Title

Cell tracking with gadophrin-2: a bifunctional contrast agent for MR imaging, optical imaging, and fluorescence microscopy

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Cell Tracking with Gadophrin-2 - A bifunctional Contrast Agent for MR Imaging, Optical Imaging and Fluorescence Microscopy

Abstract

Purpose: To assess the feasibility of Gadophrin-2 to trace intravenously injected human hematopoietic cells in athymic mice using magnetic resonance (MR) imaging, optical imaging (OI) and fluorescence microscopy.

Materials and Methods: Mononuclear peripheral blood cells from GCSF-primed patients were labeled with Gadophrin-2 (Schering AG, Berlin, Germany), a paramagnetic and fluorescent metalloporphyrin, using established transfection techniques with cationic liposomes. The labeled cells were evaluated in vitro with electron microscopy and ICP-AES. Then, 1×10^6 - 3×10^8 labeled cells were injected into 14 nude Balb/c mice and the in vivo cell distribution was evaluated with MR and OI before as well as 4, 24 and 48 hours (h) after intravenous injection (p.i.=post injection). Five additional mice served as controls: three mice were non-treated controls and two mice were investigated after injection of non-labeled cells. The contrast agent effect was determined quantitatively for MR by calculating ΔSI(%)-data. After completion of in vivo imaging studies, fluorescence microscopy of excised organs was performed.

Results: Intracellular cytoplasmatic uptake of Gadophrin-2 was confirmed by electron microscopy. Spectrometry determined an uptake of 31.56 nmol Gd per 10^6 cells. After intravenous injection, the distribution of Gadophrin-2-labeled cells in nude mice could be visualized by MR, OI and fluorescence microscopy. At 4 h p.i., the transplanted cells mainly distributed to lung, liver and spleen and, 24 h p.i., also to the bone marrow. Fluorescence microscopy confirmed the distribution of Gadophrin-2 labeled cells to these target organs.

Conclusion: Gadophrin-2 is suited as a bifunctional contrast agent for MR imaging, optical imaging and fluorescence microscopy and may be used to combine advantages of either imaging modality for an in vivo tracking of intravenously injected hematopoietic cells.

Introduction

Bone marrow transplantation using hematopoietic stem cells provides established therapeutic approaches for otherwise fatal diseases such as leukemias, lymphomas and metastasized carcinomas (1-4). New directions of the therapeutic approach to replace defective or injured tissue by stem cell derived functional tissue are anticipated to improve preventive and therapeutic care of many devastating diseases, e.g. Parkinsons disease, Alzheimers dementia and diabetes (1-4). However, the mechanisms that regulate homing, engraftment, and proliferation of marrow derived stem cells into the marrow and to organs other than the bone marrow are still poorly understood. In addition, The integration of the transplanted cells into the host parenchyma is difficult to evaluate in vivo since they cannot be distinguished on the basis of their morphological properties. Thus, cell labeling procedures might aid to identify and trace the fate of the transplanted cells. To allow a depiction of transplanted cells in vivo with imaging modalities, several methods have been designed to label stem cells with fluorescent dyes or contrast agents prior to administration (5-11). However, the optimal imaging technique to be used for an in vivo tracking of the transplanted cells is still under debate.

Bifunctional contrast agents, which could be detected at the same time by magnetic resonance (MR) imaging, optical imaging (OI) and fluorescence microscopy, would allow to integrate advantages of conceptually highly different imaging techniques (8, 12, 13): high three-dimensional anatomical resolution provided in-vivo by MR, high sensitivity provided by OI and detection at a cellular level provided by fluorescence microscopy, respectively. Furthermore, limitations of each technique, such as low sensitivity of MR, little anatomic background information obtained with OI and ex vivo information obtained with fluorescence microscopy, could be compensated by the other techniques. Thus, features invisible with one of these modalities could be rendered by the others.

Gadophrin-2 is a porphyrin based contrast agent, composed of an aromatic ring, which causes fluorescence, and covalently bound paramagnetic gadolinium chelates, which decrease T1-relaxation times of target tissues (14). The resulting MR signal enhancement of intravenously administered Gadophrin-2 has been extensively investigated (14-17), whereas little attention has been paid so far to its coexistent fluorescence capabilities. Thus, the purpose of this study was to investigate the concurrent bifunctional properties of Gadophrin-2 as a contrast agent for MR imaging, OI and fluorescence microscopy and potential applications of this agent for multimodality stem cell tracking studies in an established murine xenotransplant model.

Materials and Methods

Cell suspensions

Human peripheral blood cells were isolated by leukapheresis from nine patients, that underwent stem cell mobilization by treatment with 10 µg/kg granulocyte colony-stimulating factor for 12 to 15 days (Table 1). The study was approved by the Committee on Medical Ethics of the Technical University of Munich and consent was given by all patients. Hematopoietic mononuclear cells were isolated from the blood samples using Ficoll density centrifugation (d=1.077 g/ml, Biochrom, Berlin, Germany). After centrifugation at 1000 g for 20 minutes, the mononuclear interphase cells were collected and red cells were lysed using Ortholysis reagent (Ortho). The resulting cells were washed twice in HF/2+ (Hank's ballanced salt solution (InvitroGen), supplemented with 2% FCS and 10 mM HEPES (Gibco)). Prior to the labeling procedure, the cells were again centrifuged, and the cell pellet was resuspended with 1 ml of Dulbecco`s Modified Eagles Medium (DMEM, Gibco). Cells were counted by two independent observers (HED, MR) in a cell counter prior to each experiment.

Contrast medium

Gadophrin-2 (kindly provided by H.J. Weinmann; Schering AG, Berlin, Germany) is composed of a porphyrin ring and two covalently linked gadolinium chelates (Fig. 1) (14). It has a molecular weight of 1759.38 kDa. The r_1 relaxivity (Plasma, 20MHz, 37°) is 19.8±0.1 $l*mmol^{-1}*s^{-1}$ and the r_2 relaxivity (Plasma, 20MHz, 37°) is 30.0±1.0 $l*mmol^{-1}*s^{-1}$. The porphyrin ring provides fluorescence of the agent with an excitation of 499 nm and a maximal emission at 617 nm. For this study, Gadophrin-2 was supplied as a solution with a concentration of 25 mmol Gd per L. Due to its negative net charge, Gadophrin-2 could be encapsulated with positively charged liposomes using established transfection techniques as described in detail before (6). For this study, 20 µl of liposome formulation (Lipofectin, Life Technologies, Gibco BRL), containing 1:1 cationic lipid N-[1-(2,3-dioleyloxy)propyl]-n,n,n-trimethylammonium chloride (DOTMA) and dioleoyl phosphatidylethanolamine (DOPE) was suspended with 22.5 µl (0.56 mmol) Gadophrin-2 and this contrast agent formulation was subsequently incubated with samples of $1x10^6$ mononuclear cells in 800 µl DMEM in a CO_2 incubator at 37° C for 2 hours. With this procedure, two to four liposomes associate with a single contrast agent molecule, the lipid complex fuses with the cell plasma membrane and delivers the contrast agent to the cell cytosol (18).

In vitro studies:

Imaging: Initially, a series of eleven dilutions of Gadophrin-2 (from 600 - 6 nmol Gd/ml) as

well as normal saline as a control were investigated with MR imaging and OI. Then, 1 x 10^6 mononuclear peripheral blood cells, labeled with Gadophrin-2 as described above, were centrifuged and three sets of cell probes each were investigated with MR, OI and fluorescence microscopy.

Cell viability was determined by Trypan blue exclusion. Viability experiments were performed at least three times for both cell types by an independent observer (R.A.J.O.), who was blinded to the labeling procedure.

Electron microscopy was performed for Gadophrin-2 labeled mononuclear cells and non-labeled controls. The cell probes were fixed in 3% glutaraldehyde/cocadylate buffer overnight, desiccated, embedded in Epon (arteficial resin) and processed for electron microscopy. The cell probes were evaluated unstained, i.e. without uranyl or lead supplement, in order to prevent false positive findings. Electron microscopy was performed at a Zeiss EM 10 electron microscope at 60-80 kV.

Inductively coupled plasma atomic emission spectrometry (ICP-AES) was performed in 10^6 Gadophrin-2 labeled hematopoietic cells to determine the gadolinium-concentration within the transplanted cells. This method has a repeated sensitivity of less than one ppm.

In-vivo studies:

The study was approved by the committee on animal research responsible for our institution. Fourteen 12-week old nude Balb/c-AnNCrl mice were examined with MR imaging and optical imaging before as well as 10 minutes (n=4), 4 hours (all), 24 hours (n=10) and 48 hours (n=4) after intravenous injection of $1x10^6$ - $3x10^8$ Gadophrin-labeled hematopoietic cells. Five additional mice underwent the same imaging procedures as controls: three mice served as non-treated controls, two mice were investigated after intravenous injection of $1x10^7$ non-labeled cells. The animals were anaesthetized for the cell injection with ether and for the imaging procedures (MRI, OI) with an antagonized intraperitoneal narcosis of 0.5 mg/kg Medetomidin (Dormitor), 5 mg/kg Midazolam (Dormicum) and 0.05 mg/kg Fentanyl (Fentanyl) for anaesthesia as well as 2.5 mg/kg Atipamezol (Antisedan), 0.5 mg/kg Flumazenil (Anexate) and 1.2 mg/kg Naloxon (Narcanti) as antagonists to awake the animals after the imaging procedures. For tissue analysis, animals were sacrificed after the imaging procedures at 4 h p.i. (n=2), 24 h p.i. (n=5) or 48 h p.i. (n=4) by an intracardial injection of an overdose of narcoren (pentobarbital). Specimens from liver, spleen, lungs, thyroid as well as bone marrow from spine and femur were evaluated with optical imaging, then frozen at –80° C and processed for fluorescence microscopy.

Magnetic Resonance Imaging

MR imaging was performed using a clinical 1.5 Tesla MR scanner (Philips ACS Intera,

Best, The Netherlands) and a dedicated birdcage coil for high resolution MR imaging (Medical Advances, Milwaukee, WI, U.S.A.).

In vitro studies: Test tubes with centrifuged cell pellets were placed in a water-containing plastic container. R1-relaxation rates of labeled cells and non-labeled controls were measured with a mixed inversion recovery (IR)- spinecho (SE)-sequence, initiated with an IR-experiment with a repetition time (TR) of 5000 ms, an echo time (TE) of 18 ms and an inversion time (TI) of 600 ms, followed by a SE experiment with a TR of 600 ms and a TE of 24 ms. The R1-relaxation times of the labeled cells were subsequently derived from T1-maps, which are calculated by the scanner software. R2*-relaxation rates of the cells were measured with a T2*-FFE-EPI sequence with a flip angle α of 90°, a TR of 2000 ms and 20 increasing TEs from 4 – 36 ms. Both sequences were acquired with a field of view (FOV) of 160 x 48 mm, a matrix of 256^2 pixel and a slice thickness of 1.5 mm. The multiple echo T2*-FFE-EPI images were transferred to a personal computer running a Linux operating system. R2*-relaxation rates were calculated from the negative slope of the logarithm of the signal intensities as described before (6) using a dedicated IDL program (Interactive Data Language by Research Systems, Boulder, CO, U.S.A.).

In vivo studies: For the in-vivo studies, the animals were placed prone into the coil and evaluated with coronal T1-weighted 3D FFE 40°/25/2.7 (α/TR/TE)-sequences with a field-of-view (FOV) of 100 x 80 mm, a 512^2 pixel matrix, 2 acquisitions and an effective slice thickness of 400 μm. The in-plane spatial resolution of the MR images was 200 x 150 μm. Liver and spleen could be well depicted on the coronal images. For evaluation of the bone marrow, the 3D data sets were reconstructed along the long axis of the femur and the spine. Average signal intensities (SI) of liver, spleen and bone marrow were measured by one observer using operator-defined regions-of-interests (ROI). The size of the ROIs depended on the cell pellets or organ diameter with a minimum of 30 pixels per region. Signal-to-noise ratios were calculated by correction of SI data by the ”image noise” (random fluctuations in signal intensity), which was measured in the background anterior to the depicted object (19). $SNR = SI_{target\ organ}$ / background noise

Optical Imaging

Optical imaging was performed using a commercially available high resolution optical imager (Hamamatsu ORCA II-ER, Herrsching, Germany), providing more than 70% quantum efficiency, an 80 dB dynamic range and less than 4e- read out noise (rms). Images were obtained using a –60° Peltier-cooled digital black and white camera with 1.280 (H) x 1.024 (V) effective pixel resolution. For fluorescence excitation, a CF-1000 lamp (Illumination Technologies, USA) was used. The lamp setup was slightly modified from its original version and included a 25 nm excitation bandpass filter (500 nm, Eosin, Sp. gold) for Gadophrin. The light was led into the dark box (Unit One, USA) by an optical

fiber. To distribute the light as homogeneously as possible within the field of view, the light outputs were arranged in a ring structure. For fluorescence detection, a dedicated 40 mm emission bandpass filter (617 nm, TRITC) was used. The nude mice were subsequently placed prone and supine into the imager, cell probes were placed in microtiter plates and postmortem excised organs were placed on black cardboard. Acquisition times were 100 to 700 ms. Hamamatsu simple PCI software was used for image processing. Bright field images were merged with the corresponding fluorescence images and the obtained calibrated light emission was subsequently transformed to pseudo-colors.

Fluorescence Microscopy

Deparaffinated specimens of mouse tissues were sectioned in 2 µm thick slices and cell nuclei were stained with Diamidino-2-phenylindole (DAPI, BIOMOL Research Laboratories, Plymouth, USA) at a dilution of 1:10000 and an incubation time of 1 min. Afterwards, the tissue slices were washed three times with phosphate-buffered saline (PBS, pH=7.4) and covered with glass using fluorsave (Merck, Darmstadt, Germany). Then, cell-associated fluorescence was determined by fluorescence microscopy using a Zeiss Axioplan 2 microscope (Jena, Germany). A standard fluoresceine filter was used to detect green fluorescence, a rhodamine filter was used to detect red fluorescence and a UV filter was applied to detect blue fluorescence.

Statistical Analysis

R1 relaxation rates, ΔSI data and fluorescence data were presented as means and standard deviations of the means. To compare differences in quantitative data at different time points before and after injection of Gadophrin-targeted cells in the same animals, a two tailed paired t test was used. Statistical significance was assigned if $p < 0.05$. All statistical computations were processed using Statview 4.1 software (Abacus, Berkeley, CA, U.S.A.).

Results

Gadophrin-2: MR signal intensity and OI fluorescence

Dilution series of Gadophrin-2 revealed a positive signal enhancement of the contrast agent on T1-weighted FFE-images: this signal enhancement linearly declined down to a concentration of 20 nmol Gd/ml (Fig. 2). Lower concentrations of the contrast agent could not be detected with MR imaging. With OI, a higher sensitivity could be achieved: a fluorescence of the contrast agent could be detected down to a minimal concentration of 2 nmol Gd/ml (Fig. 2). However, OI revealed a non-linear relationship between contrast agent concentration and fluorescence: a maximal fluorescence was observed at 75 nmol Gd/ml, whereas higher and lower concentrations caused a declining fluorescence. The diminished fluorescence with relatively high contrast agent concentrations is most likely due to self quenching effects.

Cell Viability, Electron Microscopy and Labeling Efficiency

After two hours of incubation with Gadophrin-2 liposomes, the cell viability of the labeled cells (87±7%) was slightly, but not significantly lower compared to non-labeled controls (94±5%; p=0.242). Electron microscopy did not reveal any structural changes due to the labeling procedure (Fig. 3). The contrast agent was internalized into the cells by endocytosis and subsequently accumulated in lysosomes in the cytoplasm and not in the nucleus (Fig. 3). ICP-AES spectrometry revealed an internalization of 31.555 nmol Gd per 10^6 Gadophrin-2 labeled cells. No detectable gadolinium was found in non-labeled control cells.

In vitro studies of Gadophrin-2 labeled cells

Optical imaging and fluorescence microscopy revealed a strong fluorescence of Gadophrin-2 labeled cells and no fluorescence of controls (Fig. 4). In addition, the Gadophrin-2 labeled cells exhibited a strong and positive signal enhancement on T1-weighted MR images (Fig. 5). Consequently, R1-relaxation rates of Gadophrin-2 labeled cells (1.25±0.19 s^{-1}) were significantly higher compared to non-labeled controls (0.55±0.07 s^{-1}; p=0.009), whereas R2*-relaxation rates did not change significantly before (61.83±6.63 s^{-1}) and after (85.27±12.47 s^{-1}; p=0.167) the labeling procedure.

In vivo distribution of Gadophrin-2 labeled cells

Two animals, that received intravenous injections of $1*10^6$ cells demonstrated a detectable fluorescence signal with OI , but novisible changes in MR signal intensity. All other twelve animals, that received $1*10^7$-$3*10^8$ labeled cells, showed visible fluorescence resp. signal changes with both, OI and MR. Within this range of $1*10^7$-$3*10^8$ injected cells, no significant correlation was found between the number of transplanted cells and quantitative

MR enhancement data of the host organs ($p>0.05$). Directly after cell injection, OI demonstrated an increased fluorescence of the lungs, apparently as a function of transient pulmonary cell accumulation (Fig. 6). MR revealed no detectable signal changes (Fig. 7). At 4 hours post injection (h p.i.), OI fluorescence and MR signal enhancement was noted in liver, spleen and thyroid gland, apparently as a function of cell accumulation in these organs. MR derived quantitative SNR data increased significantly in these organs (Table 2, $p<0.05$). OI additionally depicted a persistent fluorescence of the lungs and a fluorescence of the vertebrae, the pelvis and femora (Fig. 6). At 24 h, both, OI and MR depicted a fluorescence resp. enhancement of femoral and pelvic bone marrow, indicating homing of the transplanted cells to these organs (Fig. 6, 7). Corresponding MR derived SNR data of the bone marrow were significantly increased compared to imaging studies at previous time points (Table 2, $p<0.05$). OI detected additional cell accumulations in the vertebrae (Fig. 7). At 48 h p.i., no further changes in OI fluorescence or MR signal intensities occurred. Control animals, that received no cells or non-labeled cells, did not reveal any detectable fluorescence resp. signal intensity changes of organs with subsequent OI or MR studies.

Investigations of postmortem excised organs

OI of excised organs showed time-dependent changes in fluorescence of lungs, liver, spleen, thyroid gland and bone marrow, which generally corresponded to the results of the in vivo studies (Fig. 8). However, the fluorescence of the organs was stronger ex vivo than in vivo, apparently due to emission light absorption in surrounding tissues in vivo. Thus, a decreasing fluorescence of lung and liver as well as an increasing fluorescence of the bone marrow at 24 and 48 h p.i. could be better depicted ex vivo (Fig.8). Corresponding fluorescence microscopy studies confirmed the distribution of Gadophrin-2 labeled cells to the described target organs (Fig.9).

Discussion

Data showed, that Gadophrin-2 is a bifunctional contrast agent suited not only for MR imaging (14), but also for optical imaging and fluorescence microscopy. Thus, in-vivo cell tracking studies with Gadophrin-2 as a cell marker may integrate advantages of either imaging technique: OI can provide an overview over the general in vivo distribution of all transplanted major and minor cell subpopulations while MR imaging can specify the accumulation of major cell populations in host organs with submillimeter anatomical resolution. Of note, MR provides three dimensional data sets as opposed to the two dimensional OI surface data sets, thereby localizing transplanted cells more exactly and more deeply within tissues as opposed to OI. In vivo findings by OI and MR imaging can be subsequently confirmed on a cellular level by fluorescence microscopy of biopsy specimens or postmortem excised organs, providing a direct visualization of labeled donor cells in host tissues without the need of antibodies or colocalization of an antibody with the contrast agent.

Alternative attempts of MR contrast agents designed to combine the advantages of fluorescence and magnetic resonance imaging techniques have been described before (8, 12, 13). These comprised the lanthanide chelators DTPA and DOTA, which were bound to Gadolinium and covalently attached to fluorescent dyes (8, 13) and arginyl peptides, which were cross-linked to magnetic nanoparticles and attached to the indocyanine dye Cy 5.5 (12).

A potential advantage of our labeling technique compared to above mentioned alternative bifunctional contrast agents is, that Gadophrin-2 accumulated only in the cytoplasm of the labeled cells, whereas at least the peptide based contrast agents are known to accumulate in the cell nucleus as well (12), where they could cause potential interactions with the DNA. The cyanine dyes in particular could cause toxic effects to the cell nucleus (20). In addition, the use of gadolinium based MR contrast agents (like gadophrin-2) may be advantageous to iron oxide based MR contrast agents for the design of bifunctional probes as the attachment of fluorescein to magnetic iron oxide nanoparticles can be associated with a profound quenching effect due to nonradiative energy transfer or collisions between the dye and the iron oxide (12). Though we also observed a quenching effect of Gadophrin-2 in vitro (Fig. 2), this occured with contrast agent concentrations, which exceeded the concentrations achieved in vivo (22-24).

The fluorescence of gadophrin-2 covered a green-red-light spectrum, which was more favorable compared to the spectrum covered by green fluorescent protein (21), because the background signal and the apparent interaction with hemoglobin were considerable smaller with gadophrin-2. Of note, previous porphyrin formulations provided fluorescence with an infrared spectrum. These formulations, however, were associated with a higher

toxicity as compared to subsequently developed probes like gadophrin-2. New generation porphyrins, like gadophrin-3, on the other hand, provide fluorescence of markedly lower intensity compared to gadophrin-2, making it inadequate for bifunctional studies (unpublished own data 2002).
The fluorescence obtained from Gadophrin-2 could not be quantified because of the following general limitations of this imaging method (21):
(1) light is scattered and absorbed in tissues. Both, the excitation light and the emission light are attenuated by a factor of two. The amount of light detected depended on the depth, thickness, composition and density of the evaluated tissues.
(2) light excitation is never homogenous. Thus, intra- and interindividual variabilities had to be considered due to the well plate and slight differences in the positioning of the animals
(3) the obtained images were planar and surface weighted
(4) there is always a certain background fluorescence, which would have to be subtracted.
Thus, optical imaging should be considered as a highly sensitive, fast and easy applicable modality for cell detection, while other imaging methods, such as MR, may be advantageous to provide 3D associations with anatomy and data quantification.

Hematopoietic cell labeling with gadophrin-2 provided a reliable identification of transplanted human donor cells in the murine target organs by OI, MR and fluorescence microscopy. Within the range of $1*10^7$-$3*10^8$ administered Gadophrin-2 labeled human hematopoietic progenitor cells, no significant correlation was found between the number of transplanted cells and the quantitative MR enhancement data of the host organs. Standard errors in the results of cell countings by the cell counter and MR signal intensity measurements along with a well known interindividual variability of the in vivo distribution of intravenously injected cells to as many as 16 different organs may have caused this insensitivity of our imaging method to minor variations in the applied cell number (22-26).
The vitality of the investigated cells was not impaired due to labeling with Gadophrin-2 and the in vivo distribution of intravenously injected Gadophrin-2 labeled cells in mice was not different compared to previous investigations with no or different cell labeling procedures (5, 22-25). While the distribution of intravenously injected cells to liver, spleen and bone marrow was previously demonstrated in vivo with other MR techniques as well (25), our combined OI and MR cell tracking technique provided additional information: Shortly after intravenous injection, a major population of transplanted cells accumulated in the lung. This observation is in accordance with the literature (26), but was, to the best of our knowledge, so far not depicted by an in vivo imaging technique.

In conclusion, the porphyrin based gadolinium compound Gadophrin-2 proved to be suited as a bifunctional contrast agent for *in vitro* labeling and *in vivo* tracking of transplanted stem cells using magnetic resonance imaging, optical imaging, and fluorescence

microscopy. The use of this bifunctional agent allows for the integration of MRI's submillimeter resolution and OI's high sensitivity, and enables direct visualization of host tissue with fluorescence microscopy without the need for antibodies. The ability to trace the in vivo cell distribution simultaneously with these different techniques may improve our understanding of how stem cells mediate recovery of injured organs and may be ultimately applied as an important mode for serial assessments of the transplantation procedure.

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References

1. Cao J, Rao M. Stem cell and precursor cell therapy. *Neuromolecular Med* 2002; 2:233-249.
2. Molina A, Popplewell L, Kashyap A, Nademanee A. Hematopoietic stem cell transplantation in the new millennium: report from city of hope national medical center. *Ann N Y Acad Sci* 2001; 938:54-61.
3. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson S, Li B, Pickel J, McKay R, Nadal-Ginard B, Bodine D, Leri A, Anversa P. Bone marrow cells regenerate infarcted myocardium. *Nature* 2001; 410:701-704.
4. Strauer B, Karnowski R. Stem Cell Therapy in Perspective. *Circulation* 2003; 107:929-934.
5. Oostendorp R, Ghaffari S, Eaves C. Kinetics of in vivo homing and recruitment into cycle of hematopoietic cells are organ-specific but CD44-independent. *Bone Marrow Transplantation* 2000; 26:559-566.
6. Daldrup-Link H, Rudelius M, Oostendorp R, Settles M, Piontek G, Metz S, Heinzmann U, Rummeny E, Schlegel J, Link TM. Targeting of Hematopoietic Progenitor Cells with MR Contrast Agents. *Radiology* 2003; 228: 760-767.
7. Fawwaz R, Oluwole T, Wang N, Kuromoto N, Iga C, Hardy M, Alderson P. Biodistribution of radiolabeled lymphocytes. *Radiology* 1985; 155:483-486.
8. Modo M, Cash D, Melledew K, Williams S, Fraser S, Meade T, Price J, Hodges H. Tracking transplanted stem cell migration using bifunctional, contrast agent-enhanced, Magnetic Resonance Imaging. *Neuroimage* 2002; 17:803-811.
9. Rudelius M, Daldrup-Link H, Heinzmann U, Piontek G, Settles M, Link T, Schlegel J. Highly efficient paramagnetic labeling of embryonic and neuronal stem cells. Journal of *Nuclear Medicine and Molecular Imaging* 2003; 30: 1038-1044. 10.
10. Schoepf U, Marecos E, Melder R, Jain R, Weissleder R. Intracellular magnetic labeling of lymphocytes for in vivo trafficking studies. *BioTechniques* 1998; 24:642-651.
11. Weissleder R, Cheng H, Bogdanova A and Bogdanov A. Magnetically labeled cells can be detected by MR imaging. *J Magn Reson Imaging* 1997; 7:258-263.
12. Josephson L, Kircher M, Mahmood U, Tang Y, Weissleder R. Near-infrared fluorescent nanoparticles as combined MR/optical imaging probes. *Bioconjugate Chem* 2002; 13:554-560.
13. Hüber M, Staubli A, Kustedjo K, Gray M, Shih J, Fraser S, Jacobs R and Maede T. Fluorescently detectable magnetic resonance imaging agents. *Bioconjugate Chem* 1998; 9:242-249.
14. Hofmann B, Bogdanov JA, Marecos E, Ebert W, Semmler W, Weissleder R. Mechanism of gadophrin-2 accumulation in tumor necrosis. *J Magn Res Imag* 1999; 9:336-341.

15. Kim T, Choi B, Par S, Lee W, Han J, Han M, Weinmann H. Gadolinium mesoporphyrin as an MR imaging contrast agent in the evaluation of tumors: an experimental model of VX2 Carcinoma in Rabbits. *Am J Roentgenol* 2000; 175:227-234.
16. Young S, Sidhu M, Qing F, Muller H, Neuder M, Zanassi G, Mody T, Hemmi G, Dow W, Mutch J, Sessler J, Miller R. Preclinical evaluation of gadolinium(III) texaphyrin complex: a new paramagnetic contrast agent for magnetic resonance imaging. *Invest Radiol* 1994; 29:330-338.
17. Young S, Fan Q. Imaging of human colon cancer xenograft with gadolinium-texaphyrin. *Invest Radiol* 1996; 31:280-283.
18. Felgner P, Gadek T, Holm M et al. Lipofectin: a highly efficient, lipid mediated DNA-transfection procedure. Proc Natl Acad Sci USA 1987;84:7413-7417.
19. Wolff S, Balaban R. Assessing contrast on MR images. *Radiology* 1997; 202:25-29.
20. Kassab K. Photophysical and photosensitizing properties of selected cyanines. J Photochem Photobiol B 2002;68(1):15-22
21. Rice BW, Cable MD, Nelson MB. In vivo imaging of light-emitting probes*. J Biomed Opt* 2001; 6(4):432-40.
22. Kerre T, de Smet G, de Smedt M, Offner F, de Bosscher J, Plum J, Vandekerckhove B. Both CD34+38- and CD34+38- cells home specifically to the bone marrow of NOD/LtSZ scid(scid mice but show different kinetics in expansion. *J Immunol* 2001;167: 3692-3698.
23. Kollet O, Spiegel A, Peled A, Petit I, Byk T, Hershkoviz R, Guetta E, Barkai G, Nagler A, Lapidot T. Rapid and efficient homing of human CD34+CD38-/lowCXCR4+ stem and progenitor cells to the bone marrow and spleen of NOD/SCID and NOD/SCID/B2mnull mice. *Blood* 2001; 97:3283-3291.
24. van Hennik P, de Koning A, Ploemacher R. Seeding efficiency of primitive human hematopoietic cells in nonobese siabetic/severe combined immune deficiency mice: implications for stem cell frequency assessment. *Blood* 1999; 94:3055-3061.
25. Daldrup-Link H, Rudelius M, Piontek G, Metz S, Bräuer R, Debus G, Corot C, Schlegel J, Link T, Peschel C, Rummeny E, Oostendorp R. Migration of iron oxide labeled hematopoietic progenitor cells in a xenotransplant model: in vivo monitoring using 1.5 Tesla magnetic resonance imaging equipment. accepted for publication in *Radiology*
26. Devine SM, Cobbs C, Jennings M, Bartholomew A, Hoffman R: Mesenchymal stem cells distribute to a wide range of tissues following systemic infusion into nonhuman primates. *Blood* 2003;101(8):2999-3001

Tables:

Patient	Gender	Age (years)	Diagnosis
1	M	61	prepre-B ALL
2	M	66	plasmacytoma
3	M	65	large cell anaplastic NHL
4	F	43	breast cancer
5	M	59	diffuse, large cell B-NHL
6	M	21	Ewings sarcoma
7	F	67	plasmacytoma
8	M	67	plasmacytoma
9	M	65	large cell anaplastic NHL

Tab. 1) Donor Patients of Hematopoietic Progenitor Cells

	SNR pre	SNR 4 h p.i.	SNR 24 h p.i.
liver	57.903±8.683	66.570±10.316*	72.604±12.703*#
spleen	48.535±7.156	53.653±5.265*	57.273±6.761*#
bone marrow	45.545±3.496	45.518±3.912	52.018±5.682*#

Tab. 2) MR signal intensities of liver, spleen and bone marrow, quantified as “signal-to-noise ratios“ (SNR), before and after injection of $1*10^7$-$3*10^8$ Gadophrin-2 labeled human hematopoietic cells. Data are displayed as means with standard deviations for organs of twelfe mice. *indicates significant differences between SNR data before (SNR pre) and after cell injection, # indicates significant differences between SNR data at 4h and 24 h p.i. (paired t-test; $p<0.05$).

Figures:

Fig. 1) Molecular Structure of Gadophrin-2

Fig. 2) Dilution series of decreasing concentrations of 600-0. 6 μmol Gd/ml Gadophrin-2 and a control sample of normal saline, imaged with T1-weighted fast field echo MR imaging (left) and Optical Imaging (right). MR depicted a positive signal enhancement down to a minimal concentration of 20 nmol Gd/ml, while OI depicted fluorescence down to a minimal concentration 0.2 nmol Gd/ml.

Fig. 3) Electron microscopy of non-labeled (A) and Gadophrin-2 labeled (B, C) human hematopoietic cells. Gadophrin-2 (arrows) is internalized and stored in the cytoplasm of the cells.

Fig. 4) Fluorescence microscopy of Gadophrin-2 labeled cells (630fold magnification). Cell nuclei are displayed blue by DAPI. Gadophrin-2 within the cytoplasm of the cells can be depicted by green fluorescence using a standard fluorescein filter (A), by red fluorescence using a rhodamine filter (B) and by yellow fluorescence using both filters (C). Control cells, which were only incubated with the transfection agent, did not show any fluorescence of the cytoplasm (D).

Fig. 5) Representative T1-weighted MR images of centrifuged cell pellets in test tubes, placed in a waterbath. Each cell pellet contains 10^6 cells, before labeling (A) and after labeling with Gadophrin-2 (B). Note the increased MR signal intensity of Gadophrin-2 labeled cells. The supernatant above the cells in the test tubes shows an identical signal intensity as compared to the surrounding water bath, indicating absence of free contrast agent in the solution.

Fig. 6) Optical imaging of a nude Balb/c AnNCrl mouse before and at various time points after intravenous injection of Gadophrin-2 labeled human hematopoietic cells reveals an initial cell accumulation in the lung and subsequent distribution to the bone marrow of the spine and pelvis.

Fig. 7) Representative T1-weighted 3D FFE 40°/25/2.7 MR images of a nude Balb/c AnNCrl mouse before and after injection of Gadophrin-2 labeled hematopoietic cells show a progressive cell accumulation in liver, spleen and bone marrow.

Fig. 8) Optical imaging of organs, excised at different time points after injection of Gadophrin-2 labeled cells shows an initial fluorescence in lung, liver and spleen and a slowly increasing fluorescence in the bone marrow over time, apparently as a function of a distribution and homing of the labeled cells to the bone marrow.

Fig. 9) Representative fluorescence microscopy of spleen specimens 24 h p.i. of Gadophrin-2 labeled progenitor cells (400fold magnification). The transplanted, labeled cells (arrows) can be delineated by green fluorescence using a standard fluorescein filter (A), by red fluorescence using a rhodamine filter (B) and by yellow fluorescence (C) using both filters. Cell nuclei are displayed blue by DAPI.