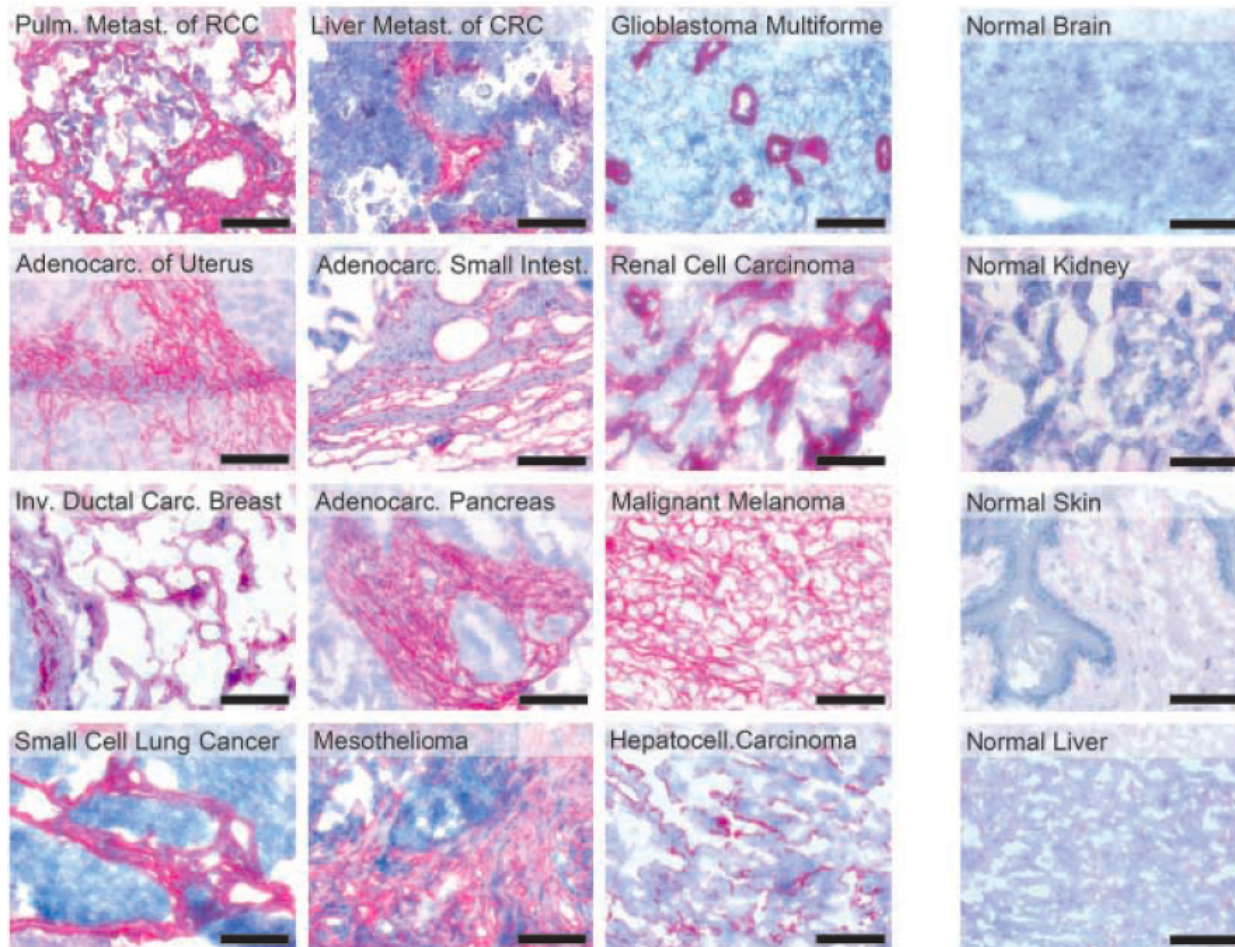
Oncofetal fibronectin has a restricted expression in normal tissue



*Expression only in placenta and in the endometrium in the proliferative phase
(sites of physiological angiogenesis)*

Multi-tumour reactivity

Oncofetal fibronectins are over-expressed in many different cancers, including breast, lung, pancreatic, lymphoma and many others.

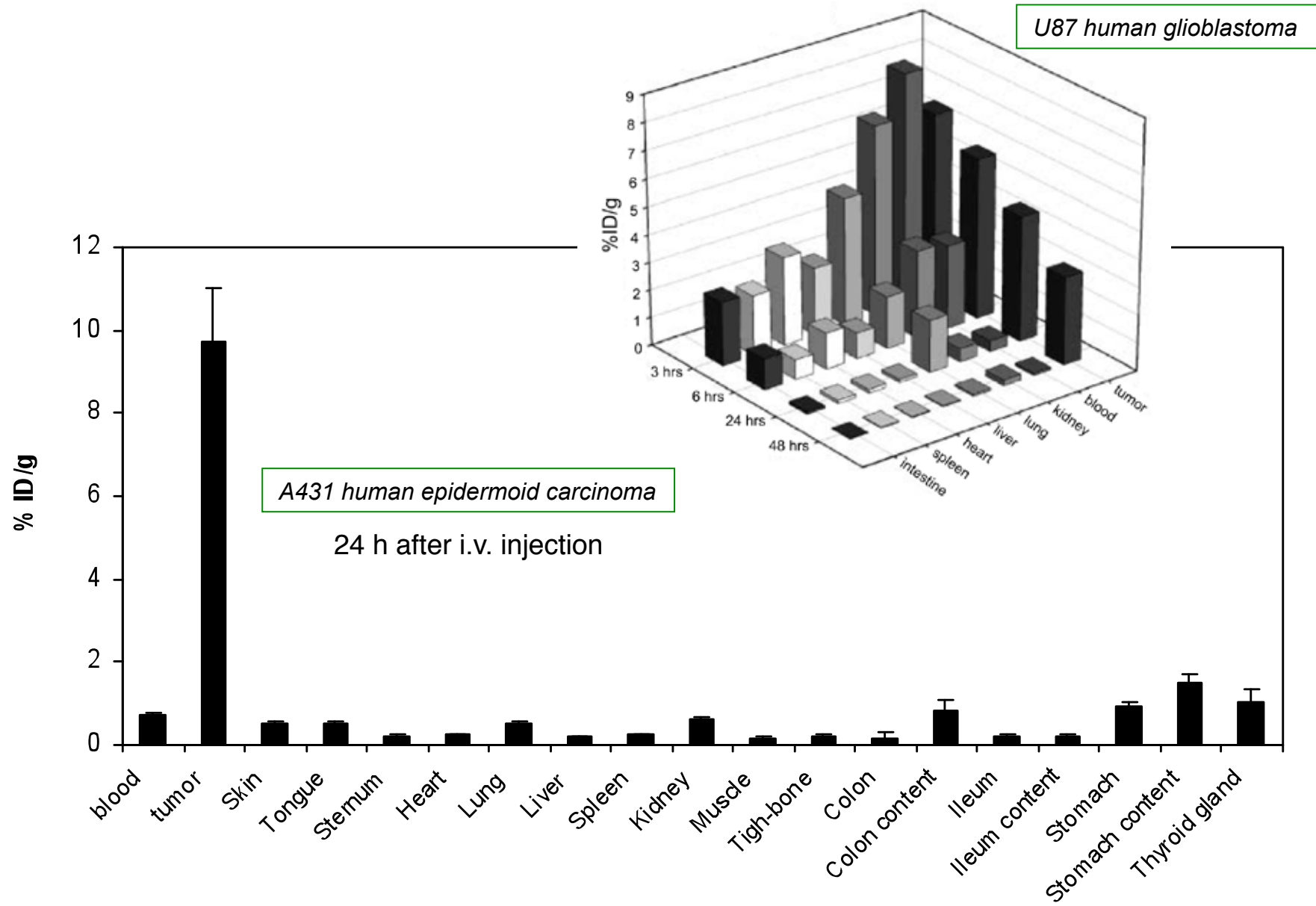


Human tumour tissues

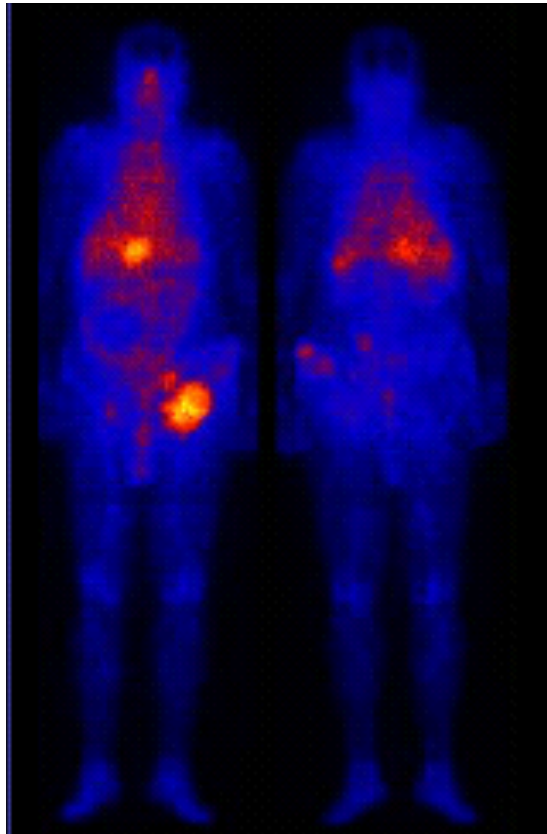
Human normal tissues

Rybak *et al.* (2007)
Cancer Res., 67, 10948

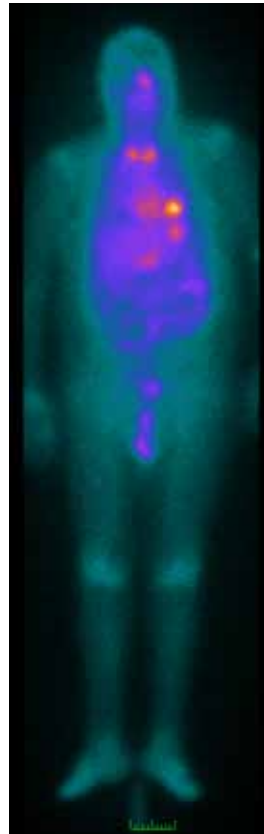
Tumor targeting with the F16 antibody



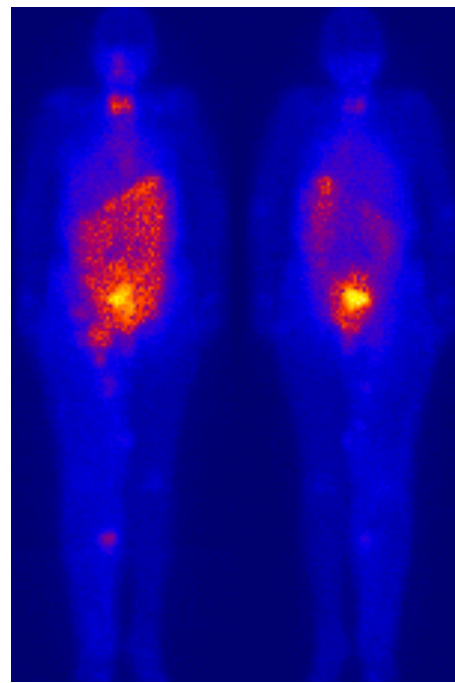
L19 uptake in Metastatic Melanoma



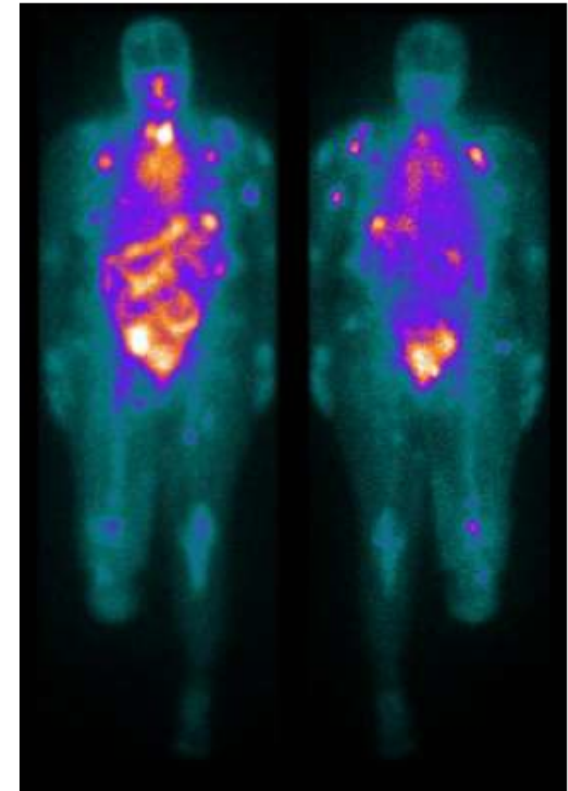
Pt. 01005
131I-L19sip
total body planar scan
90h post RIT



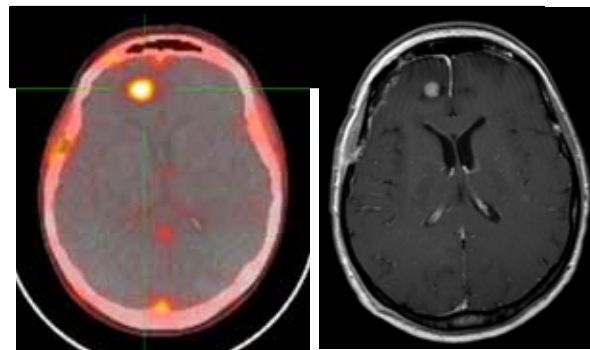
Pt. 18.001.J.001
131I-L19sip
total body planar scan
4 days post RIT



Pt. 01015
131I-L19sip
total body planar scan
11 days post RIT

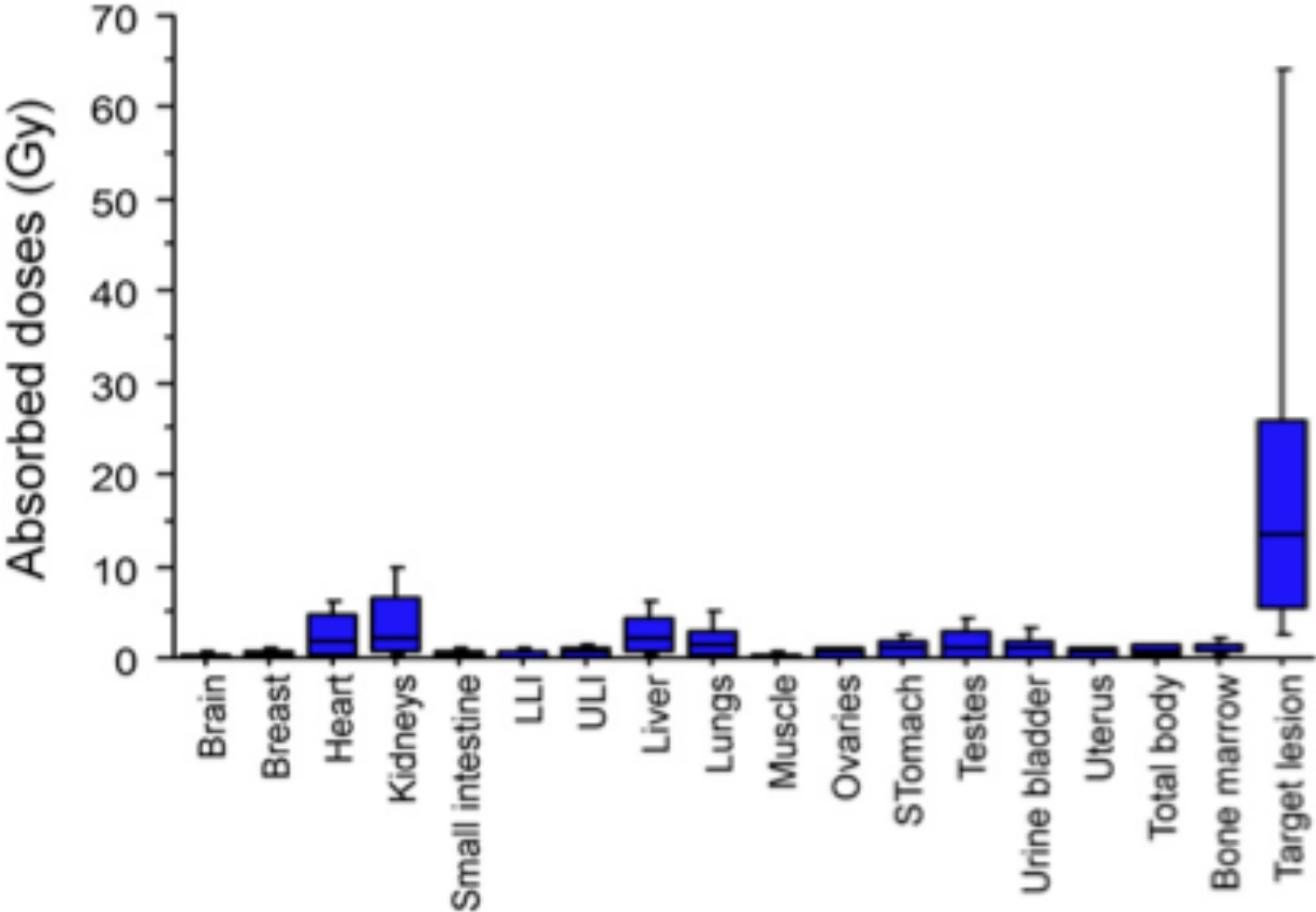


Pt. 06.002.J.033
131I-L19sip
total body planar scan
12 days post RIT

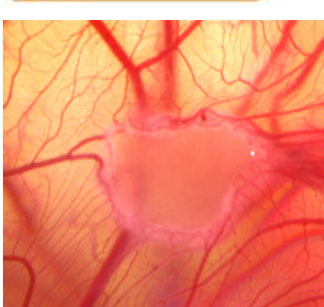
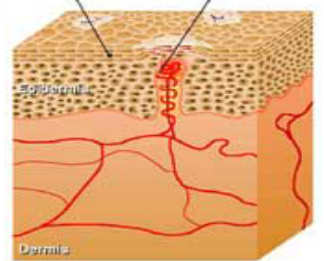
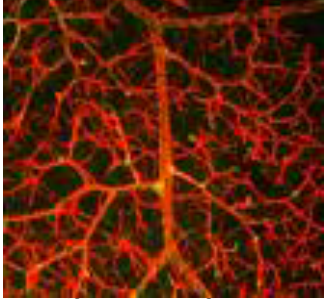


Pt. 18.008.J.914
124I-L19sip PET vs MRI
24h post DIA

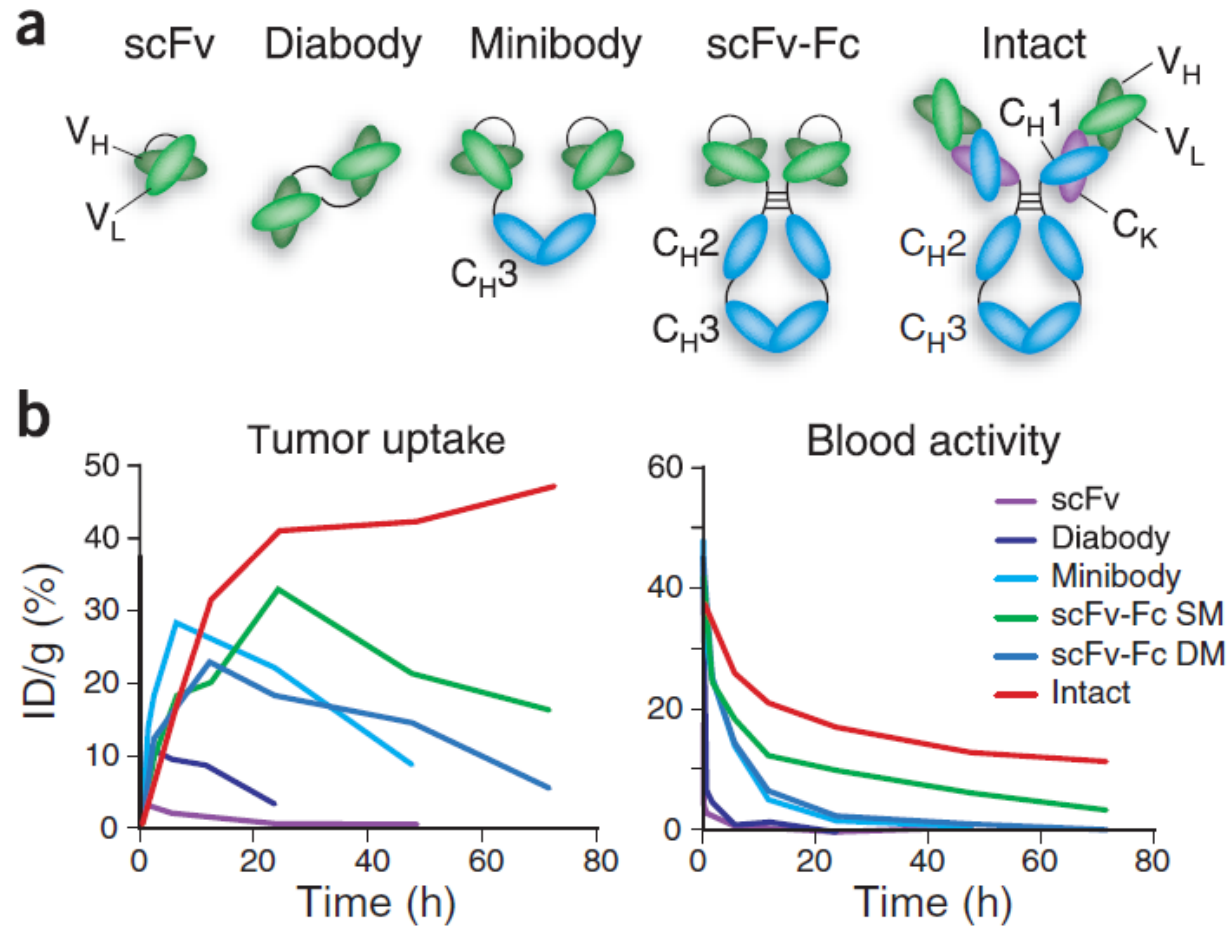
Radretumab (L19-¹³¹I): dosimetry in lymphoma



CHOICE OF ANTIBODY FORMAT AND OF PAYLOAD

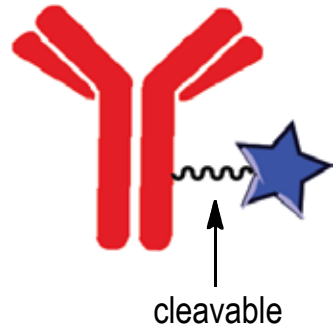


Antibody formats and pharmacokinetics

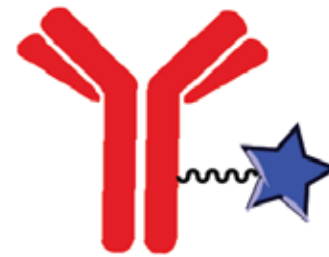


- A. M. Wu and P. D. Senter, *Nature Biotechnology* **2005**, 23, 1137-1146
- L. Borsi et al., *International Journal of Cancer* **2002**, 102, 75-85

Armed Antibodies



Drugs



Cytokines



Bispecific mAbs



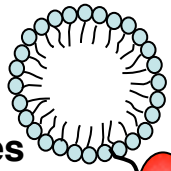
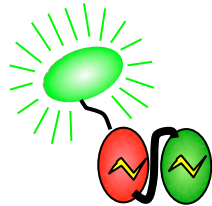
Radionuclides

PET, SPECT

Brack *et al.* (2005) *EJNM*
Santimaria (2003) *Clin. Cancer Res.*
Borsi *et al.* (2002) *Int. J. Cancer*
Berndorff (2006) *J. Nucl. Med.*

NIR Fluorophores

Neri *et al.* (1997) *Nat. Biot.*



Microbubbles

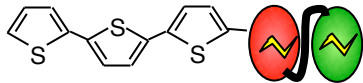
Joseph *et al.* (2004)
Pharm. Res.

drug

cleavable



Bernardes *et al.* (2012) *Angew. Chemie*



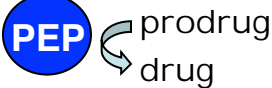
Photosensitizers

Birchler *et al.* (1999) *Nat. Biot.*
Fabbrini *et al.* (2006) *Int J. Cancer*



Therapeutic radionuclides

Demartis *et al.* (2001) *EJNM*
Berndorff (2005) *Clin. Cancer Res.*
Tijink *et al.* (2006) *J. Nucl. Med.*

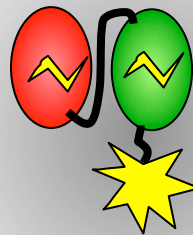
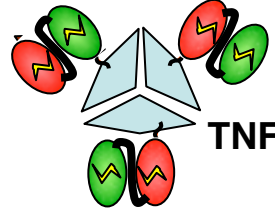


Enzymes

Heinis *et al.* (2004)
Biochemistry

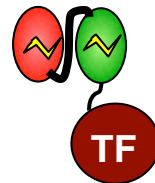
TNF

Borsi *et al.* (2003) *Blood*
Balza (2006) *Clin. Cancer Res.*



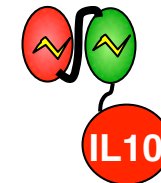
active moiety

Therapy



Procoagulants

Nilsson *et al.* (2000)
Cancer Res.



IL-10

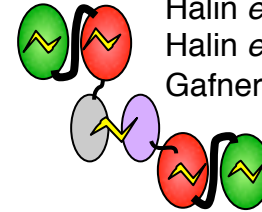
Trachsel *et al.* (2006) *J. Inv. Dermatol.*
Trachsel *et al.* (2007) *Arthritis Res. Ther.*

VEGFs

Halin *et al.* (2002) *Int. J. Cancer*

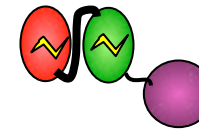
IL12

Halin *et al.* (2002) *Nat. Biot.*
Halin *et al.* (2003) *Cancer Res.*
Gafner *et al.* (2006) *Int. J. Cancer*



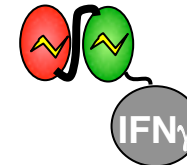
IL-2

Carnemolla *et al.*
(2002) *Blood*



IL-15 and GM-CSF

Kaspar *et al.* (2007)
Cancer Res.



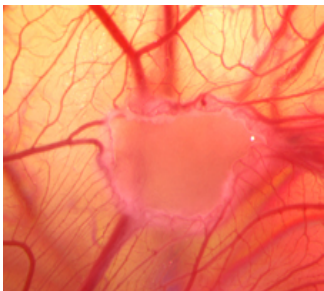
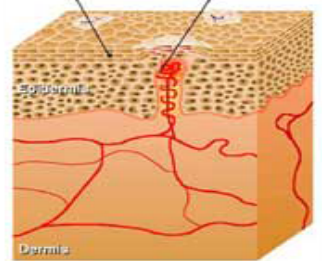
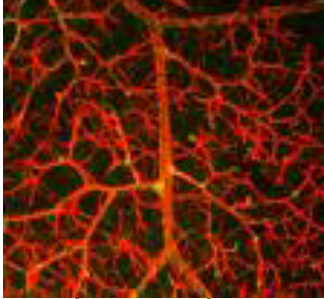
Interferon γ

Ebbinghaus *et al.* (2005)
Int. J. Cancer



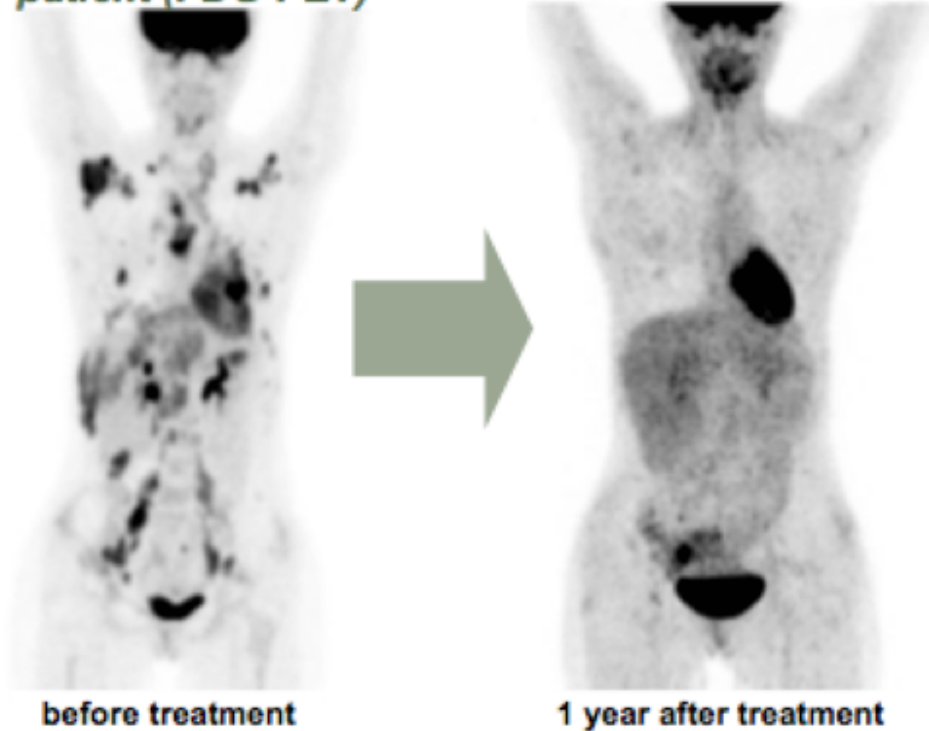
RADIOLABELED ANTIBODIES:

L19-¹³¹I



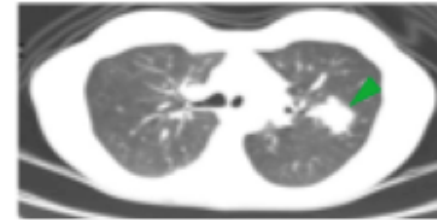
Radretumab (L19-¹³¹I): selected clinical data

Impressive response in a Hodgkin lymphoma patient (FDG-PET)

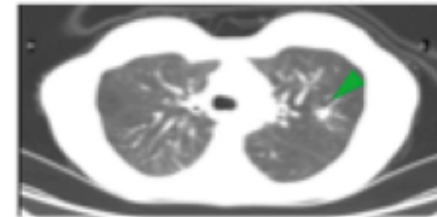


CT scans

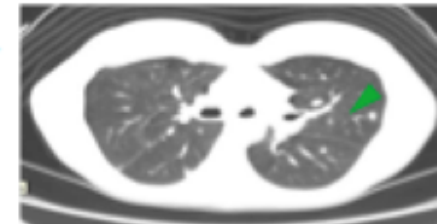
Baseline



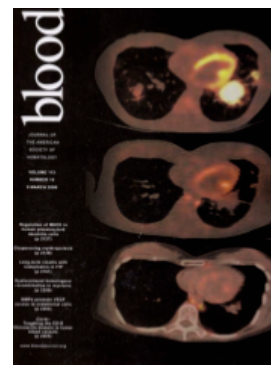
5 weeks later



15 weeks later

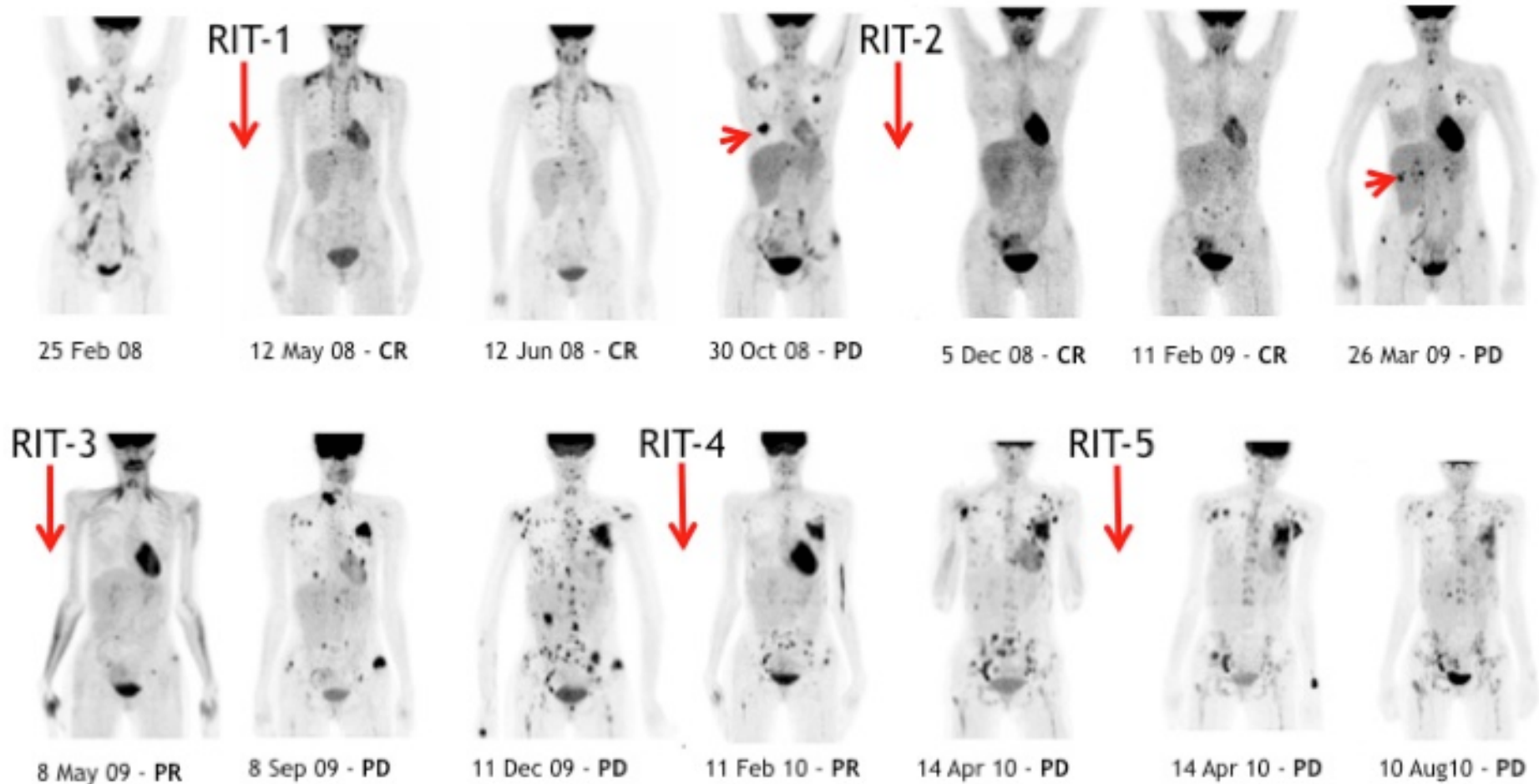


Sauer et al. (2009) *Blood*, 113, 2265



Radretumab [L19-¹³¹I]

Detailed analysis of Patient 44 (Hodgkin Lymphoma)

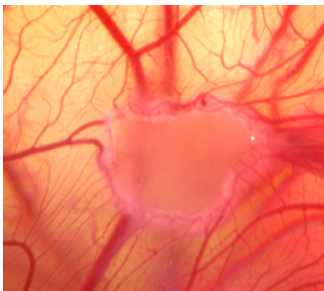
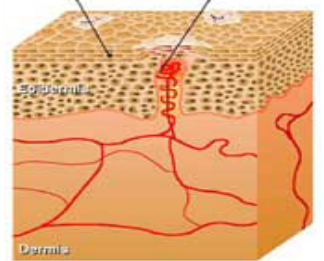
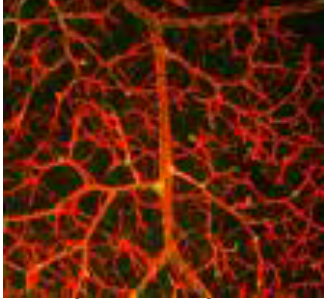


Erba et al. (2012) *J. Nucl. Med.*, 53, 922

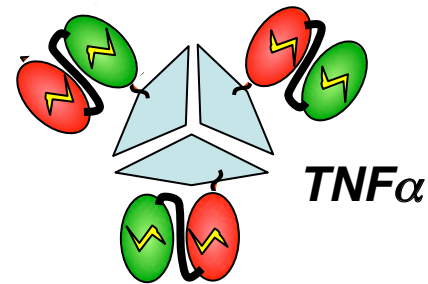
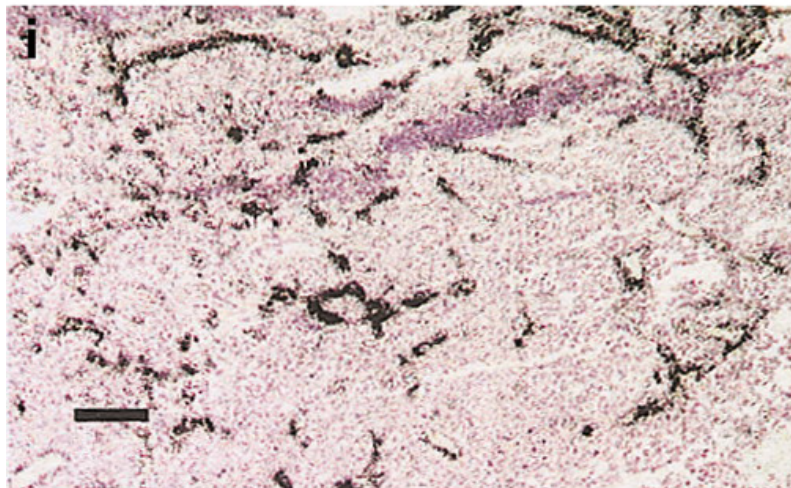
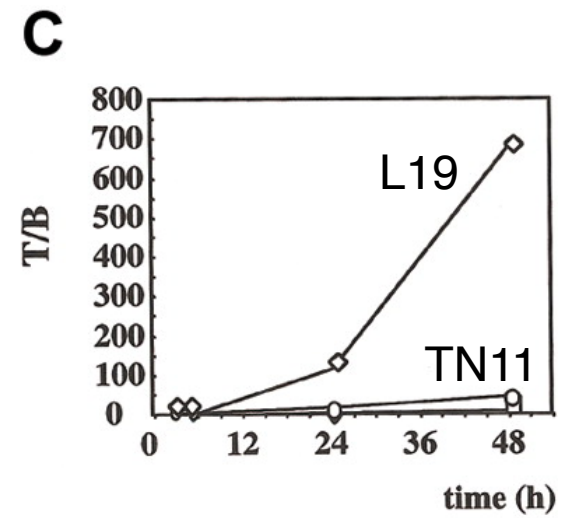
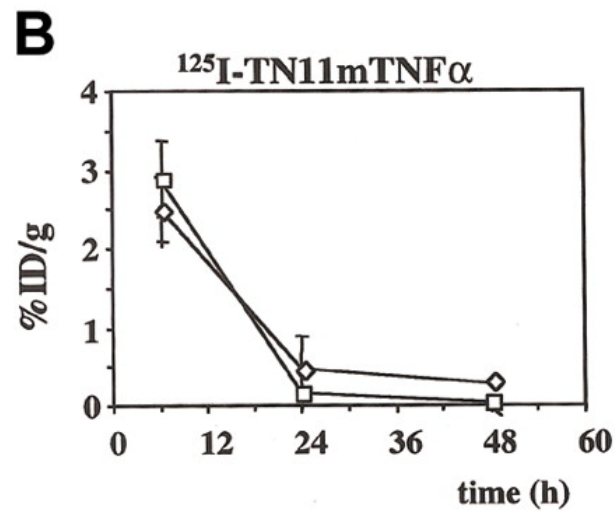
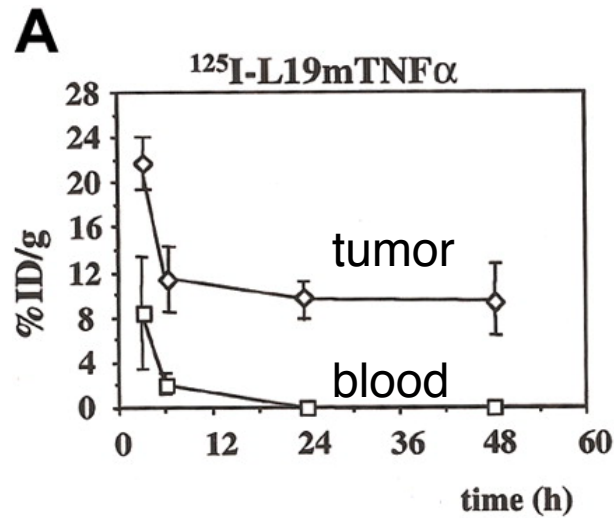


IMMUNOCYTOKINES:

L19-TNF

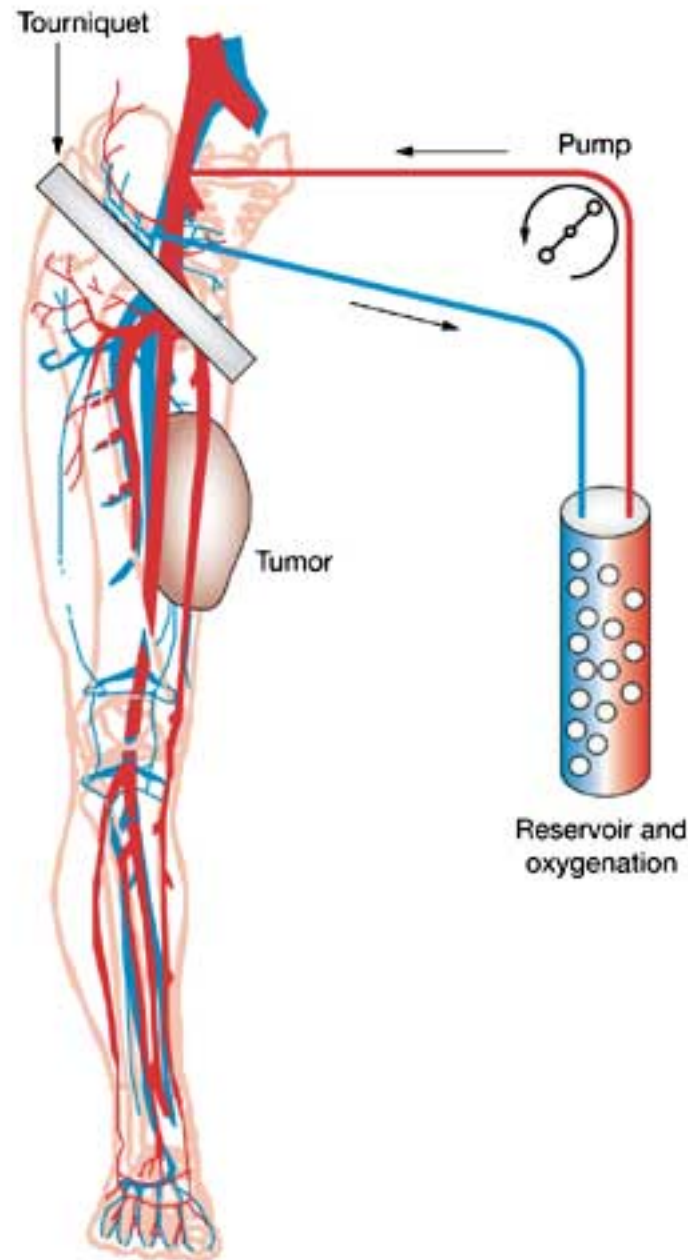


Tumour targeting with scFv-TNF fusions



Borsi *et al.*, *Blood* **2003**, *102*, 4384

Application of L19-TNF in isolated limb perfusion



Recombinant TNF (Beromun™) is administered ILP procedures at a dose of **4 mg**

If the tourniquet is not tight, the procedure becomes life-threatening, since the MTD of TNF is **300 μg**

Thanks to its targeting properties, L19-TNF can be used at a dose of **650 μg** (**220 μg** of TNF equivalents)

Therapeutic effect of L19-TNF



Pre-treatment



After ILP

Papadia et al. (2012) *J. Surg. Oncol.*, in press

Patient 14.005.H.008 - ILP study

Therapeutic effect of L19-TNF



Baseline



Week 6

Papadia et al. (2012) *J. Surg. Oncol.*, in press

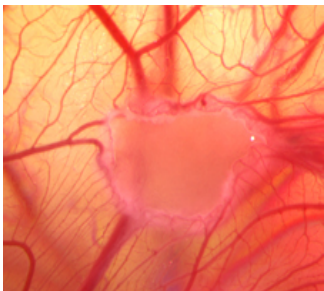
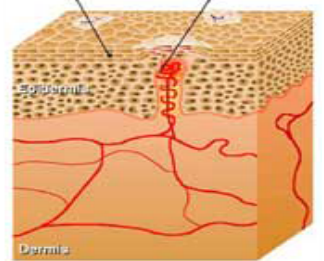
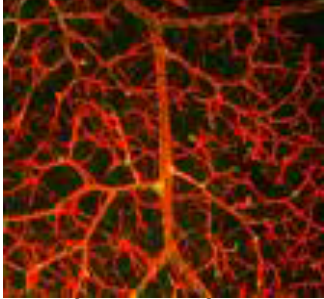
Therapeutic effect of L19-TNF





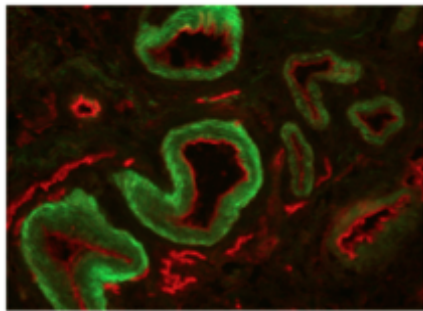
TARGETING INFLAMMATION:

F8-IL10

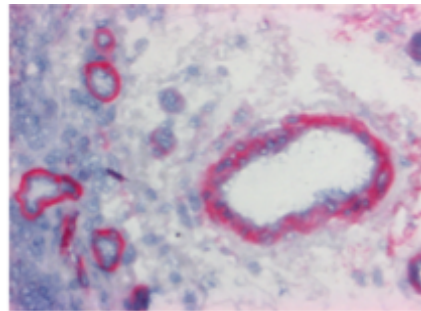


Angiogenesis is a rare process in the adult

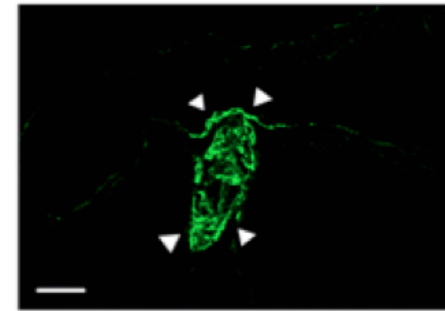
... but a **common feature** of aggressive **tumours** and of other serious pathological conditions



Endometriosis



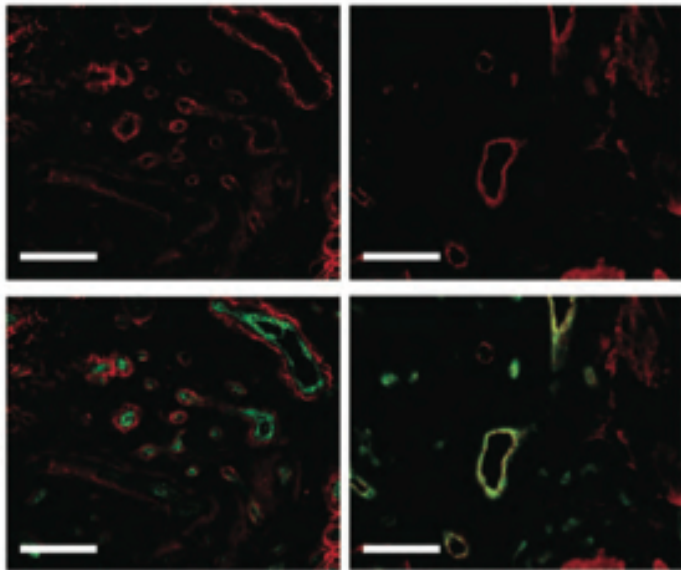
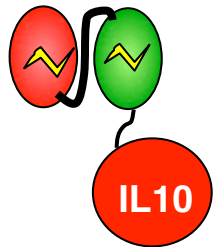
Arthritis



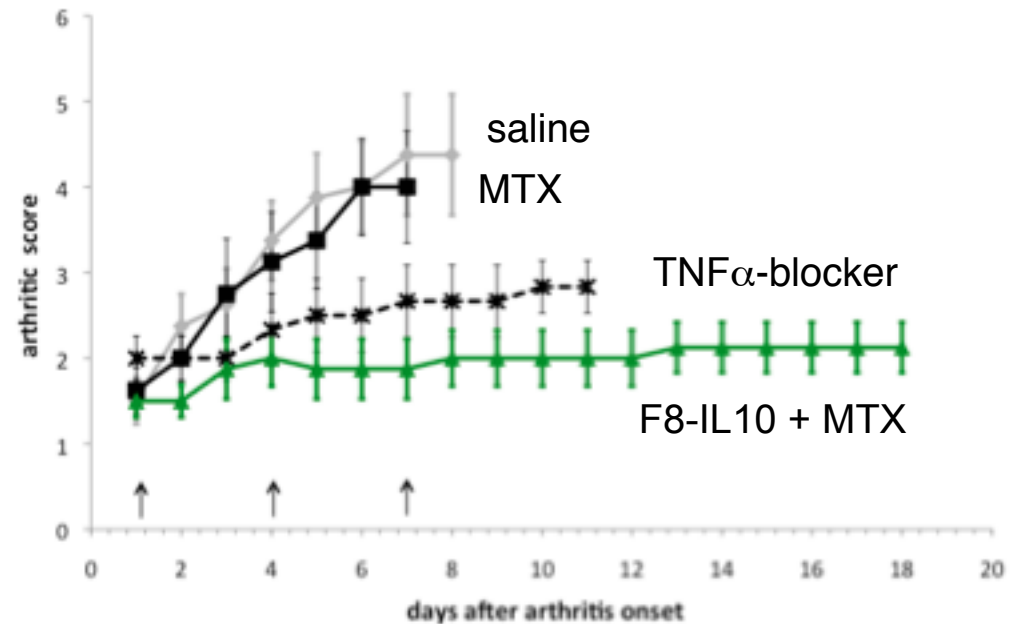
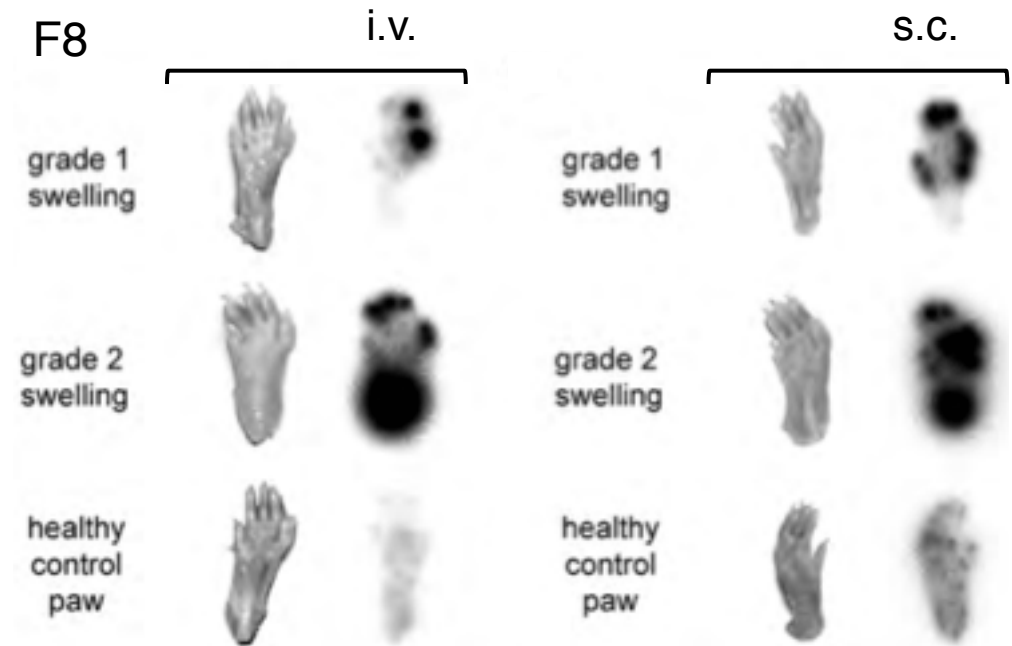
Atherosclerosis

The EDA domain of fibronectin is expressed in various inflammatory diseases

Targeting and inhibition of arthritis



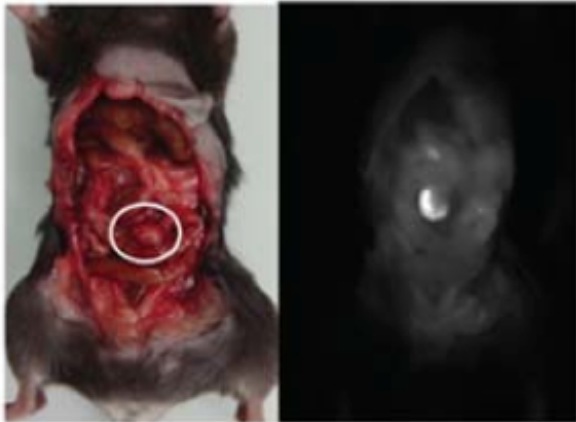
vWF, F8 (EDA)



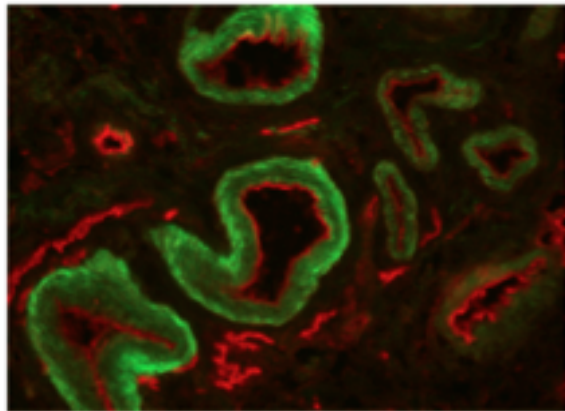
Trachsel et al. (2007) *Arthritis Res. Ther.*, 9, R9;
Schwager et al. (2009) *Arthritis Res. Ther.*, 11, R142

Targeting and inhibition of endometriosis

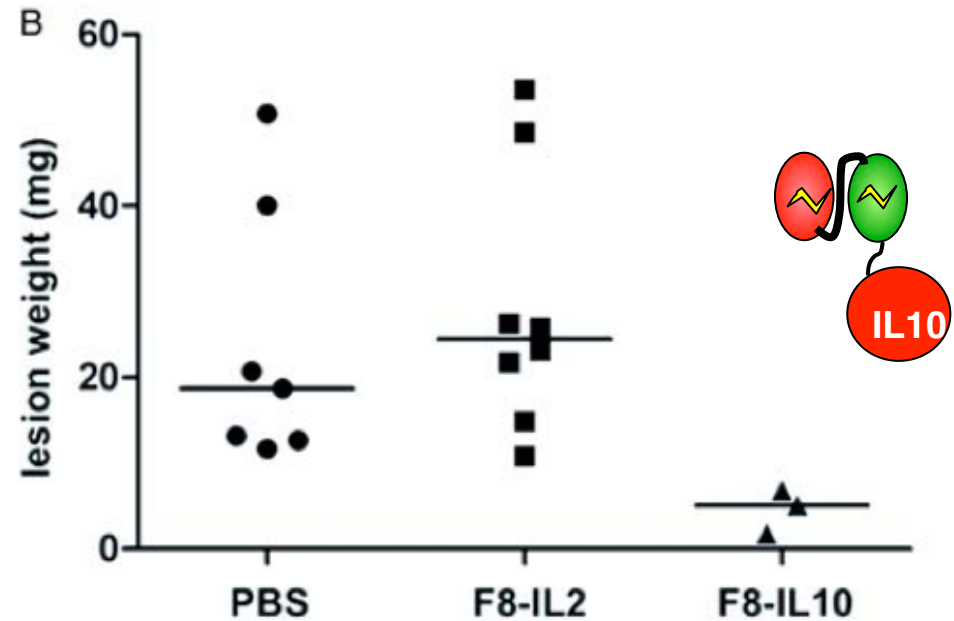
SIP(F8)-ALEXA750



SIP(unspecific)-ALEXA750



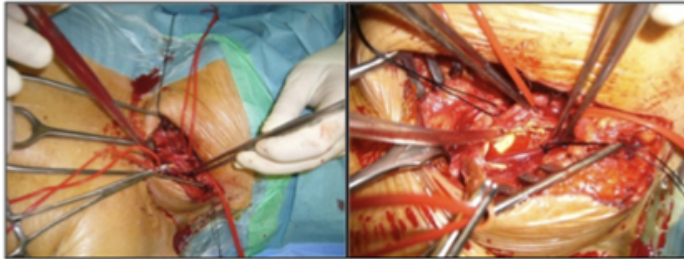
Green = F8 (EDA of fibronectin)
Red = CD31



Targeting atherosclerotic plaques

Humans:

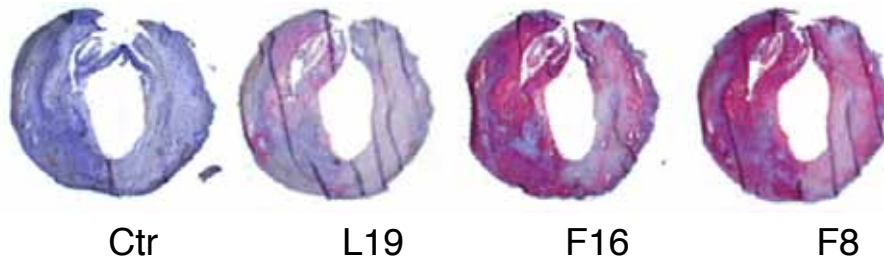
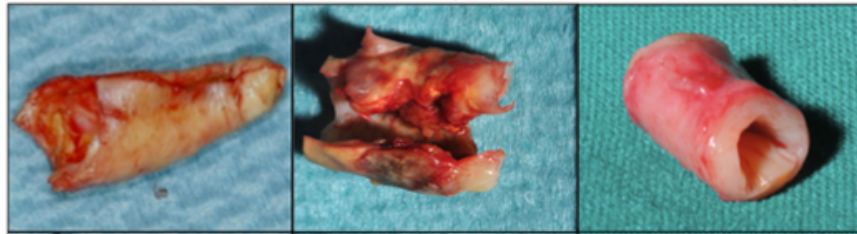
Carotid endarterectomy (CEA)



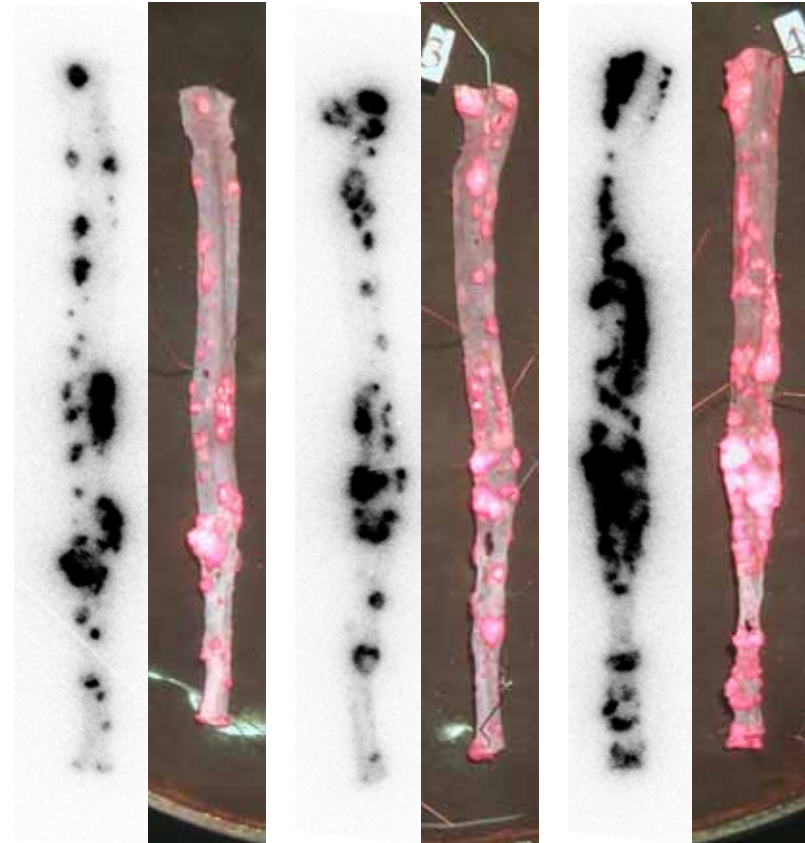
Stable plaque

Unstable plaque

Normal artery



ApoE^{-/-} mice:



Pedretti et al. (2009) *Atherosclerosis*, 208, 382-389

Matter et al. (2004) *Circulation Res.*, 95, 1225-1233
Fiechter et al. (2011) *Atherosclerosis*, 208, 382-389

SUMMARY

Tumor blood vessels express markers (e.g., Bst-2, splice isoforms of fibronectin and of tenascin-C) which are not found on the quiescent vasculature

Tumor blood vessels can be selectively targeted *in vivo* using monoclonal antibody derivatives

Promising therapeutic results can be obtained using antibodies armed with radionuclides or cytokines

There is a potential to use vascular targeting immunocytokines for applications beyond oncology

