Molecular Magnetic Resonance Imaging

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Molecular MRI (mMRI) is a special implementation of molecular imaging for the non-invasive visualization of biological processes at the cellular and molecular level. More specifically, mMRI comprises the contrast agent-mediated alteration of tissue relaxation times for the detection and localization of molecular disease markers (such as cell surface receptors, enzymes, or signaling molecules), cells (e. g., lymphocytes, stem cells) or therapeutic drugs (e. g., liposomes, viral particles). MRI yields topographical, anatomical maps, functional MRI (fMRI) provides rendering of physiologic functions, and magnetic resonance spectroscopy (MRS) reveals the distribution patterns of some specific metabolites. mMRI provides an additional level of information at the molecular or cellular level, thus extending MRI further beyond the anatomical and physiological level. These advances brought by mMRI are mandatory for MRI to be competitive in the age of molecular medicine. mMRI is already today increasingly used for research purposes; e. g., to facilitate the examination of cell migration, angiogenesis, apoptosis, or gene expression in living organisms. In medical diagnostics, mMRI will pave the way toward a significant improvement in early detection of disease, therapy planning, or monitoring of outcome and will therefore bring significant changes in the medical treatment of patients.

Introduction

In general, molecular imaging demands high-sensitivity equipment, capable of quantitative measurements to detect probes that interact with targets at the pico- or nanomolar level. The challenge to detect such sparse targets can be exemplified with cell surface receptors, a common target for molecular imaging. At high expression levels (bigger than 10^6 per cell) the receptor concentration is approx. 10^{15}/ml; i. e., the concentration is in the micromolar range [1]. Many targets, however, are expressed in even considerably lower concentrations. Therefore, the most sensitive modalities, namely nuclear imaging (PET and SPECT), have always been at the forefront of molecular imaging, and many nuclear probes in clinical use today are already designed to detect molecular mechanisms (such as FDG to detect high glucose metabolism). In recent years, however, molecular imaging has commanded attention from beyond the field of nuclear medicine. Further imaging modalities to be considered for molecular imaging primarily include MRI, optical imaging, and ultrasound.

Contrast Agents for mMRI

Clinical MRI scanners offer a spatial resolution of 250 µm in-plane (small-bore experimental systems allow for 50 µm isotropic voxels for in vivo measurements), unlimited depth penetration, and exceptionally good soft tissue contrast. The above-mentioned concentration of molecular imaging targets in the micromolar range is challenging and requires sophisticated imaging strategies. Improvements in MRI design to reduce the lower detection limit are possible only to a certain extent. Hence, biophysical amplification mechanisms to enhance the signal from the label are necessary. For MRI, two different classes of contrast agents exist: agents that influence mainly the signal in T2- (negative contrast agents, reducing the signal, Figure 1) or in T1-weighted images (positive contrast agents, increasing the signal, Figure 2). For both classes, methods for signal amplification have been developed. In general, both take advantage of either very high relaxivity probes, background reduction (SNR optimization) via induced changes in relaxivity by the targeted molecular marker, or pronounced tissue accumulation. The latter is possible only with a very restricted number of highly expressed molecular markers (e. g., fibrin for thrombosis imaging).

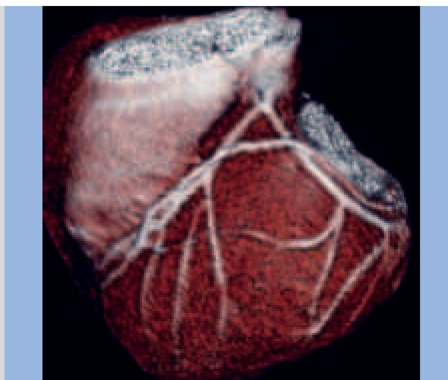
Negative Contrast Agents

For T2 contrast agents, the most prominent labels are iron oxide nanoparticles – Superparamagnetic Iron Oxide (SPIO), Very Small Paramagnetic Iron Oxide (VSPIO) or Ultrasmall Superparamagnetic Iron Oxide (USPIO); (Figure 3). These particles usually consist of a crystalline iron oxide core, surrounded by polymer coating, often dextran, polyethyleneglycol, or ionic sheath (e. g., citrate). The advantage of these preparations is that each particle contains thousands of iron atoms, resulting in very high T2 relaxivities of up to 200 $(mMs)^{-1}$ [1], which makes detection of even low concentrations of contrast agents (µmol to nmol range) possible. Noteworthy, particles smaller than 300 nm also produce a substantial T1 relaxation.

Various agent types are under investigation as potential treatments for neoplastic, myocardial, or neuronal dysfunctions. The spatial distribution of immunocompetent cells into tumors over time, as well as the movement of neuronal or (blood) stem cells has to be investigated for research and maybe therapy monitoring (Figure 4). Once delivered to the target organ, transplanted cells are difficult to image in vivo. Fluorescent labeling is depth limited. Nuclear cell tracking is limited by poor spatial resolution and radionuclide half-life. Most promising is the labeling of a specific population of cells with iron oxide and MRI. Magnetic nanoparticles are used to generate MRI contrast by disturbing the local magnetic field near excited spins (T2* relaxation) [2]. A widely accepted protocol for cell labeling using SPIO and polyamines has been published by Frank et al. [3]. Key concerns

(Figure 1)
Reconstruction of a pig heart with VSOP-C184 (60 µmol Fe/kg) acquired with a Siemens MAGNETOM Sonata (Multiphase 3D FLASH, 0.8 x 0.8 x 0.8 mm) with navigator and 12-channel RF coil; acquisition time 80 ms per RR interval.

(Courtesy of M. Taupitz, Charité, Berlin, Germany)

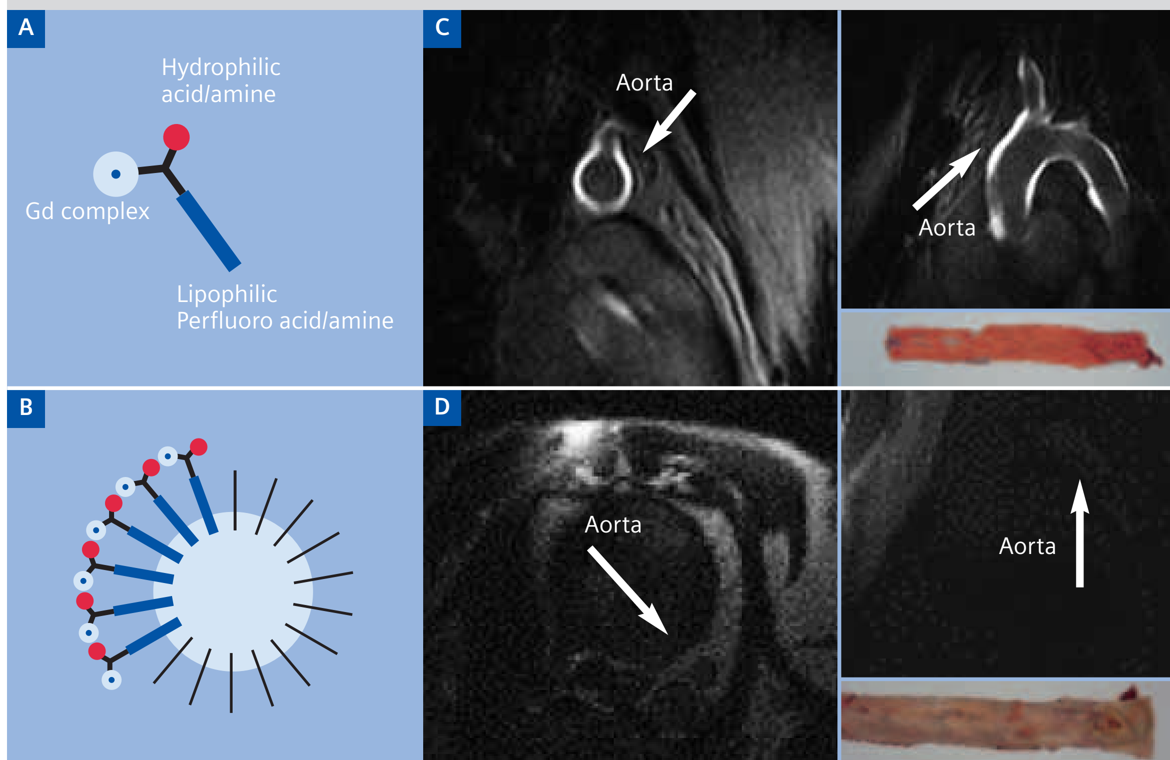


(Figure 2)
Plaque Imaging with Gadofluorine M. Structure of monomeric Gadofluorine M ***(A)*** *and Gadofluorine M micelle* ***(B)****.*

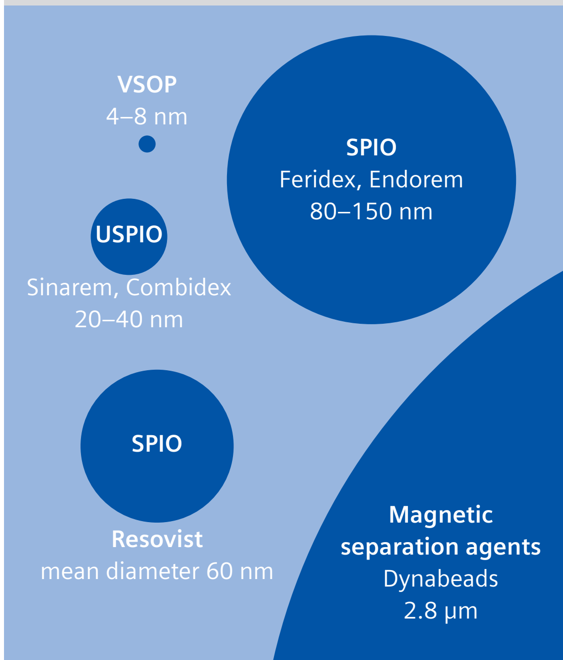
(C) *Plaques-bearing WHHL rabbit imaged with 100 µmol/kg Gadofluorine M 24 h p.i. imaged with Siemens 1.5T, IR turbo flash (300/4/150/20°) and corresponding histology.*

(D) *New Zealand white rabbit (control).*

(Courtesy of B. Misselwitz, Schering AG, Berlin, Germany)



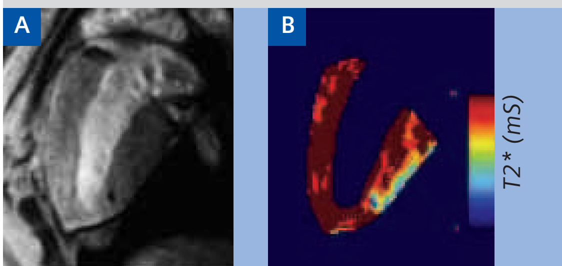
(Figure 3)
Mean diameters of various iron oxide particles.



(Figure 4)
***(A)** Still frame from a cinematic SSFP (Siemens, MAGNETOM Sonata) of iron fluorescent particle labeled mesenchymal stem cells (10^5 cells injected in vivo under X-ray fluoroscopy; Multistar, Siemens).*

***(B)** Labeled cells visualized by a T2* parametric map of the same slice (lowest T2* in the region of the injection). Typical susceptibility SSFP artifacts are in the posterobasal epicardial surface.*

(Courtesy of Alexander J. Dick, Richard B. Thompson, and Robert Lederman [2])



with cell tracking are viability, differentiability, and chromosomal stability of the labeled cells.

Similar to PET imaging, MRI can be used to image gene expression and to assess the efficiency of gene delivery. Viral or nonviral gene therapy schemes require targeting of the gene therapy vector to the tissue or cells of interest. Delivery of a therapeutic gene can be monitored by imaging of a marker gene, which is introduced into the gene therapy vector and expressed together with the therapeutic gene. The marker gene can be e. g. the transferrin receptor (accumulating iron within the cell) or another reporter system such as tyrosinase [4]. Subsequent catalysis of the synthesis of melanin, which has a high iron-binding capacity, results in a high signal intensity of the henceforth melanin-containing cells in T1-weighted images [5]. Compartmentalization of iron oxide particles within targeted cells can produce a significant signal amplification as well. Taking advantage of cellular uptake processes via endocytosis or phagocytosis clearly exceeds the contrast agent concentration that can be achieved using cell surface receptor targeting alone.

Positive Contrast Agents

Typical T1 contrast agents are small molecular weight compounds containing a single Lanthanide chelate as contrast-producing element (e. g., Gadolinium-DTPA). The tissue concentration necessary to image with these T1 contrast agents on a molecular level is considerably higher than the required concentration of iron oxide particles; it has to be in the order of mMols, since T1 relaxivity values are usually only in the 5–80 $(mMs)^{-1}$ range [1]. Evaluated relaxivity can be accomplished by restricting the free rotation by means of increasing the molecular weight. This can be achieved by using polymeric backbones or in vivo binding of small-sized contrast agents to serum proteins [6]. Multilabeled macromolecular T1 contrast agents for mMRI take advantage of the fact that relaxivity is a linear function of the number of lanthanide ions per contrast agent [1]. Various labeling concepts, including Gadolinium-loaded liposomes or polymers with thousands of Gadolinium atoms, have been developed to overcome this sensitivity limitation.

For instance, the detection of angiogenetic endothelium was achieved by large Gadolinium-loaded liposomes,

targeted to $\alpha_v\beta_3$ integrin receptors by peptides or antibodies ligands [7]. Advantages on the MRI properties may be counterbalanced by drawbacks in pharmacokinetics and bioavailability. High molecular weight compounds have a slower diffusion rate, which restricts delivery to certain tissues, such as necrotic tumor centers or the CNS.

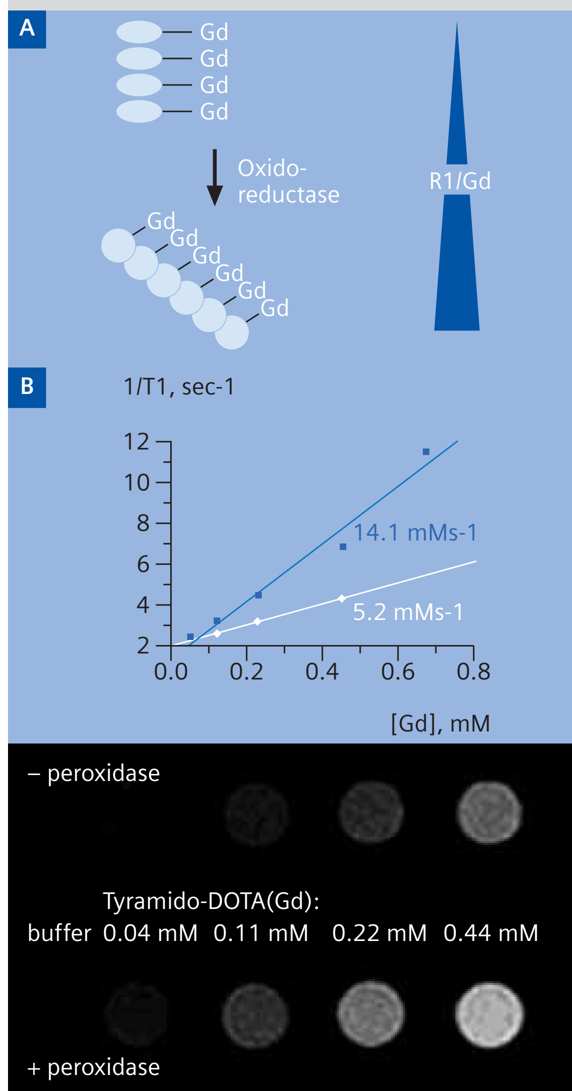
Similar to T2 contrast agents, T1 contrast probes can be activated, thus increasing the relaxivity of the agent. Target-mediated increase of relaxivity leads to an increased contrast in the presence of disease markers. An enzymatic polymerization by polymerases and oxidoreductases (e. g., by myeloperoxidases which are upregulated in vulnerable plaques) of paramagnetic substrates into oligomers represents one possible approach. In contrast to the small monomeric substrates, these polymers have a larger hydrodynamic diameter, slower rotation of paramagnetic metal chelates, and higher longitudinal relaxivity. Additionally, the elimination of the oligomeres is much slower than the elimination of the monomers. After a certain time, all signal will come from the oligomeres, retained at the target site [8] (Figure 5).

Another method utilizes conformational changes in the chelates. Monitoring of gene expression with T1 contrast agents can be achieved with a carbohydrate-modified Gadolinium chelate to image the activity of the enzyme ß-galactosidase as marker gene. The enzyme cleaves the carbohydrate residue from the chelate, inducing an increase in relaxivity by allowing access of water to the Gadolinium [9].

Chemical-exchange saturation transfer (CEST) agents are another emerging class of MRI contrast agents, which facilitate an activatable contrast. The principle is based on the fact that many molecules in the body exchange protons with bulk water. Exchanging protons can be selectively RF excited. After irradiating a millimolar pool of metabolite, the energy is transferred to a nearby pool of water, producing a strong increase in enhancement by means of chemical exchange. With CEST and its newer development paraCEST, specific metabolites can be detected. Using this method, the concentration of molecules such as glucose can be evaluated noninvasively in vivo in all organs. It furthermore allows for the measurement of the pH or the

(Figure 5)
***(A)** Polymerization of monomeric GdDOTA-Serotonin results in oligomers with higher atomic relaxivity (R1/Gd) by slower rotation of paramagnetic atoms.*

***(B)** Phantom experiment: Enzyme-mediated r_1 change. By adding tyramido-DOTA(Gd) to peroxidase in the presence of hydrogen peroxide, a three-fold increase of relaxivity and a visible increase of MR signals were observed. (Courtesy of A. Bogdanov, University of Massachusetts Medical School, Worcester MA, U.S.A.)*



temperature. Multiparametric mMRI might become feasible, as CEST agents with different absorption frequencies of the exchanging protons can be designed. The sensitivity of CEST can be improved further by incorporating larger numbers of exchangeable protons into a (polymeric) CEST agent. This has been accomplished by LIPOCEST agents, liposomes containing a paramagnetic shift reagent for water protons in their aqueous inner cavity [10] (Figure 6).
Finally, an "old friend" in MRI is attracting increasing attention in the course of molecular imaging: Fluorine-19. Xeno-labels (i.e., elements with low physiological concentration in situ but appropriate gyromagnetic properties) proved to provide superb traceability for mMRI.

Translational mMRI

To date, molecular imaging is increasingly used in laboratory studies. High-resolution MRI is well suited to screen mouse models for tumors and other abnormalities and can be applied to noninvasively follow up new experimental treatment strategies. While dedicated animal systems are available, clinical scanners are also very capable of obtaining high-resolution images. To further speed up translational research, a small-animal MR scanner based on a clinical console has been intro-

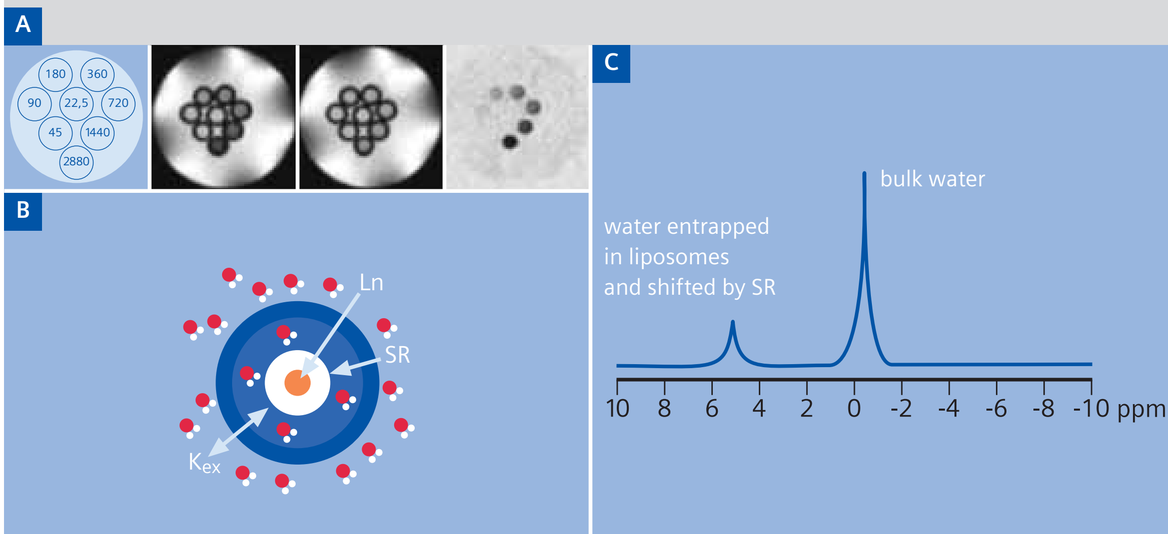
(Figure 6)
***(A)** LIPOCHEST agents. Phantom study with LIPOCHEST agents in various concentrations (22.5–2880 pM). From left to right: phantom design, irradiation +930 Hz from bulk water, irradiation –930 from bulk water, difference image.*

***(B)** CEST spectrum.*

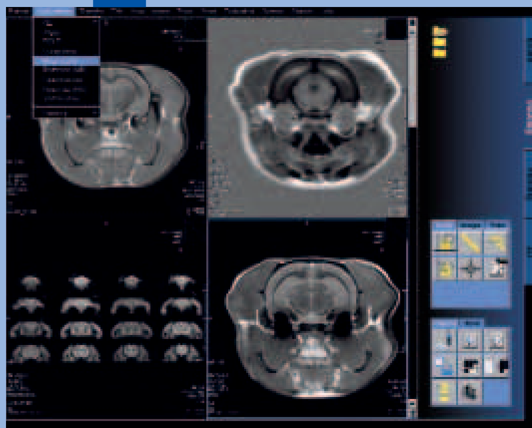
K_{ex}: Exchange rate
SR: Swift reagent
Ln: Lanthanides

***(C)** Design of liposomes containing paramagnetic shift reagent in their aqueous inner cavity.*

(Courtesy of S. Amies, University of Turin, Turin, Italy)



(Figure 7)
ClinScan syngo® is the missing link for translational mMRI. The syngo user interface (prototype) facilitates straightforward transfer of protocols from benchtop to bedside and vice versa.



duced just recently (ClinScan™, a joint Bruker and Siemens development, Figure 7). This system comes with an adapted clinical user interface and therefore supports most of the features and sequences used on clinical scanners. Standardization of the system ensures easy protocol transfer from and to clinical scanners. Applications being developed for humans (on clinical scanners) will be available on ClinScan. On the other hand, any application being developed for animals can be easily transferred to human diagnostics. In addition, adaptations for clinical scanners are under way for experimental molecular imaging. These include, but are not limited to, implementing dedicated small-animal protocols, (quantitative) analysis tools, or dedicated small-animal RF coils and gradient coils. In conclusion, MRI is increasingly suited to carry molecular imaging from animal models into clinical practice.

A wide variety of imaging agents for mMRI is emerging. The question remains when molecular imaging will enter clinical medicine. Hot (hyperintense) spots, which add information on the molecular mechanisms underlying a disease, could guide radiologists and would be highly beneficial. Hyperintense hot spots can be caused by highly specific accumulation of paramagnetic contrast agents. Most macromolecular Gadolinium chelates are in an early, preclinical research stage and the time yet required to reach clinical applicability is considerable. Furthermore, toxicity concerns due to the prolonged retention of heavily Gadolinium-loaded molecules within the organism must be disproved. Superparamagnetic contrast agents can give an easily detectable positive signal in combination with imaging techniques such as off-resonance imaging.

Superparamagnetic iron-oxide-based contrast agents are easily detectable on T2-weighted sequences, even better so on T2*-weighted images due to susceptibility effects. Iron oxide particles currently in clinical use can be utilized for cellular imaging of phagocyting cells, if combined with peptides, helping to traverse the membrane; this applicability can even be expanded to any other cell type. Iron oxide particles can furthermore be functionalized with a wide variety of biological active molecules such as targeting moieties of peptides, antibody (derivatives), nucleic acids, or aptamers to increase specific binding. Translational research with

iron oxide particles will take advantage of a growing basis of installed high-field MRI scanners, since the lower detection limit decreases with increasing field strength.

The Big Picture

Molecular imaging is one out of three pillars of molecular medicine; i. e., the translation of basic molecular biology into medicine. The other two are in vitro diagnostics (IVD) and knowledge-driven healthcare. To determine genetic predisposition, in vitro genomic or proteomic screening procedures will be used, such as DNA chip technologies or mass spectroscopy. IT tools will be mandatory as complex molecular information, be it in vivo or in vitro diagnostic, has to be integrated by IT and has to be supplemented by knowledge bases to leverage it into clinical applications. This means that diagnostic data has to be converted into meaningful medical knowledge.

The financial feasibility and medical practicability of molecular medicine, including comprehensive diagnostics, are debatable. The trial-and-error method will certainly continue to be the most reasonable approach for cost-effective therapies without side effects. However, this is not the case with therapies with a potential for serious side effects, cost-intensive (molecular) treatment schemes (such as cell or gene therapy), or therapies of chronic diseases. In such instances, a rational therapy selection based on comprehensive diagnostic data is a decisive factor for efficient and cost-sensitive patient care. One of the main cost drivers in medicine is inappropriate treatment over a prolonged time. From a medical point of view, many arising molecular therapy concepts are based on individualized drugs. These treatments must be tailored to the individual biochemical setup or disease stage of each respective patient with the support of diagnostic data. Equipped with these patient-specific data, a therapy regime is selected, taking into account the different molecular defects for each disease as well as the particular clinical history and condition of a patient.
An example of how mMRI could contribute to this scope is radiation treatment planning by semiautomated lymph nodal cancer staging using a nanoparticle-enhanced lymphotropic mMRI. Preclinical data prove that mMRI demonstrates great promise for improving quality of diagnosis in general for oncologic (tumor angiogenesis imaging), cardiovascular (vulnerable plaque imaging), and neurological (Alzheimer's plaque imaging) diseases.

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