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MRI Unit - Core facilities

Istituto Superiore di Sanità, Rome, Italy

MRI in preclinical models: a focus on nanomedicine

Nanomaterials in diagnostic and theranostic imaging

nanomaterials

MDPI

Review

Application of Nanomaterials in Biomedical Imaging and Cancer Therapy

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Received: 12 August 2020; Accepted: 27 August 2020; Published: 29 August 2020

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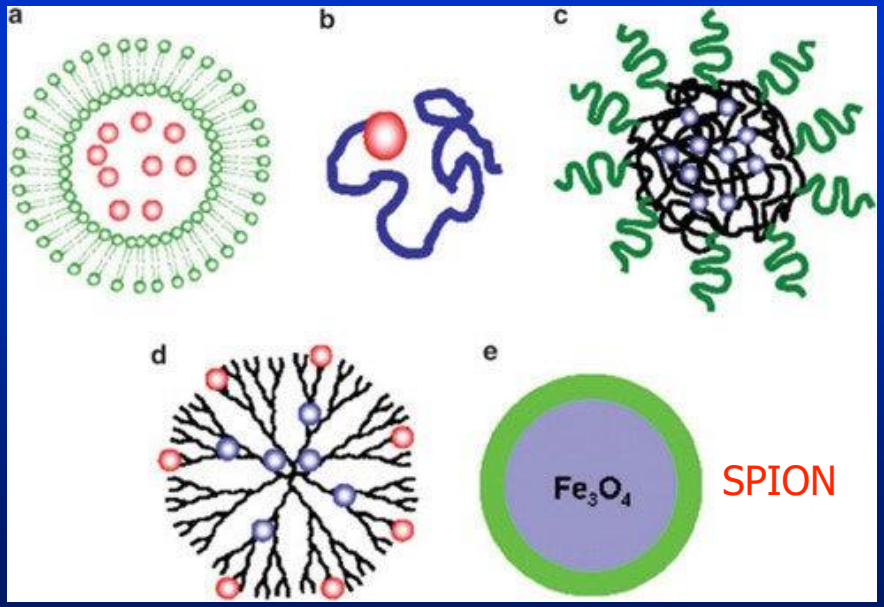
Nanoparticles in Medicine: Therapeutic Applications and Developments

L Zhang[1], FX Gu[1], JM Chan[2], AZ Wang[3,4], RS Langer[1] and OC Farokhzad[4]



Nanomaterials are indispensable tools in diagnostic and theranostic imaging for cancer detection and for the identification of novel therapeutic strategies.

Excellent drug carriers and imaging contrast agents, nanoparticles are commonly used in biomedical imaging and cancer therapy.

Superparamagnetic iron oxide nanoparticles (SPION) have a hydrodynamic diameter ranging from 1 to 100 nm. In general, large SPIONs function as T2 contrast agents, whereas small SPIONs function as T1 in MRI.

These nanoparticles are formed by small crystals of iron oxide, coated by organic compounds. Three types of iron oxide may make up the core of SPIONs : hematite (α-Fe2O3), magnetite (Fe3O4), and maghemite (γ-Fe2O3).

Superparamagnetic iron oxide nanoparticles can be conjugated to a variety of particles, such as antibodies for selective targeting.

Passive and active tumor targeting

NPs can be selectively delivered to tumors by passive and/or active targeting.

Passive targeting is likely due to enhanced permeability and retention, determined by

1) extravasation of large molecules through a leaky neo-vascular tumor system and
2) lack of functional lymphatics resulting in the accumulation of nano-materials at the tumor site.

Active targeting is mediated by NP conjugated ligands that bind with high-affinity, high-selectivity target molecules overexpressed by tumor cells as compared to healthy tissues. Targeting ligands explored to date include peptides, small organic molecules, oligosaccharides and antibodies.

The progression of most cancers is associated with the altered expression of cell surface proteins such as adhesion molecules and receptors.

Article

Journal of Biomedical Nanotechnology
Vol. 11, 81–92, 2015
www.aspbs.com/jbn

In Vivo Targeting of Cutaneous Melanoma Using an Melanoma Stimulating Hormone-Engineered Human Protein Cage with Fluorophore and Magnetic Resonance Imaging Tracers

Luca Vannucci[1,†], Elisabetta Falvo[2,†], Cristina Maria Failla[3], Miriam Carbo[2], Manuela Fornara[4], Rossella Canese[5], Serena Cecchetti[5], Lenka Rajsiglova[1], Dmitry Stakheev[1], Jiri Krizan[1], Alberto Boffi[2,4,6], Giulia Carpinelli[5], Veronica Morea[2], and Pierpaolo Ceci[2,*]

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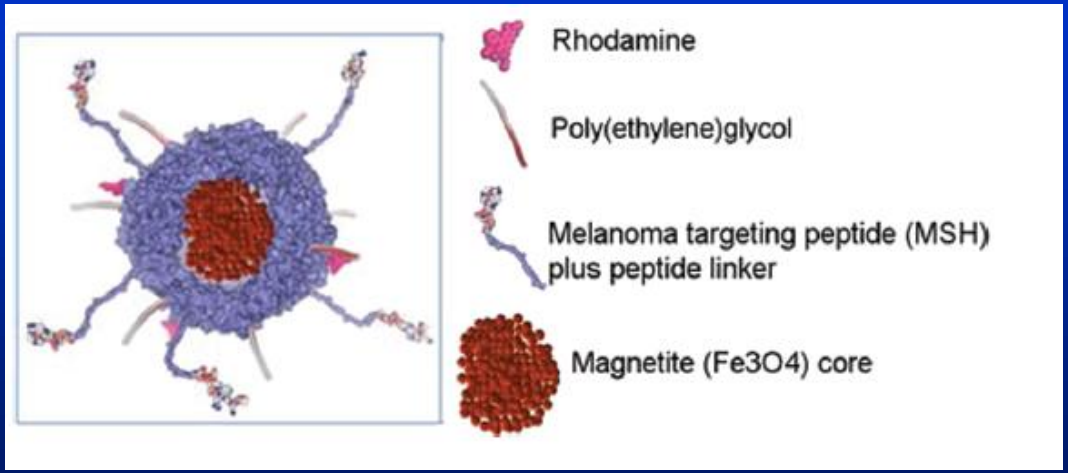
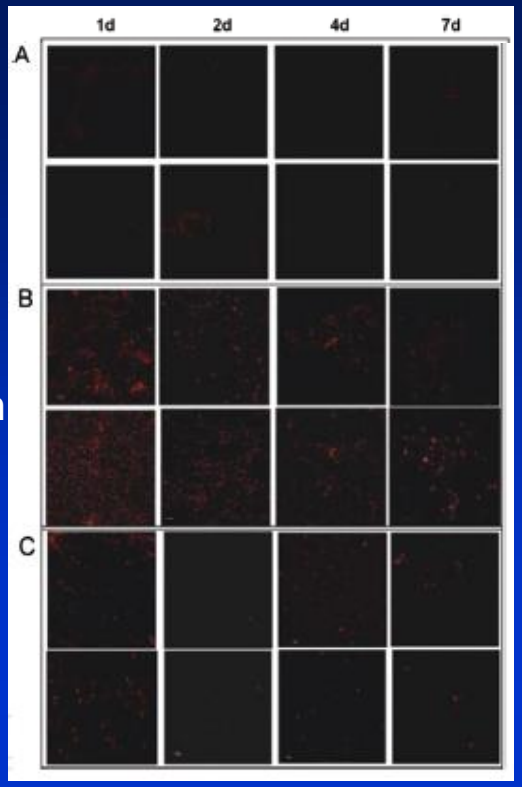
The combined use of different and complementary techniques, such as whole-mount confocal microscopy and MRI, demonstrated the efficient, specific, long-term targeting of primary melanoma by HFt-MSH-PEG NPs after systemic administration to melanoma bearing mice.



This construct is based on the human ferritin protein (HFt), a highly symmetrical assembly of 24 subunits enclosing a hollow cavity.
Functionalities:
1) alpha-melanocyte-stimulating hormone (MSH), a potent agonist of melanocortin receptor 1 (MC1R), as a targeting moiety;
2) poly(ethylene glycol) (PEG) molecules as stabilizing and long circulation- enabling agents;
3) rhodamine fluorophores for tracking diffusion and localization of NPS;
4) magnetite-maghemite encapsulation



skin

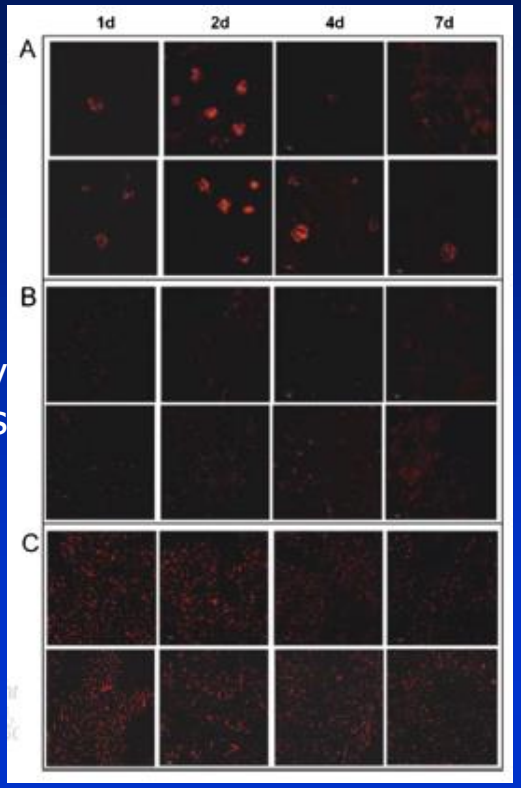
spleen

lung

kidney glomeruli

kidney tubuls

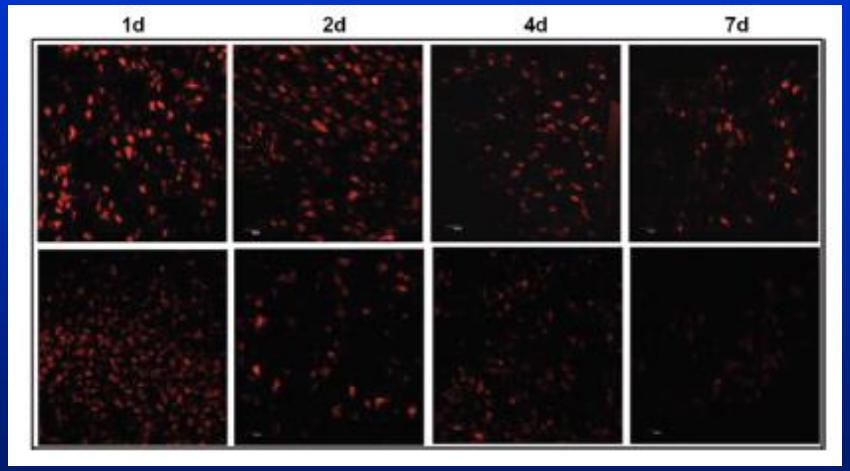
liver



In vivo localization of targeted (HFt-MSH-PEG, top panels) and untargeted (HFt-PEG, bottom panels) NPs 1, 2, 4, and 7 days after intracardial injection in B16F10 melanoma-bearing mice.
Specimens were analyzed by whole-mount confocal microscopy and localization of rhodamine-labeled HFt-based NPs is shown in red.

The intense fluorescence signal detected in the melanoma, and the absence (skin) or limited presence and fast disappearance of fluorescence in other examined tissues (lung, kidney, spleen and liver) indicated that the HFt-MSH-PEG NPs were able to target primary melanoma with high selectivity with respect to other organs and untargeted NPs.

B16F10 melanoma



24h after melanoma targeting HFt-MSH-PEG NPs

24h after untargeted HFt-PEG NPs

from Vannucci et al, J Biomedical Nanotech 2015

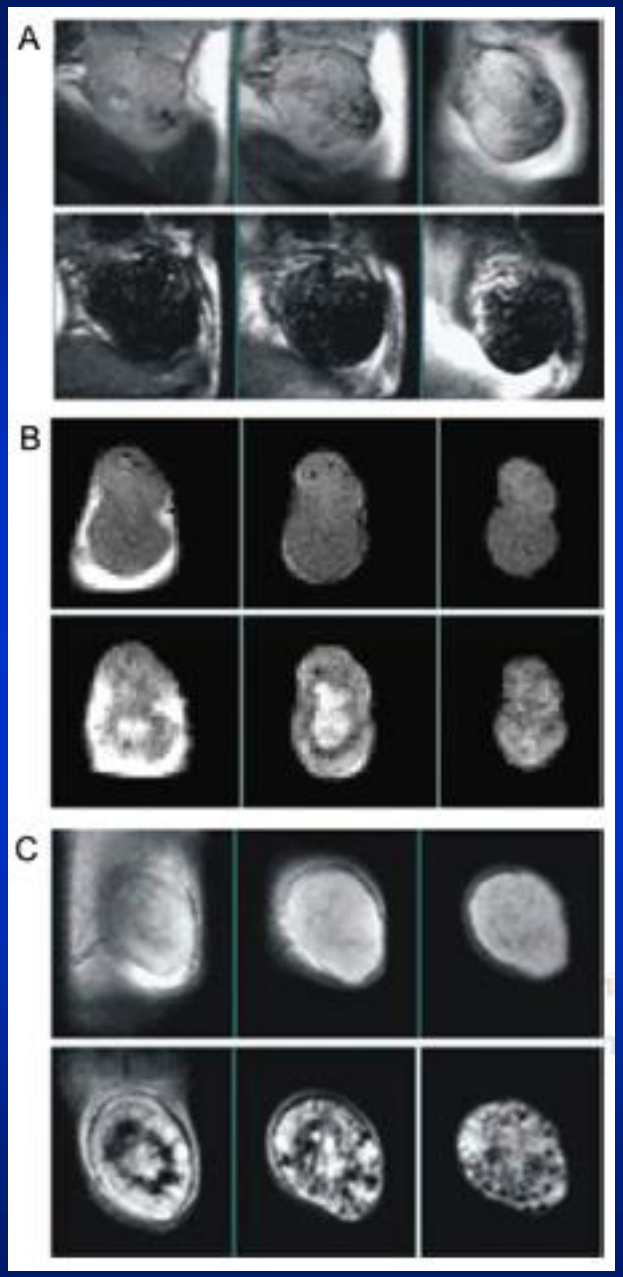
In vivo T2-weighted MRI of primary tumors.

Tumor bearing mice were analyzed by MRI after intracardial administration of magnetite-loaded HFt-based NPs.

Accumulation of NPs appears as dark areas in the tumor sections.

Only NPs possessing the MSH ligand were seen to accumulate in melanomas. The untargeted NPs were confined to vessels.

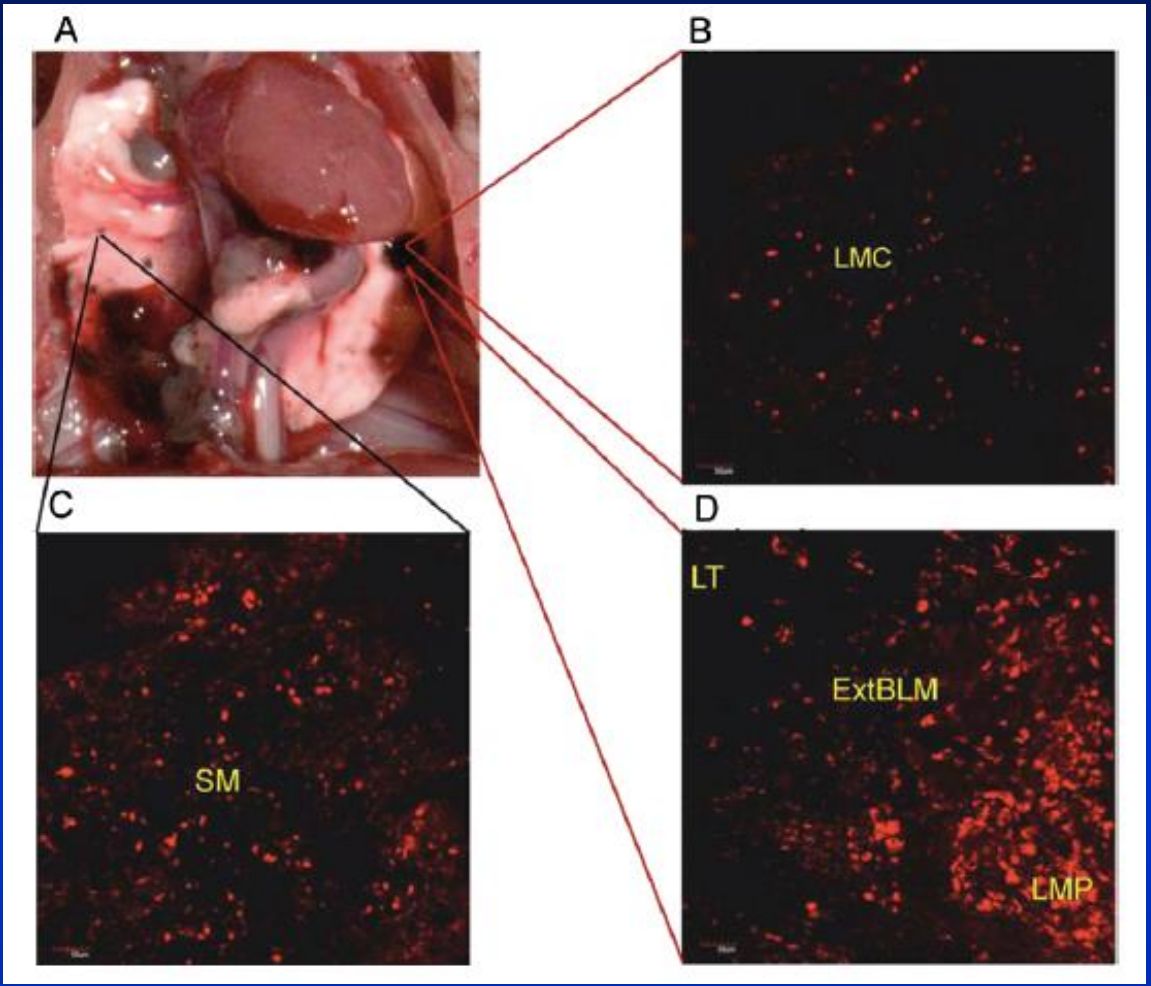
The melanoma targeting NPs did not accumulate within the adenocarcinoma but remained confined to the vascular bed.



from Vannucci et al, J Biomedical Nanotech 2015

Targeted NP localization in metastasis of < 1 mm was mostly homogeneous, while in larger metastases (≥ 1 mm), NPs accumulated more in the periphery than in the central section

Untargeted HFt-NPs were only found in the vascularized periphery of a large metastasis



HFt-MSH-PEG NP localization in a spontaneous model of B16F10 melanoma lung metastasis.
(A) In situ lungs showing metastatic spread with metastases of various dimensions.
(B) NP localization in the central section of a large metastasis (LMC; 2.5 mm).
(C) Homogeneous distribution of NPs in a small metastasis (SM; 0.5 mm)
(D) Vascularized periphery (LMP) and external border (ExtBLM) of a large metastasis and lung tissue (LT).

from Vannucci et al, J Biomedical Nanotech 2015

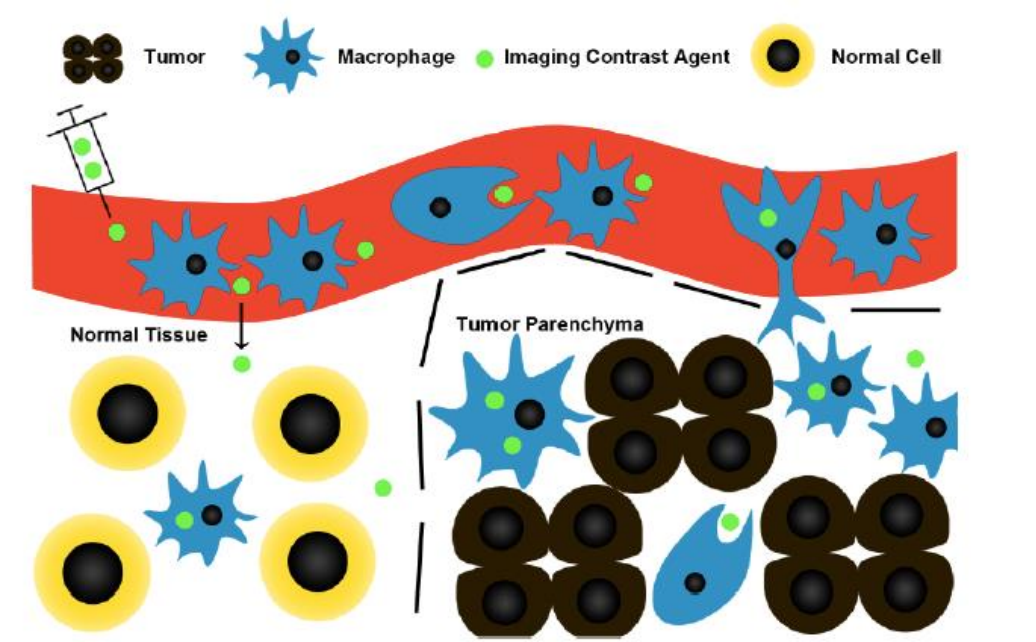
In Vivo MR Imaging of Tumor-Associated Macrophages: The Next Frontier in Cancer Imaging

Runze Yang[1,2,3], Susobhan Sarkar[2,3], V Wee Yong[2,3,4] and Jeff F Dunn[1,2,3]

[1]Department of Radiology, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada. [2]Hotchkiss Brain Institute, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada. [3]Department of Clinical Neurosciences, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada. [4]Department of Oncology, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada.

Magnetic Resonance Insights
Volume 11: 1–8

DOI: 10.1177/1178623X18771974
SAGE



Contrast agents are preferentially phagocytosed by monocytes and macrophages, and can be used to label TAMs. Contrast agents are injected intravenously where they are phagocytosed by circulating monocytes. Some of these monocytes migrate into the tumor and differentiate into macrophages. As tumors have often increased vascular permeability, contrast agents could also leak into the tumor and be picked up by macrophages.

Post-contrast images are usually acquired at least 24 hours after administration of contrast, to allow time for phagocytosis/cell migration, and wash out of non-phagocytosed contrast agent.

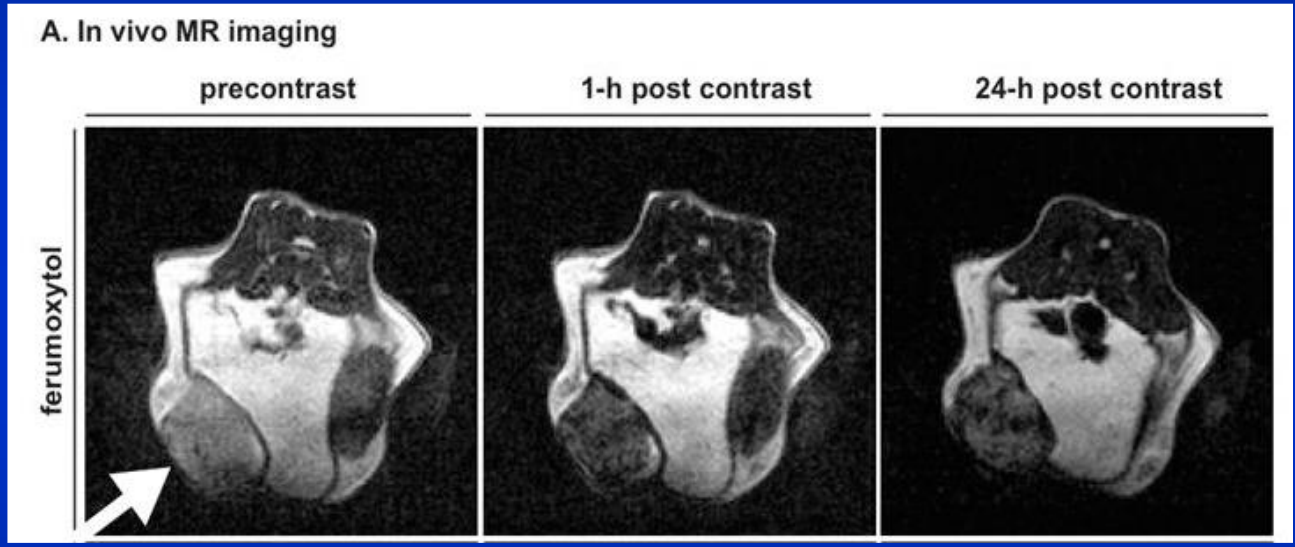
In vivo MRI probes to visualize Tumor-Associated Macrophages, TAMs

1H MRI **Ferumoxytol (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) to treat anemia and recently used to monitor TAMs in a glioblastoma	**Gadolinium** is not readily phagocytosed by macrophages, so it 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

MR Imaging of Tumor-Associated Macrophages with Clinically- Applicable Iron Oxide Nanoparticles

From Daldrup-Link et al, Clin Cancer Res 2011 17:5695-5704

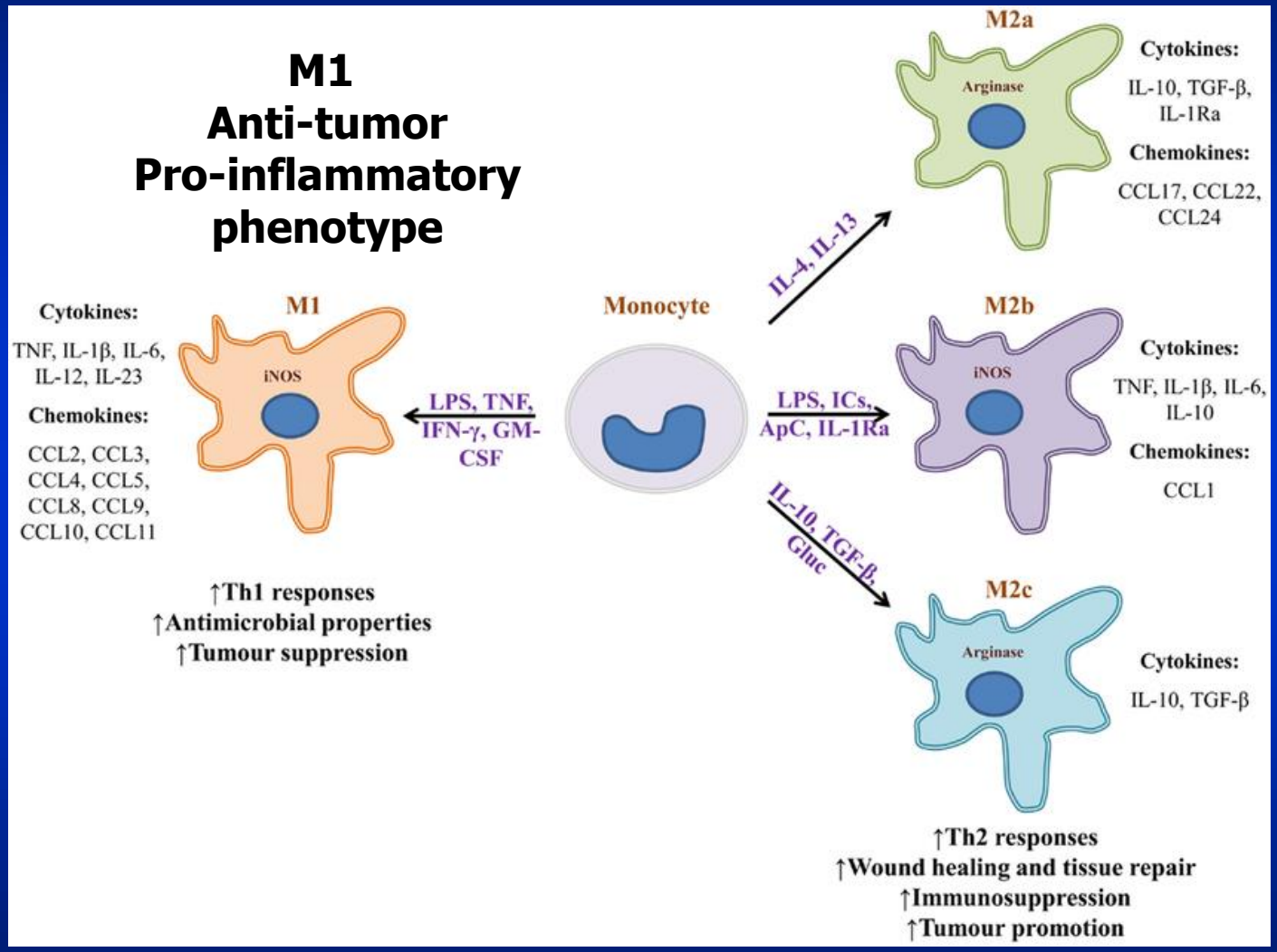
In vivo, ferumoxytol administration was associated with an initial tumor perfusion, followed by tumor retention and persistent MR-enhancement 24 hours after i.v. administration, which correlated with phagocytosed nanoparticles in TAMs.



FDA-approved iron oxide nanoparticle compound ferumoxytol (Feraheme) is preferentially phagocytosed by TAMs, but not by neoplastic tumor cells.

Ferumoxytol-enhancement may serve as a new biomarker for long-term prognosis and related treatment decisions that will support ongoing development of new immune-targeted therapies.

Immune escape mechanisms in cancer

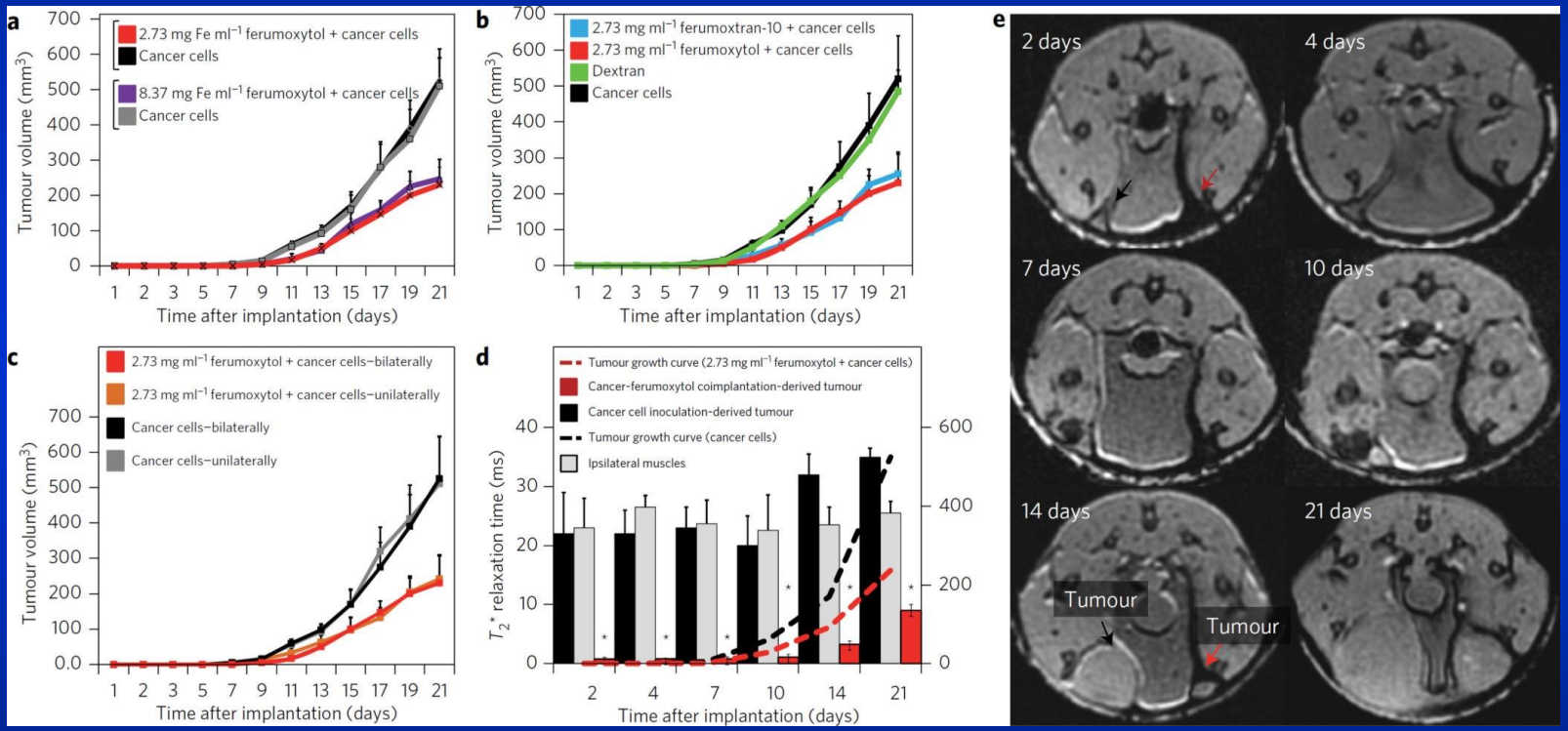


M2
Pro-tumor
Anti-inflammatory
phenotype

Ferumoxytol polarizes TAMs into an anti-tumor phenotype

Iron oxide nanoparticles inhibit tumor growth by inducing pro- inflammatory macrophage polarization in tumor tissues

From Zanganeh et al, *Nat Nanotechnol* 2016



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

- Intravenous injection of ferumoxytol can suppress tumor growth in several mouse tumor models.
- Ferumoxytol metabolization by macrophages (but not monocytes) can polarize them into an anti-tumor phenotype [Histology showed an increase in pro-inflammatory macrophage marker (CD80), and a decrease in anti-inflammatory marker (CD206) within the tumor].

PFC emulsion detects macrophage distribution and density

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

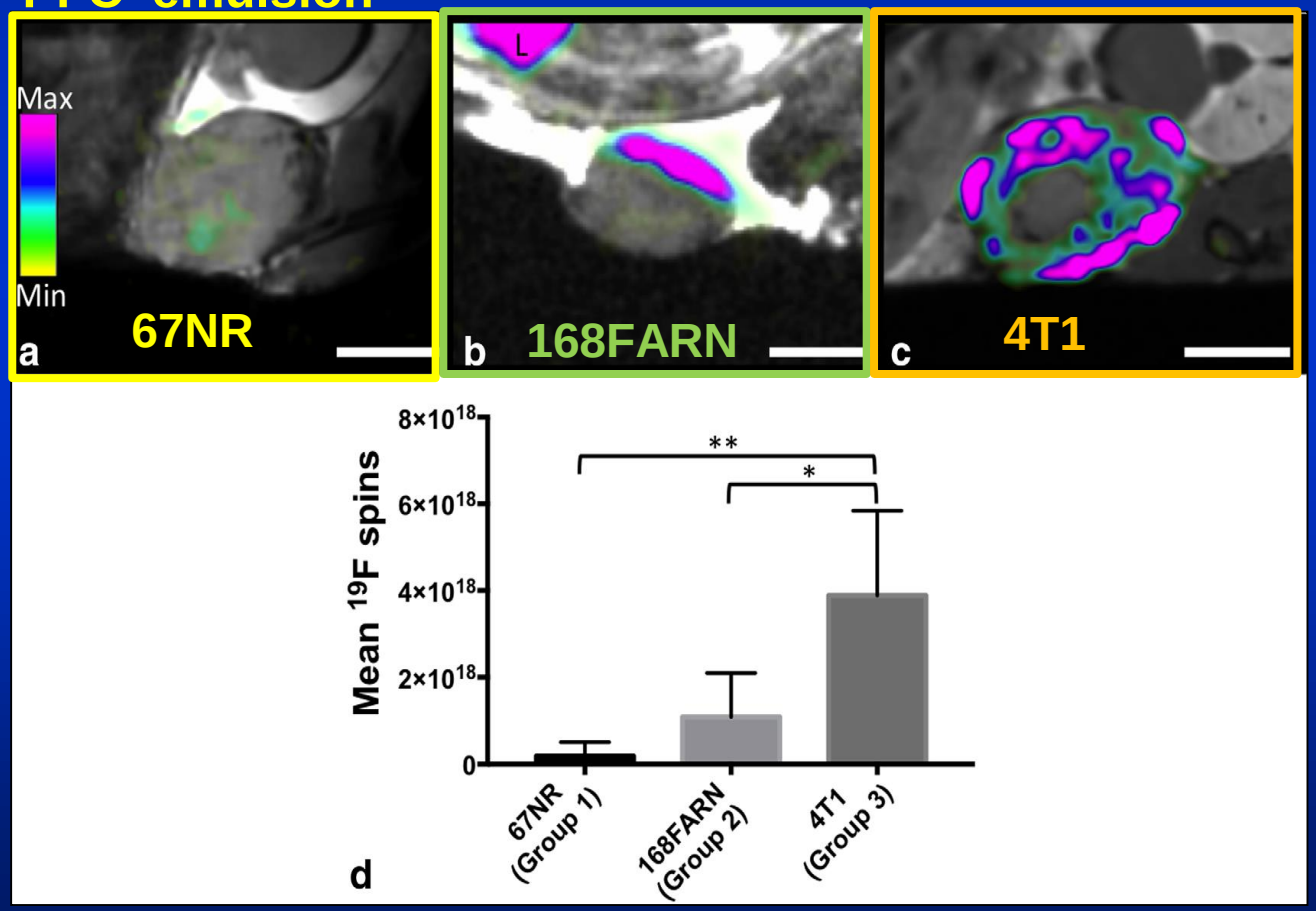
a) A very low 19F signal was detected over few voxels in **non-metastatic 67NR tumors**.

b) A moderate19F signal was seen in **168FARN tumors**, typically along the tumor edge.

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

This technique can be used to discriminate between breast tumours of varying levels of aggressiveness

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



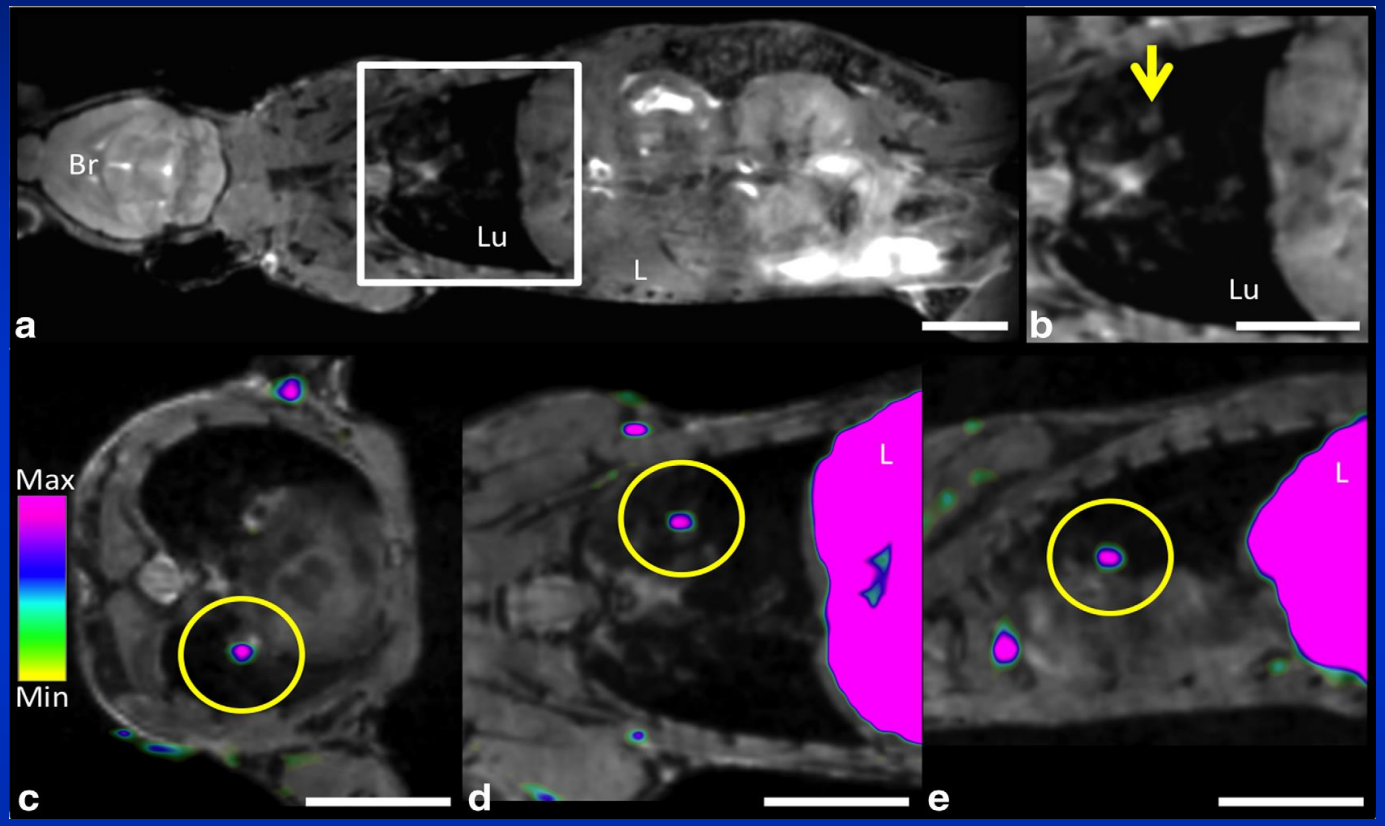
Makela et al, Magn Reson Med 80:1138–1147, 2018.

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.

Considering that non tumour-bearing mice have ^{19}F signal coming from liver, spleen, lymph nodes and bone marrow due to uptake of PFC by resident phagocytic cells, other spots of PFC accumulation are due to the presence of metastasis.

19F signal was observed within lung metastases in mice with 4T1 tumours, and fluorescence microscopy confirmed the presence of PFC-positive macrophages.



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

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

Magnetic Resonance Targeting (MRT) increased the anti-tumor 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

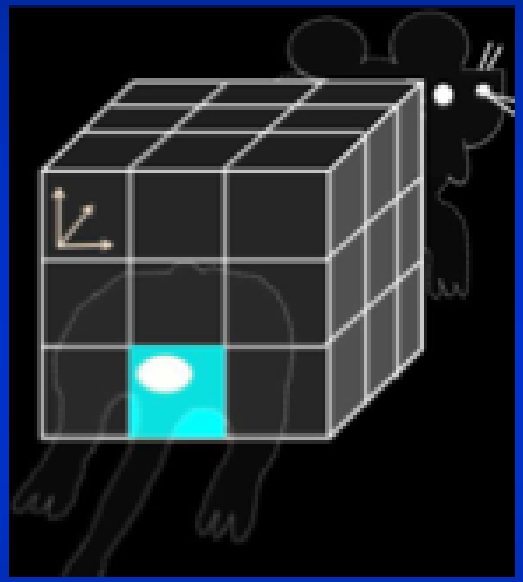
An MRI system is used to direct macrophages carrying an oncolytic virus, **Seprehvir,** into primary and metastatic tumor sites in mice.

Macrophages were magnetically labeled with super-paramagnetic iron oxide nanoparticles (SPION), and pulsed magnetic field gradients were applied in the direction of the tumor sites.

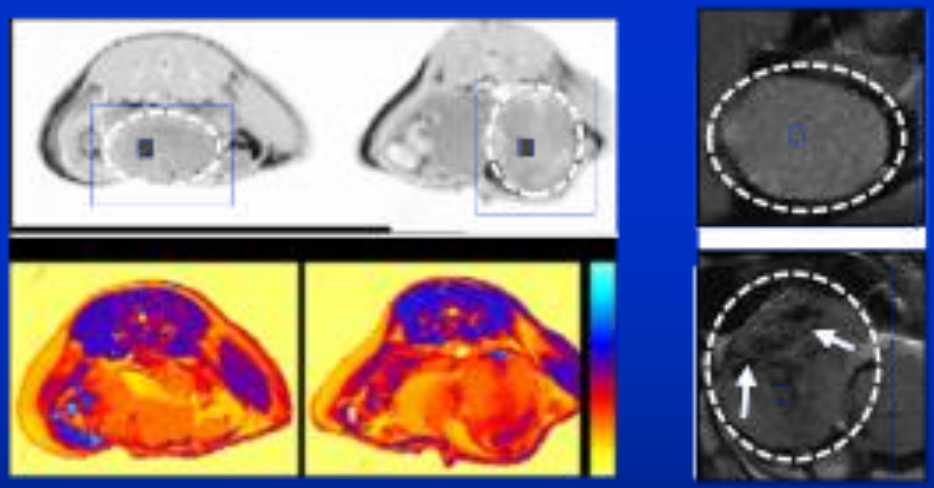
MRT guides macrophages from the bloodstream into tumors, which results in increased tumor macrophage infiltration and a reduction in tumor burden and metastasis.

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

Magnetic resonance targeting (MRT) 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 tumors.
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, to poorly vascularized, relatively inaccessible tumor areas.

From Muthana et al Nature Communication 2014

Theranostics 2019, Vol. 9, Issue 3 778

IVYSPRING INTERNATIONAL PUBLISHER

Theranostics

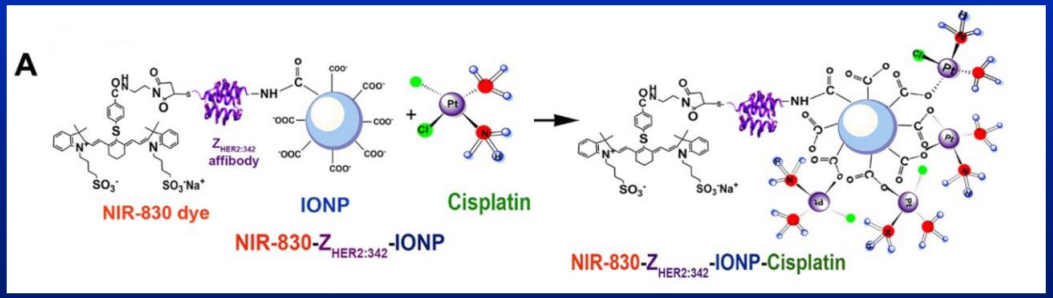
2019; 9(3): 778-795. doi: 10.7150/thno.29964

Research Paper

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

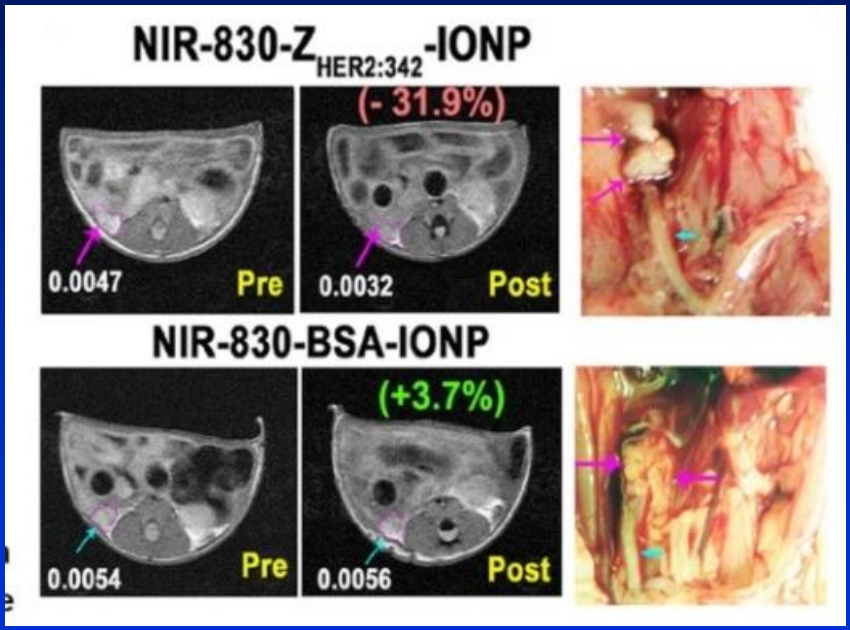
Minati Satpathy[1], Liya Wang[2], Rafal J. Zielinski[3], Weiping Qian[1], Y. Andrew Wang[4], Aaron M. Mohs[5], Brad A. Kairdolf[5], Xin Ji[4], Jacek Capala[6], Malgorzata Lipowska[2], Shuming Nie[5], Hui Mao[2], Lily Yang[1]

HER2-targeted theranostic nanoparticles carrying cisplatin in ovarian cancer



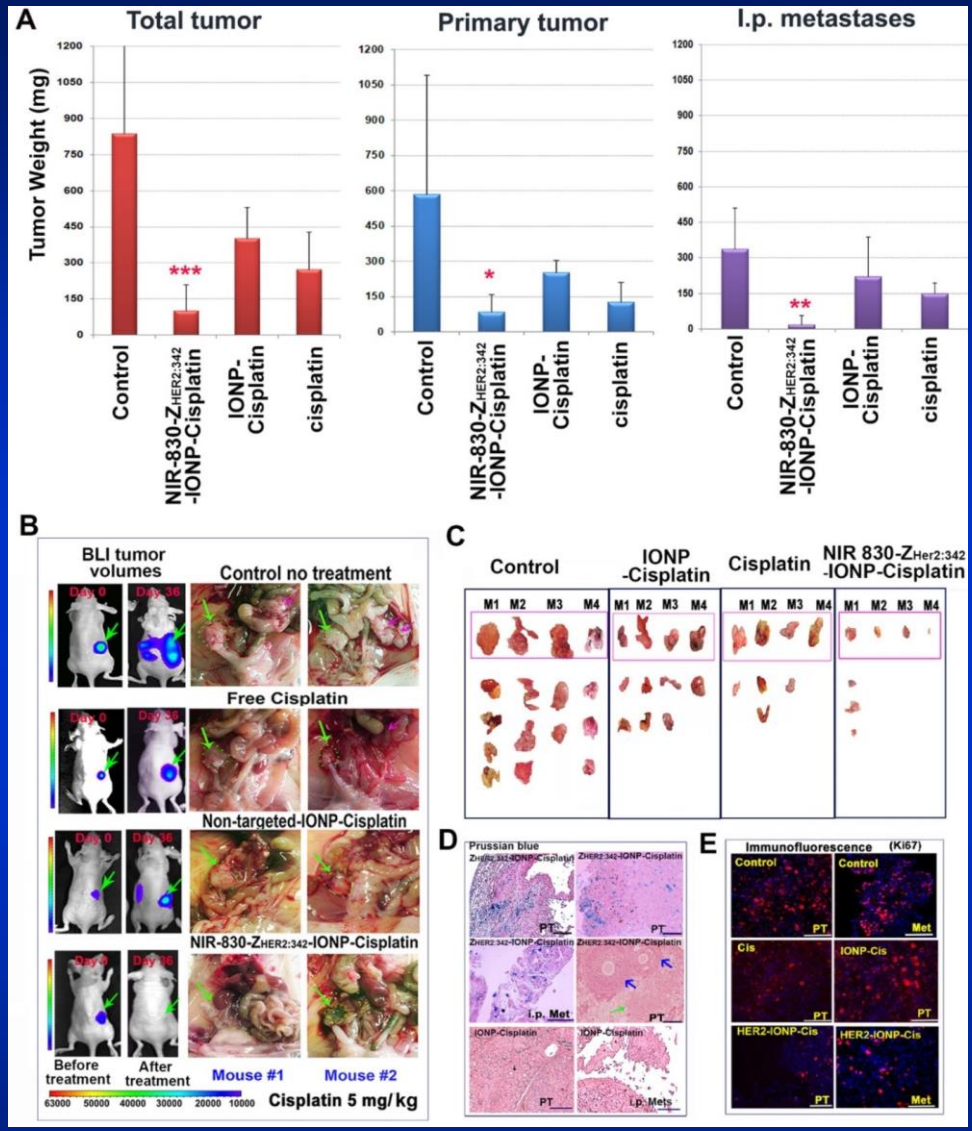
Another innovative strategy with the ability to both deliver drug and to image tumours have been described in this paper.

This is a promising platform for the development of image-guided cancer therapy of heterogeneous and drug-resistant human cancers



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.

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.



From Satpathy et al Theranostics 2019

In the Core facilities of the Istituto Superiore di Sanità (ISS)

From a 4.7 T Varian system ….

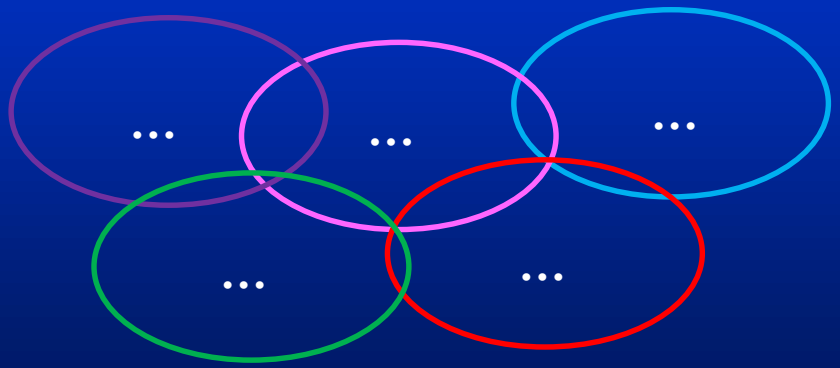
….to a 7 T Bruker system equipped with a cryo probe

Oncology

Neuroscience

Metabolic diseases

New applications

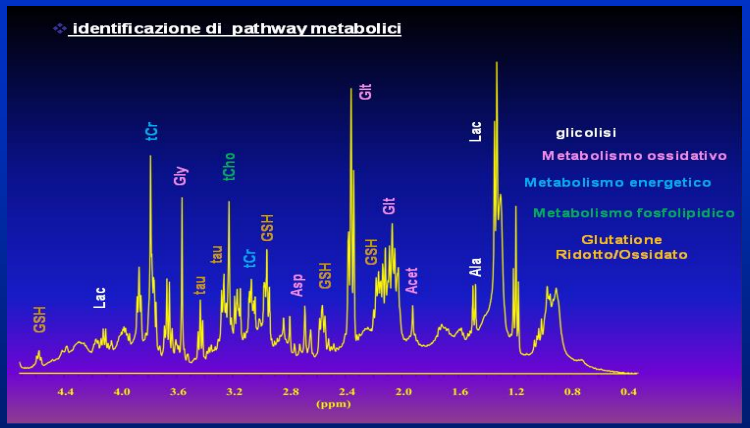


NMR and MRI Area

High resolution NMR (9.4T)

Egidio Iorio
Maria Elena Pisanu
Mattea Chirico

Servizio Grandi strumentazioni e Core facilities
Istituto Superiore di Sanità, Roma



MRI in preclinical models: a focus on nanomedicine

Research on well-characterized, controlled animal models is an essential step for fundamental discoveries in oncology.
Among medical imaging modalities, MRI is the technique that most benefits for the development of innovative and targeted contrast agents.

Nanomaterials, such as nanoparticles, in diagnostic and theranostic imaging are indispensable tools for cancer detection, and for the identification of novel therapeutic strategies. Continuous discovery in nanotechnology is expected to significantly influence future cancer therapy and medical imaging.

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Thank you for your attention