Preclinical Imaging: Oncology

DGN Summer School, 04.09.2014

Dr. Christoph Grießinger, Dipl. Biology

Werner Siemens Imaging Center
Department of Preclinical Imaging and Radiopharmacy,
University of Tübingen

Head: Prof. Bernd J. Pichler

Agenda Preclinical Imaging Oncology

PET-Imaging with conventional tracers

→ Therapy monitoring

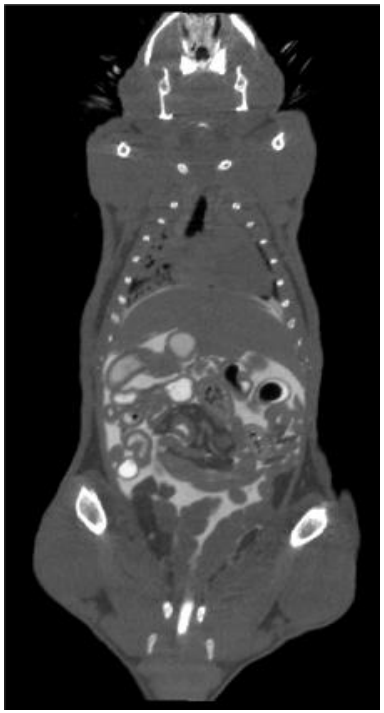
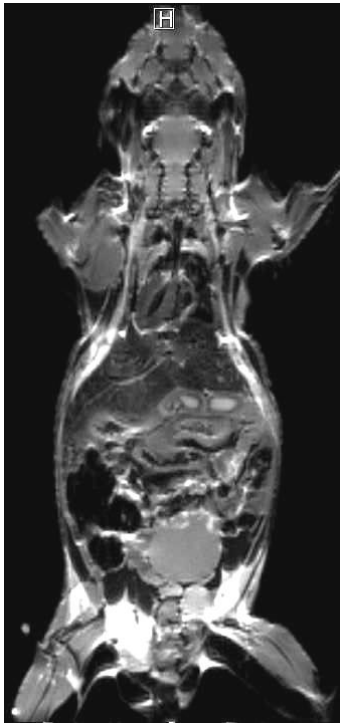
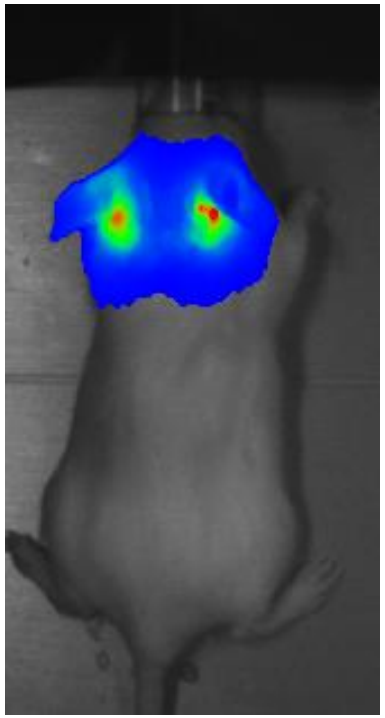
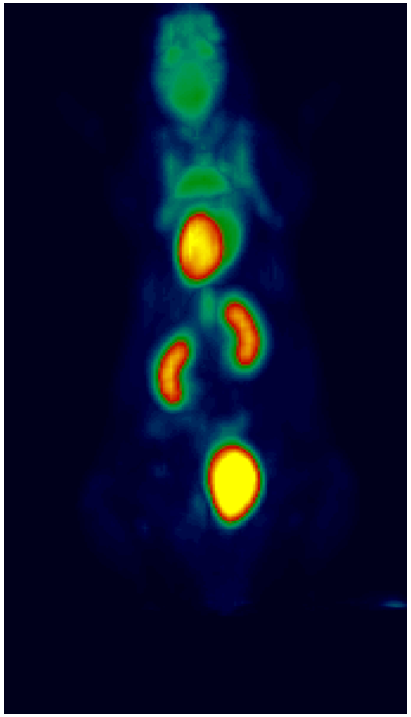
→ PET and MR-spectroscopy

Immuno-PET → antibodies and antibody fragments

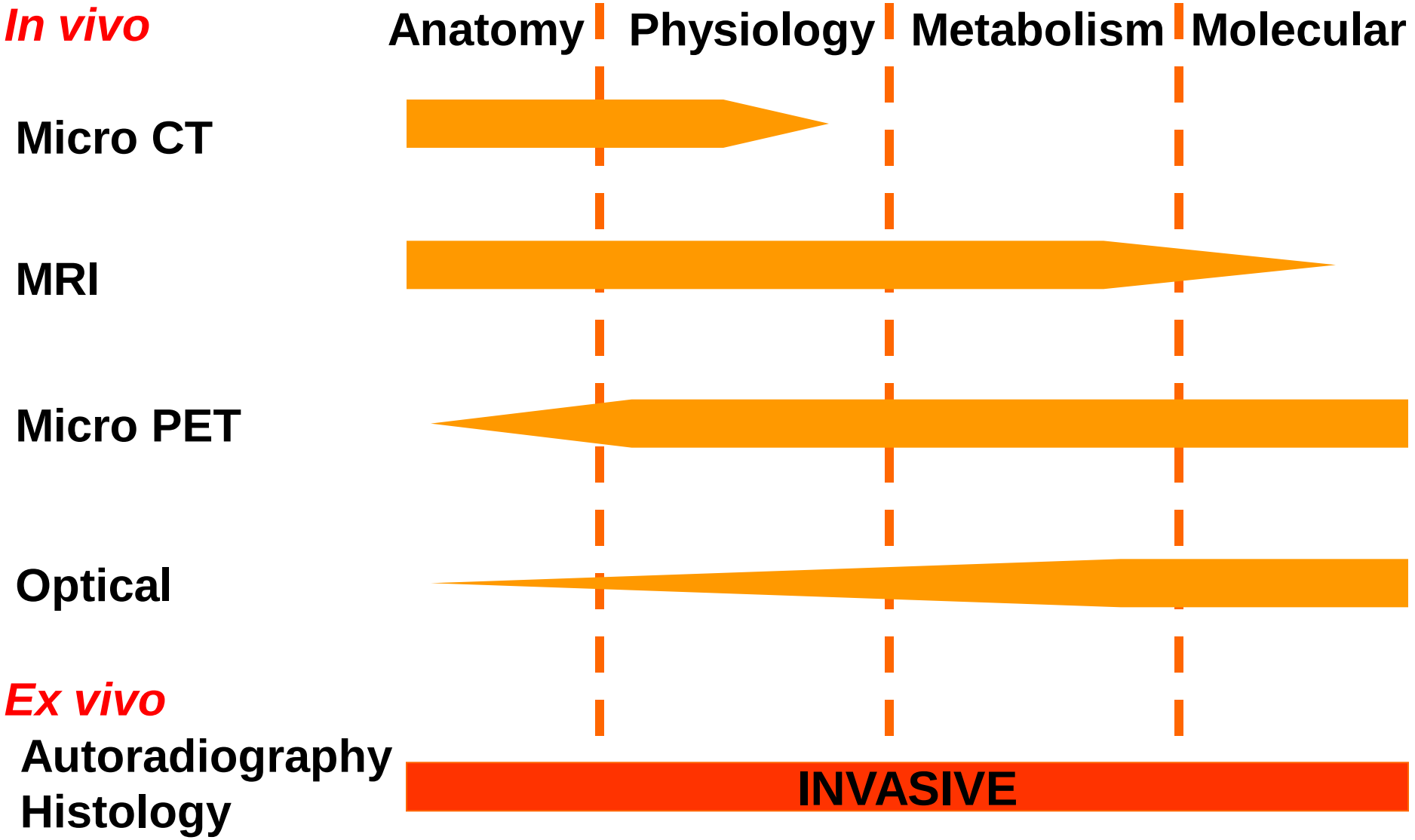
MRI of peritoneal tumors → pancreatic tumours and immunotherapy

Metastasis-detection → Sequential PET/MRI of rhabdomyosarcoma metastasis

Imaging Modalities

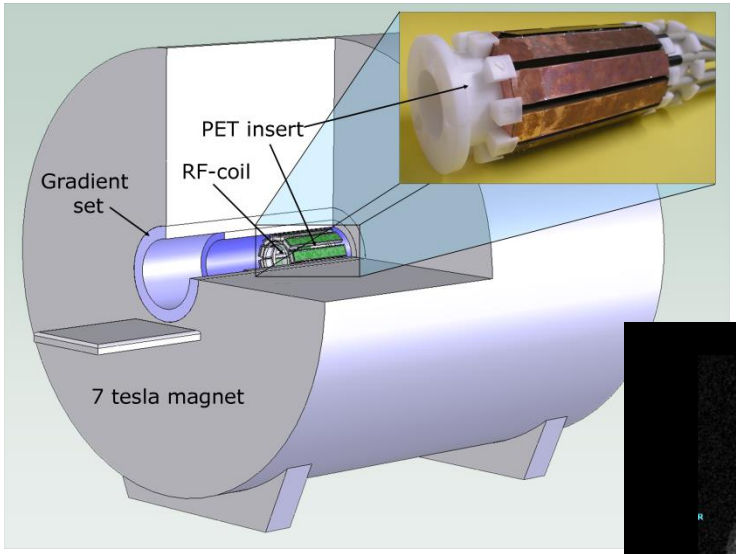
CT	MRI	Optical Imaging	PET
			
Morphology	**Morphology (Function)**	**Function**	**Function**

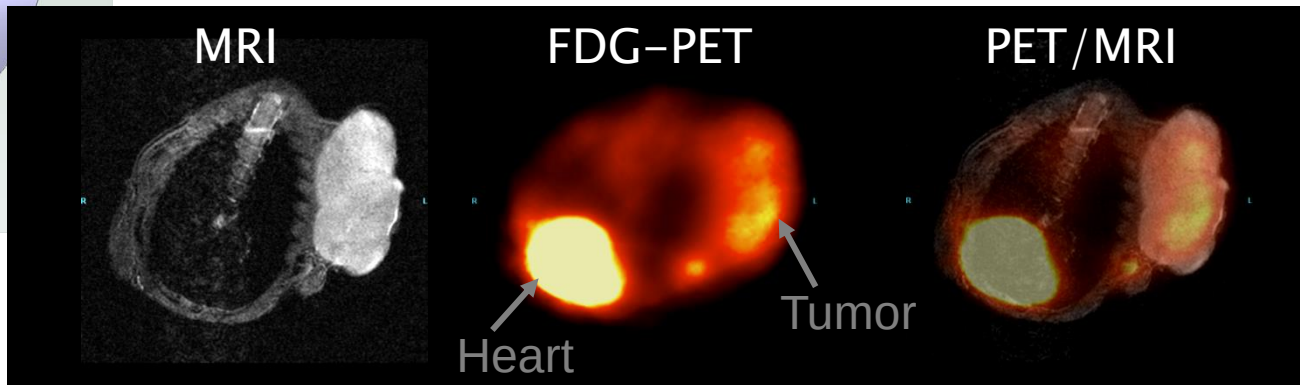
Imaging Modalities



Simultaneous PET/MRI

- Simultaneous acquisition of the anatomical information of the MRI and molecular information by PET
- Basic technology for the clincial combined PET/MRI systems (Siemens)





Judenhofer et al, Nat. Med. 2008

Therapymonitoring with conventional tracers

- **Characterization of a novel c-met inhibitor BAY853474**

- **c-Met:**

– proto-oncogenic receptor tyrosine kinase

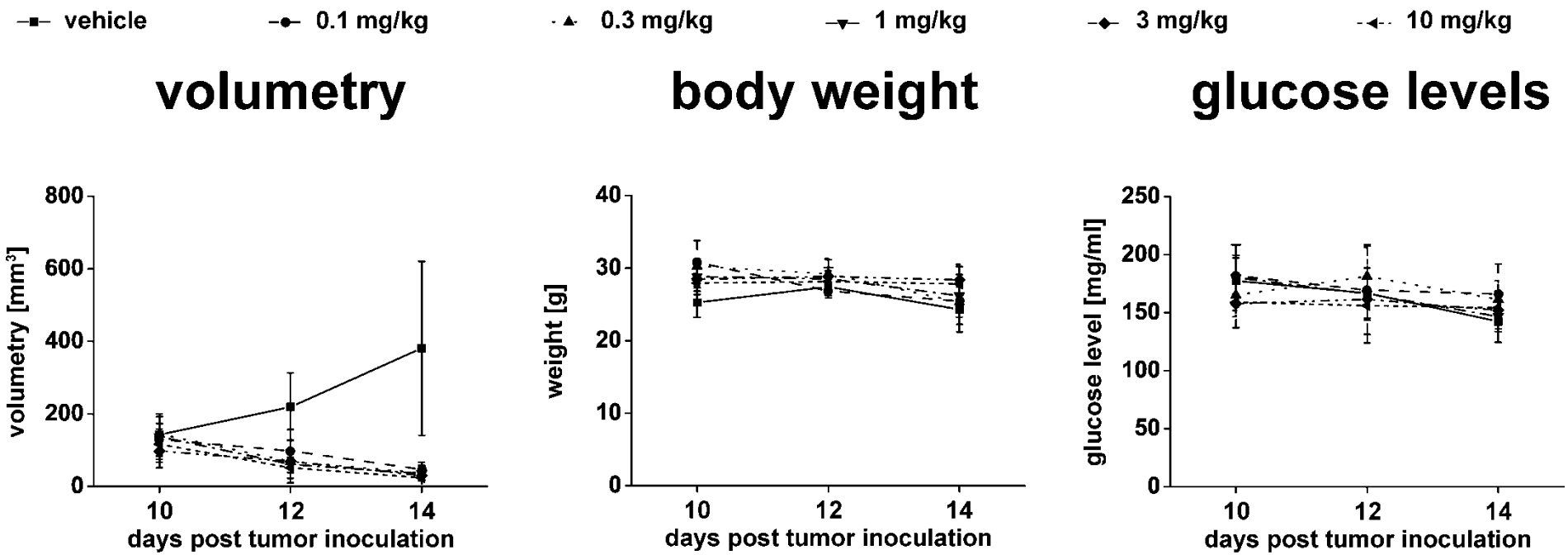
– proliferation, survival, motility, angiogenesis

– target for cancer therapy

- **Aim of the study:**

preclinial evaluation of a novel highly selective small molecule c-Met inhibitor with **[^{18}F]FDG**- or **[^{18}F]FLT**-PET and biomolecular analysis in the Hs746T gastric cancer xenograft model

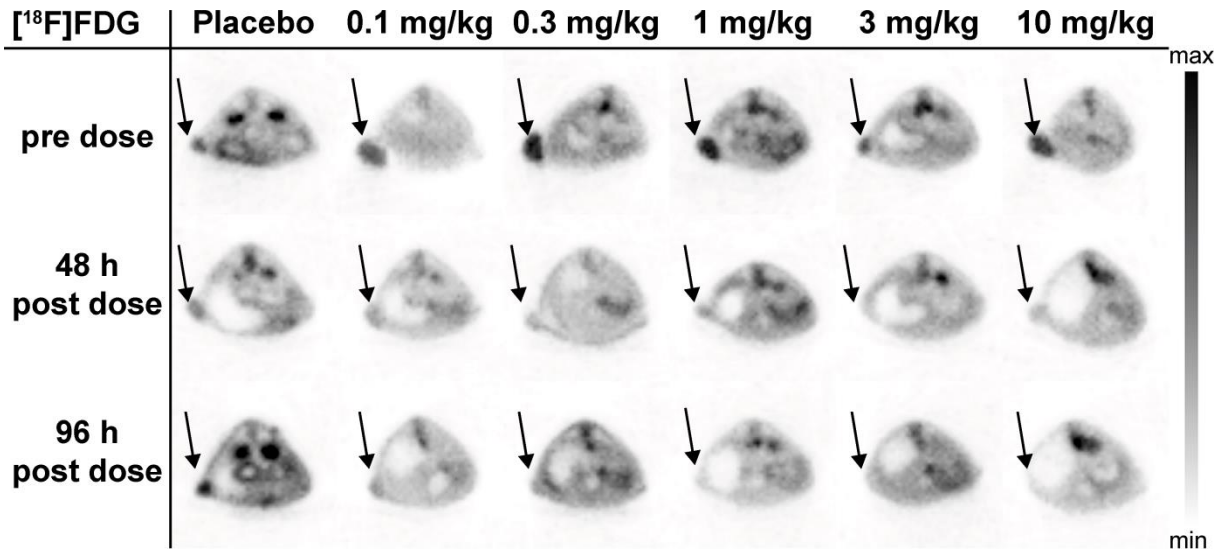
Results

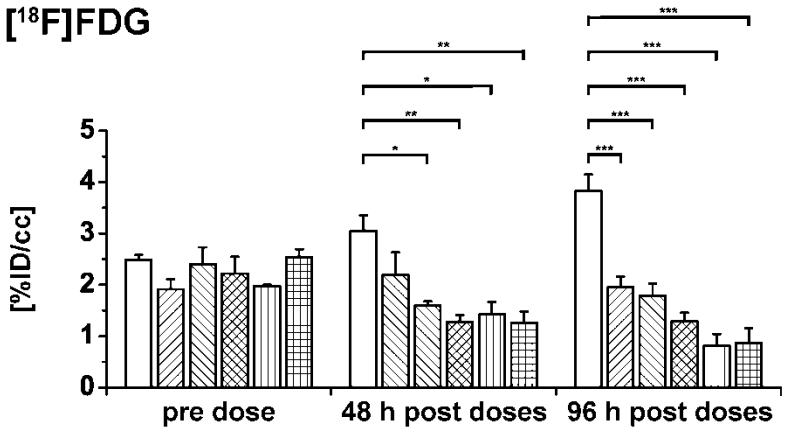


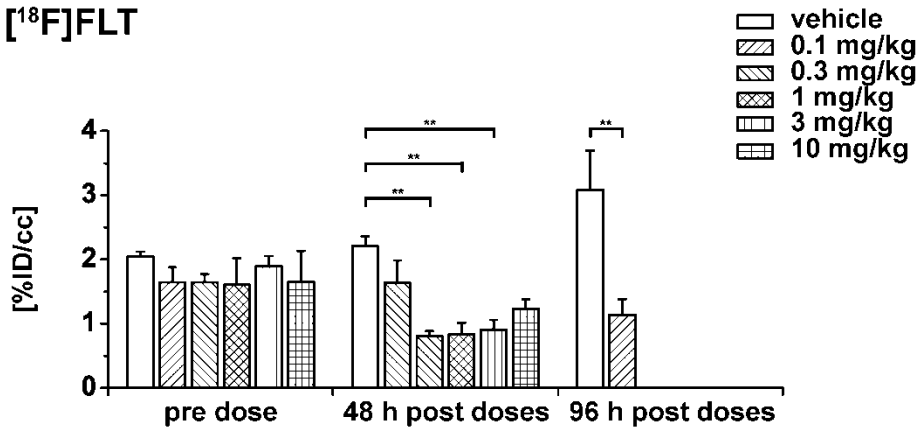
- **fast decrease of tumor volume**
- **no adverse side effects of treatment**

Wiehr et al., Mol Imaging Biol 2013

Results *in vivo* PET

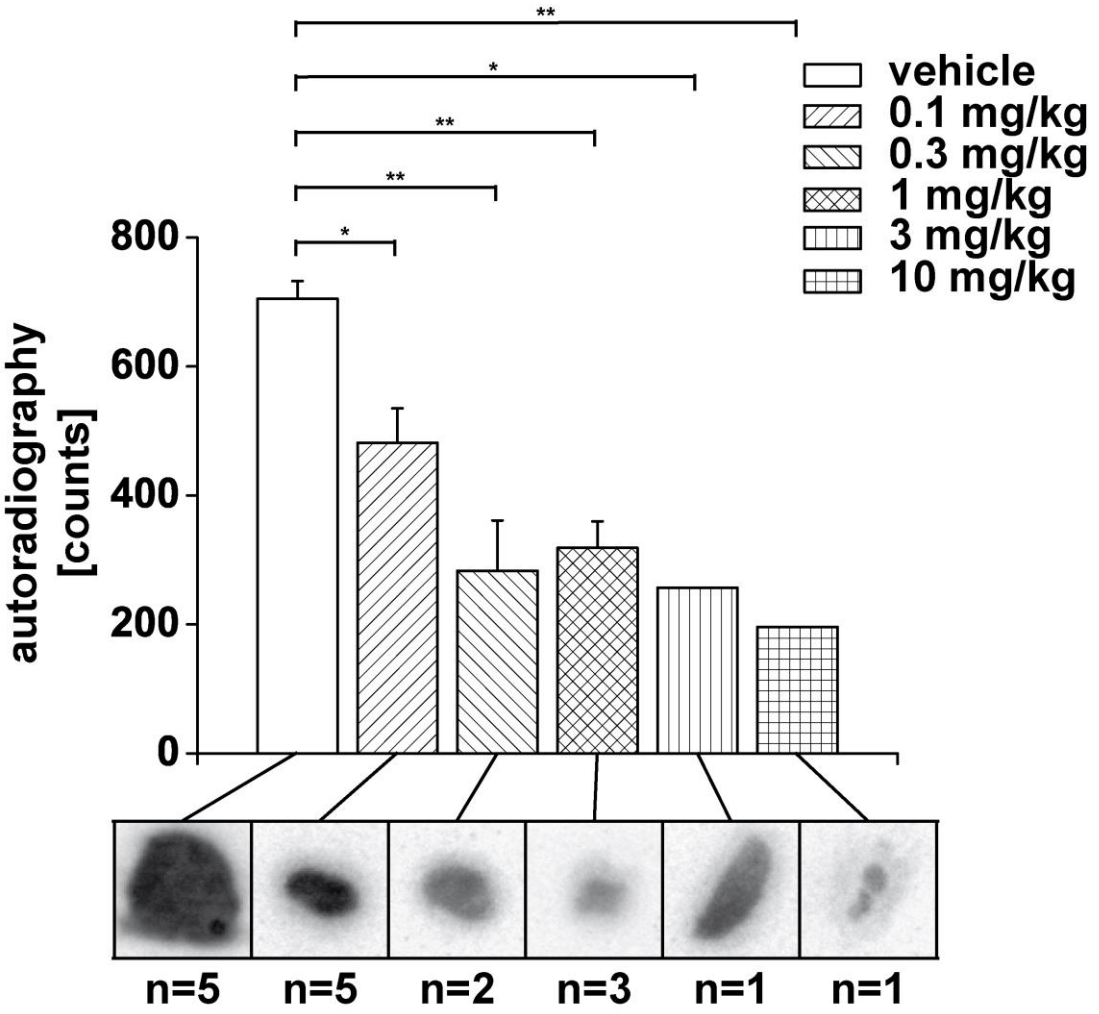






Wiehr et al., Mol Imaging Biol 2013

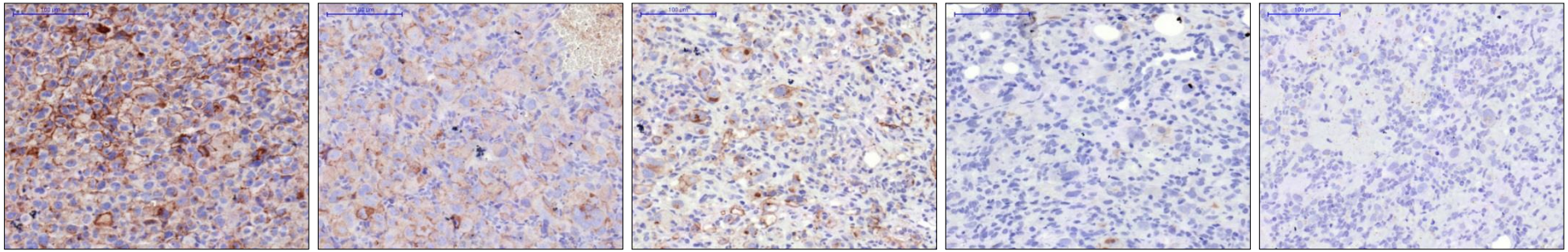
Autoradiography [^{18}F]FDG



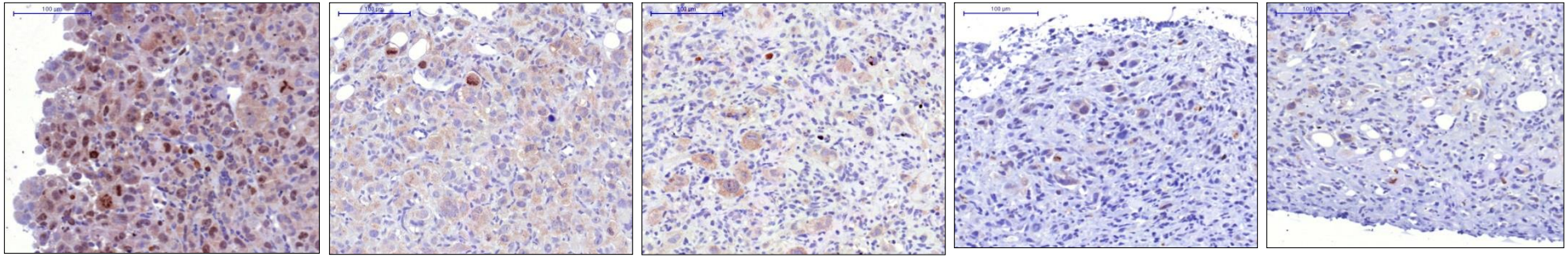
Wiehr et al., Mol Imaging Biol 2013

Ex vivo Results: Histology

vehicle	0.1 mg/kg	0.3 mg/kg	1 mg/kg	3 mg/kg



Glut1



Ki67

Wiehr et al., Mol Imaging Biol 2013

Conclusion: Therapy characterisation

- no adverse effects observed in mice
- strong tumor growth reduction
- weak [^{18}F]FDG and no [^{18}F]FLT uptake after 96h *p.i.*
- PET findings were confirmed by autoradiography and histology
- preclinical studies suggest c-Met inhibitor BAY-853474 for cancer therapy

In vivo PET/MR-spectroscopy

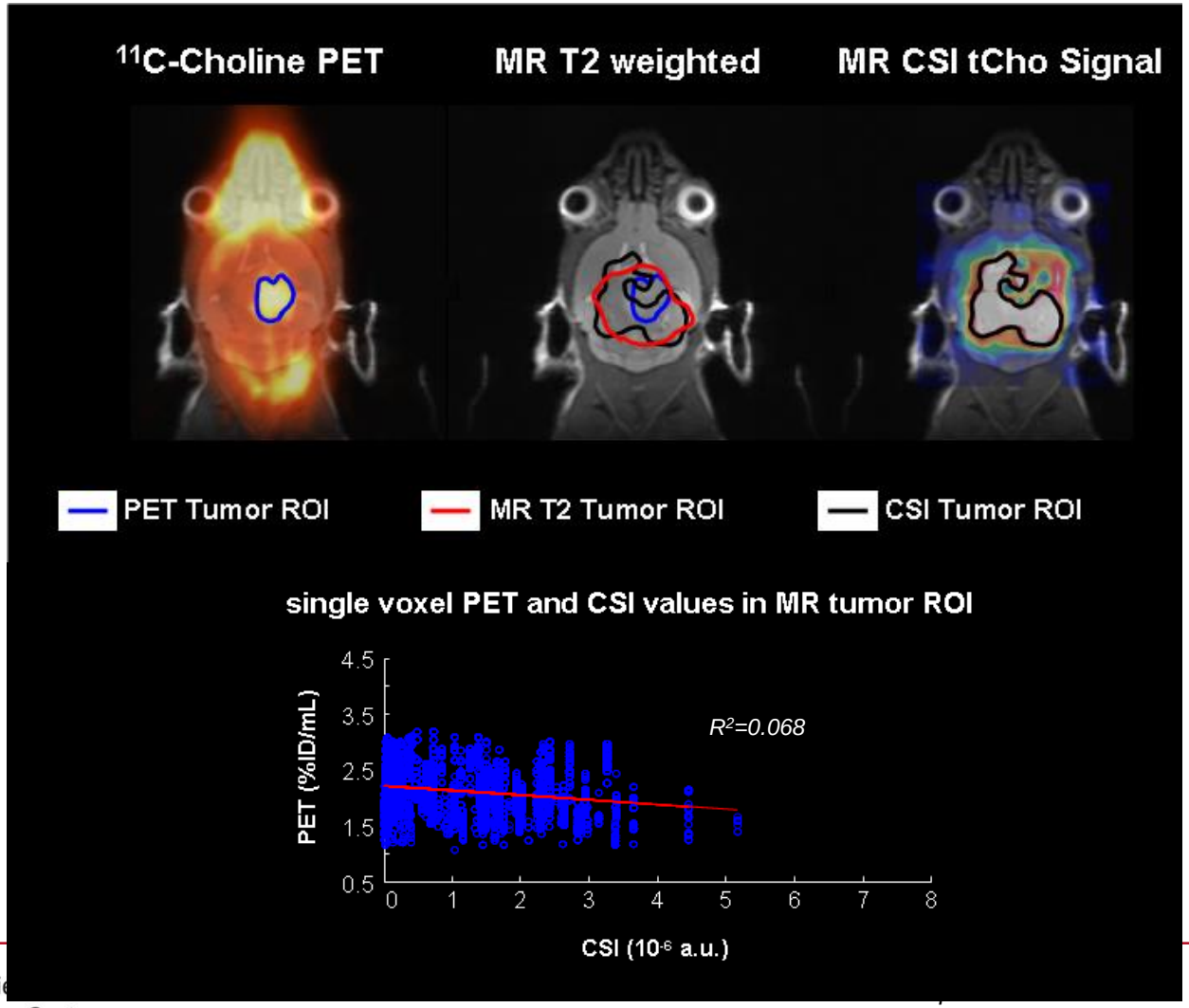
- metabolites, transporters and enzymes in choline metabolism are regarded as biomarkers for disease progression in a variety of cancers

- Comparison of magnetic-resonance spectroscopy (MRS, chemical shift imaging (CSI) of total choline (tCho)) and [^{11}C]choline PET can target these pathways

- Animal model: astrocytoma model SMA560 injected intracranially into syngeneic VM/DK mice

in vivo PET/MR spectroscopy

- *larger study performed (n=35 mice)*
- *mismatch of [^{11}C]choline and choline-CSI*



Wehrl HF et al. Cancer Research 2013 Mar 1; 73(5)

in vivo PET/MR spectroscopy

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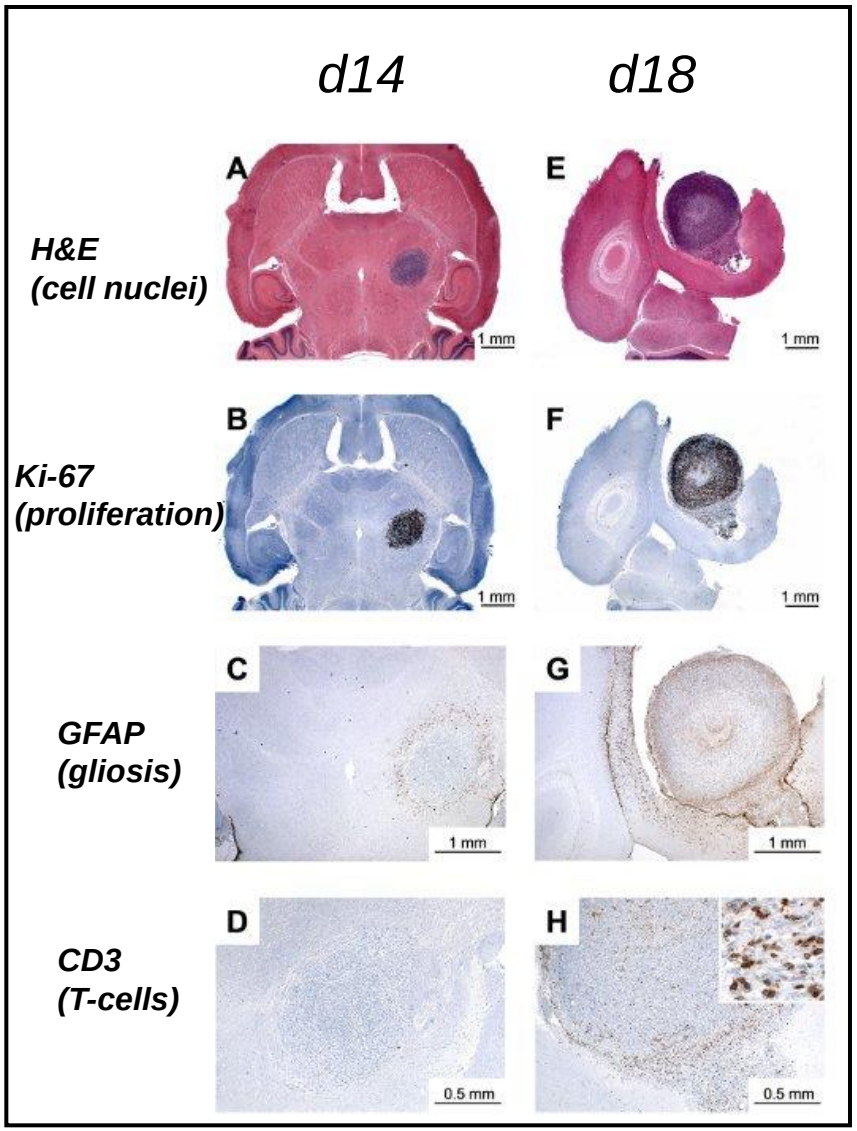
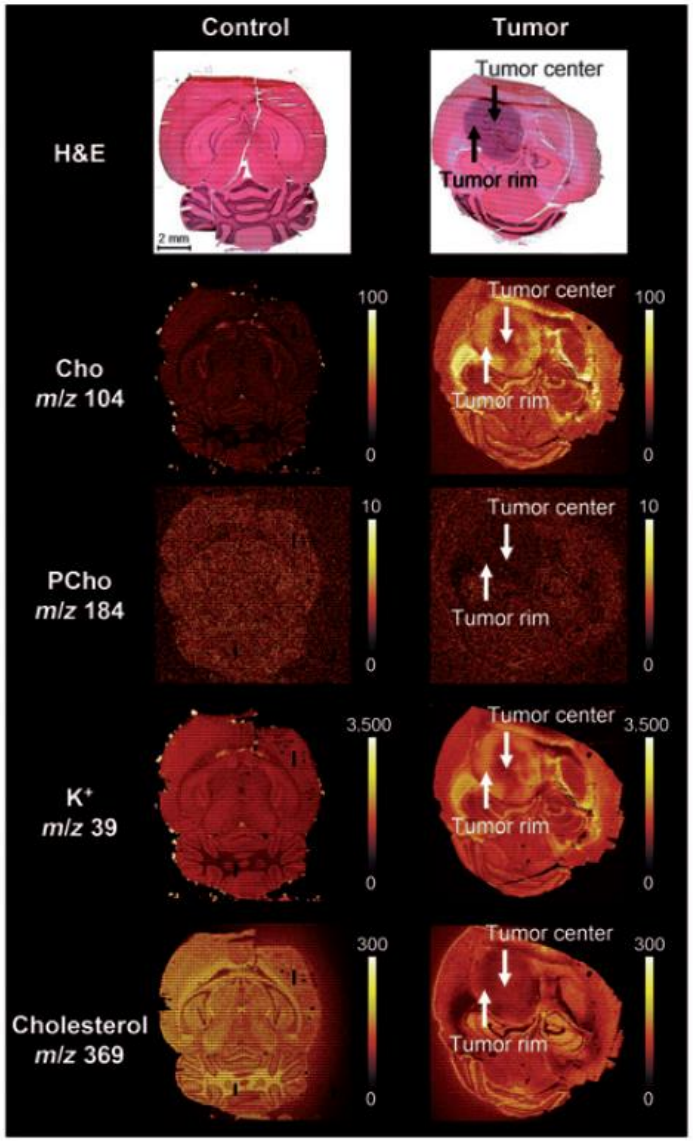
- *histological and secondary ion mass spectrometry imaging (SIMS) supports complimentarity of [^{11}C]choline-PET and tCho-CSI.*

Histology



SIMS



Summary: PET/MR-spectroscopy

- Concentration limit: PET 10^{-12} mol/L
  MRS 10^{-3} – 10^{-6} mol/L

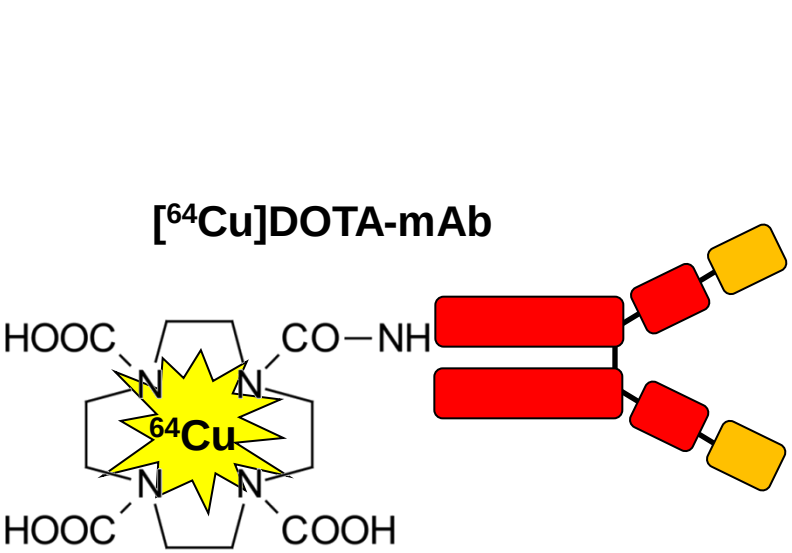
- [^{11}C]choline-PET and CSI showed complementary correlations
  PET → tumour center → proliferation
  CSI → tumour rim → inflammation

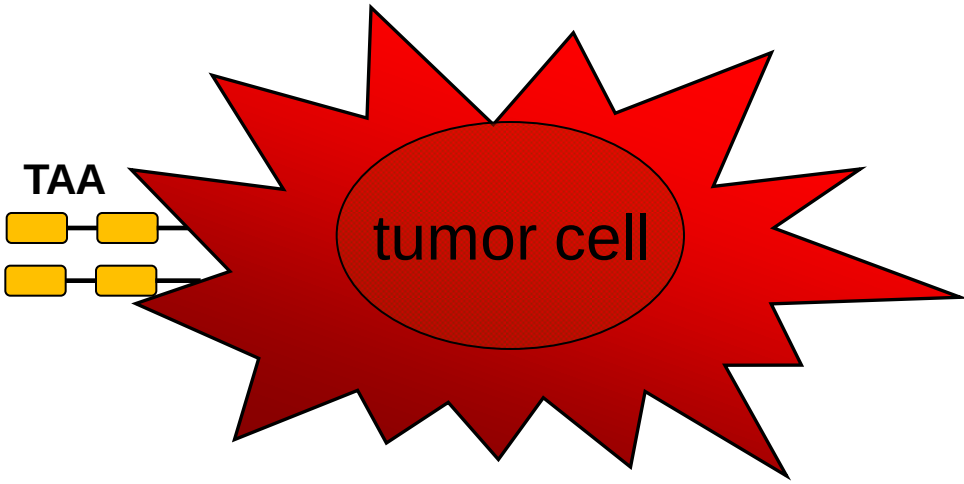
- [^{11}C]choline with higher potential as biomarker for therapy planning and monitoring

Immuno-PET

- Detection of tumors expressing tumor associated antigens (NY-Eso I, PSMA etc.)

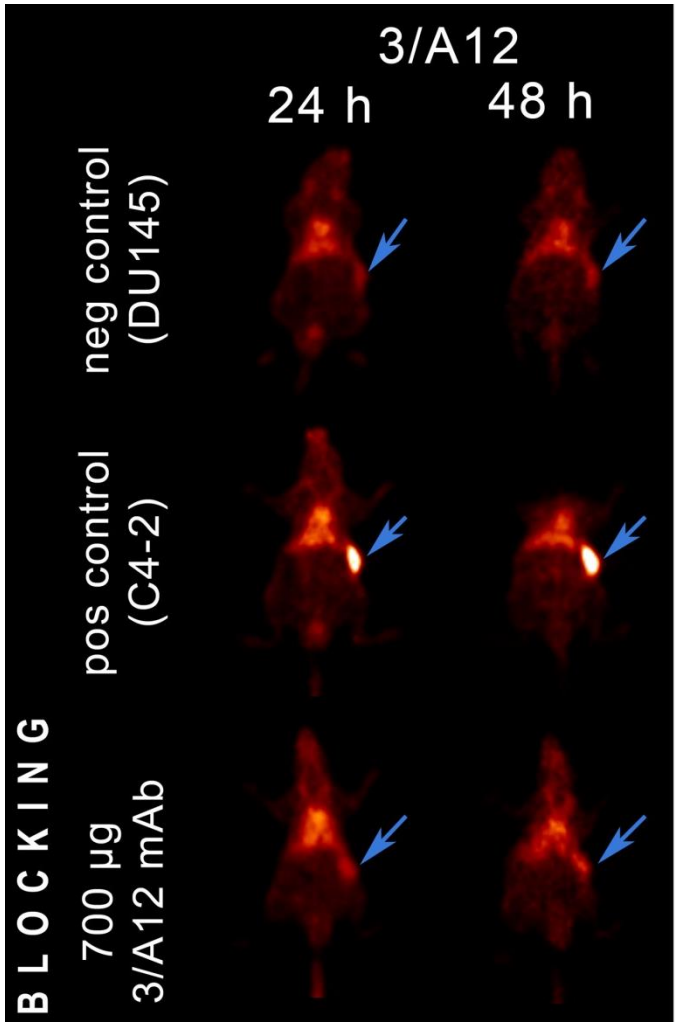
- Modifiying of monoclonal antibodies (mAb), mAb-fragments, minibodies or diabodies with chelators and subsequent radiolabeling with long living radioactive metals (^{64}Cu, ^{89}Zr).

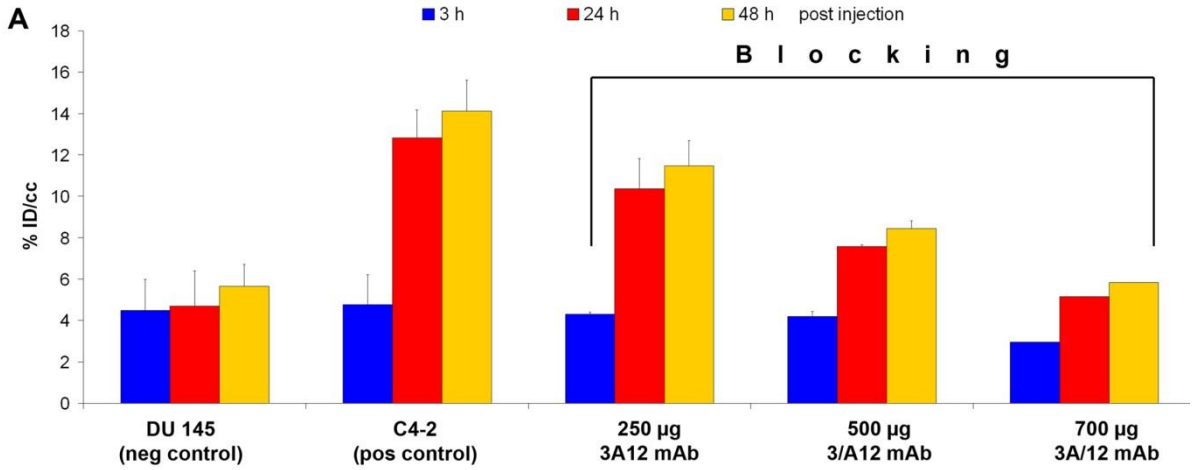




Immuno-PET

^{64}Cu-labelled PSMA-specific mAb 3/A12 for detection of subcutaneous PSMA-expressing tumors





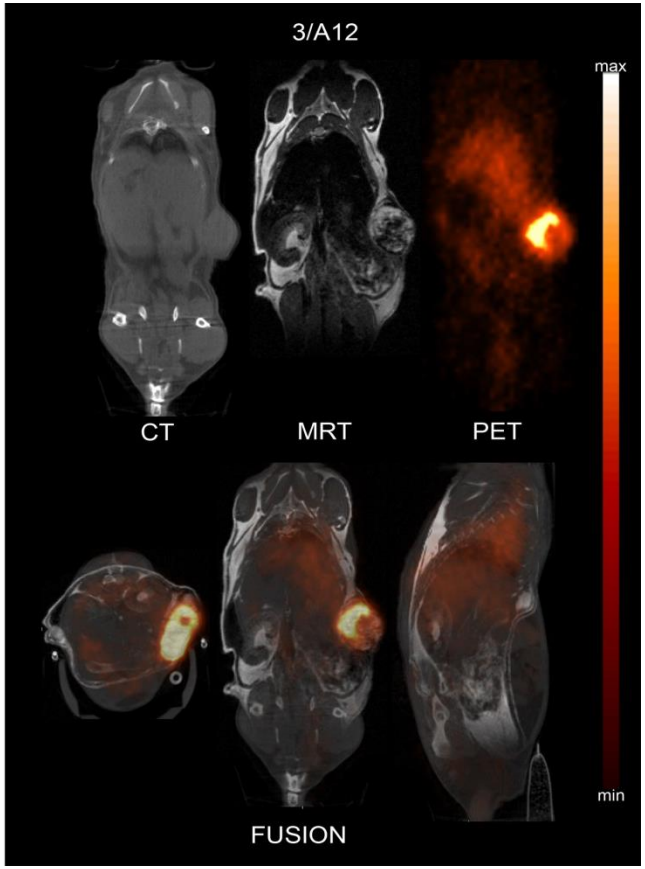
Blocking studies:

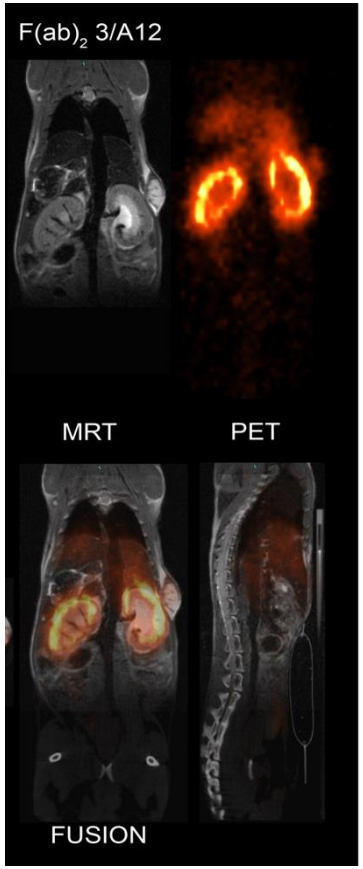
→Necessary to assess specific *in vivo* binding of the tumor

Elsässer-Beile et al., J Nucl Med 2009

PET/MRI

PET/MRI of ^{64}Cu-labelled PSMA-specific Abs / fragments





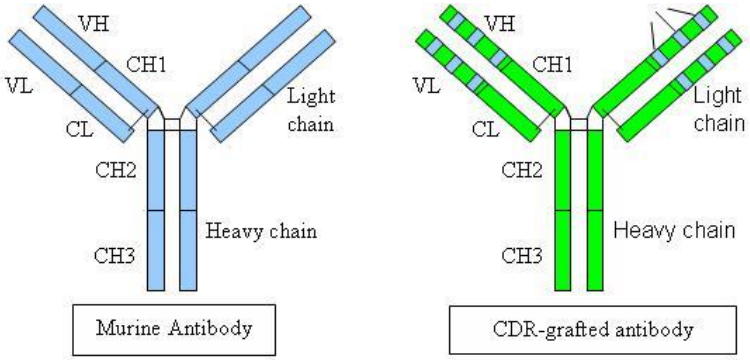
Alt et al., Prostate 2010

Pro: High uptake
Cons: slow plasma clearing
→ high background

Pro: fast plasma clearing via kidneys
→Low background
Cons: Low uptake

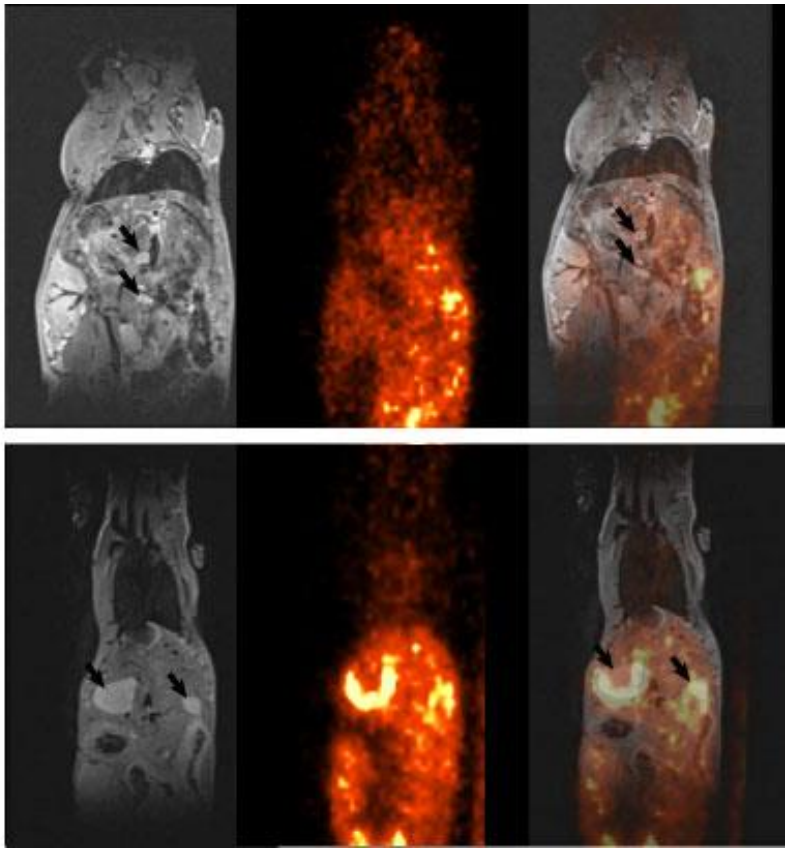
^{64}Cu-DOTA-M5A against CEA

- *CEA (CEACAM5)→ Carcinoembryonic Antigene*
  - *GPI-anchored surface protein*
  - *Upregulated in a wide range of carcinoma (colon, lung, breast, etc.)*
  - *In colon cancer, 95% of metastases of CEA-positive primary tumors show strong CEA expression*



- ***Aim:** Testing of the CEA-specific M5A-antibody in an orthotopic syngenic tumor model for detection of liver metastases*

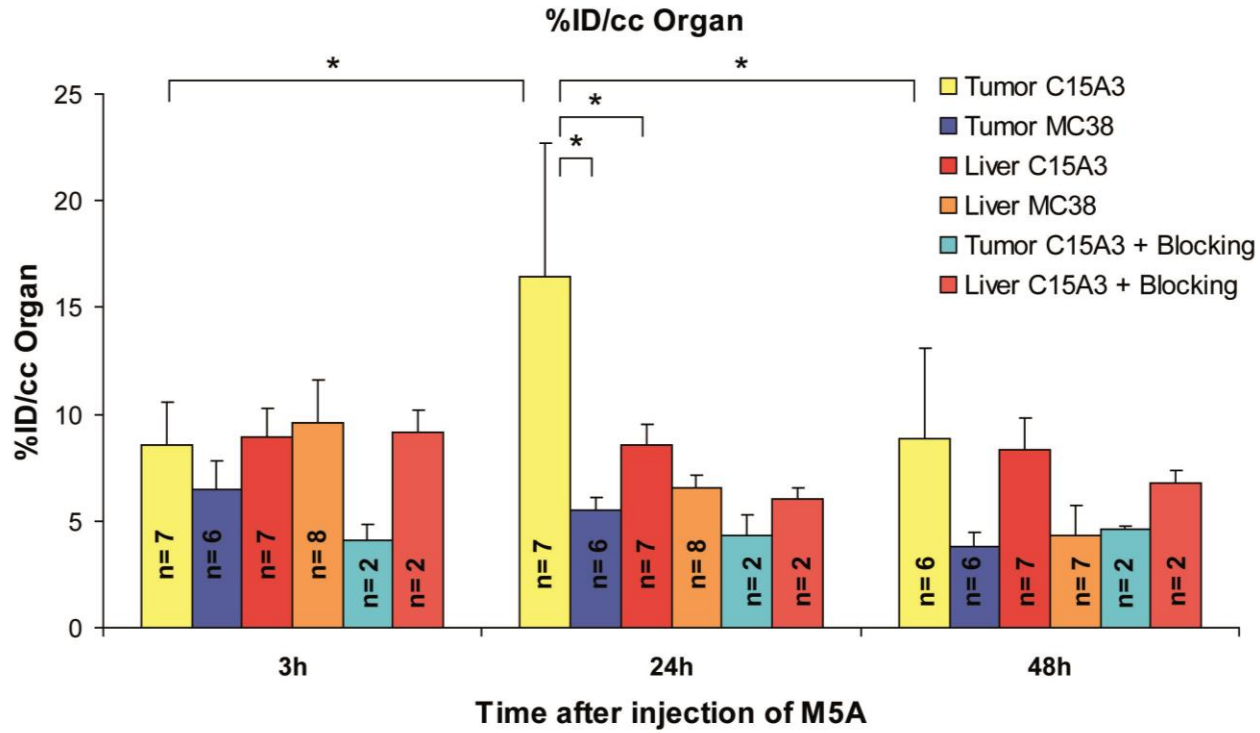
Results



24h after injection of M5A

Krueger et al. accepted

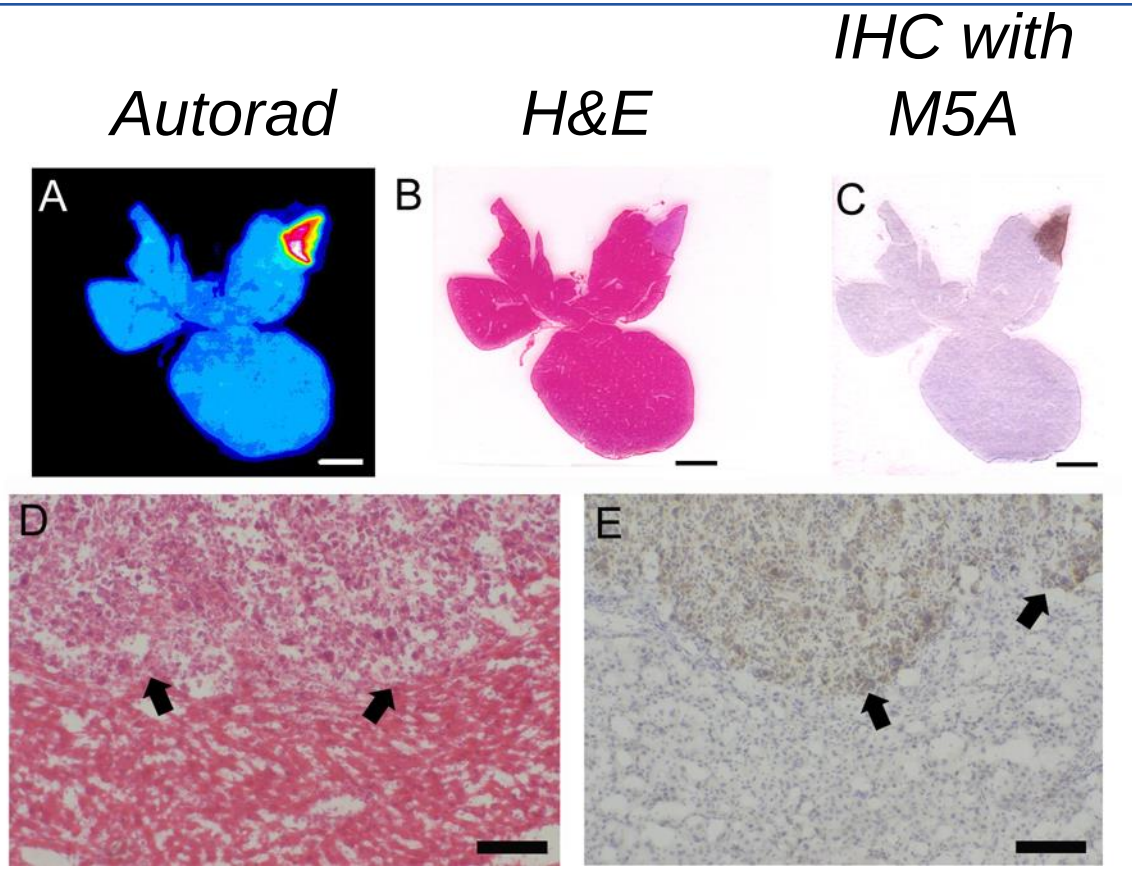
Results



- *Highest uptake after 24h*
- *Significantly higher uptake in CEA-positive metastases in comparison with CEA-negative metastases*
- *Significantly higher uptake in CEA-positive metastases in comparison to liver tissue*
- *Strong decrease after blocking*

Krueger et al. accepted

Results



- *Autoradiography supports inhomogeneous antibody distribution*
- *Immunohistochemistry shows homogeneous CEA expression*
- *H&E staining schows necrotic areas that might explain inhomogeneous antibody distribution*

Krueger et al. accepted

Summary: Immuno PET

TAA-detection to visualize tumours is easily conductible

Study 1: PSMA

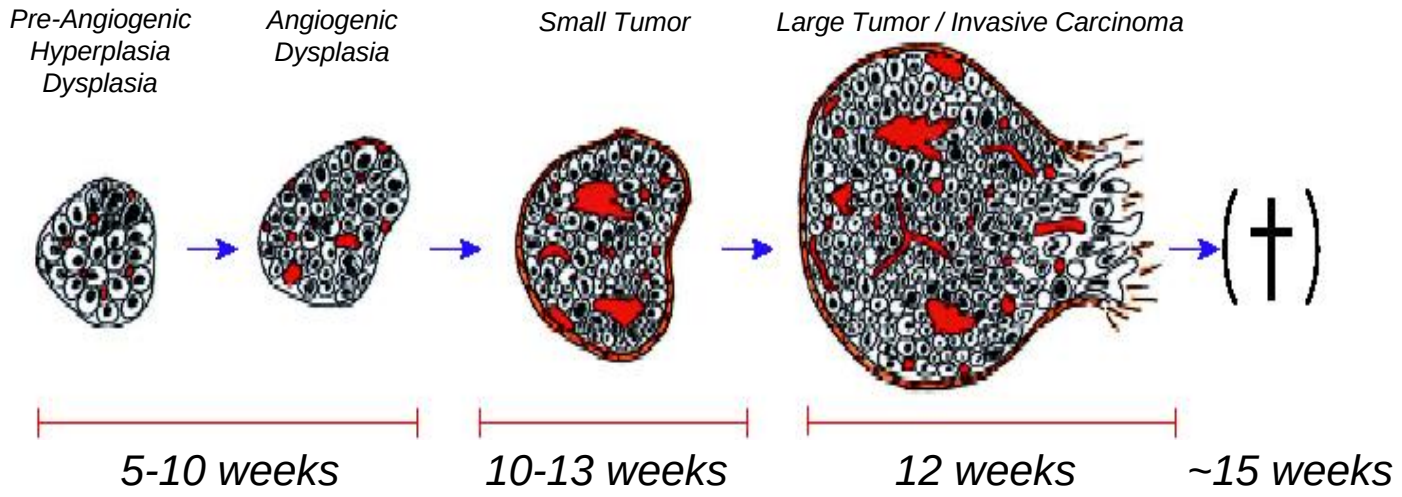
- Specific Binding of PSMA-mAb 3/A12 after 24 h
- 3/A12-mAb-fragments: low uptake, fast clearance

Study 2: CEA

- Visualization of CEA-positive liver metastasis with M5A is possible
- M5A-binding is specific as shown with CEA-metastases, blocking experiments and unspecific control antibody

Peritoneal tumor: RIP1-Tag2

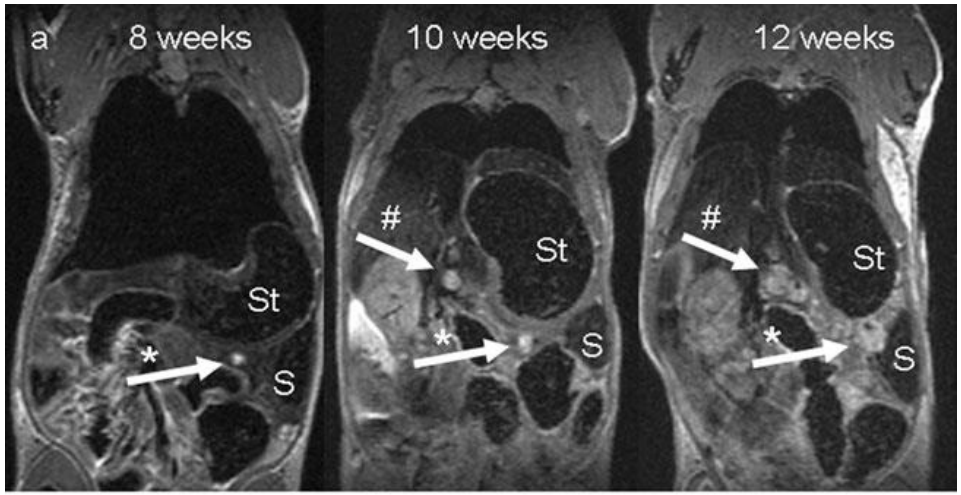
- **RIP1-Tag2 Mouse** (D.Hanahan 1985)
  - → Insulin producing β-cells of the pancreas express the oncogen T antigen (Tag2) of the Simian-Virus 40 under control of the rat insulin promoter (RIP1)
  - → Tag protein promotes malignant transformation by inhibition of the tumor suppressor genes p53 and Rb
  - → **Blood glucose level** as marker of tumor burden

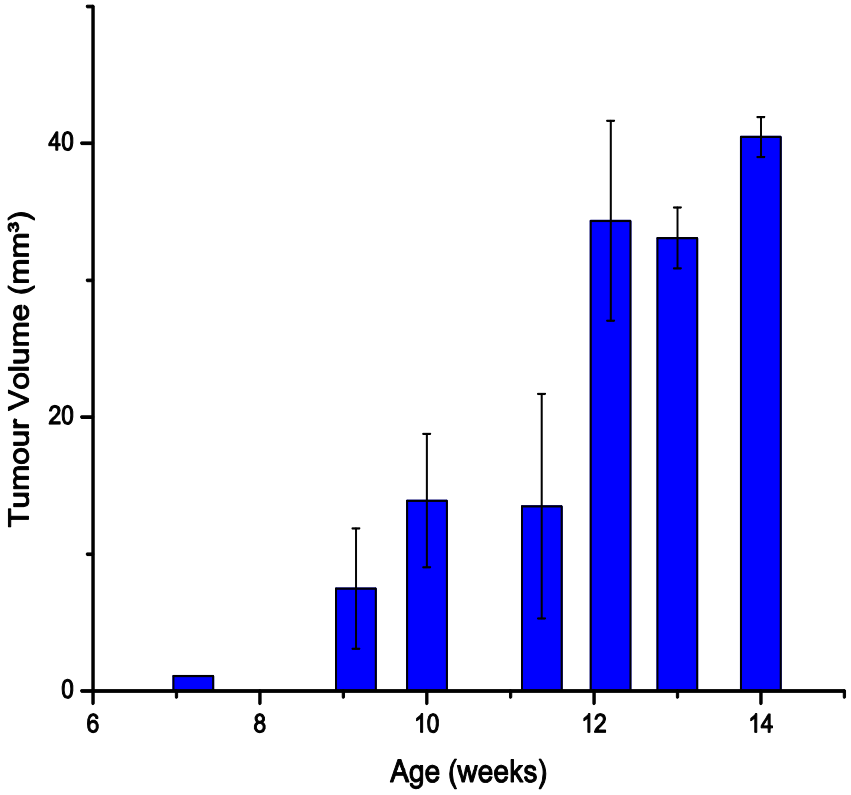


Scheme of tumor development in the RIP1-Tag2 mouse model, G.Bergers, et al.: Science 1999

Detection of Pancreatic Tumors

Monitoring tumor growth in RIP1-Tag2-transgenic mice
→ Spontaneous induction of β-cell insulinomas in the pancreas





Schmid et al., Mol imaging Biol 2013

Imaging of Metastasis

- Evaluation of an animal model for rhabdomyosarcoma (RMS) metastases
- *i.p.* transfer of mCherry/gaussiaLuciferase transgenic RMS-cells in immunedeficient mice

Evaluation of the RMS metastasis model

- Monitoring tumor growth (OI) by fluorescence, bioluminescence
- Tracer evaluation
  - $[^{18}F]$FDG → glucose consumption
  - $[^{18}F]$FLT → proliferation (TK-activity)
  - $[^{11}C]$choline → proliferation (membrane synthesis)
  - MRI → anatomy
- Therapy with the chemotherapeutic vincristine

In vivo OI

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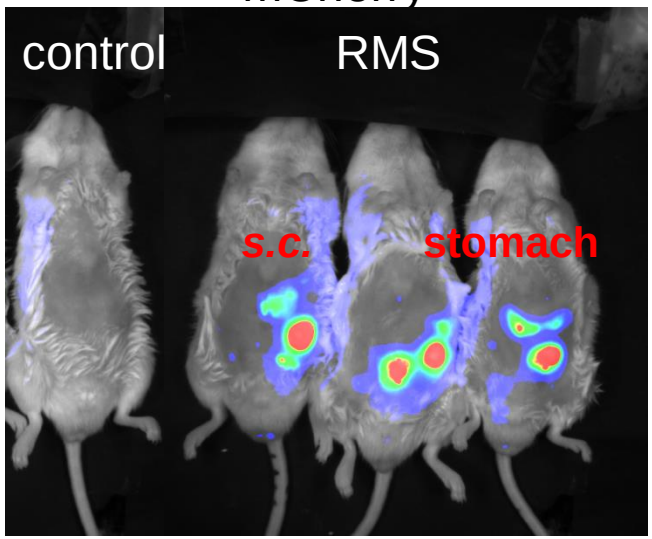
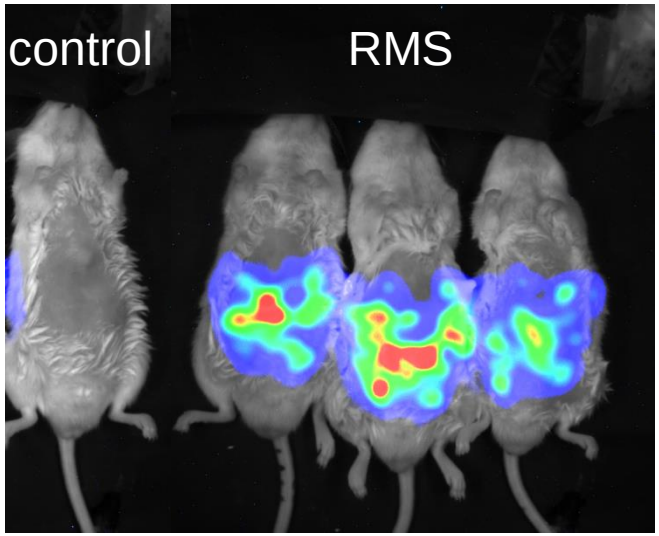
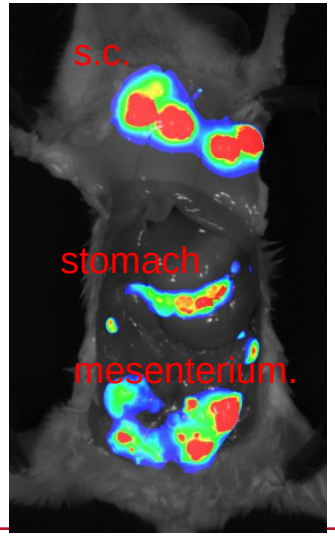
	fluorescence mCherry	bioluminescence Gaussia-Luciferase
In vivo OI		
Ex vivo OI		

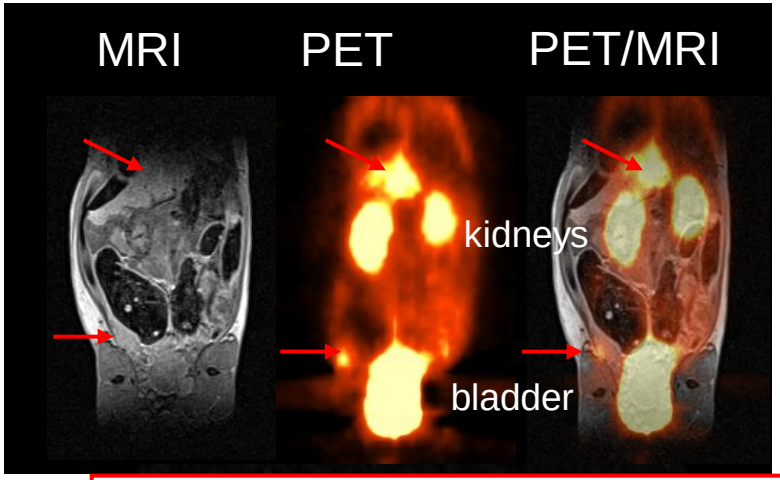
Pro: Monitoring of subcutaneous metastases
Ex vivo detection of small metastases

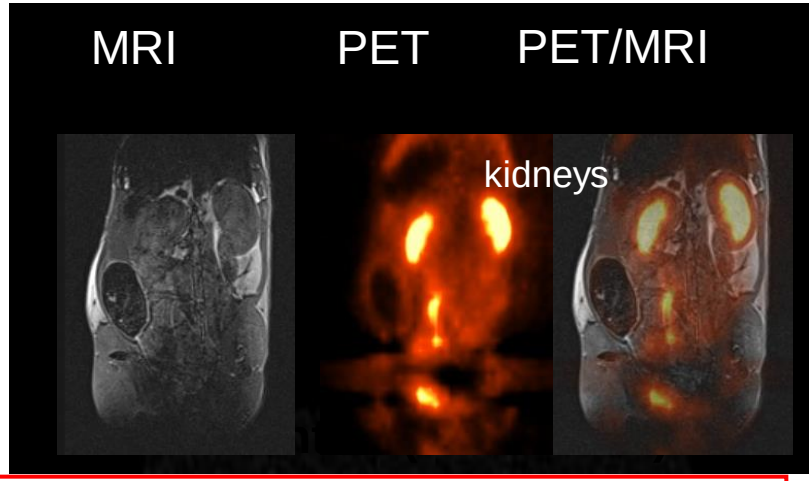
Cons: no *in vivo* detection by bioluminescence
no *in vivo* detection of peritoneal metastases

Armeanu-Ebinger, Griessinger et al., JNM 2014

Sequential PET/MRI

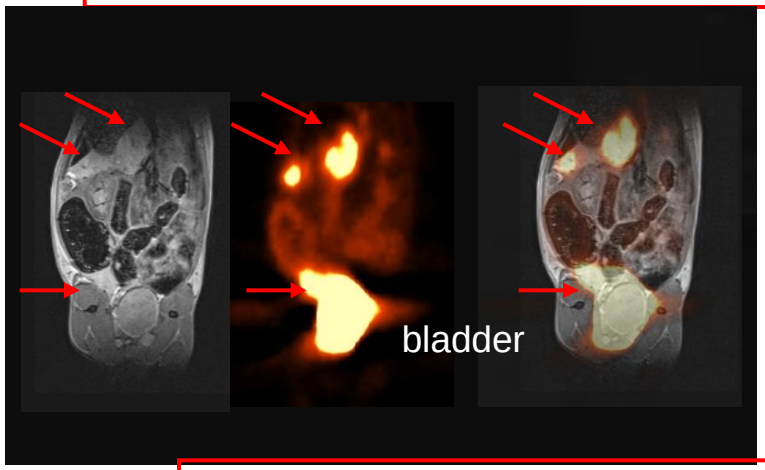
[^{18}F]FDG (glucose consumption)

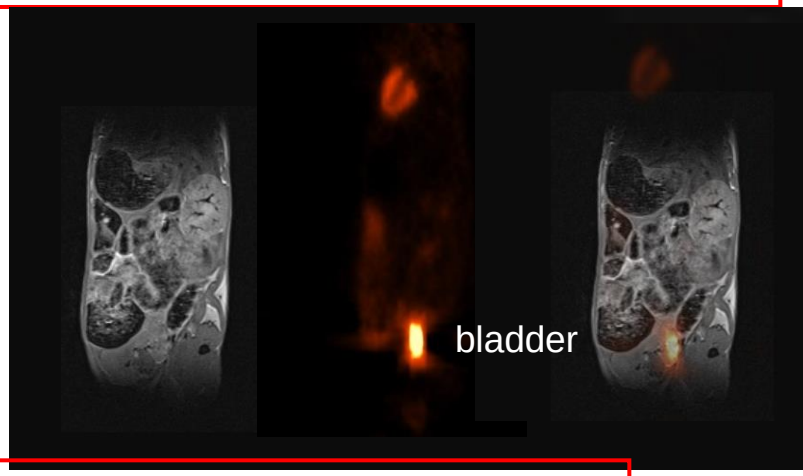




Pro: high RMS-uptake **Cons:** high background (kidneys, intestine, bladder)

[^{18}F]FLT (proliferation)

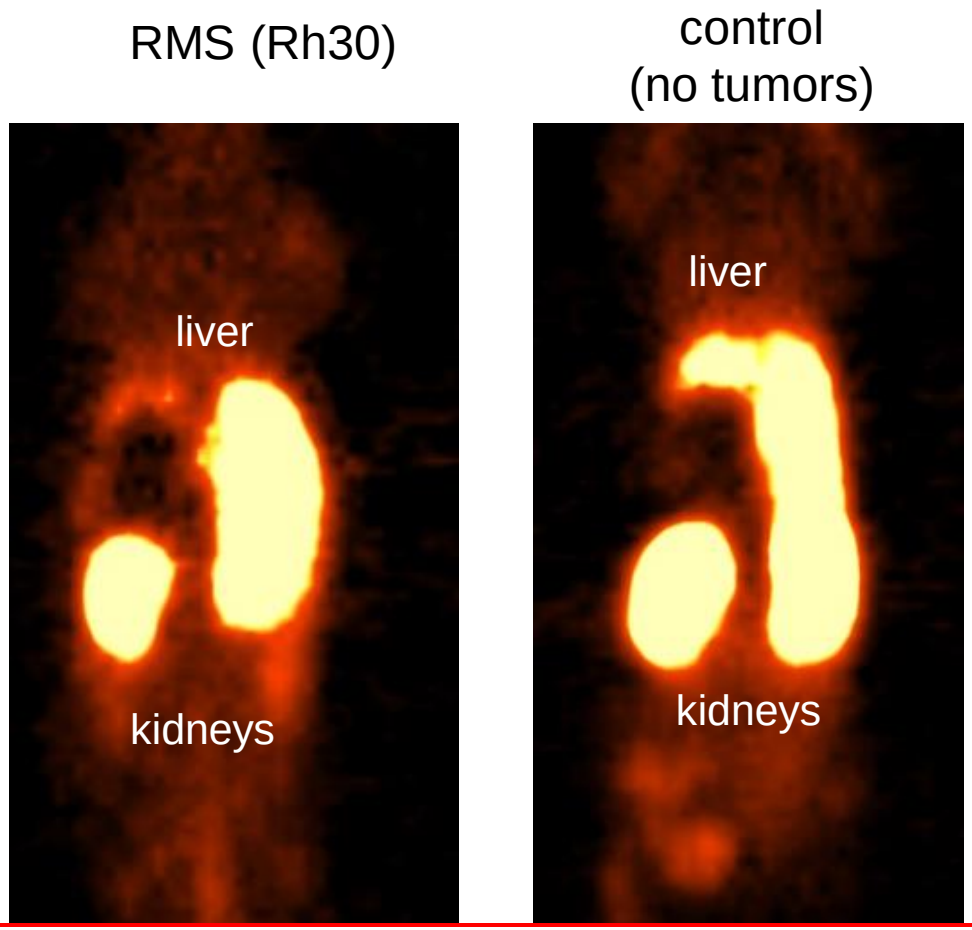




Pro: high RMS-uptake **Cons:** low background (only bladder)

Armeanu-Ebinger, Griessinger et al., JNM 2014

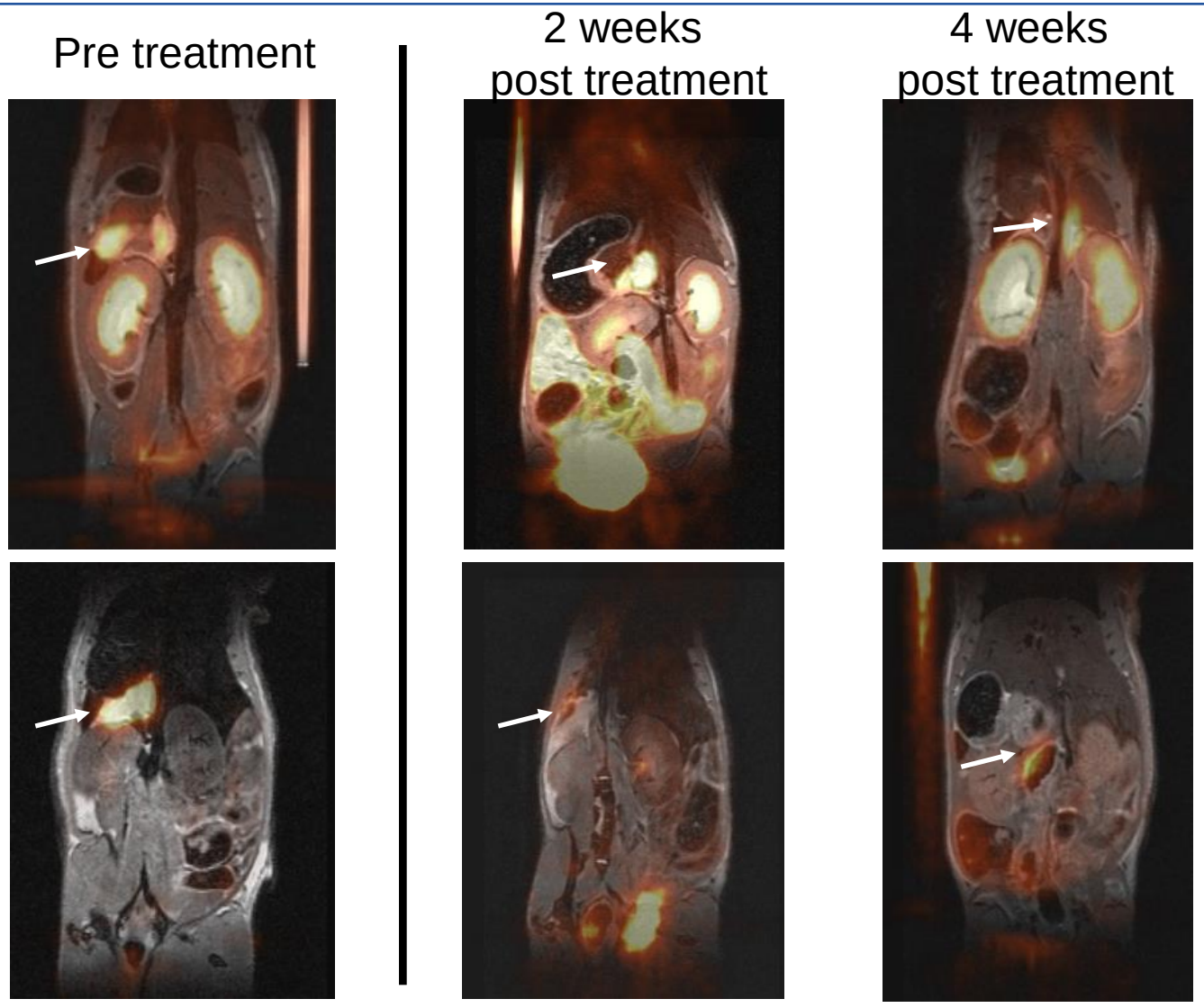
PET with [^{11}C]Choline



Pro: no bladder-uptake
Cons: no/very low RMS-uptake, high background (kidneys, liver)

Armeanu-Ebinger, Griessinger et al., JNM 2014

Therapy with vincristine



Armeanu-Ebinger, Griessinger et al., JNM 2014

Summary: Detection of RMS-metastasis

- [^{18}F]FLT is superior to [^{18}F]FDG for initial metastasis detection due to a high signal/background ratio

- After therapy [^{18}F]FDG is superior because antiproliferative therapy abolished [^{18}F]FLT uptake

General summary

- All imaging approaches provide powerful possibilities in various experimental setups
  (therapy, phenotyping, TAA-expression, metastasis, cell tracking etc.)

- but application specific limitations have to be considered
  OI: limited penetration depth, high sensitivity
  PET: high sensitivity, low resolution, very comprehensive
  MRI: high resolution, lower sensitivity, expensive

  → the combination of different modalities compensate for these limitations

- cross validation with common *ex vivo* methods (biodistribution, autoradiography, histology, PCR) necessary

Outlook:

- *implementation of spectroscopy (MALDI, NMR) as routinely used alternatives to biodistribution, autoradiography, etc.*

Thank you for your Attention!