MRI of Tumor-Associated Macrophages with Clinically Applicable Iron Oxide Nanoparticles

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Abstract

Purpose: The presence of tumor-associated macrophages (TAM) in breast cancer correlates strongly with poor outcome. The purpose of this study was to develop a clinically applicable, noninvasive diagnostic assay for selective targeting and visualization of TAMs in breast cancer, based on magnetic resonance and clinically applicable iron oxide nanoparticles.

Experimental Design: F4/80-negative mammary carcinoma cells and F4/80-positive TAMs were incubated with iron oxide nanoparticles and were compared with respect to magnetic resonance signal changes and iron uptake. MMTV-PyMT transgenic mice harboring mammary carcinomas underwent nanoparticle-enhanced magnetic resonance imaging (MRI) up to 1 hour and 24 hours after injection. The tumor enhancement on MRIs was correlated with the presence and location of TAMs and nanoparticles by confocal microscopy.

Results: In vitro studies revealed that iron oxide nanoparticles are preferentially phagocytosed by TAMs but not by malignant tumor cells. In vivo, all tumors showed an initial contrast agent perfusion on immediate postcontrast MRIs with gradual transendothelial leakage into the tumor interstitium. Twenty-four hours after injection, all tumors showed a persistent signal decline on MRIs. TAM depletion via cCSF1 monoclonal antibodies led to significant inhibition of tumor nanoparticle enhancement. Detection of iron using 3,3'diaminobenzidine-enhanced Prussian Blue staining, combined with immunodetection of CD68, localized iron oxide nanoparticles to TAMs, showing that the signal effects on delayed MRIs were largely due to TAM-mediated uptake of contrast agent.

Conclusion: These data indicate that tumor enhancement with clinically applicable iron oxide nanoparticles may serve as a new biomarker for long-term prognosis, related treatment decisions, and the evaluation of new immune-targeted therapies.

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Introduction

Although breast cancer has not historically been linked to underlying inflammation or infection, it exhibits tumor-associated inflammation marked by infiltration of leukocytes into developing tumors where increases in some leukocyte subsets parallels disease progression (1–3). In the majority of cases, however, the natural immunity to cancer that is present is not protective, but instead fosters progression. Studies in transgenic mouse models of mammary carcinogenesis revealed that tumor-associated macrophages (TAM) promote tumor growth and enhance pulmonary metastasis by high-level expression of epidermal growth factor (EGF) and activation of EGF-regulated signaling in mammary epithelial cells (MEC) critical for invasive tumor growth and metastatic dissemination (4). Histopathologic and flow cytometric evaluations have revealed that TAMs are the most abundant innate immune cell present in murine mammary carcinomas and in human breast cancers (2, 5). TAM presence in several types of human cancer, including breast, correlates with increased vascular density and worse clinical outcome (6–11). A clinically reliable noninvasive in vivo imaging test that could reliably detect and quantify TAMs could be employed as a novel, widely applicable prognostic assay for stratifying individual patients to more aggressive and/or TAM-targeted therapies.

Intravenously injected superparamagnetic iron oxide (SPIO) nanoparticles are effective contrast agents for magnetic resonance imaging (MRI). SPIO are phagocytosed by macrophages in various target tissues depending on their particle size and composition. Relatively large SPIO with
The presence of tumor-associated macrophages (TAM) in adenocarcinomas correlates strongly with poor outcome in patients with breast cancer. Our data indicate that the Food and Drug Administration (FDA)-approved iron oxide nanoparticle compound ferumoxytol (Feraheme) is preferentially phagocytosed by TAMs, but not by neoplastic tumor cells. In vivo, ferumoxytol administration was associated with an initial tumor perfusion, followed by tumor retention and persistent magnetic resonance-enhancement at 24 hours after intravenous administration, which correlated with phagocytosed nanoparticles in TAMs. Together, these data indicate that 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. Since ferumoxytol is FDA-approved as an iron supplement, this application is immediately clinically applicable as an imaging approach via an "off label" use.

Translational Relevance

- The goal of this study was to utilize novel USPIO to develop an immediately clinically applicable molecular imaging approach for enhanced imaging of TAMs in breast cancer. Our imaging technique relies on the iron supplement ferumoxytol (Feraheme), recently Food and Drug Administration–approved for intravenous treatment of iron deficiency in patients (15–17). Ferumoxytol is also a USPIO compound, providing a strong signal effect on MRIs and thus exerting properties of a magnetic resonance contrast agent (18–21). On the basis of these properties, we postulated that ferumoxytol would be phagocytosed by TAMs in breast cancer, thereby enabling selective detection of TAMs on delayed, postperfusion MRIs.

Materials and Methods

Contrast agents

Three USPIO nanoparticle compounds were investigated: (i) Ferumoxytol (Feraheme, AMAG Pharmaceuticals Inc.) is a USPIO nanoparticle applied for intravenous treatment of iron deficiency in patients with impaired renal function (15, 16, 20, 22, 23). Ferumoxytol consists of an iron oxide core and a carboxydextran coating. Ferumoxytol has a mean hydrodynamic diameter of 30 nm, an \( r_1 \) relaxivity of 38 \( s^{-1} mM^{-1} \), and an \( r_2 \) relaxivity of 83 \( s^{-1} mM^{-1} \) at 0.94 T and 37°C. Ferumoxytol was conjugated to fluorescein isothiocyanate (FITC; Ferumoxytol–FITC) for detection by immunofluorescent microscopy. (ii) P904 (Guerbet Group, Paris, France) is a USPIO compound currently in phase I clinical trials in Europe with plans for global distribution (24–27). P904 consists of an iron oxide core and a hydrophilic coating by a monomeric organic molecule with 20 hydroxyl groups. P904 has a mean hydrodynamic diameter of 21 nm, an \( r_1 \) of 14 \( s^{-1} mM^{-1} \), and an \( r_2 \) relaxivity of 92 \( s^{-1} mM^{-1} \) at 1.5 T and 37°C. (iii) P1133 (Guerbet) is a preclinical USPIO with potential future clinical development (24). P1133 is based on P904 but also incorporates 8 to 10 folate moieties per nanoparticle in its coating, added via an amino PEG derivative of folinic acid coupled on its g-carboxylic moiety to the carboxylate-bearing iron core. P1133 has a mean hydrodynamic diameter of 26 nm, an \( r_1 \) relaxivity of 12 \( s^{-1} mM^{-1} \), and an \( r_2 \) relaxivity of 95 \( s^{-1} mM^{-1} \) at 1.5 T and 37°C.

Animal model

This study was approved by the animal care and use committees at the respective institutions. MMTV-PyMT mice that spontaneously develop multifocal, multiclonal mammary adenocarcinomas were used at 12 to 14 weeks of age, weight, and tumor size were not significantly different between experimental groups that received different contrast agents \((P > 0.05)\). Additional experiments were carried out in 7 postpubertal female FVB/n mice (10–12 weeks), which received injections of 50,000 PyMT-derived tumor cells into the right lower mammary fat pad for induction of orthotopic tumors. Three of these animals were treated with anti-colony stimulating factor (CSF)-1 monoclonal antibody (mAb), clone 5A1, purified by the UCSF Hybridoma core using the ATCC hybridoma (#CRL-2702). The animals received an intraperitoneal injection of 2 mg of anti-CSF1 mAb, consisting of a 1 mg starting dose followed by 0.5 mg chaser doses on day 5 and 8, and ferumoxytol-enhanced MRI on day 9. Three additional animals served as controls and received intraperitoneal injections of PBS at the corresponding time points above, followed by ferumoxytol-enhanced MRI. One additional mouse received 3 subsequent MRIs at 0, 1, and 24 hours without any contrast agent injection to confirm that tumors did not show any changes in magnetic resonance signal over a 2 day observation period. For all animals, MRI experiments were carried out when mammary tumors reached an approximate size of 1.0 cm.

Macrophage isolation and in vitro labeling

Tumors from MMTV-PyMT mice at day 90 to 95, or PyMT-orthotopic tumors, were isolated and digested in collagenase and DNAse (Roche Applied Sciences), strained over a cell strainer (BD Falcon, BD Biosciences), and incubated with phycoerythrin (PE)-conjugated rat anti-mouse F4/80 antibody (clone CI:A3-1, Caltag). Cells were then incubated with anti-PE magnetic beads and isolated...
over a magnetic column to provide F4/80+ cells (macrophages and monocytes) and F4/80− cell fractions (malignant mammary epithelial cells and other stromal populations). In a previous study, we reported that F4/80+ cells represent Ly6C+Ly6C−CD11b+F4/80+ TAMs (2). A total of $4 \times 10^6$ of both F4/80+ and F4/80− cells were plated onto cell culture dishes in DMEM supplemented with 10% fetal calf serum. A total of 200 µg [Fe]/ml of either Ferumoxytol, P1133, or P904 were added to the cell culture medium. Additional samples were incubated with P1133 + 1.67 µmol/ml of free folic acid, a dose that corresponds to 10 times the dose of folic acid engrafted onto P1133. Cells were incubated overnight at standard cell culture conditions (37°C, 5% CO2). The next day, nonadherent cells were discarded and adherent cells were removed via a cell lifter. Removed cells were washed 3 times in PBS and resuspended in 400 µl of ficoll at a density of 1.07 g/ml and placed into 2.0 ml conical tubes for imaging. Experiments were done in duplicates.

In vitro cell imaging and data analysis

For in vitro imaging of nanoparticle-loaded cells, a clinical 3T scanner was used (Signa Excite HD, GE Medical Systems) with a standard wrist coil (USA Instruments). Test tubes were immersed in a water bath and a multiecho spin echo sequence was obtained with the following parameters: TE 15, 30, 45, 60 ms, TR 2000 ms, FOV 8 × 8 cm, matrix 256 × 196 pixels, slice thickness 2 mm, and 2 acquisitions. Image processing was done by using MRVision software (MR Vision Co.). T2 relaxation times were calculated assuming a monoeponential signal decay and using nonlinear least square curve fitting on a pixel by pixel basis.

Determination of cell iron content

After imaging, cell samples were digested overnight in trypsin and placed in 10% HNO3. Inductively coupled plasma optical emission spectroscopy (ICP-OES) was done to quantify the iron content per sample (Perkin-Elmer).

In vivo imaging

Animals were randomly assigned to MRI when their tumor reached a size of approximately 1.0 cm. Imaging of MMTV-PyMT mice before and after injection of different nanoparticles was done with a 2 T Omega CSI-II magnetic resonance scanner (Bruker Instruments) and imaging of mice before and after anti-CSF1 mAb treatment was done with a 1 T desktop magnetic resonance scanner (Aspect M2 Compact High Performance MR System). Animals were anesthetized with isoflurane and placed on a recirculating water warming pad in a dedicated radiofrequency coil for high resolution MRI. A butterfly cannula filled with heparinized saline solution was introduced into the tail vein and left in place. T1, T2, and T2* weighted imaging sequences were obtained with the following parameters: T1 Spin echo (SE): TR 500 ms, TE 12 ms; T2 SE: TR 2000-2500 ms, TE 15, 30, 45, 60 ms (2T), and TE 20, 40, 60, 80 ms (1T); T2* Gradient echo (GE): TR 240 ms, TE 10 ms, flip angle 30 degrees (2T). MRIs were obtained with a field of view (FOV) of 3 × 3 cm (2T) or 6 × 6 cm (1T), a matrix of 128 × 128 or 200 × 200 pixels, and a slice thickness of 1 to 2 mm.

Following precontrast T1, T2, and T2* weighted imaging, 24 PyMT animals received intravenous injections of 0.5 mmol [Fe]/kg ferumoxytol (n = 7), P904 (n = 7), P1133 (n = 7), P1133 + 2.35 mmol/kg free folic acid (100 times the dose of folate engrafted onto P1133; n = 3), or P1133 + 0.235 mmol/kg free folic acid (10 times the dose of folate engrafted onto P1133; n = 3). Additional tumor-bearing mice after anti-CSF1 mAb treatment (n = 3) or controls (n = 3) were injected with 0.5 mmol [Fe]/kg ferumoxytol. After contrast media injection, without repositioning the mouse, 6 subsequent multiecho T2 SE sequences were obtained over the course of an hour, followed by T1- and T2*-weighted images. Mice were removed from the scanner, allowed to wake up, and imaged 24 hours later with T1, T2, and T2* weighted sequences. T2-relaxation times of the tumor were calculated based on multiecho SE sequences and converted to R2-relaxation rates (R2 = 1/T2), which is proportional to contrast agent concentration. The relative change in R2 data between pre- and postcontrast MRIs, ΔR2 (%), was determined as a quantitative measure of tumor contrast enhancement.

Histology

After the last MRI, at 24 hours postcontrast media injection, mice were sacrificed, and mammary tumors explanted and placed in optimal cutting temperature (OCT) compound on dry ice for histologic processing. Samples were cut onto slides and warmed to room temperature, followed by fixation in 100% ice-cold acetone. Some samples were then washed in H2O2 and iron deposits in the tissue were detected using the Accustain Iron Stain Kit (Sigma-Aldrich) according to the manufacturer’s instructions, followed by signal enhancement with Fast 3,3-diaminobenzidine (DAB, Vector Laboratories) for 2 minutes. After blocking sections with PBS containing 5% goat serum and 2.5% bovine serum albumin (blocking buffer), sections were incubated overnight at 4°C with 0.5× blocking buffer containing either rabbit anti-mouse folate receptor α (1:100, Abcam) or rat anti-mouse folate receptor β (1:8, kind gift from Prof. Matsuyama, Kagoshima University, Japan; ref. 29). Staining for folate receptor β was enhanced using a biotinylated anti-rat secondary antibody (1:200, Vector Laboratories) and the Tyramide Signal Amplification kit (Perkin-Elmer). After extensive washing, sections were incubated overnight with FITC-conjugated rat anti-mouse CD68 (1:50, Serotec), followed by Alexa 488 conjugated goat anti-FITC and either Alexa 546-conjugated donkey anti-rabbit or Alexa 546-conjugated Streptavidin (1:500, Invitrogen). For detection of Ferumoxytol-FITC, sections were stained with rat anti-mouse CD68 (1:100, Serotec), washed, and then incubated with a combination of Alexa 546-conjugated donkey anti-rat and Alexa 488-conjugated goat anti-FITC. All slides were mounted using Prolong Gold with DAPI (Invitrogen) and analyzed using an LSM510 confocal microscope (Zeiss).
Statistics
Statistical analysis comparing the differences of tumor relaxation rates between mice receiving different contrast agents was done with a Wilcoxon rank sum test. A t test was used to determine the significance of differences between different cell samples and differences between age and tumor size of mice. A P value of less than 0.05 was considered significant.

Results
F4/80-positive TAMs phagocytose USPIO in vitro
Following incubation with the iron oxide nanoparticle ferumoxytol, F4/80+ TAMs showed a markedly decreased signal on T2-weighted MRIs, whereas F4/80− cells showed minimal signal changes compared with untreated controls (Fig. 1A). Calculation of changes in relaxation rates (ΔR) as quantitative measures of the magnetic resonance signal enhancement (Fig. 1B) corroborated the qualitative findings with significantly higher ΔR2 data for ferumoxytol-exposed F4/80+ TAMs compared with ferumoxytol-exposed F4/80− cells consisting primarily of carcinoma cells (P > 0.05). Determination of iron content in the samples revealed that increased iron uptake was responsible for the observed relaxation rate changes (Fig. 1C).

Since both TAMs and malignant epithelial cells highly express the folate receptor, folate-linked USPIO have been recently developed for “tumor-targeted imaging” (24, 30, 31). F4/80+ TAMs incubated with folate-engrafted P1133 nanoparticles showed significantly stronger visual and quantitative magnetic resonance signal enhancement as compared with ferumoxytol and P904 (Fig. 1). However, folate-engraftment also leads to significantly increased nanoparticle uptake and magnetic resonance enhancement of F4/80+ populations. The P1133-induced signal effect was inhibited by coinubcation with free folic acid to P904 levels (Fig. 1), thus indicating that folate-targeting mediates increased USPIO uptake in vitro.

Ferumoxytol leads to persistent tumor enhancement on delayed, postperfusion MRIs and corresponds to specific nanoparticle retention in TAMs
We investigated 90-day-old MMTV-PyMT mice bearing late-stage mammary adenocarcinomas before and after intravenous injection of ferumoxytol, as well as syngeneic mice with PyMT-derived orthotopic mammary tumors. All tumors showed an initial negative (dark) enhancement on immediate postcontrast T2-weighted MRIs, which was most pronounced in the tumor periphery and increased slowly and gradually up to 1.0-hour postinjection (p.i.). This corresponds to an initial blood pool perfusion of USPIO with slow, gradual transendothelial leakage of the nanoparticles into the tumor interstitium (32–35). At 24-hour p.i. of ferumoxytol, all tumors showed a persistent signal decline, which was most pronounced in tumor centers (Fig. 2). We used DAB-enhanced Prussian Blue staining for detection of iron, and immunodetection of CD68+ TAMs in tissue sections of mammary tumors localized ferumoxytol to CD68+ TAMs (Fig. 3A). As it was difficult to show selective uptake using DAB-generated contrast due to high background, we also generated ferumoxytol-FTTC to show colocalization by immunofluorescence using an Alexa 488-conjugated anti-FTTC antibody. As shown in Figure 3B, ferumoxytol was specifically found within CD68+ TAMs, but not keratin 18-expressing malignant epithelial cells. Although ferumoxytol was not found within all TAMs, these results indicate that the magnetic resonance signal effects on delayed MRIs were largely due to TAM-mediated uptake of contrast agent.

USPIO-mediated TAM enhancement on delayed MRIs can be increased by folate receptor targeting of nanoparticles
To determine whether folate receptor targeting could enhance the MRIs, we obtained additional MRIs of
MMTV-PyMT mice with late-stage mammary adenocarcinomas injected with the folate-engrafted USPIO P1133, the nontargeted analogue P904, or P1133 plus free folic acid. P1133 and P904 caused a nonspecific tumor-perfusion effect on T2-weighted images during the first hour p.i., which was not significantly different as compared with tumor-bearing mice injected with ferumoxytol (Fig. 2). Delayed MRIs showed a significantly stronger persistent tumor signal decline at 24-hour p.i. of P1133 compared with ferumoxytol ($P < 0.05$).

In vivo inhibition experiments with free folic acid are limited due to rapid liver uptake and renal elimination of free folic acid (36). Inhibition experiments with free folic acid at a 10 times increased dose as compared with the folate dose delivered with P1133 resulted in a minor, albeit not significant inhibition of the P1133-induced tumor enhancement ($P > 0.05$). Inhibition experiments with higher folic acid doses proved toxic in tumor-bearing mice, similar to previous reports (37). However, delayed MRIs showed significantly less tumor enhancement at 24-hour
p.i. of folate-free P904 compared with folate-linked P1133 ($P < 0.05; \text{Fig. 2}$). Because P1133 and P904 are chemically identical except for folate engraftment on P1133, this data indicates increased nanoparticle uptake via folate receptor targeting.

We next evaluated TAMs versus epithelial cells for expression of $\alpha$ and $\beta$ folate receptor in mammary tumors and revealed folate receptor $\alpha$ staining throughout epithelium, with no expression detectable on CD68$^+$ TAMs (Fig. 4A). In contrast, expression of folate receptor $\beta$ was observed exclusively on CD68$^+$ cells, although these represented only a portion of the total CD68$^+$ TAMs found within tumors (Fig. 4B) and seemed to be primarily associated with vascular and peripheral regions of mammary tumors. Consistent with the MRIs, Prussian Blue staining for iron was more prominent within tumors from mice injected with P1133 compared with P904 or ferumoxytol (Fig. 4C). Furthermore, although some iron staining was observed in areas that did not seem occupied by TAMs (blue arrow), this was minor compared with staining within stromal areas likely enriched with TAMs (red arrow). Thus, while folate engraftment did increase uptake of USPIOs by cells
other than TAMs, these results indicate that folate modification of USPIOs may still improve their clinical use as evaluators of TAM presence within tumors.

**Ferumoxytol-enhanced MRI detects TAM-depletion after anti-CSF1-mAb treatment**

Imaging data from a control mouse that underwent 3 subsequent MRIs at 0, 1, and 24 hours without any contrast agent injection confirmed that MMTV-PyMT tumors do not show any intrinsic changes in magnetic resonance signal within a 2-day observation period. Mice treated with anti-CSF1 mAb showed a similar ferumoxytol-tumor perfusion effect compared with untreated controls during the first hour after intravenous ferumoxytol-injection. However, at 24-hour p.i., anti-CSF1 mAb-treated tumors showed less magnetic resonance contrast effects and significantly smaller ΔR2 enhancement data compared with untreated controls (Fig. 5A). Corresponding confocal microscopy evaluations confirmed TAM-depletion of anti-CSF1 mAb-treated tumors (Fig. 5B), indicating that ferumoxytol-enhanced MRI is related to TAM density.

**Discussion**

Results from this study show that ferumoxytol can be used as a reliable tool to quantitatively monitor macrophage presence in tumors, suggesting that this imaging technique can be readily investigated as a surrogate measure to predict outcomes for patients with breast cancer, and applied to monitor TAM-targeted therapies now in clinical trials.
confocal microscopy evaluations confirmed TAM-depletion within anti-
enhancement data compared with untreated controls. B, corresponding
resonance signal within a 2-day observation period. Note that mice
MMTV-PyMT tumors do not show any intrinsic changes in magnetic
resonance without any contrast agent injection to confirm that
control. An additional control mouse underwent serial magnetic
imaging. MRI, on the other hand, is radiation free, estab-
ishment for breast cancer detection, and integrates near-
imaging TAM detection, or if the dose can be safely increased. Of
studies reported herein were done with ferumoxytol doses
of 0.5 mmol/kg. Previously described ferumoxytol doses
in humans were in the order of 0.035–0.072 mmol/kg
(18, 19). Iron oxide nanoparticles are generally applied
in higher doses in rodents as opposed to humans to
compensate for the relatively shorter blood half life in
rodents. However, future clinical applications must show
if the currently applied dose in patients is sufficient for
TAM detection, or if the dose can be safely increased. Of
note, ferumoxytol showed an excellent safety profile in
patients (2–4, 42–44). Although phagocytotic capacity
can be altered by in vitro polarization of macrophages, we
have no evidence that ferumoxytol uptake corresponds
to a particular TAM phenotype. Regardless, aggressive
human breast cancers have been reported to contain few
(if any) T½1-polarized macrophages (47), and in the
MMTV-PyMT transgenic model in particular, TAMs are
strongly T½2-polarized by interleukin 4 (2).

Ferumoxytol enhancement is a new, noninvasive indi-
cator for TAM-tumor infiltration, which may serve as a
novel biomarker for breast cancers with poor outcome and
may be utilized to stratify tumors with high TAM
infiltration for immune-targeted therapies. There have
been multiple approaches for specific targeting and/or
blockade of TAMs for therapeutic purposes (29, 48, 49),
some of which are currently in clinical trials based on
experimental data showing that genetic, immunologic, or
pharmacologic blockade of CSF1, or its receptor (CSF1R),
decreases TAM presence in tissues and in experimental
solid tumors, correlating with diminished tumor angio-
genesis, and reduced primary tumor growth and pulmo-
nary metastasis (50–54). Because these therapies are not
cytotoxic, biomarkers of their efficiency at inducing
macrophage depletion would be of great clinical benefit.
Moreover, since clinical trials of new therapeutic drugs
and new combination therapies are expensive and take
tears to complete, the immediate value and impact of
imaging TAMs and/or TAM-depletion via MRI would
be immense.

We recognize several limitations with this approach.
Studies reported herein were done with ferumoxytol doses
of 0.5 mmol/kg. Previously described ferumoxytol doses
in humans were in the order of 0.035–0.072 mmol/kg
(18, 19). Iron oxide nanoparticles are generally applied
in higher doses in rodents as opposed to humans to
compensate for the relatively shorter blood half life in
rodents. However, future clinical applications must show
if the currently applied dose in patients is sufficient for
TAM detection, or if the dose can be safely increased. Of
note, ferumoxytol showed an excellent safety profile in
more than 700 patients (17). Larger superparamagnetic
iron oxide nanoparticles (SPIO, diameter >50 nm) provide
higher cellular uptake via ex vivo labeling. However, SPIOs
are rapidly phagocytosed by macrophages in liver, spleen,
and bone marrow and do not reach TAMs in vivo. USPIOs,
on the other hand, are not as quickly recognized by the RES
and have a longer circulation time, and can therefore leak
into tumor interstitium, where they can be phagocytosed
by TAMs. Thus, for “in vivo TAM labeling,” USPIOs are
advantageous (32, 33).

To the best of our knowledge, this is the first report of
utilizing a clinically applicable nanoparticle for TAM-
detection by MRI. Other investigators have reported
TAM-detection with nanoparticles that are not clinically
applicable, for either MRI (38) or optical imaging (39). In
addition, there have been reports of radiotracer-based
approaches for TAM detection by positron emission tomo-
graphy (PET; ref. 39). The latter is associated with radiation
exposure and therefore not used routinely for breast
imaging. MRI, on the other hand, is radiation free, estab-
lished for breast cancer detection, and integrates near-
microscopic anatomic resolution, high sensitivity, and
excellent soft tissue contrast. Although histologic methods
for quantifying TAMs are more precise, they are invasive,
limited to one or few observations, and not representative
of the whole tumor in the case of biopsies.

Preclinical and clinical evidence indicates that chronic
presence of diverse leukocyte subsets within the stroma
of breast cancers promotes tumor growth and metastasis
(3, 40, 41). TAMs play a significant protumorigenic role
in this context by augmenting neoplastic cell survival and
motility via elaboration of cytokines, chemokines, pro-
teases, and reactive oxygen species (3, 4, 42, 43). TAMs also
potentiate pulmonary metastasis of mammary adeno-
carcinomas through enhanced angiogenesis via regulation
of VEGF bioavailability and supplying epidermal growth
factor (EGF) to mammary epithelium (5, 44), in addition
to suppression of protective adaptive immune responses
(3, 42, 43, 45, 46). Exuberant macrophage recruitment
to breast cancer has been reported to be strongly asso-
ciated with poor prognosis, both in animal models and
in patients (2–4, 42–44). Although phagocytotic capacity
can be altered by in vitro polarization of macrophages, we
have no evidence that ferumoxytol uptake corresponds
to a particular TAM phenotype. Regardless, aggressive
human breast cancers have been reported to contain few
(if any) T½1-polarized macrophages (47), and in the
MMTV-PyMT transgenic model in particular, TAMs are
strongly T½2-polarized by interleukin 4 (2).

Figure 5. Ferumoxytol-enhanced MRI detects TAM-depletion
noninvasively in vivo. A, quantitative magnetic resonance signal
enhancement (delta R2 measurement) of MMTV-PyMT mammary
tumors before and after iron oxide-nanoparticle administration,
displayed as mean ± SD of 3 mice treated with anti-CSF1 mAb or PBS
control. An additional control mouse underwent serial magnetic
resonance without any contrast agent injection to confirm that
MMTV-PyMT tumors do not show any intrinsic changes in magnetic
resonance signal within a 2-day observation period. Note that mice
treated with anti-CSF1 mAb showed significantly smaller AR2
enhancement data compared with untreated controls. B, corresponding
confocal microscopy evaluations confirmed TAM-depletion within anti-
CSF1 mAb-treated tumors.

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As shown by our data, an alternative approach to increase the sensitivity of MRI would be to utilize folate-entrapped nanoparticles. Although such particles are currently not available for clinical use, precursors of such compounds are currently entering clinical trials and thus folate-entrapped derivatives may become clinically available in the future. Folate-entrainment enhances nanoparticle uptake via the folate receptor β, which is highly expressed on TAMs (31). Several investigators including us have reported uptake of USPIO and folate-entrapped USPIO by neoplastic cells, which may be a confounding variable when aiming for TAM detection (24, 55, 56). However, data presented here reveal that the ferumoxytol and P1133 uptake in malignant epithelial cells is significantly lower as compared with macrophage uptake, leading to negligible interferences of our imaging approach.

In conclusion, we have shown that iron oxide nanoparticle-enhanced MRI can be utilized to detect TAMs in a mouse model of mammary carcinogenesis. Ferumoxytol is a clinically available nanoparticle that can be readily applied for TAM imaging in patients with breast cancer via an "off label" use. Macrophage detection may be enhanced by using folate-entrapped nanoparticles that may become available for clinical use in the near future. Clinical studies are underway to evaluate these findings in patients.

Disclosure of Potential Conflicts of Interest

C. Corot works for Guerbet Research and provided contrast agents for this study. She was not involved in data analyses. The other authors disclosed no potential conflicts of interest.

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References


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