Per quanto concerne i moderatori, relatori, formatori, tutor, docenti è richiesta dall'Accordo Stato-Regioni vigente apposita dichiarazione esplicita dell'interessato, di trasparenza delle fonti di finanziamento e dei rapporti con soggetti portatori di interessi commerciali relativi agli ultimi due anni dalla data dell'evento.
La documentazione deve essere disponibile presso il Provider e conservata per almeno 5 anni.

Dichiarazione sul Conflitto di Interessi

Il sottoscritto_____**ROSSELLA CANESE**_____ in qualità di:

□ responsabile scientifico □ moderatore □ docente □ relatore
□ tutor

dell'evento "X CONGRESSO AIRMM - RISONANZA MAGNETICA IN MEDICINA 2019:
DALLA RICERCA TECNOLOGICA AVANZATA ALLA PRATICA CLINICA"
Milano, 28-29 marzo 2019

da tenersi per conto di **Biomedia srl Provider n. 148,**

ai sensi dell'Accordo Stato-Regione in materia di formazione continua nel settore "Salute" (Formazione ECM) vigente,

Dichiara

X che negli ultimi due anni NON ha avuto rapporti anche di finanziamento con soggetti portatori di interessi commerciali in campo sanitario

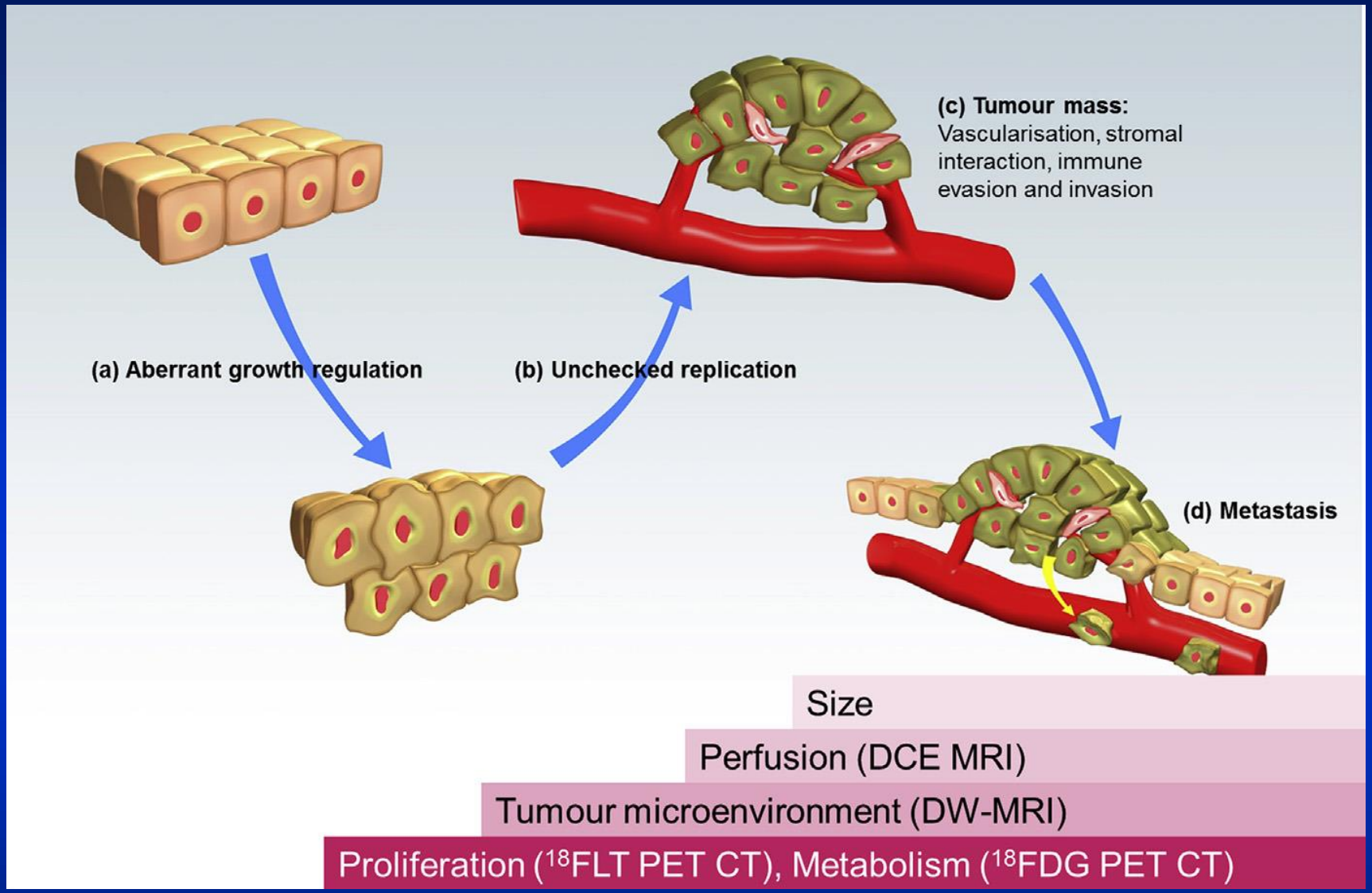
Rossella Canese

MRI Unit - Core facilities

Istituto Superiore di Sanità, Rome, Italy

MRI Biomarkers for assessment of therapeutical efficacy of experimental drugs

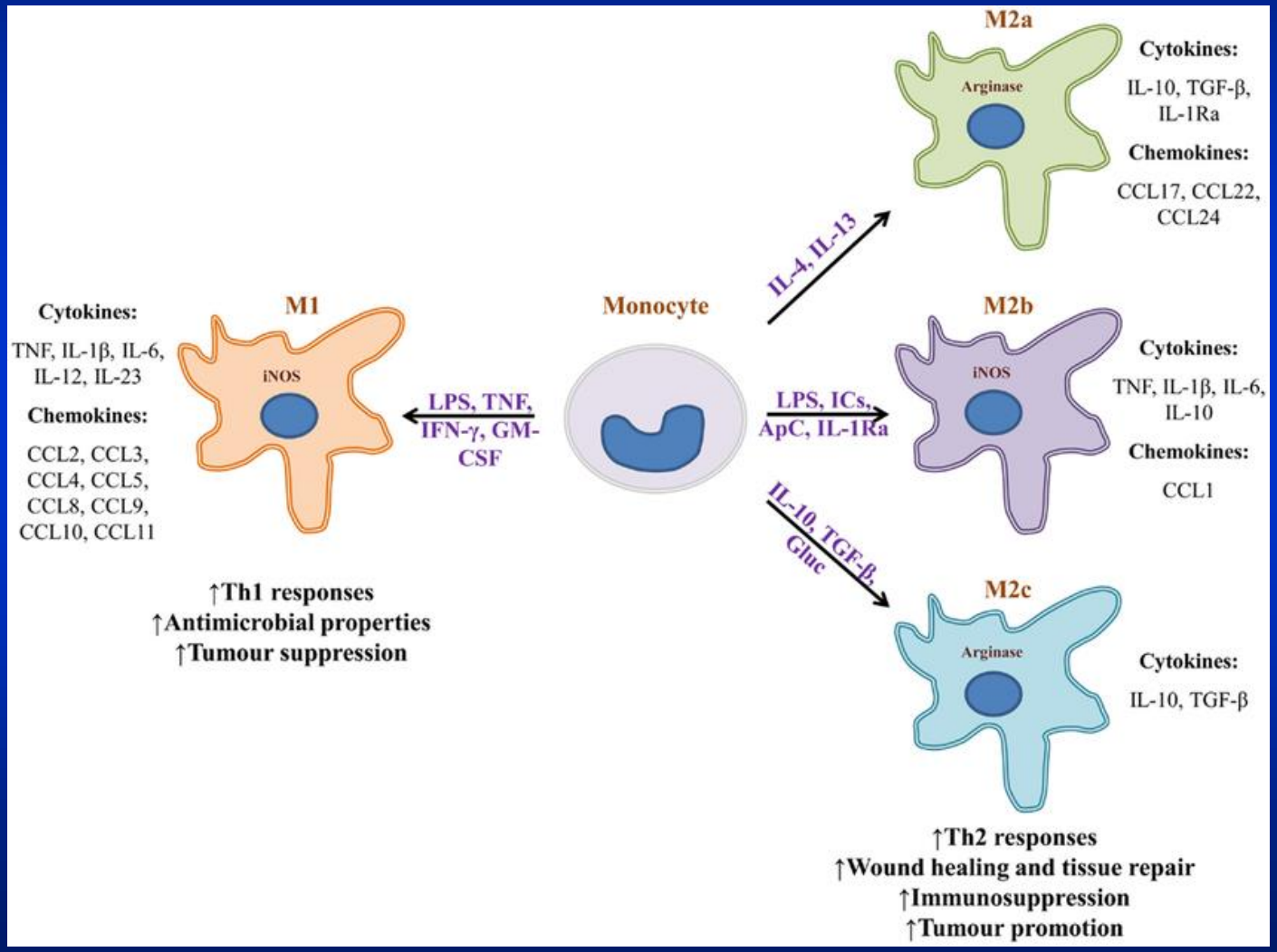
Servizio Grandi Strumentazioni e Core facilities – ISS
X congresso AIRMM - Milano 28 Marzo 2019

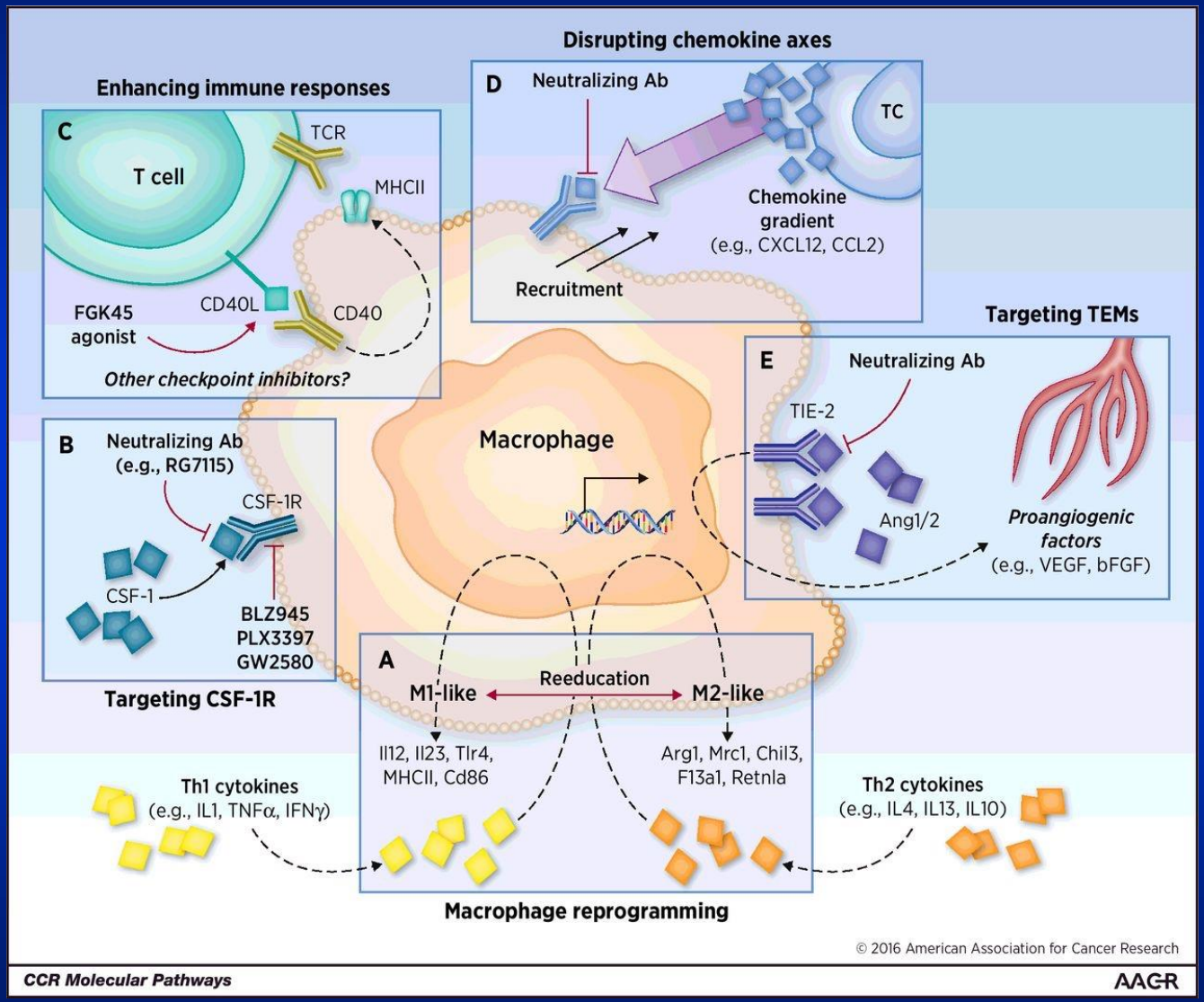


Correlation between imaging modalities and the mechanistic steps involved in tumour growth, vascularization, stromal interaction and metastasis.

From Weller et al, European Journal of Cancer 59 (2016) 65e78

Immune escape mechanisms in cancer: macrophage M_2 polarization





© 2016 American Association for Cancer Research

In vivo MRI probes to visualize TAMs

1H MRI **Ferumoxitol (USPIO)** T2*w MRI	1H MRI **Gd-DTPA + Abs** T1w MRI	19F MRI **Perfluorocarbon** (PFC) emulsion
Ferumoxytol, a FDA-approved ultra-small iron oxide nanoparticle (USPIO) used to treat anemia and recently used to monitor TAMs in a human glioblastoma	**Gadolinium** is not readily phagocytosed to macrophages, so they must be conjugated to antibodies that bind macrophage receptors	**PFC** is a perfluoropolyether that contains fluorine atoms and is formulated as an emulsion. An average droplet size of 165nm allows for passive targeting of macrophages

Ferumoxytol to monitor TAMs in GBM

MRI monitoring of monocytes to detect immune stimulating treatment respo

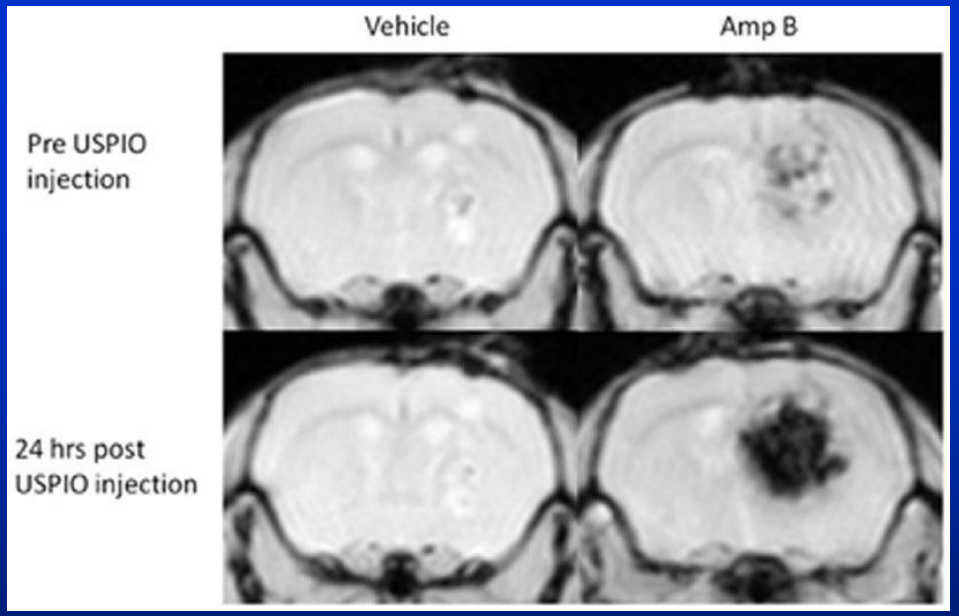
Runze Yang, Susobhan Sarkar, Daniel J. Korchinski, Ying Wu, V. Wee. Yong, Jeff F. Dunn

364 Neuro-Oncology 19(3), 364–371, 2017

The authors have shown that innate immune stimulation in an animal model of glioblastoma can be tracked using **ferumoxytol.**

The innate immune system can be stimulated with **amphotericin B (Amp B)** to reduce tumor growth *in vivo*. As few as 7 days of treatment with Amp B can cause an increase in tumor iron signal (measured with a 9.4T Bruker MRI and a multi-GE sequence to map T2*), indicating an increase in ferumoxytol and thus monocyte infiltration into the tumor

Immunohistochemistry, confirmed a significantly higher number of macrophage/microglia in the tumor of Amp B-treated animals compared to vehicle, and that macrophage/microglia were co-localized with iron staining.



Ferumoxytol polarizes TAMs into an anti-tumor phenotype

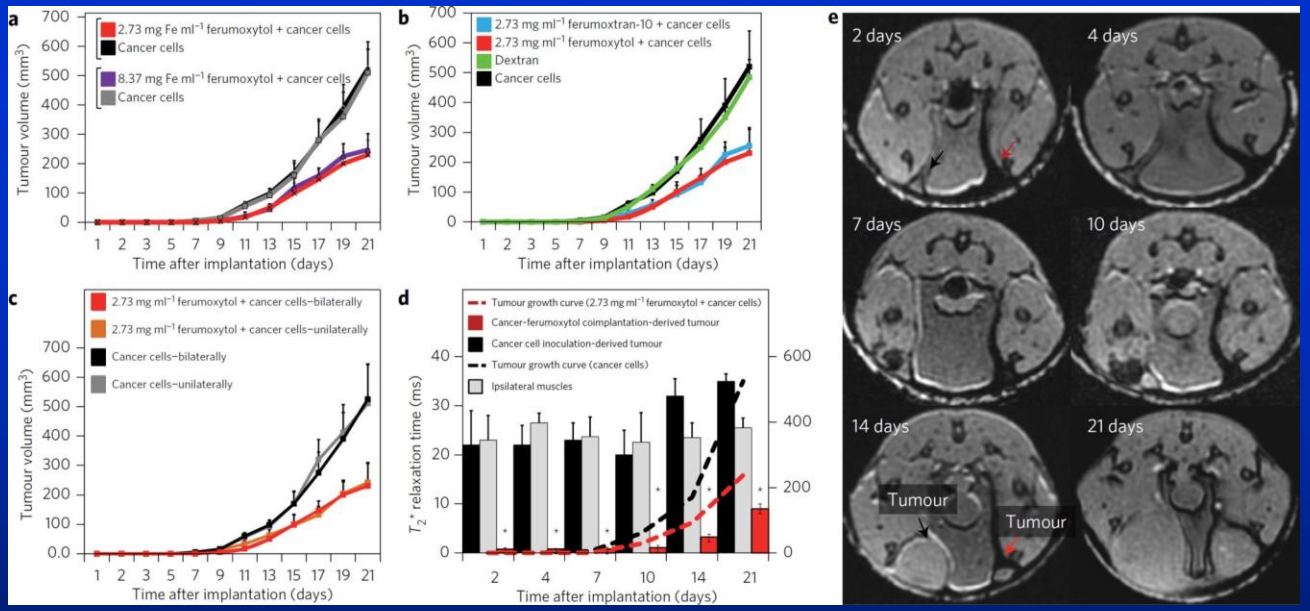
Iron oxide nanoparticles inhibit tumour growth by inducin pro-inflammatory macrophage polarization in tumour tissues

Saeid Zanganeh[1,2], **Gregor Hutter**[2,3], **Ryan Spitler**[1], **Olga Lenkov**[1,2], **Morteza Mahmoudi**[4], **Aubie Shaw**[5], **Jukka Sakari Pajarinen**[6], **Hossein Nejadnik**[1,2], **Stuart Goodman**[6], **Michael Moseley**[1], **Lisa Marie Coussens**[5], and **Heike Elisabeth Daldrup-Link**[1,2,7,*]

[1]Department of Radiology, Molecular Imaging Program at Stanford, Stanford University, 725 Welch Road, Stanford, California 94305, USA

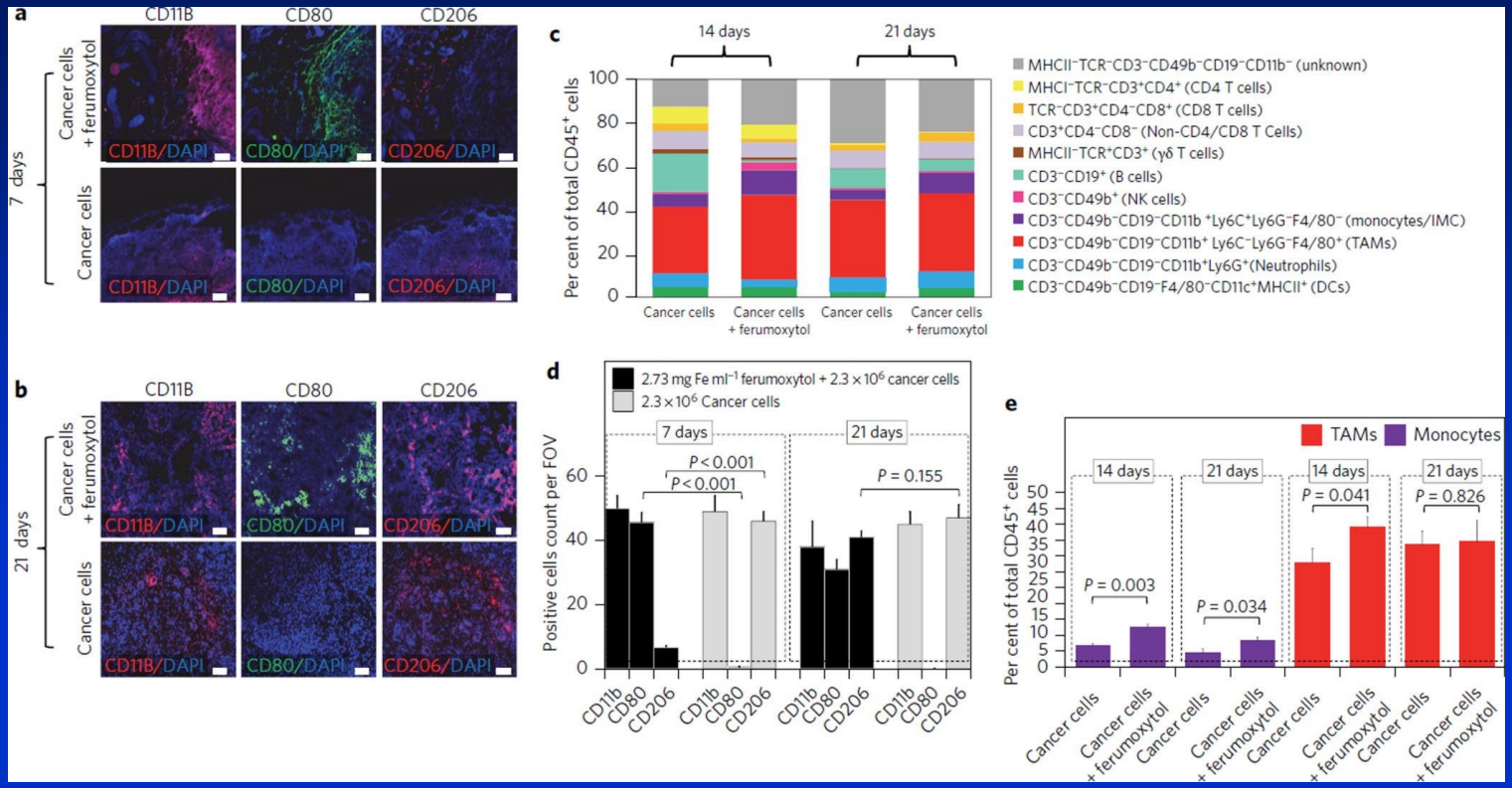
Nat Nanotechnol. 2016 November ; 11(11): 986–994.

Intravenous injection of ferumoxytol can suppress tumor growth in several mouse tumor models, and ferumoxytol allowed its own therapeutic effects to be detected by monitoring its metabolism by using a multi-GE to quantify T2* with a 7T MRI.



MMTV-PyMT-derived cancer cells into the bilateral mammary fat pads of female FVB/N

From Zanganeh et al Nat Nanotechnol. 2016



Metabolism of ferumoxytol by macrophages (and not monocytes) can polarize them into an anti-tumor phenotype.

These results were validated with histopathology and flow cytometry, showing that ferumoxytol treatment caused an increase in pro-inflammatory macrophage markers (CD80), and a decrease in anti-inflammatory marker (CD206) within the tumor. Ferumoxytol can preferentially recruit and polarize macrophages into an anti-tumor phenotype, cementing the notion that ferumoxytol is a promising agent in the treatment and monitoring of immune stimulating therapies in cancer.

PFC emulsion detects macrophage distribution and density

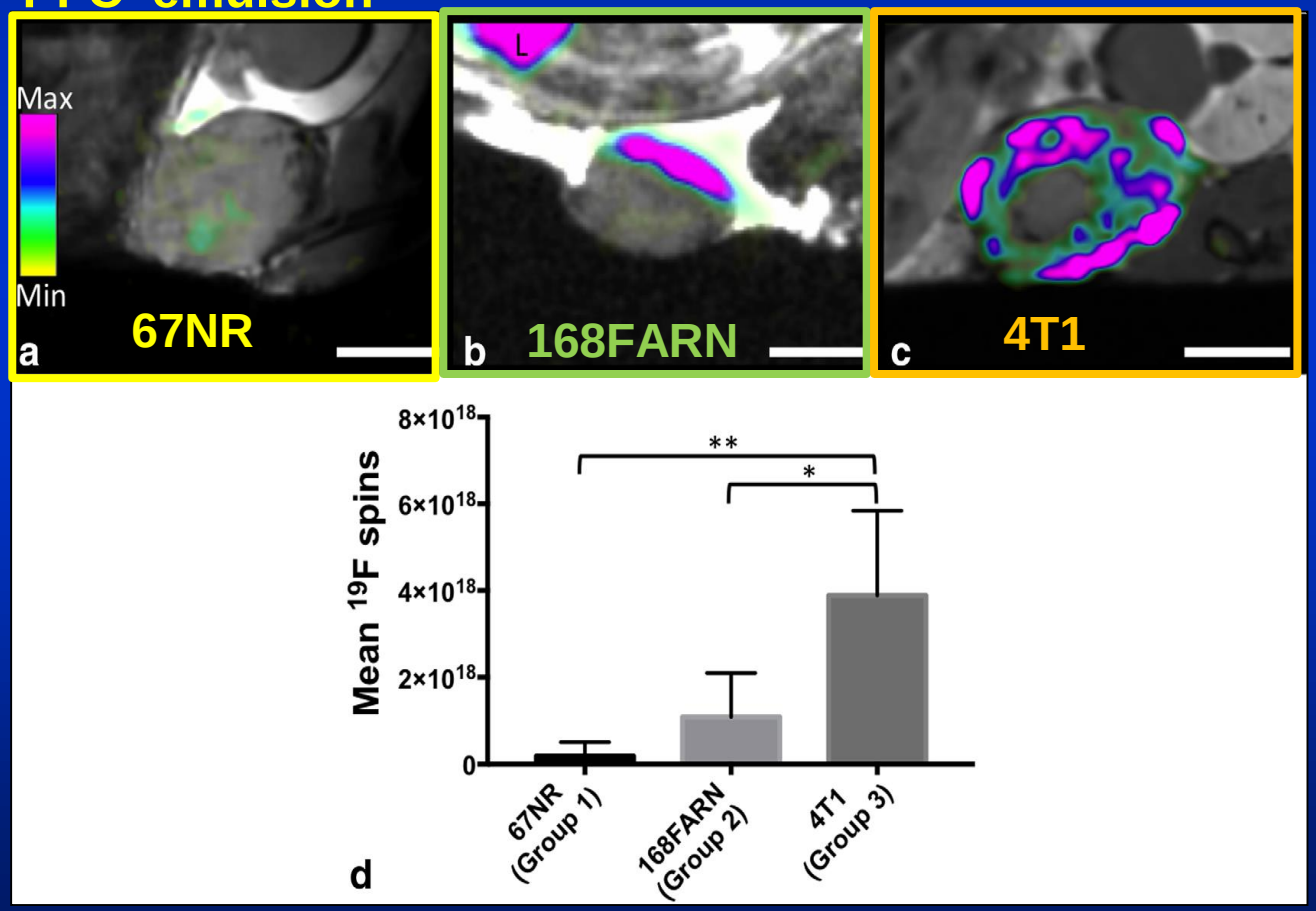
Imaging Macrophage Distribution and Density in Mammary Tumors and Lung Metastases Using Fluorine-19 MRI Cell Tracking

a) Very little 19F signal was observed in the **nonmetastatic 67NR tumors**, with low signal within voxels throughout the tumors.

b) A moderate amount of 19F signal was seen in **168FARN tumors**, typically along the tumor edge.

c) A higher amount of 19F signal was observed in the **highly metastatic 4T1 tumors** surrounding the periphery.

Primary mammary tumors, 24 h after i.v. injection of PFC emulsion

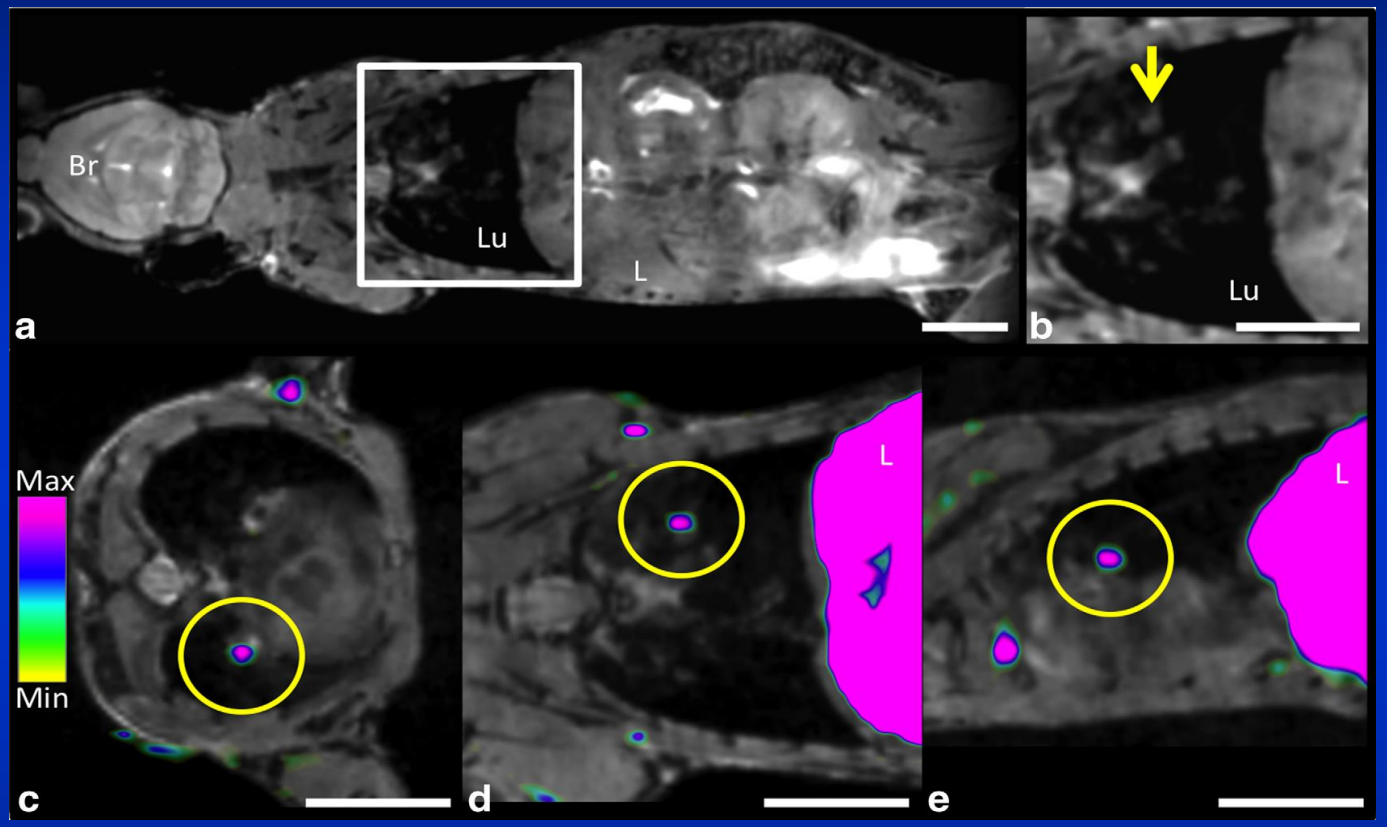


Makela et al, Magn Reson Med 80:1138–1147, 2018.

Lung metastasis from highly metastatic 4T1 primary tumors

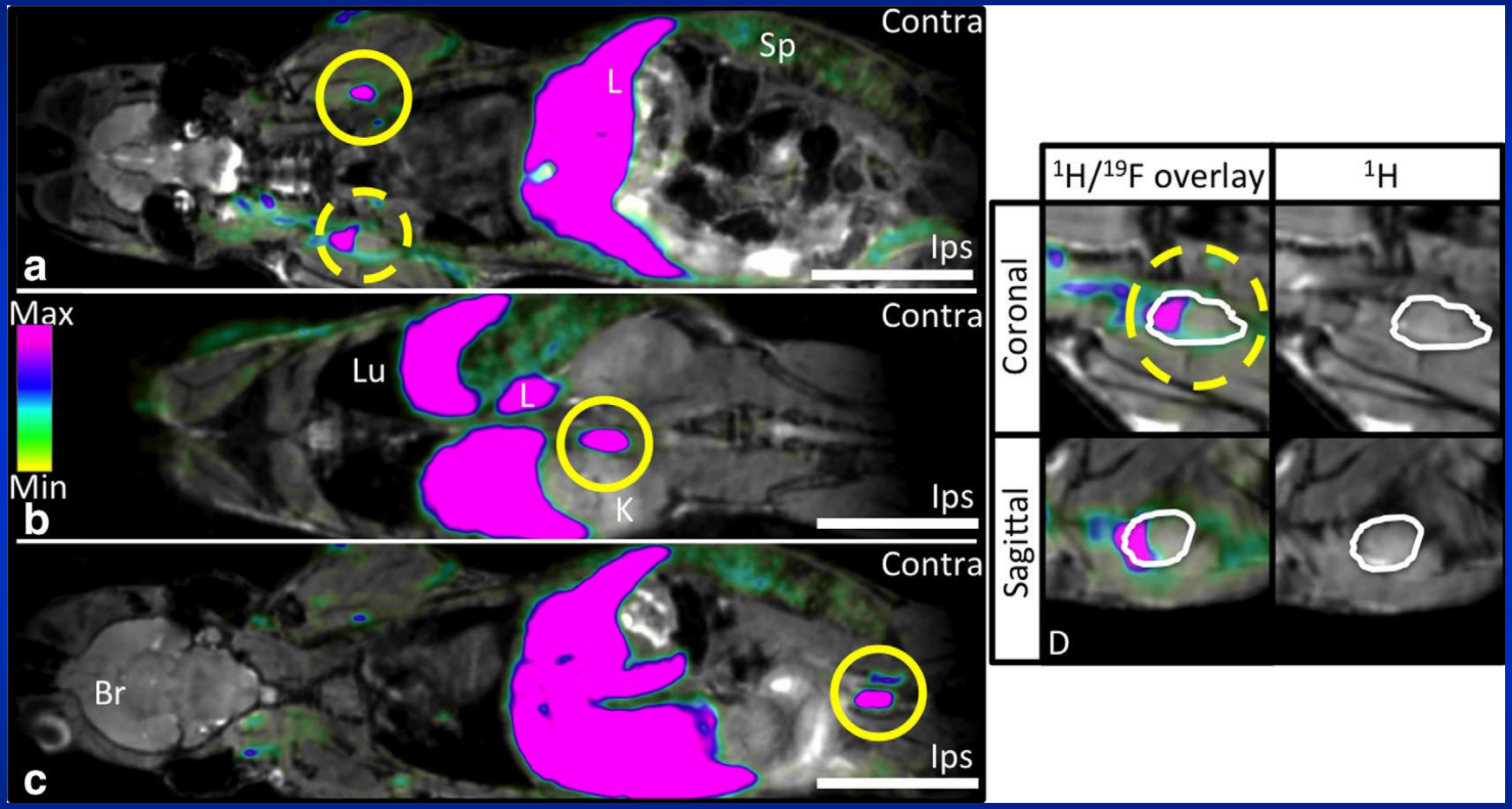
^{1}H and ^{19}F images were acquired with balanced steady-state free precession (bSSFP) pulse sequences at 9.4 T.

^{19}F images were acquired with 500 x500 x1000 um spatial resolution with the following parameters: FOV=80 40 40mm, matrix=160 80 40, flip angle=63 , bandwidth=50 kHz, TR/TE= 4.7/2.4 ms, 10 averages (30 averages for metastasis imaging), and 4 phase cycles resulting in a scan time of 10 minutes (30 minutes for metastasis imaging).



Makela et al, Magn Reson Med 80:1138–1147, 2018.

Lymph nodes from highly metastatic 4T1 primary tumors



Detection of isoflurane was decreased by using a 1 kHz Gaussian excitation pulse

Makela et al, Magn Reson Med 80:1138–1147, 2018.

19F MRI to detect the effects of oncolytic virotherapy

OPEN ACCESS Freely available online

PLOS ONE

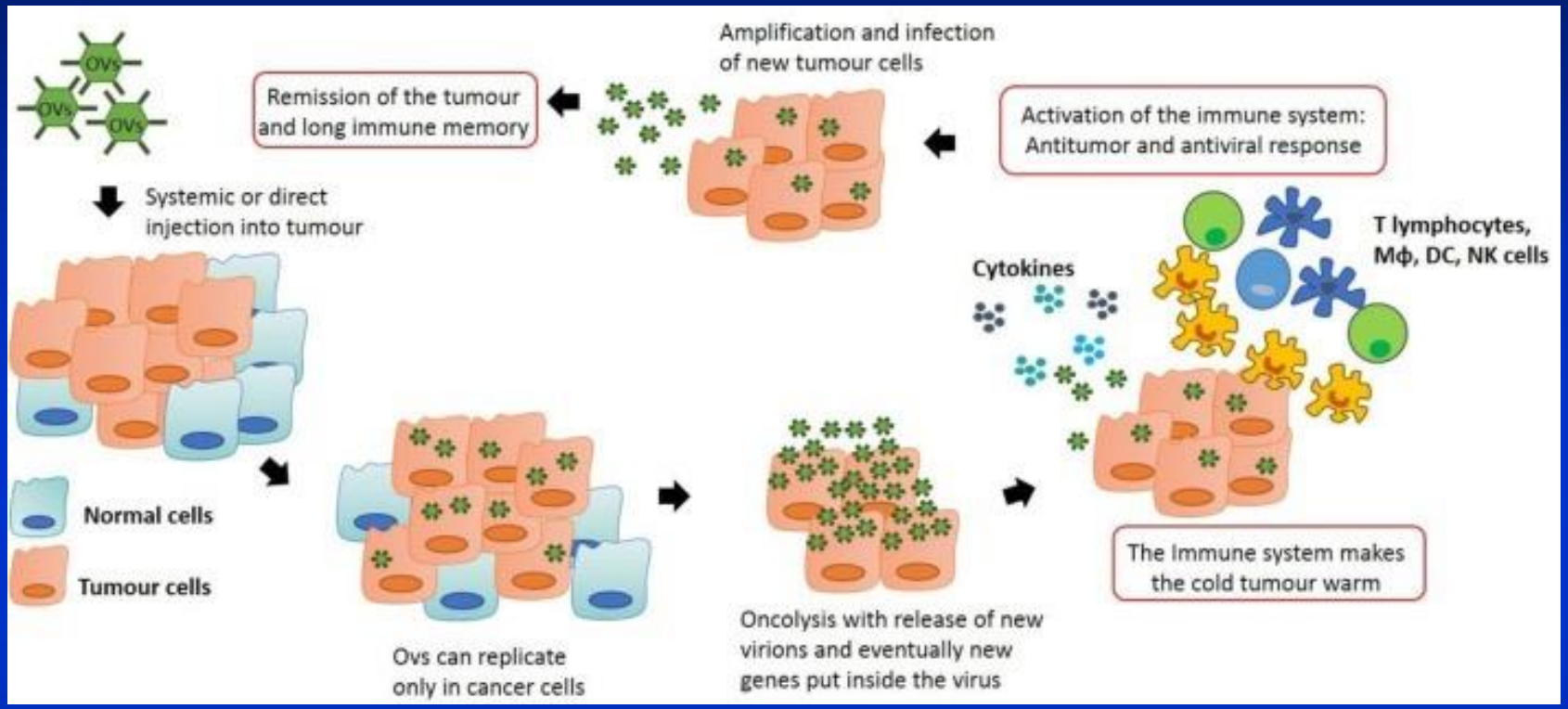
Imaging of Intratumoral Inflammation during Oncolytic Virotherapy of Tumors by ^{19}F-Magnetic Resonance Imaging (MRI)

Stephanie Weibel[1☯], Thomas Christian Basse-Luesebrink[1,2,3☯], Michael Hess[1], Elisabeth Hofmann[1], Carolin Seubert[1], Johanna Langbein-Laugwitz[1], Ivaylo Gentschev[1,7], Volker Jörg Friedrich Sturm[2], Yuxiang Ye[2], Thomas Kampf[2], Peter Michael Jakob[2,3], Aladar A. Szalay[1,4,5,6,7*]

1 Department of Biochemistry, Biocenter, University of Wuerzburg, Wuerzburg, Germany, **2** Department of Experimental Physics 5, University of Wuerzburg, Wuerzburg, Germany, **3** Research Center for Magnetic Resonance Bavaria e.V., Wuerzburg, Germany, **4** Rudolf Virchow Center, Research Center for Experimental Biomedicine, and, **5** Institute for Molecular Infection Biology, University of Wuerzburg, Wuerzburg, Germany, **6** Department of Radiation Medicine and Applied Sciences, University of California San Diego Health System, La Jolla, California, United States of America, **7** Genelux Corporation, San Diego Science Center, San Diego, California, United States of America

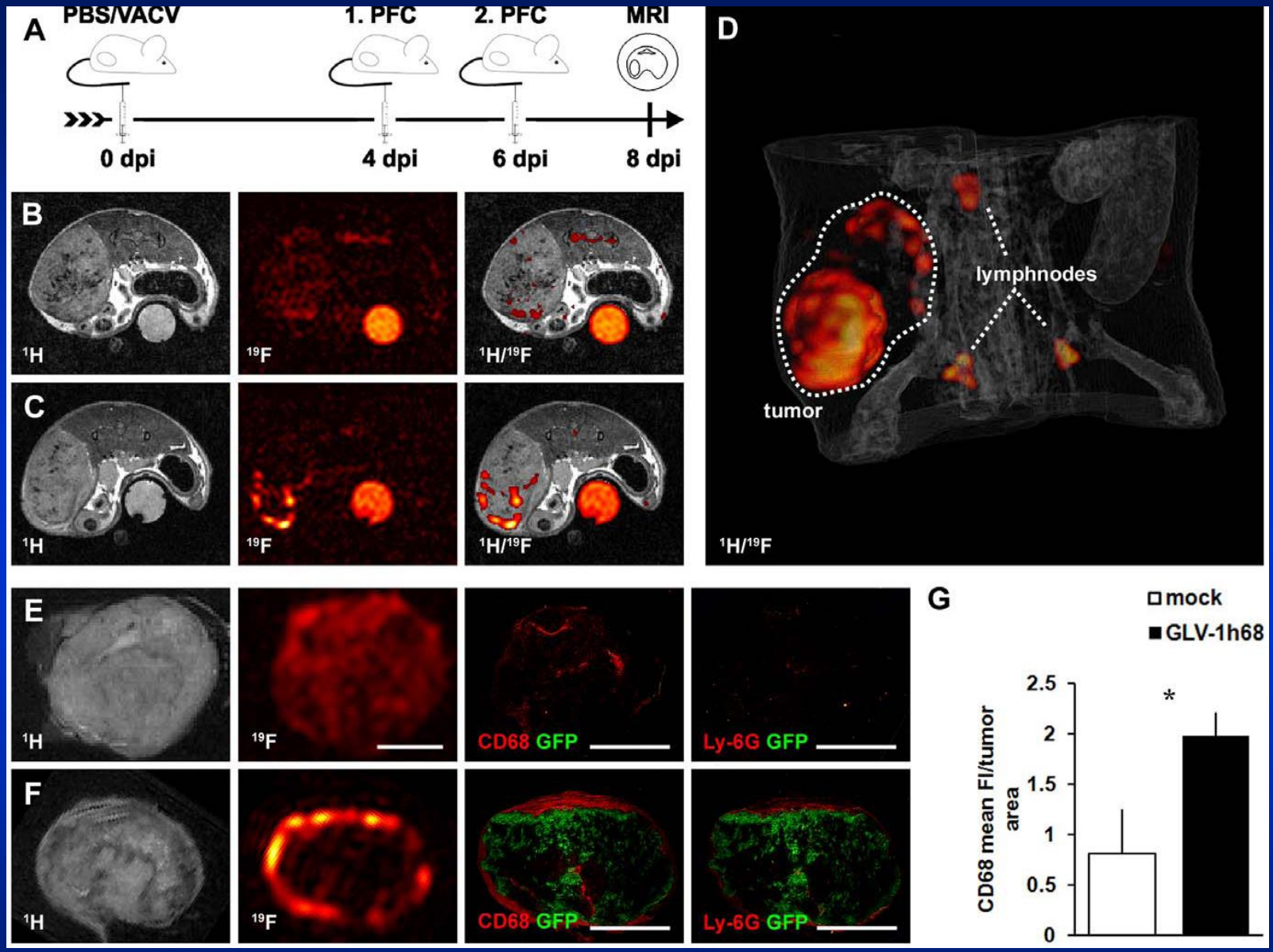
Oncolytic virotherapy of tumors is based on the lytic destruction of solid tumors mediated by infection of the malignant tissue by tumor-specific viruses.
Today, a multitude of different virus strains with oncolytic potential are described in the literature and promising pre-clinical data as well as clinical trial reports from oncolytic virotherapy are available.
In addition to the oncolytic tissue destruction, massive inflammation within the tumor microenvironment occurs, which is primarily directed against the virus. However, virus-mediated cell lysis also leads to the release of tumor-associated antigens, which may finally stimulate an anti-tumoral immune response.

Oncolytic virus (OV) therapy



Anti-tumor immunity by oncolytic virus (OV) therapy: OVs can be modified in order to let them to replicate only in transformed cells. This process stimulates the immune system which is recruited into the tumor, skewing the neoplastic mass from an immuno-suppressive environment to an inflammatory site. Macrophages and T lymphocytes are key players in this process, producing cytokines that can recruit other immune cells and actively destroy cancer cells. This action generates an immunological memory that avoids cancer recurrence and synergizes with the oncolytic action of the viruses, potentially leading to tumor remission.

Most **oncolytic virus therapies** have been tested in **patients** with melanoma or brain tumors,



1936-MEL melanoma- bearing athymic nude mice were i.v. injected with either 16107 pfu of GLV-1h68 or PBS as control.

From Weibel *et al. 2013 PlosOne 8(2): e56317*

Magnetic Resonance Targeting (MRT) increased the anti-tumour effects of macrophage virotherapy

Directing cell therapy to anatomic target sites *in vivo* with magnetic resonance targeting

Munitta Muthana[1], Aneurin J. Kennerley[2,*], Russell Hughes[3], Ester Fagnano[1], Jay Richardson[1], Melanie Paul[1], Craig Murdoch[4], Fiona Wright[1], Christopher Payne[5], Mark F. Lythgoe[5], Neil Farrow[6], Jon Dobson[7], Joe Conner[8], Jim M. Wild[9] & Claire Lewis[3]

nature COMMUNICATIONS

NATURE COMMUNICATIONS | 6:8009 | 2014

An MRI system is used to direct macrophages carrying an oncolytic virus, **Seprehvir,** into primary and metastatic tumour sites in mice.

To achieve this, macrophages were magnetically labeled with super-paramagnetic iron oxide nanoparticles (SPION) and pulsed magnetic field gradients were applied in the direction of the tumour sites.

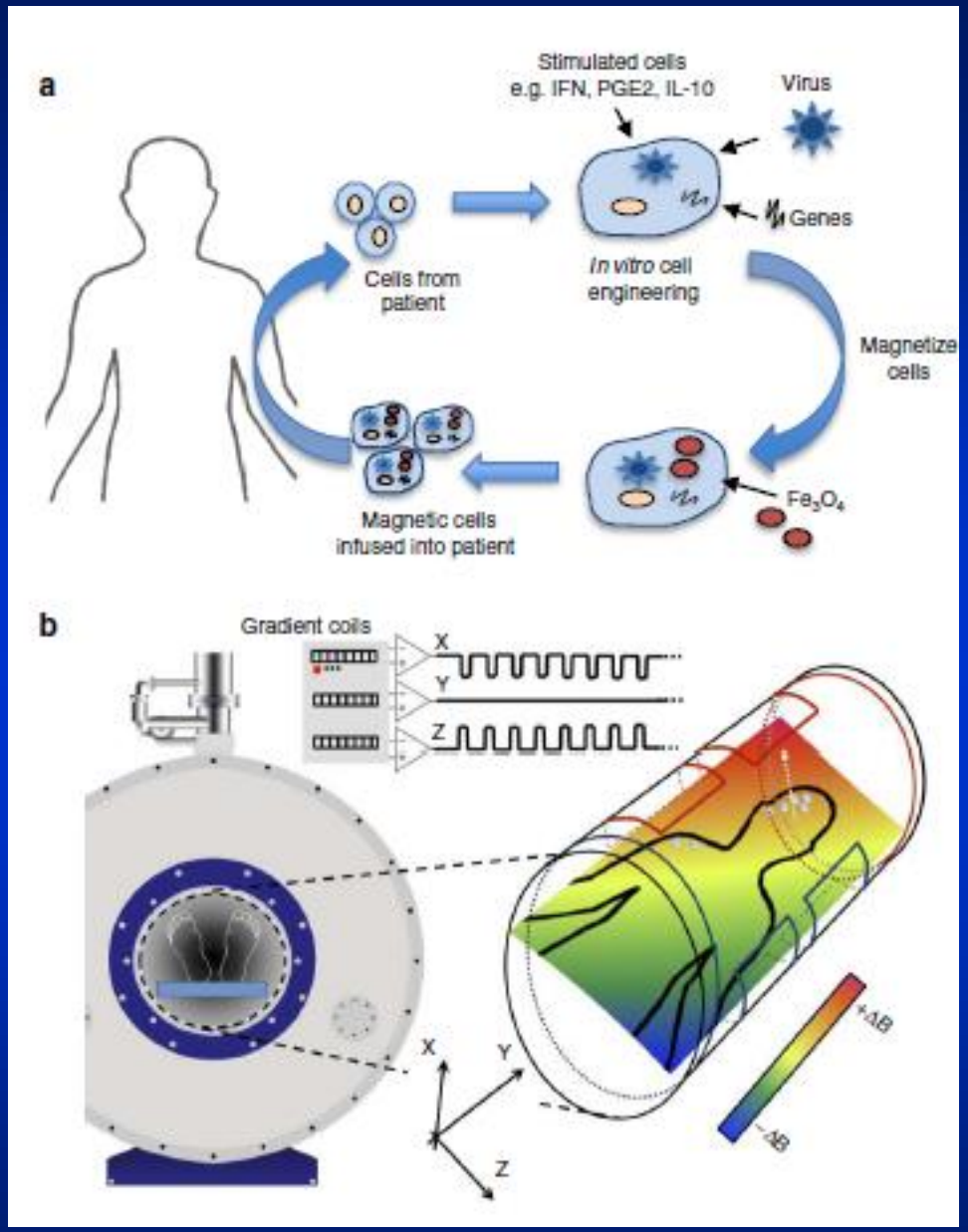
Magnetic resonance targeting guides macrophages from the bloodstream into tumours, resulting in increased tumour macrophage infiltration and reduction in tumour burden and metastasis.

Clinical MRI scanners can track the location of magnetically labelled cells and also have the potential **to steer them into one or more target tissues**.

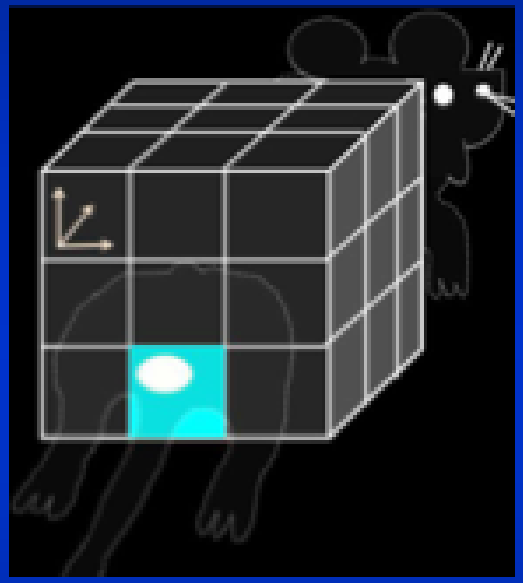
Principle of MRT to steer cell-based therapies to specific tissues

(a) The cells used for these studies are derived from monocytes isolated from patient blood, cultured in the presence of various stimuli to produce ‘therapeutic’ macrophages (for example, cytokines, therapeutic genes or viruses) and loaded with SPIOs before reinfusion back into the same patient.

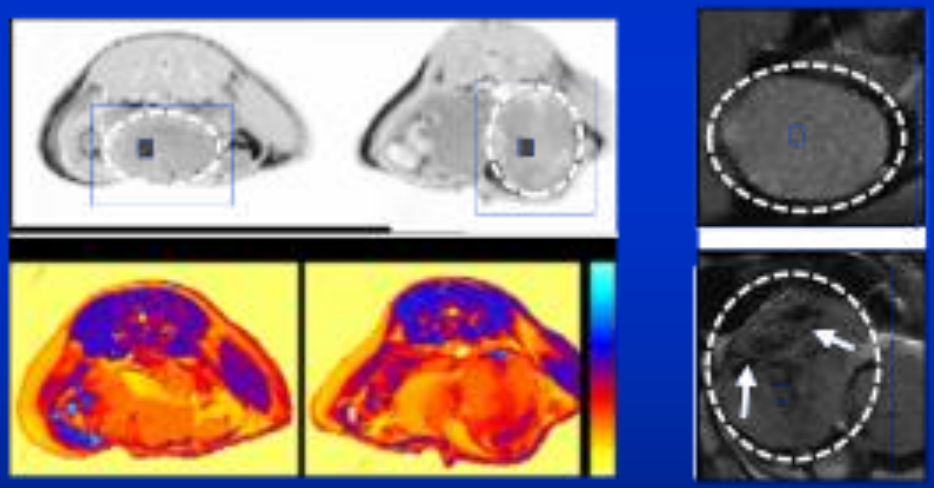
(b) (b) The subject is then placed in the centre of an MRI scanner where linear spatial encoding magnetic gradients can be used to induce a force on a magnetized body. The magnetic cells injected into the bloodstream of the subject circulate and are targeted into the diseased organ/tissue under the influence of the applied magnetic field for increased cell uptake.



Magnetic resonance targeting (MRT) that uses the magnetic field gradient coils inherent to all magnetic resonance imaging (MRI) systems, to steer ferromagnetic particles (or cells containing them) to a target site.

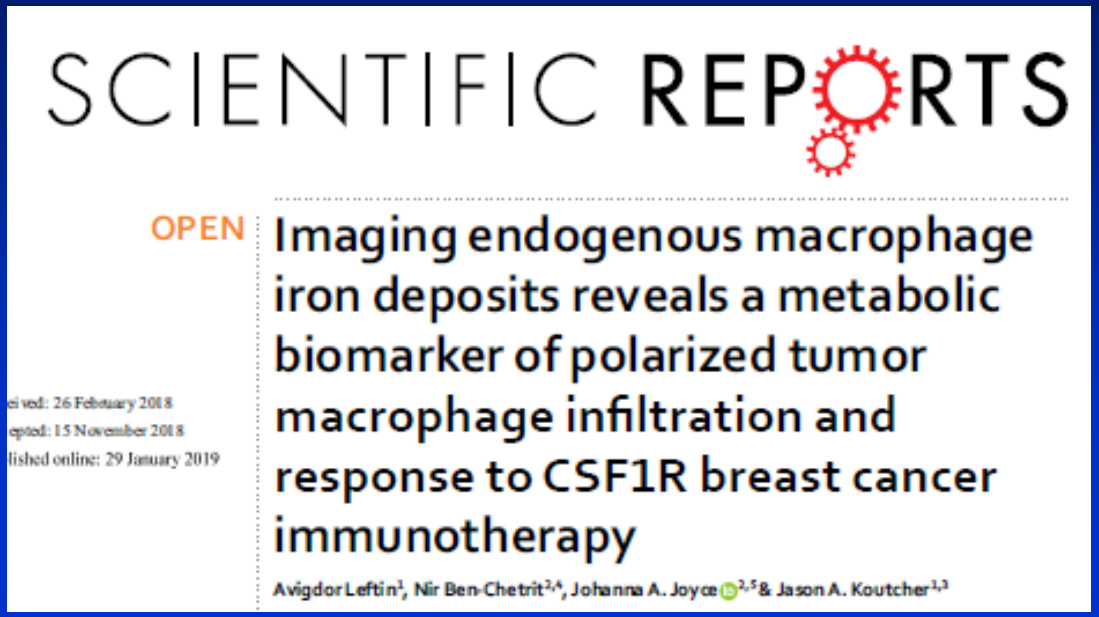


Three million SPIO-loaded macrophages were administered intravenously to mice bearing orthotopic primary and metastatic (lung) prostate tumours.
A pulsed magnetic field gradient was applied for 1 h, in the direction of the prostate, with an effective magnetic field offset, $B_{off} \cong 7mT$ on top of the static magnetic field of the scanner (B0=7 T).



MRT could be used to deliver therapeutic agents such as oncolytic viruses (OVs) to these poorly vascularized, and therefore relatively inaccessible, areas of tumours

Endogenous iron deposit in TAMs detected by FeMRI



SCIENTIFIC REPORTS

OPEN

Imaging endogenous macrophage iron deposits reveals a metabolic biomarker of polarized tumor macrophage infiltration and response to CSF1R breast cancer immunotherapy

ei ved: 26 February 2018
epted: 15 November 2018
lished online: 29 January 2019

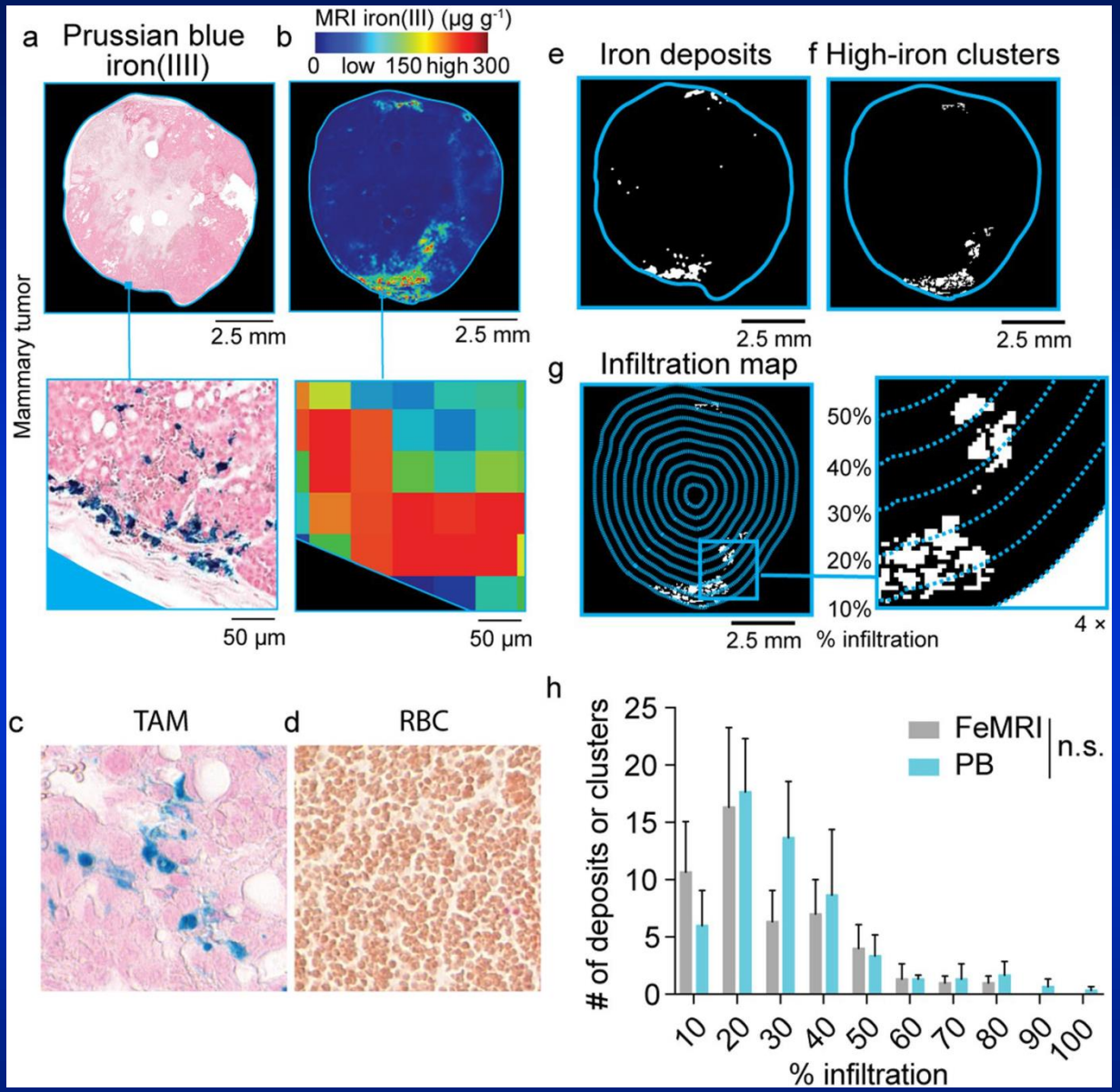
Avigdor Leftin[1], Nir Ben-Chetrit[2,4], Johanna A. Joyce[2,5] & Jason A. Koutcher[1,3]

Leftin et al ,
Scientific Reports |
(2019) 9:857

Iron deposits are a phenotypic trait of tumor-associated macrophages (TAMs). Histological iron imaging and contrast-agent free magnetic resonance imaging (MRI) can detect these deposits, but their presence in human cancer, and correlation with immunotherapeutic response is largely untested.

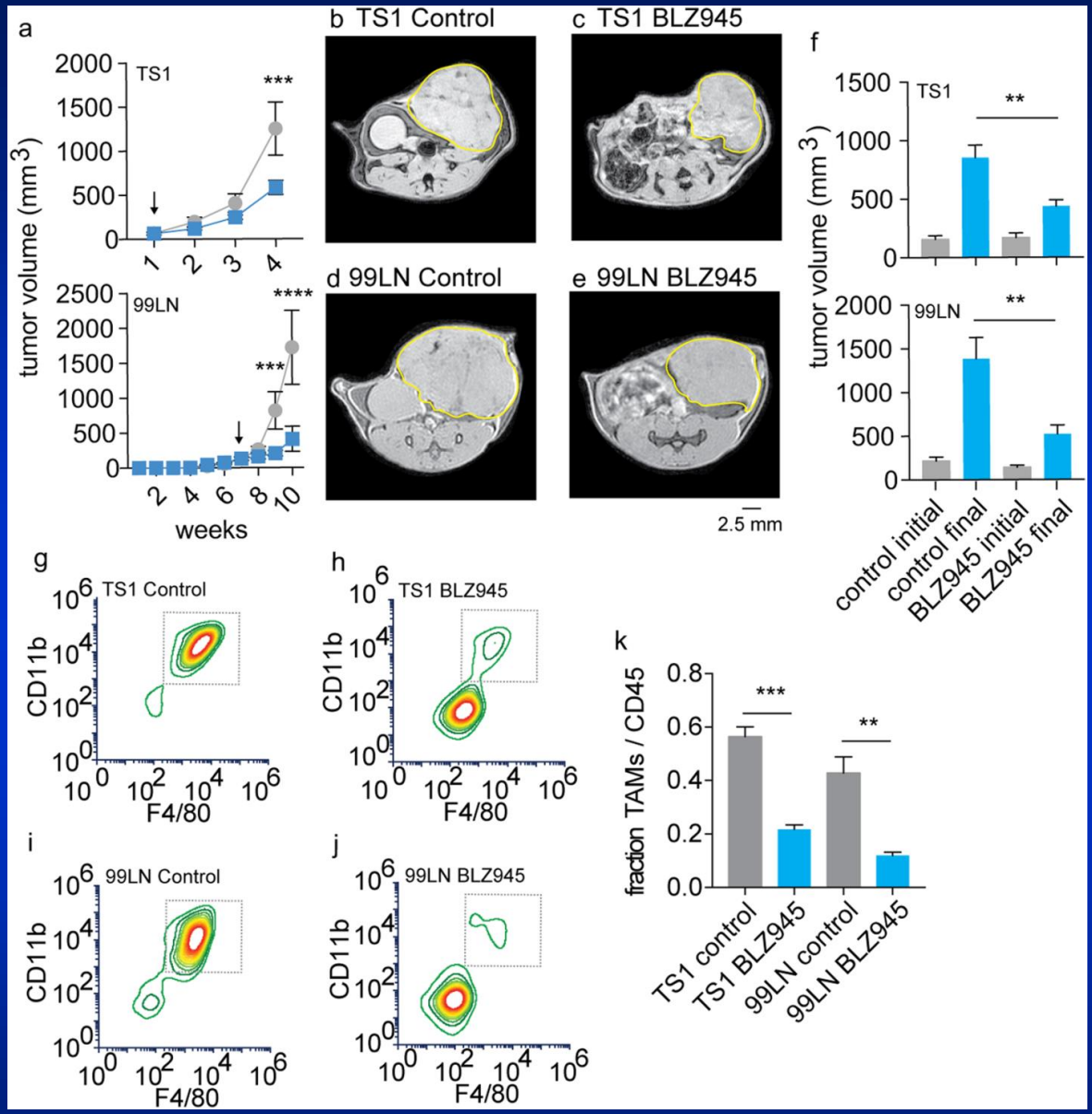
This study, using these **iron imaging approaches**, evaluated the spatial distribution of polarized macrophage populations containing high endogenous levels of iron in preclinical murine models and human breast cancer, and used them as metabolic biomarkers to correlate TAM infiltration with response to immunotherapy in preclinical trials.

2D multi-gradient echo (MGE) relaxometry pulse sequence (7T) was used with the following parameters: TR/TE 1.2s /3 ms × 16 echos, in vivo spatial resolution 0.1 mm × 0.1 mm × 1 mm, ex vivo 0.05 mm × 0.05 mm × 0.5 mm, RF flip angle 60°, and each spatial encoding phase encode acquisition was gated on the animal's respiratory cycle.



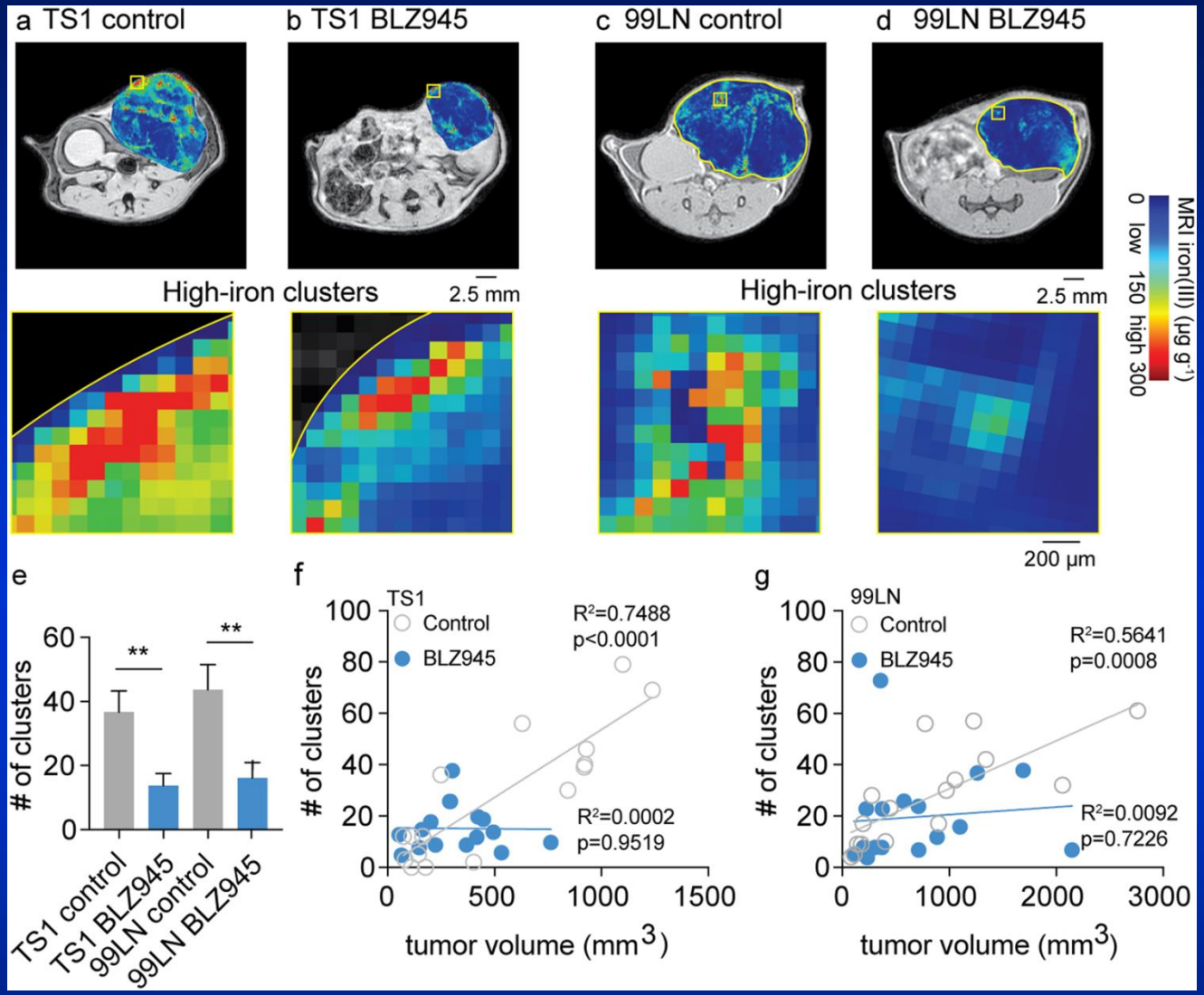
Leftin et al, Scientific Reports | *(2019) 9:857*

Immune therapy by a small molecule i**nhibitors of macrophage colony stimulating factor** 1 receptor (CSF-1R) BLZ945 has been shown to reduce the accumulation of TAM



Leftin et al, Scientific Reports | *(2019) 9:857*

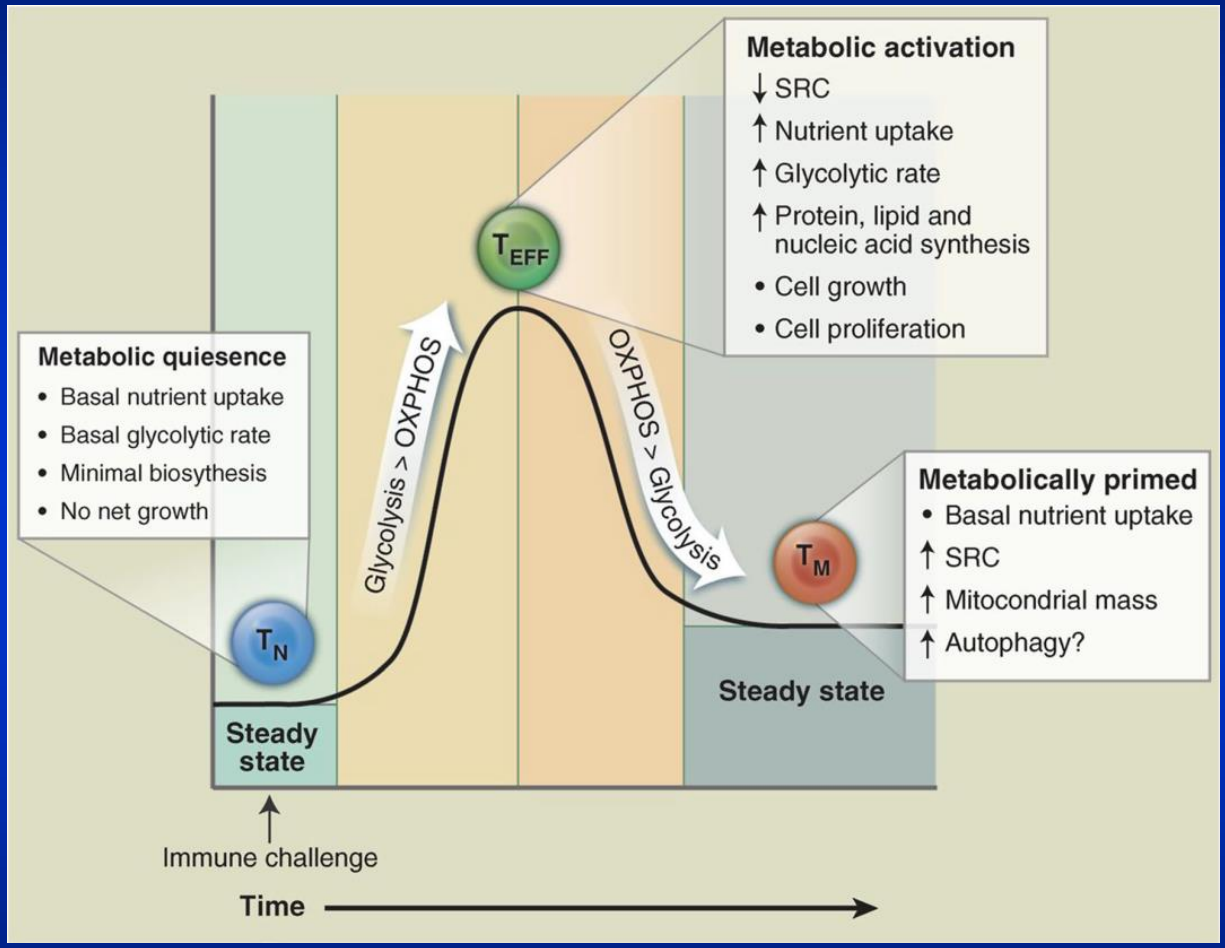
In vivo iron MRI (FeMRI) of murine macrophage iron deposits and correlation between immune and therapeutic CSF1R inhibitor response. Representative *in vivo* FeMRI axial cross sections of the mammary tumors are shown in control and BLZ945 treated (**a**,**b**) TS1, and (**c,d**) 99LN models.



Leftin et al, Scientific Reports | *(2019) 9:857*

CEST- MRI of T cell activation

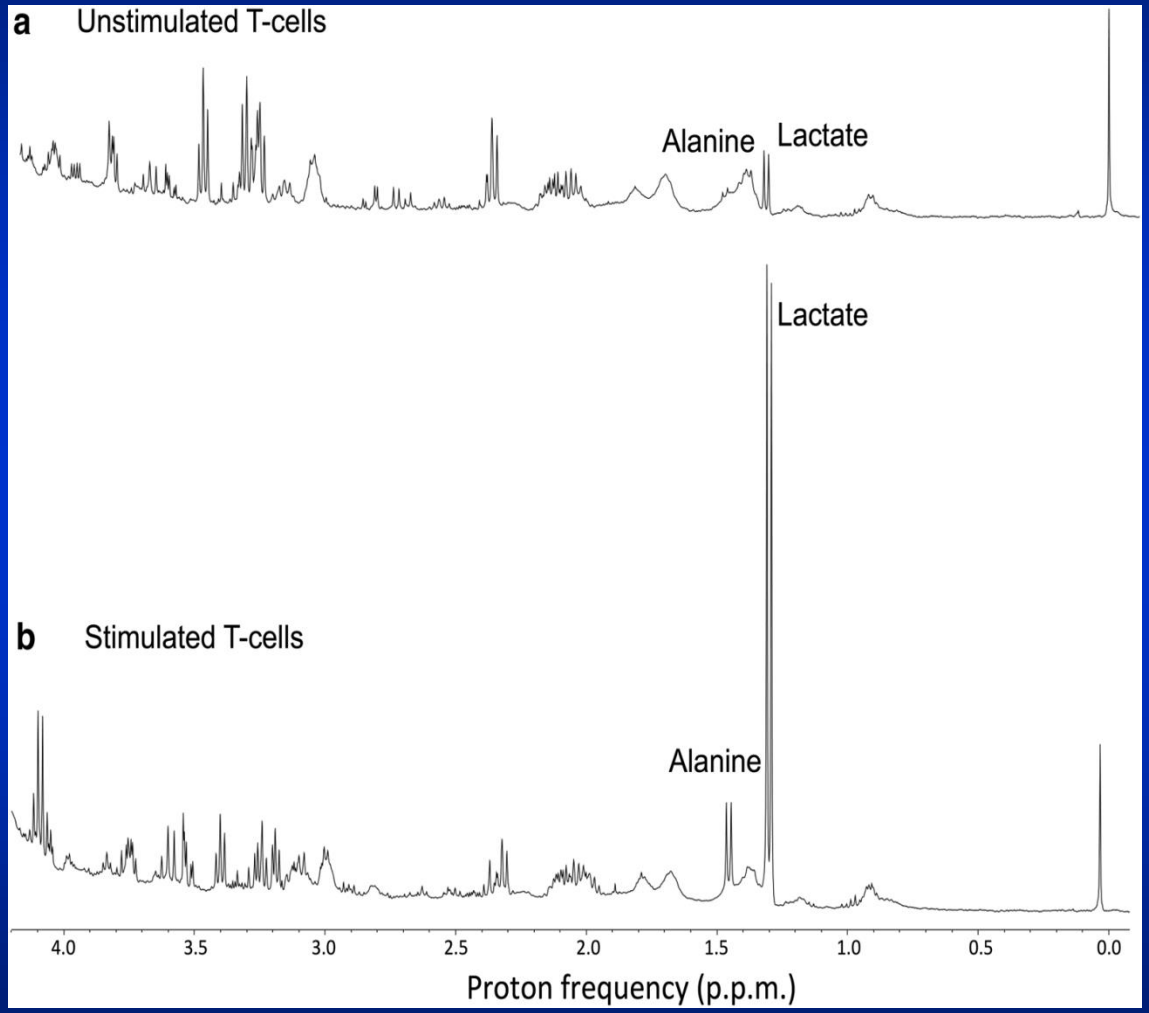
Representation of metabolic switch in different stages of T cells. Naïve T cells (TN) are predominantly depending on the oxidative phosphorylation for the energy needs. The proliferative T cells (TEFF) shifts to aerobic glycolysis to support their high energy demand. The memory Tcells (TM) use oxidative phosphorylation pathway for their metabolic requirement.



Pearce et al, Science. 2013 October 11; 342(6155)

^{1}H MR spectrum of T cells

1H NMR spectra from cultured T cells obtained at 9.4 T.
a Spectrum from previously non-stimulated resting T-cells shows **lactate and other intra cellular metabolites, and very little alanine.**
b Spectrum from stimulated T cells with CD3/CD28 obtained under identical conditions of the spectra in **a**, which shows substantially (over 12-fold increase) higher lactate and alanine (3-fold increase) compared to that from non-stimulated resting T-cells

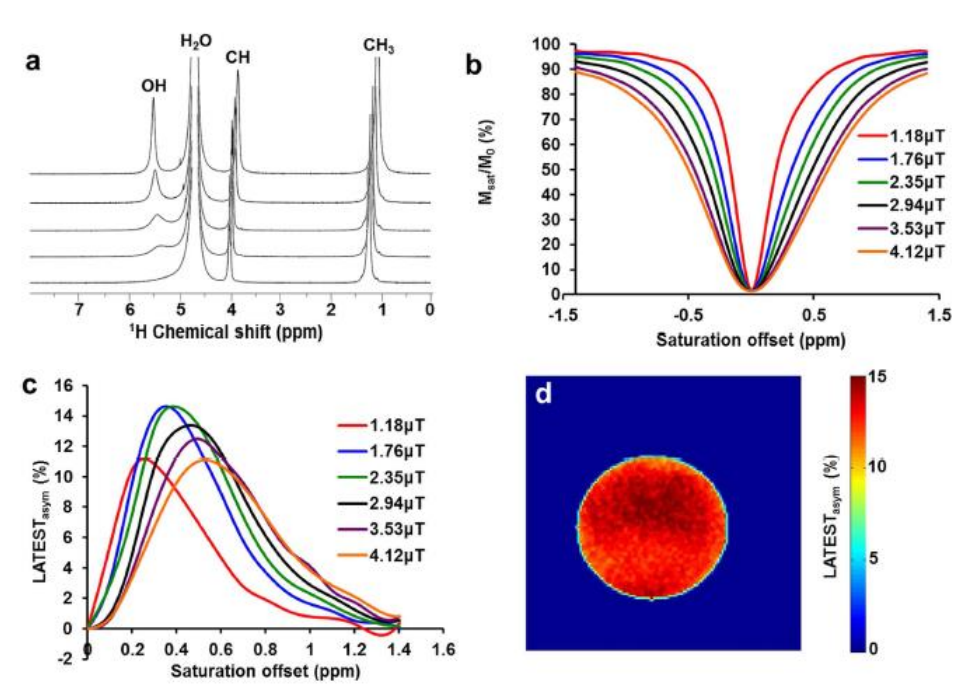


From Haris *et al. J Transl Med (2017) 15:140*

Lactate Chemical Exchange Saturation Transfer (LATEST) Imaging *in vivo* A Biomarker for LDH Activity

Catherine DeBrosse[1,*], Ravi Prakash Reddy Nanga[1,*], Puneet Bagga[1], Kavindra Nath[2], Mohammad Haris[3], Francesco Marincola[3], Mitchell D. Schnall[1], Hari Hariharan[1] & Ravinder Reddy[1]

SCIENTIFIC REPORTS | 6:19517 | 2016

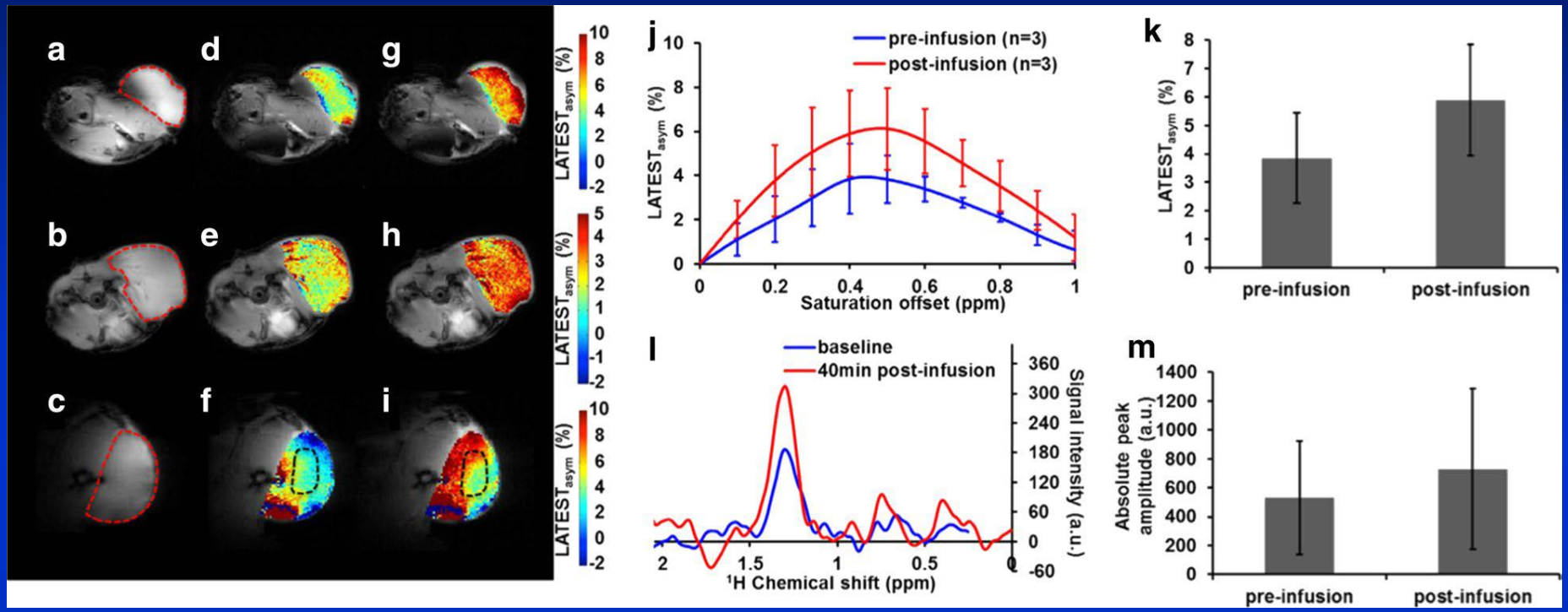


LATEST:a magnetic resonance imaging (MRI) method based on **lac**tate chemical exchange satu- ration transfer (C**EST**) to image lactate.

In this work, CEST technique is used to exploit the exchange of –OH protons on lactate with bulk water.

This method utilizes standard proton MRI and requires neither ^{13}C labeled pyruvate nor DNP polarization.

CEST MRI of lactate: a biomarker for immunotherapy



LATEST from lymphoma tumors

a–**c** Anatomical image from three animals, with flank tumor region indicated by *dotted red line*, and the LATEST maps (**d**–**f**) pre-infusion and (**g**–**i**) post-infusion with (**j**) corresponding asymmetry plots

(**k**) LATEST change at 0.4 ppm from three animals pre- and post-infusion,

(**l**) representative SEL-MQC ^{1}H-MRS pre- and 40 min post-infusion from flank tumor showing (**m**) increase in lactate peak amplitude from three animals (~40%) from spectroscopy.

From DeBrosse et al, Scientific Reports | 6:19517

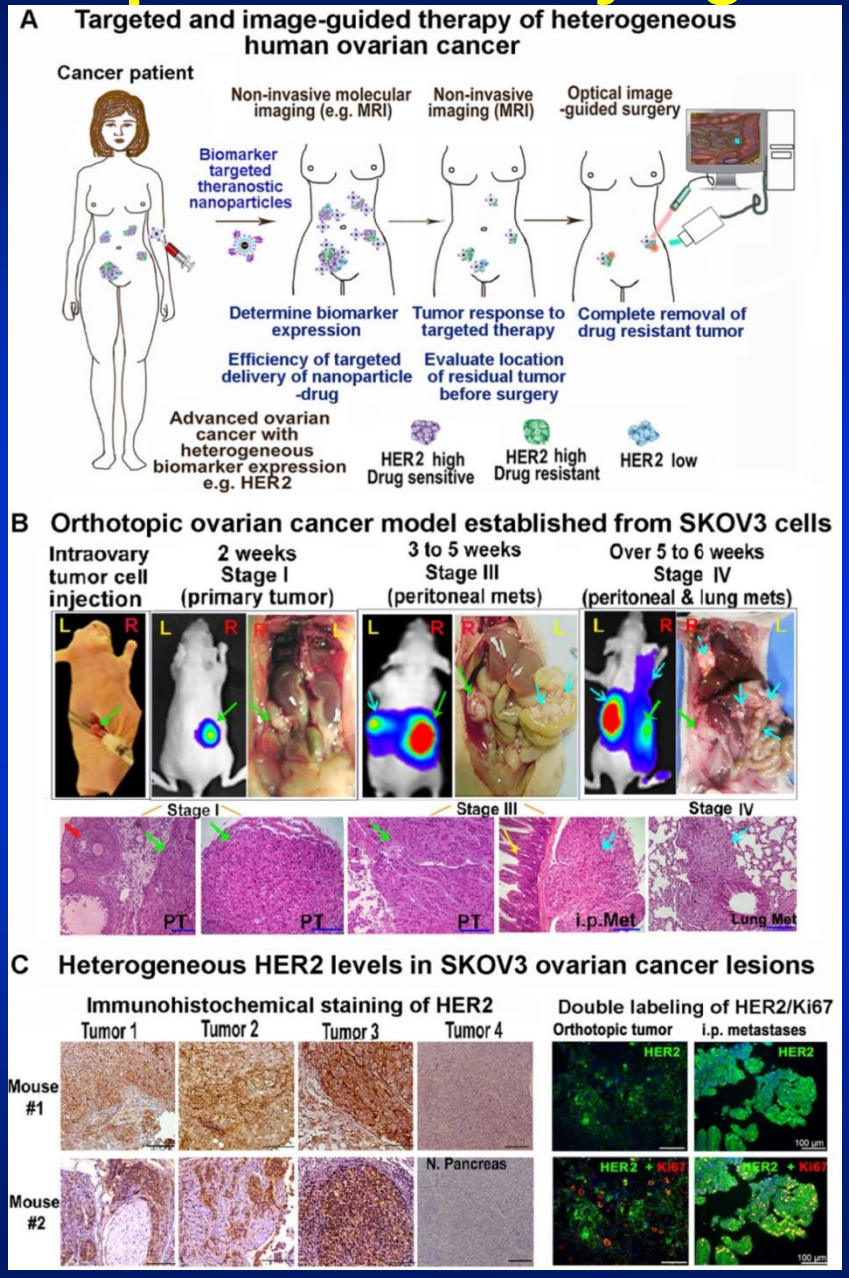
HER2-targeted theranostic nanoparticles carrying cisplatin in ovarian cancer

Targeted Drug Delivery and Image-Guided Therapy of Heterogeneous Ovarian Cancer Using HER2-Targeted Theranostic Nanoparticles

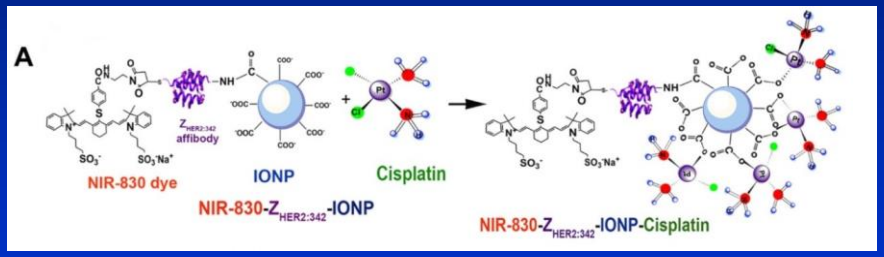
Systemic delivery of HER2-targeted magnetic iron oxide nanoparticles carrying cisplatin significantly inhibited the growth of primary tumor and peritoneal and lung metastases in the ovarian cancer xenograft model in nude mice.

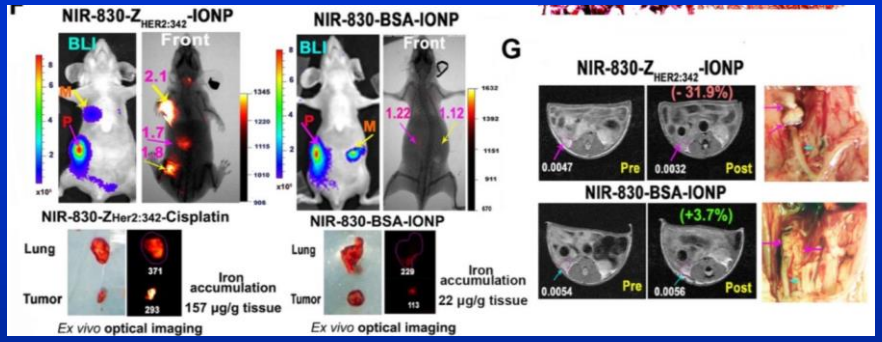
Differential delivery of theranostic nanoparticles into individual tumors with heterogeneous levels of HER2 expression and various responses to therapy were detectable by MRI.

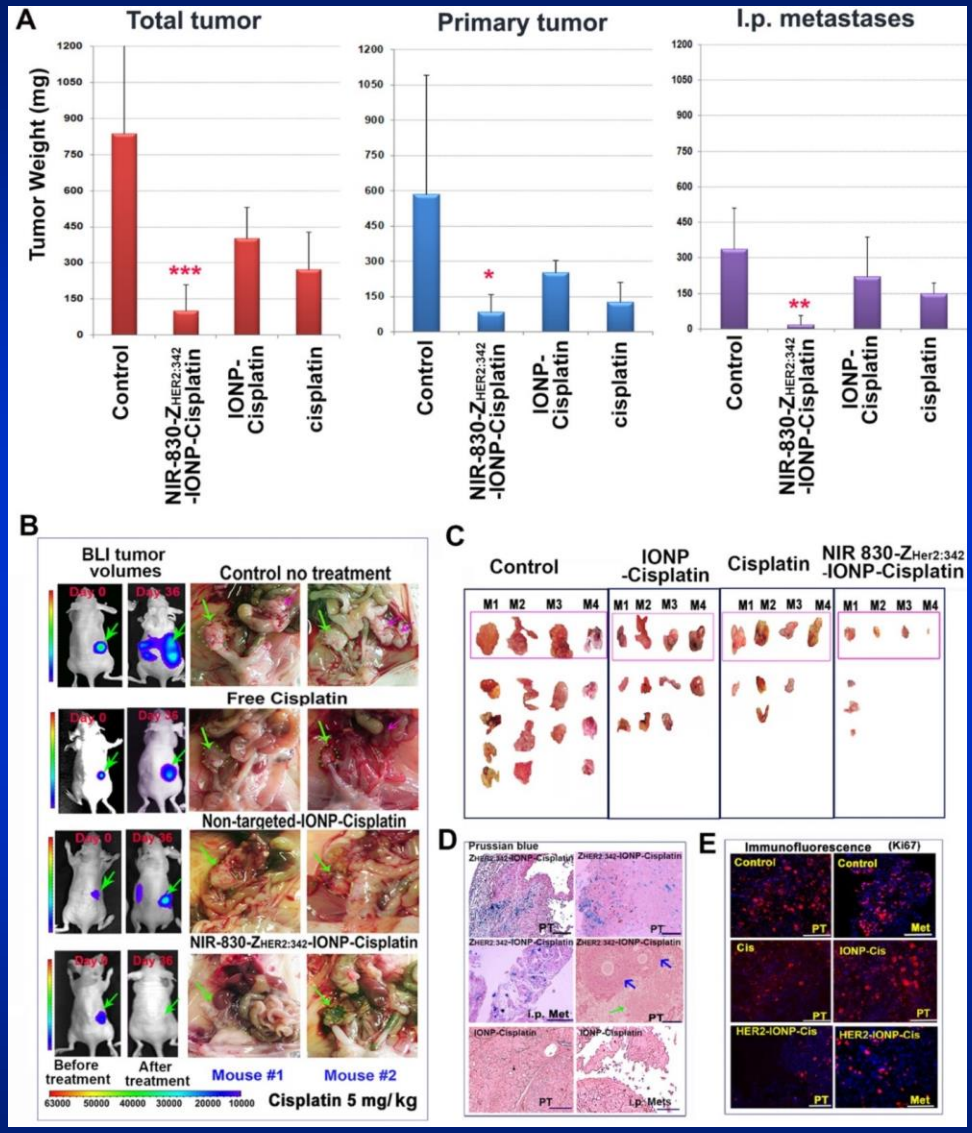
The author found also a stronger therapeutic response in metastatic tumors compared to primary tumors, likely due to a higher level of HER2 expression and a larger number of proliferating cells in metastatic tumor cells.



Satpathy et al Theranostics 2019, Vol. 9, Issue 3


A
NIR-830 dye
IONP
Cisplatin
NIR-830-Z$_{HER2:342}$-IONP
NIR-830-Z$_{HER2:342}$-IONP-Cisplatin


F
NIR-830-Z$_{HER2:342}$-IONP
NIR-830-BSA-IONP
BLI
Front
NIR-830-Z$_{Her2:342}$-Cisplatin
NIR-830-BSA-IONP
Lung
Tumor
Iron accumulation
157 μg/g tissue
Iron accumulation
22 μg/g tissue
Ex vivo optical imaging
Ex vivo optical imaging
G
NIR-830-Z$_{HER2:342}$-IONP
(-31.9%)
0.0047
Pre
0.0032
Post
NIR-830-BSA-IONP
(+3.7%)
0.0054
Pre
0.0056
Post


A
Total tumor
Primary tumor
l.p. metastases
Tumor Weight (mg)
Control
NIR-830-Z$_{HER2:342}$-IONP-Cisplatin
IONP-Cisplatin
cisplatin
B
BLI tumor volumes
Control no treatment
Free Cisplatin
Non-targeted-IONP-Cisplatin
NIR-830-Z$_{HER2:342}$-IONP-Cisplatin
Before treatment
After treatment
Mouse #1
Mouse #2
Cisplatin 5 mg/kg
C
Control
IONP -Cisplatin
Cisplatin
NIR 830-Z$_{Her2:342}$ -IONP-Cisplatin
M1 M2 M3 M4
D
Prussian blue
I.p. Met
IONP-Cisplatin
I.p. Mets
E
Immunofluorescence (KI67)
Control
Met
Cis
IONP-Cis
HER2-IONP-Cis
PT

ADC to detect collagen I fraction in PDX models

www.impactjournals.com/oncotarget/ Oncotarget, 2017, Vol. 8, (No. 62), pp: 105682-105691

Research Paper

Diffusion-weighted MRI-derived ADC values reflect collagen I content in PDX models of uterine cervical cancer

Anette Hauge[1], Catherine S. Wegner[1], Jon-Vidar Gaustad[1], Trude G. Simonsen[1], Lise Mari K. Andersen[1] and Einar K. Rofstad[1]

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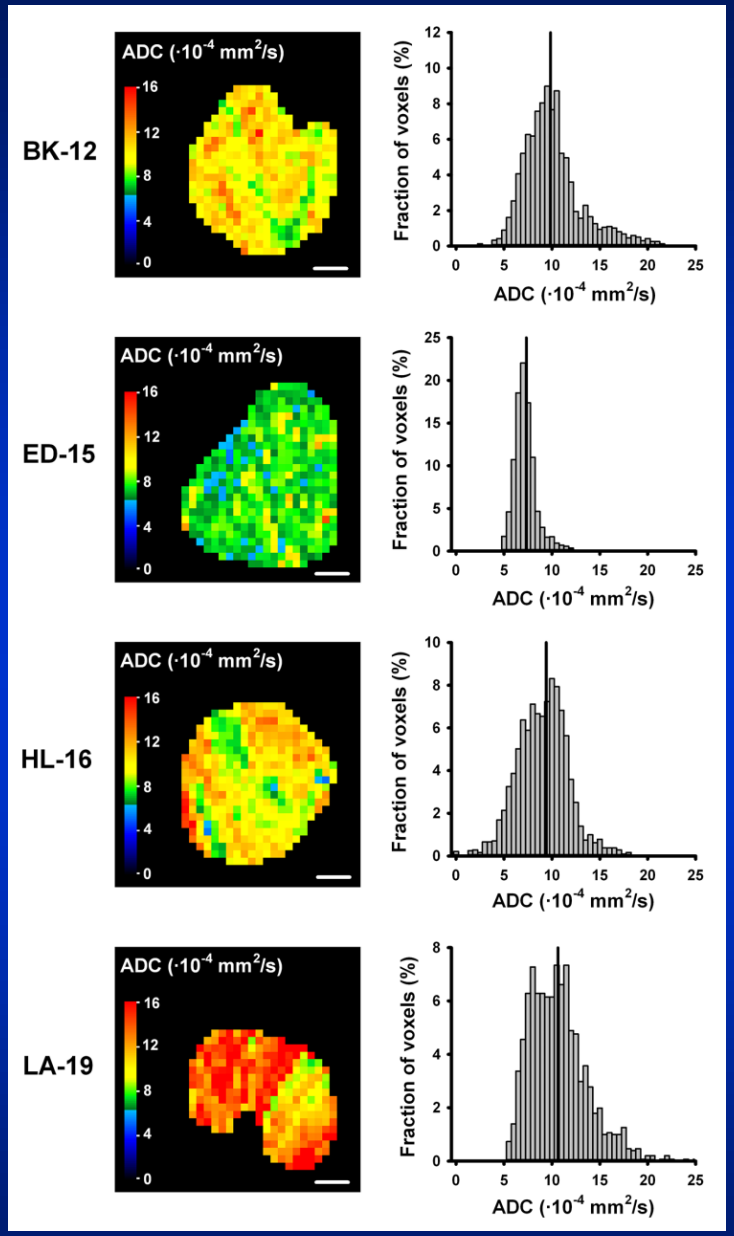
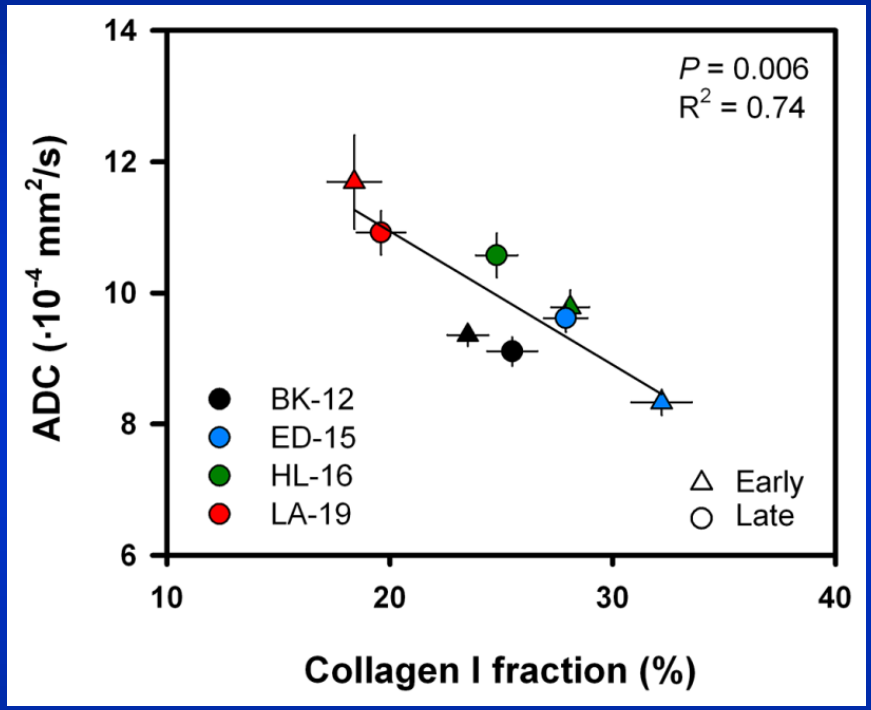
Tumor tissue is composed of malignant parenchymal cells embedded in a supportive connective tissue basis; the tumor stroma.
The stroma comprises blood vessels, immune cells, and activated connective tissue cells, *e.g.* fibroblasts, producing extracellular matrix constituents, such as polysaccharides and various fiber proteins. Amongst them, **type I collagen** is the most abundant.

Tumor to stroma ratio is a recognized prognostic factor in many solid tumours because collagen and other ECM proteins may serve as steric barriers to the interstitial diffusion of therapeutic agents and because of its influence in signalling pathways..



ADC from DWI with
b_{value}= 200, 400, 700, and 1000 s/mm²

Four patient-derived xenograft (PDX) models of **squamous cell carcinoma of the uterine cervix**. Early and late generation tumors of the BK-12, ED-15, HL-16, and LA-19 model were studied



Across four biologically dissimilar PDX models of cervical cancer, representing four different patient tumors, the authors found a significant inverse correlation between ADC and the fraction of collagen I-positive tissue.

DCE-MRI to detect the hypoxic fraction of tumour

Hauge *et al. J Transl Med (2017) 15:225*
DOI 10.1186/s12967-017-1331-4

Journal of Translational Medicine

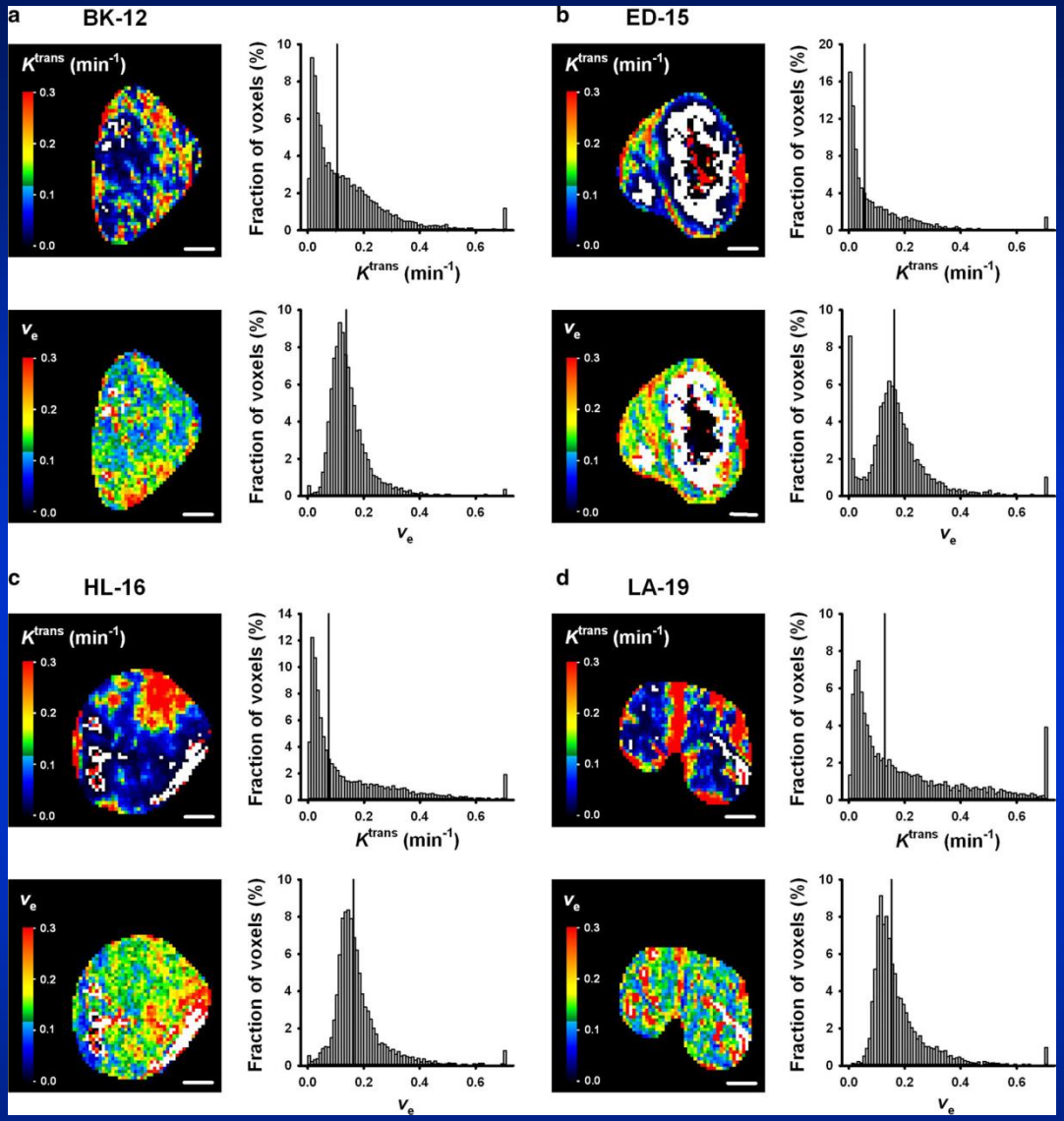
RESEARCH Open Access

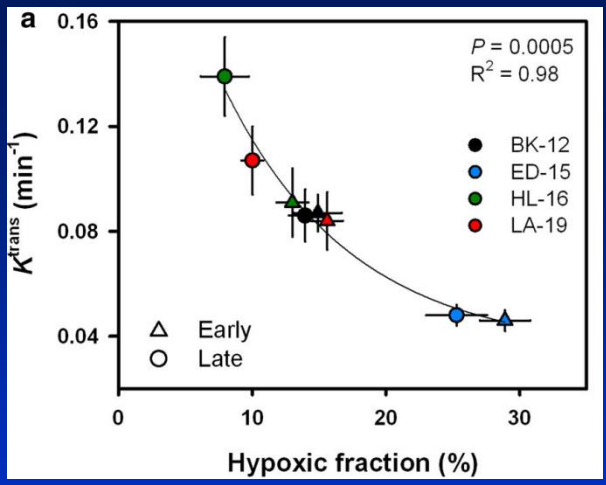
DCE-MRI of patient-derived xenograft models of uterine cervix carcinoma: associations with parameters of the tumor microenvironment

Anette Hauge, Catherine S. Wegner, Jon-Vidar Gaustad, Trude G. Simonsen, Lise Mari K. Andersen and Einar K. Rofstad*

Abnormalities in the tumor microenvironment are associated with resistance to treatment, aggressive growth, and poor clinical outcome in patients with advanced cervical cancer.

The potential of dynamic contrast enhanced (DCE) MRI to assess the microvascular density (MVD), interstitial fluid pressure (IFP), and hypoxic fraction of patient-derived cervical cancer xenografts of uterine cervix carcinoma was investigated.





Strong correlations were detected between *K*trans and the amount of hypoxic tumor tissue in PDX cervix carcinoma models, supporting the development and integration of this imaging method in clinical management of cervical cancer.

Moreover, as hypoxia represents a major obstacle for the cure of a wide range of cancers, these results may prove to be valuable also for patients with other diagnoses than squamous cell carcinoma of the uterine cervix.

Hauge *et al. J Transl Med (2017) 15:225*

MRI Biomarkers for assessment of therapeutical efficacy of experimental drugs

I biomarcatori di imaging possono

- **rappresentare una risorsa unica nella caratterizzazione cellulare della lesione tumorale**
- **contribuire ad una migliore stratificazione dei pazienti che possono beneficiare delle nuove terapie**

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Thank you for your attention