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Magnetic Resonance Imaging of Stem Cell-Macrophage Interactions with Ferumoxytol and Ferumoxytol-Derived Nanoparticles

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Abstract

“Off the shelf” allogeneic stem cell transplants and stem cell nano-composites are being used for the treatment of degenerative bone diseases. However, major and minor histocompatibility antigens of therapeutic cell transplants can be recognized as foreign and lead to their rejection by the host immune system. If a host immune response is identified within the first week post-transplant, immune modulating therapies could be applied to prevent graft failure and support engraftment. Ferumoxytol (Feraheme™) is an FDA approved iron oxide nanoparticle preparation for the treatment of anemia in patients. Ferumoxytol can be used “off label” as an MR contrast agent, as these nanoparticles provide measurable signal changes on MRI. In this focused review article, we will discuss three methods to localize and identify innate immune responses to stem cell transplants using ferumoxytol-enhanced MRI, which are based on tracking stem cells, tracking macrophages or detecting mediators of cell death:

(1) monitor MRI signal changes of ferumoxytol-labelled stem cells in the presence or absence of innate immune responses, (2) monitor influx of ferumoxytol-labeled macrophages into stem cell implants and (3) monitor apoptosis of stem cell implants with caspase-3 activatable nanoparticles.

These techniques can detect transplant failure at an early stage, when immune-modulating interventions can potentially preserve the viability of the cell transplants and thereby improve bone and cartilage repair outcomes. Approach 1 and 2 are immediately translatable to clinical practice.

Summary

In this review we showed that ferumoxytol can be used “off label” as an MR contrast agent to provide measurable signal changes on MRI. The ferumoxytol-enhanced MRI methods that can be used to localize and identify innate immune responses to stem cell transplants are: (1) monitoring MRI signal changes of ferumoxytol-labelled stem cells in the presence or absence of innate immune responses, (2) monitoring influx of ferumoxytol-labeled macrophages into stem cell implants, and (3) monitoring apoptosis of stem cell implants with caspase-3 activatable nanoparticles. These techniques can detect transplant failure at an early stage, when immune-modulating interventions can potentially preserve the viability of the cell transplants and thereby improve the transplant repair outcomes.

INTRODUCTION

Bone and cartilage injuries are costly and debilitating to both individuals and our society. They can result from osteoarthritis, trauma or tumor surgery, and often do not heal without significant medical intervention (Brooks, 2002; Chimutengwende-Gordon & Khan, 2012; Ciapetti, Granchi, & Baldini, 2012; Jorgensen, Gordeladze, & Noel, 2004). Achieving successful repair of bone and cartilage defects requires complex surgical interventions and causes medical costs in the order of \$21 billion every year (Buza & Einhorn, 2016). Unlike many other tissues, bone and cartilage defects can regenerate completely if bridged with appropriate graft material for mechanical support and repair. For this purpose, more than two million bone grafts and osteochondral autograft systems (OATS) are transplanted each year, representing the second most commonly transplanted materials after blood products (Campana et al., 2014; Shegarfi & Reikeras, 2009). Considering escalating demands and limited availability and efficacies of bone allografts and OATS, additional solutions are needed.

Stem cell transplants and stem cell nano-composites are attractive alternatives for bone and cartilage repair. Stem cells represent “live” tissue sources for bone and cartilage engineering, providing a number of advantages over bone allografts and Osteochondral Autograft or Allograft Transfer System (OATS), including higher tissue regeneration potential, immediate availability, potentially unlimited quantities and potentially better engraftment outcomes (Chimutengwende-Gordon & Khan, 2012; Ciapetti et al., 2012; Jorgensen et al., 2004). A major concern about using allogeneic adult stem cells (O’Sullivan, Vegas, Anderson, & Weir, 2011; Preynat-Seauve & Krause, 2011; Yang, 2007; Zangi et al., 2009), embryonic stem cell-derived progenitors (English & Wood, 2010; Swijnenburg et al., 2005; Swijnenburg, van der Bogt, Sheikh, Cao, & Wu, 2007; Thompson & Manilay, 2011; van der Bogt, Swijnenburg, Cao, & Wu, 2006), or induced pluripotent stem cells (Boyd, Rodrigues, Lui, Fu, & Xu, 2012; Zhao, Zhang, Rong, & Xu, 2011) is that they can differ in the major and minor histocompatibility antigens present on host cells, causing them to be recognized as foreign and be rejected by the host immune system. Furthermore, as many physicians advocate for the use of allogeneic (“off the shelf”) stem cells (Charron, Suberbielle-Boissel, & Al-Daccak, 2009; Chimutengwende-Gordon & Khan, 2012; Ciapetti et al., 2012; Jorgensen & Noel, 2011; Polak & Mantalaris, 2008; Sherman et al., 2011; Z. Y. Zhang et al., 2012), rejection may become a common occurrence. Therefore, a non-invasive *in vivo* diagnostic test to detect stem cell acceptance or rejection would be immediately valuable.

A variety of imaging methods have been developed to improve our understanding of the *in vivo* fate of therapeutic cells. Labeling therapeutic cells with superparamagnetic iron oxide nanoparticles enables cell tracking with magnetic resonance imaging (MRI) for several weeks and at relatively high anatomical resolution (Beeres et al., 2007; Sheikh & Wu, 2006; S. J. Zhang & Wu, 2007; Zhou, Acton, & Ferrari, 2006). Labeling therapeutic cells with radiotracers enables detection with photon emission computed tomography (SPECT) and positron emission tomography (PET) at high sensitivity (Beeres et al., 2007; Sheikh & Wu, 2006; S. J. Zhang & Wu, 2007; Zhou et al., 2006). Transduction of therapeutic cells with reporter genes can enable cell detection based on luminescence or radiotracer entrapment with little to no background signal and for several cell generations (Beeres et al., 2007;

Sheikh & Wu, 2006; S. J. Zhang & Wu, 2007; Zhou et al., 2006). Optical and bioluminescence imaging provides relatively straight forward tools for cell tracking over multiple time points in animal models (Schroeder, 2008; Zhou et al., 2006). Labeling therapeutic cells with markers that can be detected with ultrasound guided photoacoustic (US/PA) imaging adds quantification capability to the inherent anatomical data (Nam, Ricles, Suggs, & Emelianov, 2012). Iron oxide nanoparticle labeled cells can be also tracked with magnetic particle imaging (MPI), a new imaging modality which provides highly sensitive cell detection and quantification without background signal (Lemaster, 2018; Nejadnik, Pandit, et al., 2018). The main limitations for the above mentioned imaging techniques are sensitivity for MRI, ionizing safety issues and (depending on the applied tracer) a potentially short tracking phase for PET and SPECT, and penetration depth and photobleaching for optical imaging techniques (Beeres et al., 2007; Kraitchman et al., 2003; S. C. Li et al., 2010; Schroeder, 2008; Sheikh & Wu, 2006; S. J. Zhang & Wu, 2007; Zhou et al., 2006).

This focused review article will concentrate on recent developments in clinically translatable MRI approaches for detection of stem cell-macrophage interactions with ferumoxytol and ferumoxytol-derived nanoparticles.

PATHOPHYSIOLOGY OF STEM CELL–MACROPHAGE INTERACTIONS

There are three possible scenarios of macrophage interactions with stem cell transplants (Figure 1):

i) The normal wound healing process leads to a physiologic macrophage response:

Every wound in an immunocompetent living subject leads to an inflammatory reaction with influx of monocytes and other immune cells. These cells first create a pro-inflammatory environment, where macrophages remove dead cells and debris, followed by an anti-inflammatory environment, which supports tissue repair (Wynn & Vannella, 2016). If stem cells are transplanted into a bone or cartilage defect and survive the initial pro-inflammatory environment, then macrophages can support tissue regeneration in the regenerative phase by providing soluble growth and/or survival factors to the transplanted cells (Boyd et al., 2012; Charron et al., 2009; Duffy, Ritter, Ceredig, & Griffin, 2011; English & Wood, 2010; Heidt, Segundo, Chadha, & Wood, 2010; Karabekian, Posnack, & Sarvazyan, 2011; Kofidis et al., 2005; Swijnenburg et al., 2005; Yang, 2007; Zangi et al., 2009; Zhao et al., 2011). From these observations, it is important to note that every wound will contain a “baseline” level of monocytes and macrophages.

ii) Stem cell death in a hostile environment leads to macrophage influx:

If stem cells are implanted into a hostile pro-inflammatory microenvironment, and the metabolic switch to an anti-inflammatory environment does not occur, then the stem cells might not survive. If the transplanted cells undergo apoptosis, then this might attract further influx of local macrophages and monocytes from the blood into the implant, which phagocytose and remove dead cells and debris. From a pathophysiological point of view, it is important to note that in this scenario, the stem cells develop apoptosis first and are

subsequently phagocytosed by macrophages (Bulte, 2017; Khurana et al., 2012; Ma et al., 2015; Nejadnik et al., 2016). Therapeutic interventions to improve stem cell survival in this setting include (1) advanced scaffolds with minimized immunogenicity and optimized biodegradability; (2) preconditioning of MSC by culturing them in a hypoxic environment to prepare them for the hypoxic wound environment; (3) genetic manipulation of MSC e.g. with BCL-2 to decrease apoptosis mediators in the therapeutic cells; (4) combined administration of MSC with target tissue cell types to increase the differentiation and apoptosis of the implanted cells or co-administration of anti-inflammatory drug to achieve minimum immune reaction to the transplant (Hyun et al., 2013; L. Li, Chen, Wang, & Zeng, 2016).

iii) Immune-mismatched stem cell transplants can trigger a macrophage response:

Allogeneic, human-leukocyte antigen (HLA)-mismatched stem cell transplants can trigger a macrophage response, which can lead to an acute or chronic allograft rejection (Ikezumi, Hurst, Masaki, Atkins, & Nikolic-Paterson, 2003; Mannon, 2012). Contrary to T cell-mediated immune responses against solid organ transplants, immune rejections of stem cell transplants are initiated by the innate immune system (Nilsson, Korsgren, Lambris, & Ekdahl, 2010). Monocytes and macrophages recognize “foreign” cell transplants and release pro-inflammatory cytokines, leading to a vicious cycle of tissue inflammation, cell damage and ultimately, loss of the cell transplant (English & Wood, 2013; Griffin et al., 2013). New immune-modulating therapeutic agents can suppress macrophage infiltration in allografts (Julier, Park, Briquez, & Martino, 2017).

In order to improve our understanding of successful tissue regeneration processes, recognize complications of the engraftment process and monitor responses to immune-modulating therapies, it is essential to develop imaging techniques that can diagnose the above mentioned stem cell–macrophage interactions (Daldrop-Link et al., 2017; Khurana et al., 2012; Nejadnik et al., 2016). Although blood and serum assays are available to detect markers of pro- or anti-inflammatory reactions within the body, they can only generate indirect and non-specific information about innate immune responses in stem cell transplants. Some investigators have studied pre-clinical imaging tools to detect immune responses to stem cell transplants, either by tracking the long-term fate of labelled stem cells (Kim et al., 2006; Swijnenburg et al., 2008; Zangi et al., 2009) or by tracking the migration of T-cells (Cahalan, 2011; Celli, Albert, & Bousso, 2011; Negrin & Contag, 2006) or macrophages into the transplant (Christen et al., 2009; Hitchens et al., 2011; Kanno et al., 2001; Y. Zhang, Dodd, Hendrich, Williams, & Ho, 2000). However, these previous approaches were not immediately translatable to clinical practice, because they used probes that are not approved for use in patients. Ferumoxytol-enhanced MRI has a distinct advantage in that it is immediately clinically applicable.

MR IMAGING OF STEM CELL–MACROPHAGE INTERACTIONS

The FDA-approved iron supplement ferumoxytol (Feraheme™) is composed of iron oxide nanoparticles used for the intravenous treatment of patients with iron deficiency (Lu, Cohen, Rieves, & Pazdur, 2010). Our group has shown that ferumoxytol can be used as an MR

contrast agent, as these nanoparticles provide measurable signal changes on MRI (Khurana, Nejadnik, et al., 2013). Specifically, when ferumoxytol is phagocytosed by macrophages or stem cells, an MR signal decline is detected on T2-weighted MR images (Khurana, Chapelin, et al., 2013; Khurana, Nejadnik, et al., 2013; Khurana et al., 2012). This can be used to either track the fate of ferumoxytol labeled stem cells (Nejadnik et al., 2016) or directly track the degree of macrophage infiltration in the target tissue (Khurana et al., 2012). Ferumoxytol is currently the only nanoparticle compound that is FDA approved and readily clinically available as an imaging agent for this purpose via an “off-label” use (Khurana, Nejadnik, et al., 2013; Thu et al., 2012).

There are at least three approaches to image stem cell-macrophage interactions with ferumoxytol-enhanced MRI: One can (a) track the fate of ferumoxytol-labelled stem cells with MRI, (b) track the fate of ferumoxytol-labelled macrophages with MRI, or (c) modify ferumoxytol nanoparticles to generate caspase-3 activatable nanoprobes:

A) Tracking Ferumoxytol Labelled Stem Cells:

Current clinical MR imaging studies diagnose stem cell graft failure several months after therapeutic cell delivery based on lack of tissue repair (Chang, Sherman, Madelin, Recht, & Regatte, 2011; Choi, Potter, & Chun, 2008; Trattinig et al., 2011). However, stem cell apoptosis in a hostile, pro-inflammatory microenvironment typically occurs during the first few days after therapeutic cell transfer due to lack of growth factors, pro-inflammatory conditions or immune rejection (Daldrup-Link & Nejadnik, 2014; Toma, Wagner, Bowry, Schwartz, & Villanueva, 2009; von Bahr et al., 2012). We found that ferumoxytol labelling enabled us to differentiate MR signal characteristics of viable and apoptotic cell transplants in arthritic joints (Daldrup-Link & Nejadnik, 2014; Henning et al., 2011; Khurana et al., 2012; Nedopil et al., 2010). Our studies in rodents showed that a rapid decline in T2-signal of iron-labelled MSCs at two weeks after Matrix Associated Stem Cell Implantation (MASI) correlated with lack of cartilage repair at 12 weeks (Nejadnik et al., 2016). Apoptotic ferumoxytol-labelled MSCs (and not viable, labelled MSCs) were cleared by macrophages (Nejadnik et al., 2016). This is in accordance with previous investigations that showed that apoptosis leads to macrophage recognition and phagocytosis (Poon, Lucas, Rossi, & Ravichandran, 2014). We found that a loss of the ferumoxytol MRI signal of failed therapeutic cell transplants at 2 weeks after their implantation could predict incomplete tissue repair and unfavourable outcomes six weeks later (Nejadnik et al., 2016).

Ferumoxytol labeling of stem cells can be achieved through the following approaches:

(i) *Ex vivo* stem cell labeling: Van Buul et al. and Thu et al. labelled human bone marrow stromal cells with ferumoxide nanoparticles by using protamine as a clinically translatable transfection agent (Thu et al., 2012; van Buul et al., 2009). Our team showed that this approach could also be applied to labelling stem cells with ferumoxytol (Khurana, Nejadnik, et al., 2013). Thu and Joe Frank et al. also found that adding heparin further increased the uptake of ferumoxytol-heparin-protamine (HPF) nanocomplexes into hematopoietic stem cells, bone marrow stromal cells, neural stem cells, and T-cells (Thu et al., 2012).

Our team developed an immediately clinically translatable approach for labelling autologous (“off the shelf”) mesenchymal stromal cells (MSC) with ferumoxytol nanoparticles without any transfection agent (Nejadnik, Taghavi-Garmestani, et al., 2018). This approach leveraged the process of MSC expansion in cell cultures and presence of proteins in clinical MSC expansion media. We found that when ferumoxytol nanoparticles are incubated in protein-containing media, proteins in the media form a protein corona around the nanoparticles. This protein corona increases the hydrodynamic diameter of the nanoparticles and facilitates uptake by MSCs through phagocytosis (Nejadnik, Taghavi-Garmestani, et al., 2018).

(ii) *In vivo* stem cell labelling: We developed a new *in vivo* cell labelling approach for autologous cell transplants that does not require *ex vivo* manipulation of therapeutic cells (Khurana, Chapelin, et al., 2013). We showed that intravenous injection of ferumoxytol into a living subject leads to ferumoxytol uptake by MSCs in bone marrow. After harvest from bone marrow and transplantation into osteochondral defects, ferumoxytol-labelled MSCs could be tracked with clinical MR imaging (Khurana, Chapelin, et al., 2013). This new and patented approach only required an intravenous injection of an FDA-approved iron supplement before cell transplantation and did not require any manipulation of the harvested cells (Patent US14/161,315). We recently applied this concept to track ferumoxytol labelled bone marrow cells in osteonecrotic lesions (ON) of patients, who underwent a decompression surgery (Theruvath et al., 2018). The surgery involved drilling a track through the major trochanter of the femur to the ON in the epiphysis in order to “decompress” presumed increased interstitial pressure in an epiphyseal ON. Our orthopedic surgeons recently augmented this procedure by harvesting bone marrow cells from the iliac crest, enriching the bone marrow aspirate for MSCs and osteoprogenitor cells, and delivering these cells through the drilled track into the osteonecrotic area (Goodman, 2013; Lee & Goodman, 2009). Since the standard clinical decompression procedure does not involve a cell transplant, potential lack of engraftment or death of the transplanted cells would not affect clinical outcomes. This represented an opportunity to evaluate the MR signal effects and engraftment outcomes of *in vivo* labelled cell transplants in a clinical setting. In a “first-in-man” clinical trial, we injected ferumoxytol 24–48 hours prior to a clinically scheduled decompression surgery (Figure 2). Our orthopaedic surgeons then harvested bone marrow aspirates from the iliac crest and transplanted the iron labelled bone marrow cells into osteonecrotic bone lesions. The ferumoxytol labelled MSCs could be tracked with MR imaging over several weeks (Theruvath et al., 2018).

Our experimental studies showed that the MRI signal kinetics of ferumoxytol labelled therapeutic cells could reveal information that was not attainable with unlabeled cell transplants: the ferumoxytol signal disappeared faster in stem cells that underwent apoptosis compared to stem cells that survived (Figure 3). Possible explanations include a more rapid iron metabolism in macrophages that engulfed ferumoxytol-labeled dead MSC (compared to iron in viable MSCs) and a migration of ferumoxytol-MSC-loaded macrophages from the transplant site to other sites. We previously noted a faster metabolism of ferumoxytol nanoparticles in macrophages that had engulfed dead ferumoxytol-labeled MSC compared to ferumoxytol metabolism in viable MSC and migration of macrophages from apoptotic

MASt to lymph nodes in rat knee joints (Nejadnik et al., 2016). Several other investigators noted macrophage migration to draining lymph nodes after they cleared debris and dead cells in inflamed peritoneum (Bellingan, Caldwell, Howie, Dransfield, & Haslett, 1996; Bellingan et al., 2002; C. Cao, Lawrence, Strickland, & Zhang, 2005; Lan, Nikolic-Paterson, & Atkins, 1993), experimental glomerulonephritis (Lan et al., 1993), or spinal cord injury (Shakhbazov et al., 2015).

B) Tracking Ferumoxytol Labelled Macrophages:

Immune rejection or death of transplanted stem cells can lead to an influx of macrophages into the transplant (Grinnemo et al., 2004). Various investigators reported that macrophages migrate into stem cell transplants in response to stem cell death and/or inflammatory reactions (Abdel-Hamid, Hussein, Ahmad, & Elgezawi, 2005; Dupont et al., 2010; Luong-Van, Grondahl, Song, Nurcombe, & Cool, 2007). We have shown that MR imaging can diagnose immune rejection non-invasively by tracking macrophage migration into cell transplants in arthritic joints (Khurana et al., 2012). This approach is based on intravenously injected iron oxide nanoparticles, which are phagocytosed by macrophages in the reticuloendothelial system (Daldrop-Link, Rummeny, Ihssen, Kienast, & Link, 2002; Metz et al., 2006; Simon et al., 2005). Similarly to the above described *in vivo* labelling approach for tracking other bone marrow cells, macrophages in the bone marrow can be pre-loaded with an intravenous injection of the FDA-approved iron supplement ferumoxytol (Khurana et al., 2012). Migration of iron-loaded macrophages into the transplant can be diagnosed by a significant decline in T2 relaxation times at two and four weeks post-transplant compared to pre-transplant images (Khurana et al., 2012) (Figure 4).

We utilized this approach for non-invasive monitoring of engraftment outcomes of cell transplants in a mouse model of calvarial defect repair. We created 5 mm calvarial defects in Jax C57BL/6-Tg(Csf1r-EGFP/NGFR/FKBP1A/ TNFRSF6) 2Bck/J mice, which express an enhanced green fluorescent protein (EGFP) fusion protein under the control of the *Csf1r* promoter, enabling mononuclear immune cells (i.e., monocytes, macrophages, and dendritic cells) to be detected using fluorescence microscopy. The calvarial defects do not heal spontaneously but can be repaired by implantation of mesenchymal stem cells (MSCs) or adipose fat-derived stem cells (ADSCs) in growth factor-enriched scaffold (Behr et al., 2012; Levi et al., 2012; Levi et al., 2011). We tracked macrophage responses to immune-matched and immune-mismatched stem cell transplants with MRI and intravital microscopy (IVM) (Daldrop-Link et al., 2017). Serial MRI images of matched mADSC transplants demonstrated only slightly declining T2-signal over time whereas mismatched transplants showed a stronger decline in MRI signal at the internal, dura-facing edge of the transplant. At days 14 and 21, T2 relaxation times of mismatched transplants were significantly lower compared to matched transplants. Immediately after each MRI scan, we acquired IVM images, which demonstrated an increasing accumulation of GFP-positive macrophages in both matched and mismatched ADSC transplants over time. Immune-mismatched transplants showed a significantly higher mean quantity of macrophages at day 14 compared to immune-matched transplants.

Other investigators utilized the *in vivo* macrophage labelling approach to study *in vivo* recruitment of ferumoxytol-labelled macrophages to melanoma (MDA-MB-435R) tumors in mice in response to Abraxane therapy (Q. Cao et al., 2018).

Innate immune responses involve anti-inflammatory M2 macrophage phenotypes and pro-inflammatory M1 macrophage phenotypes. Stem cell engraftment and wound healing requires predominant M2 phenotypes in the wound environment and stem cell rejection involves predominant M1 macrophages (Luo et al., 2018). Ferumoxytol is phagocytosed by both macrophage phenotypes and cannot distinguish between M1 and M2 phenotypes (Kirschbaum et al., 2016). However, our studies show that an immune rejection involves significantly higher total macrophage quantities compared to successful engraftment processes and that this difference can be detected with intravital microscopy (IVM) and MRI (Daldrup-Link et al., 2017). We can not discriminate between M1 and M2 macrophages using ferumoxytol MR imaging. Alternatively, several investigators are developing nano-probes that specifically target M1 macrophages (Shimizu et al., 2017) or M2 macrophages (C. Zhang et al., 2017). In addition, the nanoparticles themselves might change macrophage phenotype polarizations: we showed that high doses of ferumoxytol could activate macrophages and thereby cause intrinsic pro-inflammatory effects (Zanganeh et al., 2016).

Currently, the M1/M2 classification has provided a beneficial framework. However, the definition of M1 and M2 macrophage paradigm originates from the pre-genomic period, when a few markers were studied to determine differences and similarities in macrophage reactions to stimuli. Recent research about macrophage functions, cytokine signaling pathways, and immune-relevant ligands and receptors demonstrate a far more complex immunological system and a need for more comprehensive classification (Martinez & Gordon, 2014).

C) Detecting Signaling Molecules of Apoptosis with Modified Ferumoxytol Nanoparticles:

Matrix associated stem cell implants (MASI) are hampered by high failure rates, in part because of a lack of biomarkers for successful MSC engraftment (Hyun et al., 2013). Unfortunately, current MR imaging findings within the first one to two weeks after MASI cannot distinguish between grafts that will or will not repair the defect (K. Li et al., 2018). While the above-described approaches to diagnosing stem cell apoptosis based on rapid loss of a ferumoxytol label over time or the diagnosis of an influx of ferumoxytol labelled macrophages into an unlabelled stem cell transplant can provide indirect information about the functional status of the cell transplant, the above-described techniques do not directly diagnose biomarkers of engraftment failure.

To address this problem and increase the specificity of our imaging approach, we recently developed a novel ferumoxytol-based bimodal imaging probe, which has an attached caspase-3 sensor to detect immune rejection-mediated cell death (K. Li et al., 2018). Combined, dual-modality imaging probes can provide information about cell location and cell function at the same time. For example, in case of ferumoxytol nanoparticles with an attached caspase-activatable fluorescent probe, the “always on” iron oxide moiety can help to locate cells with MRI while the activatable fluorescent moiety can provide information about the viability of the cells. Our probe has been designed by conjugating caspase-3

cleavable peptides containing AFC fluorophore (KKKKDEVD-AFC) to ferumoxytol nanoparticles. The surface modification did not compromise the T2-relaxivity of the resultant ferumoxytol ferumoxytol-AFC nanoparticles for MRI. We showed that ferumoxytol-AFC can be easily internalized into living macrophages and MSCs, as confirmed by Prussian blue stains and inductively coupled plasma mass spectrometry. Apoptotic cells showed significant green fluorescence due to caspase-3-mediated cleavage of DEVD and release of AFC fluorophores with a maximum at 500 nm (Figure 5). This probe has the advantage to detect stem cell location and stem cell injury during pro-inflammatory rejection processes, and monitor response to novel immune-modulating interventions.

Other new nanoparticles are designed to increase the sensitivity and accuracy of disease detection. Thorek et al. produced a multimodal nanoparticle, (89)Zr-ferumoxytol, for the enhanced detection of lymph nodes with positron emission tomography combined with magnetic resonance imaging (PET/MRI). To increase the chance of clinical translational potential, they altered ferumoxytol with minimal modification for radiolabelling. They showed that (89)Zr-ferumoxytol can be used for high-resolution tomographic studies of lymphatic drainage in preclinical disease models which can be used to improve preoperative planning for nodal resection and tumour staging (Thorek et al., 2014).

However, although this and other bifunctional probes have higher specificity compared to unmodified ferumoxytol nanoparticles, they have the disadvantage of a longer path to clinical translation.

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References:

- Abdel-Hamid M, Hussein MR, Ahmad AF, & Elgezawi EM (2005). Enhancement of the repair of meniscal wounds in the red-white zone (middle third) by the injection of bone marrow cells in canine animal model. *Int J Exp Pathol*, 86(2), 117–123. doi:10.1111/j.0959-9673.2005.00420.x [PubMed: 15810983]
- Beeres SL, Bengel FM, Bartunek J, Atsma DE, Hill JM, Vanderheyden M, ... Bax JJ (2007). Role of imaging in cardiac stem cell therapy. *J Am Coll Cardiol*, 49(11), 1137–1148. doi:10.1016/j.jacc.2006.10.072 [PubMed: 17367656]
- Behr B, Sorkin M, Lehnhardt M, Renda A, Longaker MT, & Quarto N (2012). A comparative analysis of the osteogenic effects of BMP-2, FGF-2, and VEGFA in a calvarial defect model. *Tissue Eng Part A*, 18(9–10), 1079–1086. doi:10.1089/ten.TEA.2011.0537 [PubMed: 22195699]
- Bellingan GJ, Caldwell H, Howie SE, Dransfield I, & Haslett C (1996). In vivo fate of the inflammatory macrophage during the resolution of inflammation: inflammatory macrophages do not die locally, but emigrate to the draining lymph nodes. *J Immunol*, 157(6), 2577–2585. [PubMed: 8805660]
- Bellingan GJ, Xu P, Cooksley H, Cauldwell H, Shock A, Bottoms S, ... Laurent GJ (2002). Adhesion molecule-dependent mechanisms regulate the rate of macrophage clearance during the resolution of peritoneal inflammation. *J Exp Med*, 196(11), 1515–1521. [PubMed: 12461086]

- Boyd AS, Rodrigues NP, Lui KO, Fu X, & Xu Y (2012). Concise review: Immune recognition of induced pluripotent stem cells. *Stem Cells*, 30(5), 797–803. doi:10.1002/stem.1066 [PubMed: 22419544]
- Brooks PM (2002). Impact of osteoarthritis on individuals and society: how much disability? Social consequences and health economic implications. *Curr Opin Rheumatol*, 14(5), 573–577. [PubMed: 12192258]
- Bulte JWM (2017). Science to Practice: Can MR Imaging Cell Tracking of Macrophage Infiltration Be Used as a Predictive Imaging Biomarker for Transplanted Stem Cell Rejection? *Radiology*, 284(2), 307–309. doi:10.1148/radiol.2017170536 [PubMed: 28723288]
- Buza JA 3rd, & Einhorn T (2016). Bone healing in 2016. *Clin Cases Miner Bone Metab*, 13(2), 101–105. doi:10.11138/ccmbm/2016.13.2.101 [PubMed: 27920804]
- Cahalan MD (2011). Imaging transplant rejection: a new view. *Nat Med*, 17(6), 662–663. doi:10.1038/nm0611-662 [PubMed: 21647143]
- Campana V, Milano G, Pagano E, Barba M, Cicione C, Salonna G, ... Logroscino G (2014). Bone substitutes in orthopaedic surgery: from basic science to clinical practice. *J Mater Sci Mater Med*, 25(10), 2445–2461. doi:10.1007/s10856-014-5240-2 [PubMed: 24865980]
- Cao C, Lawrence DA, Strickland DK, & Zhang L (2005). A specific role of integrin Mac-1 in accelerated macrophage efflux to the lymphatics. *Blood*, 106(9), 3234–3241. doi:10.1182/blood-2005-03-1288 [PubMed: 16002427]
- Cao Q, Yan X, Chen K, Huang Q, Melancon MP, Lopez G, ... Li C (2018). Macrophages as a potential tumor-microenvironment target for noninvasive imaging of early response to anticancer therapy. *Biomaterials*, 152, 63–76. doi:10.1016/j.biomaterials.2017.10.036 [PubMed: 29111494]
- Celli S, Albert ML, & Bousso P (2011). Visualizing the innate and adaptive immune responses underlying allograft rejection by two-photon microscopy. *Nat Med*, 17(6), 744–749. doi:10.1038/nm.2376 [PubMed: 21572426]
- Chang G, Sherman O, Madelin G, Recht M, & Regatte R (2011). MR imaging assessment of articular cartilage repair procedures. *Magn Reson Imaging Clin N Am*, 19(2), 323–337. doi:10.1016/j.mric.2011.02.002 [PubMed: 21665093]
- Charron D, Suberbielle-Boissel C, & Al-Daccak R (2009). Immunogenicity and allogenicity: a challenge of stem cell therapy. *J Cardiovasc Transl Res*, 2(1), 130–138. doi:10.1007/s12265-008-9062-9 [PubMed: 20559977]
- Chimutengwende-Gordon M, & Khan WS (2012). Advances in the use of stem cells and tissue engineering applications in bone repair. *Curr Stem Cell Res Ther*, 7(2), 122–126. [PubMed: 22023632]
- Choi YS, Potter HG, & Chun TJ (2008). MR imaging of cartilage repair in the knee and ankle. *Radiographics*, 28(4), 1043–1059. doi:10.1148/rg.284075111 [PubMed: 18635628]
- Christen T, Nahrendorf M, Wildgruber M, Swirski FK, Aikawa E, Waterman P, ... Libby P (2009). Molecular imaging of innate immune cell function in transplant rejection. *Circulation*, 119(14), 1925–1932. doi:10.1161/CIRCULATIONAHA.108.796888 [PubMed: 19332470]
- Ciapetti G, Granchi D, & Baldini N (2012). The combined use of mesenchymal stromal cells and scaffolds for bone repair. *Curr Pharm Des*, 18(13), 1796–1820. [PubMed: 22352754]
- Daldrup-Link HE, Chan C, Lenkov O, Taghavigarmestani S, Nazekati T, Nejadnik H, ... Gambhir SS (2017). Detection of Stem Cell Transplant Rejection with Ferumoxylol MR Imaging: Correlation of MR Imaging Findings with Those at Intravital Microscopy. *Radiology*, 161139. doi:10.1148/radiol.2017161139
- Daldrup-Link HE, & Nejadnik H (2014). MR Imaging of Stem Cell Transplants in Arthritic Joints. *J Stem Cell Res Ther*, 4(2), 165. doi:10.4172/2157-7633.1000165 [PubMed: 25068075]
- Daldrup-Link HE, Rummeny EJ, Ihssen B, Kienast J, & Link TM (2002). Iron-oxide-enhanced MR imaging of bone marrow in patients with non-Hodgkin's lymphoma: differentiation between tumor infiltration and hypercellular bone marrow. *Eur Radiol*, 12(6), 1557–1566. doi:10.1007/s00330-001-1270-5 [PubMed: 12042968]
- Duffy MM, Ritter T, Ceredig R, & Griffin MD (2011). Mesenchymal stem cell effects on T-cell effector pathways. *Stem Cell Res Ther*, 2(4), 34. doi:10.1186/scrt75 [PubMed: 21861858]

- Dupont KM, Sharma K, Stevens HY, Boerckel JD, Garcia AJ, & Guldberg RE (2010). Human stem cell delivery for treatment of large segmental bone defects. *Proc Natl Acad Sci U S A*, 107(8), 3305–3310. doi:10.1073/pnas.0905444107 [PubMed: 20133731]
- English K, & Wood KJ (2010). Immunogenicity of embryonic stem cell-derived progenitors after transplantation. *Curr Opin Organ Transplant*. doi:10.1097/MOT.0b013e3283424faa
- English K, & Wood KJ (2013). Mesenchymal stromal cells in transplantation rejection and tolerance. *Cold Spring Harb Perspect Med*, 3(5), a015560. doi:10.1101/cshperspect.a015560 [PubMed: 23637312]
- Goodman SB (2013). Cell-based therapies for regenerating bone. *Minerva Ortop Traumatol*, 64(2), 107–113. [PubMed: 24436510]
- Griffin MD, Ryan AE, Alagesan S, Lohan P, Treacy O, & Ritter T (2013). Anti-donor immune responses elicited by allogeneic mesenchymal stem cells: what have we learned so far? *Immunol Cell Biol*, 91(1), 40–51. doi:10.1038/icb.2012.67 [PubMed: 23207278]
- Grinnemo KH, Mansson A, Dellgren G, Klingberg D, Wardell E, Drvota V, ... Le Blanc K (2004). Xenoreactivity and engraftment of human mesenchymal stem cells transplanted into infarcted rat myocardium. *J Thorac Cardiovasc Surg*, 127(5), 1293–1300. doi:10.1016/j.jtcvs.2003.07.037 [PubMed: 15115985]
- Heidt S, Segundo DS, Chadha R, & Wood KJ (2010). The impact of Th17 cells on transplant rejection and the induction of tolerance. *Curr Opin Organ Transplant*, 15(4), 456–461. doi:10.1097/MOT.0b013e32833b9bfb [PubMed: 20616728]
- Henning TD, Gawande R, Khurana A, Tavri S, Mandrusow L, Golovko D, ... Daldrup-Link HE (2011). Magnetic Resonance Imaging of Ferumoxide-Labeled Mesenchymal Stem Cells in Cartilage Defects: In Vitro and In Vivo Investigations. *Mol Imaging* doi:10.2310/7290.2011.00040
- Hitchens TK, Ye Q, Eytan DF, Janjic JM, Ahrens ET, & Ho C (2011). ¹⁹F MRI detection of acute allograft rejection with in vivo perfluorocarbon labeling of immune cells. *Magn Reson Med*, 65(4), 1144–1153. doi:10.1002/mrm.22702 [PubMed: 21305593]
- Hyun J, Grova M, Nejadnik H, Lo D, Morrison S, Montoro D, ... Longaker MT (2013). Enhancing in vivo survival of adipose-derived stromal cells through Bcl-2 overexpression using a minicircle vector. *Stem Cells Transl Med*, 2(9), 690–702. doi:10.5966/sctm.2013-0035 [PubMed: 23934910]
- Ikezumi Y, Hurst LA, Masaki T, Atkins RC, & Nikolic-Paterson DJ (2003). Adoptive transfer studies demonstrate that macrophages can induce proteinuria and mesangial cell proliferation. *Kidney Int*, 63(1), 83–95. doi:10.1046/j.1523-1755.2003.00717.x [PubMed: 12472771]
- Jorgensen C, Gordeladze J, & Noel D (2004). Tissue engineering through autologous mesenchymal stem cells. *Curr Opin Biotechnol*, 15(5), 406–410. doi:S0958-1669(04)00109-0 [pii] 10.1016/j.copbio.2004.08.003 [PubMed: 15464369]
- Jorgensen C, & Noel D (2011). Mesenchymal stem cells in osteoarticular diseases. *Regen Med*, 6(6 Suppl), 44–51. doi:10.2217/rme.11.80 [PubMed: 21999261]
- Julier Z, Park AJ, Briquez PS, & Martino MM (2017). Promoting tissue regeneration by modulating the immune system. *Acta Biomater*, 53, 13–28. doi:10.1016/j.actbio.2017.01.056 [PubMed: 28119112]
- Kanno S, Wu YJ, Lee PC, Dodd SJ, Williams M, Griffith BP, & Ho C (2001). Macrophage accumulation associated with rat cardiac allograft rejection detected by magnetic resonance imaging with ultrasmall superparamagnetic iron oxide particles. *Circulation*, 104(8), 934–938. [PubMed: 11514382]
- Karabekian Z, Posnack NG, & Sarvazyan N (2011). Immunological barriers to stem-cell based cardiac repair. *Stem Cell Rev*, 7(2), 315–325. doi:10.1007/s12015-010-9202-x [PubMed: 21086071]
- Khurana A, Chapelin F, Beck G, Lenkov OD, Donig J, Nejadnik H, ... Daldrup-Link HE (2013). Iron administration before stem cell harvest enables MR imaging tracking after transplantation. *Radiology*, 269(1), 186–197. doi:10.1148/radiol.13130858 [PubMed: 23850832]
- Khurana A, Nejadnik H, Chapelin F, Lenkov O, Gawande R, Lee S, ... Daldrup-Link HE (2013). Ferumoxytol: a new, clinically applicable label for stem-cell tracking in arthritic joints with MRI. *Nanomedicine (Lond)*, 8(12), 1969–1983. doi:10.2217/nmm.12.198 [PubMed: 23534832]
- Khurana A, Nejadnik H, Gawande R, Lin G, Lee S, Messing S, ... Daldrup-Link HE (2012). Intravenous ferumoxytol allows noninvasive MR imaging monitoring of macrophage migration

- into stem cell transplants. *Radiology*, 264(3), 803–811. doi:10.1148/radiol.12112393 [PubMed: 22820731]
- Kim DE, Tsuji K, Kim YR, Mueller FJ, Eom HS, Snyder EY, ... Schellingerhout D (2006). Neural stem cell transplant survival in brains of mice: assessing the effect of immunity and ischemia by using real-time bioluminescent imaging. *Radiology*, 241(3), 822–830. doi:10.1148/radiol.2413050466 [PubMed: 17114629]
- Kirschbaum K, Sonner JK, Zeller MW, Deumelandt K, Bode J, Sharma R, ... Breckwoldt MO (2016). In vivo nanoparticle imaging of innate immune cells can serve as a marker of disease severity in a model of multiple sclerosis. *Proc Natl Acad Sci U S A*, 113(46), 13227–13232. doi:10.1073/pnas.1609397113 [PubMed: 27799546]
- Kofidis T, de Bruin JL, Yamane T, Tanaka M, Lebl DR, Swijnenburg RJ, ... Robbins RC (2005). Stimulation of paracrine pathways with growth factors enhances embryonic stem cell engraftment and host-specific differentiation in the heart after ischemic myocardial injury. *Circulation*, 111(19), 2486–2493. doi:10.1161/01.CIR.0000165063.09283.A8 [PubMed: 15883216]
- Kraitchman DL, Heldman AW, Atalar E, Amado LC, Martin BJ, Pittenger MF, ... Bulte JW (2003). In vivo magnetic resonance imaging of mesenchymal stem cells in myocardial infarction. *Circulation*, 107(18), 2290–2293. doi:10.1161/01.CIR.0000070931.62772.4E [PubMed: 12732608]
- Lan HY, Nikolic-Paterson DJ, & Atkins RC (1993). Trafficking of inflammatory macrophages from the kidney to draining lymph nodes during experimental glomerulonephritis. *Clin Exp Immunol*, 92(2), 336–341. [PubMed: 8485918]
- Lee K, & Goodman SB (2009). Cell therapy for secondary osteonecrosis of the femoral condyles using the Collect DBM System: a preliminary report. *J Arthroplasty*, 24(1), 43–48. doi:10.1016/j.arth.2008.01.133 [PubMed: 18534437]
- Lemaster JEC, F.; Kim T; Hariri A; Jokerst VJ;. (2018). Development of a Trimodal Contrast Agent for Acoustic and Magnetic Particle Imaging of Stem Cells. *ACS Appl. Nano Mater*, 1(3), 1321–1331. doi:10.1021/acsanm.8b00063
- Levi B, Nelson ER, Hyun JS, Glotzbach JP, Li S, Nauta A, ... Longaker MT (2012). Enhancement of human adipose-derived stromal cell angiogenesis through knockdown of a BMP-2 inhibitor. *Plast Reconstr Surg*, 129(1), 53–66. doi:10.1097/PRS.0b013e3182361ff5 [PubMed: 21915082]
- Levi B, Nelson ER, Li S, James AW, Hyun JS, Montoro DT, ... Longaker MT (2011). Dura mater stimulates human adipose-derived stromal cells to undergo bone formation in mouse calvarial defects. *Stem Cells*, 29(8), 1241–1255. doi:10.1002/stem.670 [PubMed: 21656608]
- Li K, Chan CT, Nejadnik H, Lenkov OD, Wolterman C, Paulmurugan R, ... Daldrup-Link HE (2018). Ferumoxytol-based Dual-modality Imaging Probe for Detection of Stem Cell Transplant Rejection. *Nanotheranostics*, 2(4), 306–319. doi:10.7150/ntno.26389 [PubMed: 29977742]
- Li L, Chen X, Wang WE, & Zeng C (2016). How to Improve the Survival of Transplanted Mesenchymal Stem Cell in Ischemic Heart? *Stem Cells Int*, 2016, 9682757. doi: 10.1155/2016/9682757 [PubMed: 26681958]
- Li SC, Tachiki LM, Luo J, Dethlefs BA, Chen Z, & Loudon WG (2010). A biological global positioning system: considerations for tracking stem cell behaviors in the whole body. *Stem Cell Rev*, 6(2), 317–333. doi:10.1007/s12015-010-9130-9 [PubMed: 20237964]
- Lu M, Cohen MH, Rieves D, & Pazdur R (2010). FDA report: Ferumoxytol for intravenous iron therapy in adult patients with chronic kidney disease. *Am J Hematol*, 85(5), 315–319. doi:10.1002/ajh.21656 [PubMed: 20201089]
- Luo Y, Shao L, Chang J, Feng W, Liu YL, Cottler-Fox MH, ... Zhou D (2018). M1 and M2 macrophages differentially regulate hematopoietic stem cell self-renewal and ex vivo expansion. *Blood Adv*, 2(8), 859–870. doi:10.1182/bloodadvances.2018015685 [PubMed: 29666049]
- Luong-Van E, Grondahl L, Song S, Nurcombe V, & Cool S (2007). The in vivo assessment of a novel scaffold containing heparan sulfate for tissue engineering with human mesenchymal stem cells. *J Mol Histol*, 38(5), 459–468. doi:10.1007/s10735-007-9129-y [PubMed: 17694276]
- Ma N, Cheng H, Lu M, Liu Q, Chen X, Yin G, ... Zhao S (2015). Magnetic resonance imaging with superparamagnetic iron oxide fails to track the long-term fate of mesenchymal stem cells transplanted into heart. *Sci Rep*, 5, 9058. doi:10.1038/srep09058 [PubMed: 25762186]

- Mannon RB (2012). Macrophages: contributors to allograft dysfunction, repair, or innocent bystanders? *Curr Opin Organ Transplant*, 17(1), 20–25. doi:10.1097/MOT.0b013e32834ee5b6 [PubMed: 22157320]
- Martinez FO, & Gordon S (2014). The M1 and M2 paradigm of macrophage activation: time for reassessment. *F1000Prime Rep*, 6, 13. doi:10.12703/P6-13 [PubMed: 24669294]
- Metz S, Lohr S, Settles M, Beer A, Woertler K, Rummeny EJ, & Daldrup-Link HE (2006). Ferumoxtran-10-enhanced MR imaging of the bone marrow before and after conditioning therapy in patients with non-Hodgkin lymphomas. *Eur Radiol*, 16(3), 598–607. doi:10.1007/s00330-005-0045-9 [PubMed: 16284770]
- Nam SY, Ricles LM, Suggs LJ, & Emelianov SY (2012). In vivo ultrasound and photoacoustic monitoring of mesenchymal stem cells labeled with gold nanotracers. *PLoS One*, 7(5), e37267. doi:10.1371/journal.pone.0037267 [PubMed: 22615959]
- Nedopil A, Klenk C, Kim C, Liu S, Wendland M, Golovko D, ... Daldrup-Link HE (2010). MR signal characteristics of viable and apoptotic human mesenchymal stem cells in matrix-associated stem cell implants for treatment of osteoarthritis. *Invest Radiol*, 45(10), 634–640. doi:10.1097/RLI.0b013e3181ed566c [PubMed: 20808236]
- Negrin RS, & Contag CH (2006). In vivo imaging using bioluminescence: a tool for probing graft-versus-host disease. *Nat Rev Immunol*, 6(6), 484–490. doi:10.1038/nri1879 [PubMed: 16724101]
- Nejadnik H, Lenkov O, Gassert F, Fretwell D, Lam I, & Daldrup-Link HE (2016). Macrophage phagocytosis alters the MRI signal of ferumoxytol-labeled mesenchymal stromal cells in cartilage defects. *Sci Rep*, 6, 25897. doi:10.1038/srep25897 [PubMed: 27174199]
- Nejadnik H, Pandit P, Lenkov O, Lahiji AP, Yerneni K, & Daldrup-Link HE (2018). Ferumoxytol Can Be Used for Quantitative Magnetic Particle Imaging of Transplanted Stem Cells. *Mol Imaging Biol* doi:10.1007/s11307-018-1276-x
- Nejadnik H, Taghavi-Garmestani SM, Madsen SJ, Li K, Zanganeh S, Yang P, ... Daldrup-Link HE (2018). The Protein Corona around Nanoparticles Facilitates Stem Cell Labeling for Clinical MR Imaging. *Radiology*, 286(3), 938–947. doi:10.1148/radiol.2017170130 [PubMed: 29091749]
- Nilsson B, Korsgren O, Lambris JD, & Ekdahl KN (2010). Can cells and biomaterials in therapeutic medicine be shielded from innate immune recognition? *Trends Immunol*, 31(1), 32–38. doi:10.1016/j.it.2009.09.005 [PubMed: 19836998]
- O'Sullivan ES, Vegas A, Anderson DG, & Weir GC (2011). Islets transplanted in immunoisolation devices: a review of the progress and the challenges that remain. *Endocr Rev*, 32(6), 827–844. doi:10.1210/er.2010-0026 [PubMed: 21951347]
- Polak JM, & Mantalaris S (2008). Stem cells bioprocessing: an important milestone to move regenerative medicine research into the clinical arena. *Pediatr Res*, 63(5), 461–466. doi:10.1203/10.1203/PDR.0b013e31816a8c1c [PubMed: 18427288]
- Poon IK, Lucas CD, Rossi AG, & Ravichandran KS (2014). Apoptotic cell clearance: basic biology and therapeutic potential. *Nat Rev Immunol*, 14(3), 166–180. doi:10.1038/nri3607 [PubMed: 24481336]
- Preynat-Seauve O, & Krause KH (2011). Stem cell sources for regenerative medicine: the immunological point of view. *Semin Immunopathol*, 33(6), 519–524. doi:10.1007/s00281-011-0271-y [PubMed: 21598094]
- Schroeder T (2008). Imaging stem-cell-driven regeneration in mammals. *Nature*, 453(7193), 345–351. doi:10.1038/nature07043 [PubMed: 18480816]
- Shakhbazov A, Mishra M, Chu TH, Brideau C, Cummins K, Tsutsui S, ... van Minnen J (2015). Fluorescent Phosphorus Dendrimer as a Spectral Nanosensor for Macrophage Polarization and Fate Tracking in Spinal Cord Injury. *Macromol Biosci*, 15(11), 1523–1534. doi:10.1002/mabi.201500150 [PubMed: 26175127]
- Shegarfi H, & Reikeras O (2009). Review article: bone transplantation and immune response. *J Orthop Surg (Hong Kong)*, 17(2), 206–211. doi:10.1177/230949900901700218 [PubMed: 19721154]
- Sheikh AY, & Wu JC (2006). Molecular imaging of cardiac stem cell transplantation. *Curr Cardiol Rep*, 8(2), 147–154. [PubMed: 16524542]

- Sherman LS, Munoz J, Patel SA, Dave MA, Paige I, & Rameshwar P (2011). Moving from the laboratory bench to patients' bedside: considerations for effective therapy with stem cells. *Clin Transl Sci*, 4(5), 380–386. doi:10.1111/j.1752-8062.2011.00283.x [PubMed: 22029813]
- Shimizu Y, Hanzawa H, Zhao Y, Fukura S, Nishijima KI, Sakamoto T, ... Kuge Y (2017). Immunoglobulin G (IgG)-Based Imaging Probe Accumulates in M1 Macrophage-Infiltrated Atherosclerotic Plaques Independent of IgG Target Molecule Expression. *Mol Imaging Biol*, 19(4), 531–539. doi:10.1007/s11307-016-1036-8 [PubMed: 27981470]
- Simon GH, Raatschen HJ, Wendland MF, von Vopelius-Feldt J, Fu Y, Chen MH, & Daldrup-Link HE (2005). Ultrasmall superparamagnetic iron-oxide-enhanced MR imaging of normal bone marrow in rodents: original research original research. *Acad Radiol*, 12(9), 1190–1197. doi:S1076-6332(05)00449-6 [pii] 10.1016/j.acra.2005.05.014 [PubMed: 16099684]
- Swijnenburg RJ, Schrepfer S, Cao F, Pearl JI, Xie X, Connolly AJ, ... Wu JC (2008). In vivo imaging of embryonic stem cells reveals patterns of survival and immune rejection following transplantation. *Stem Cells Dev*, 17(6), 1023–1029. doi:10.1089/scd.2008.0091 [PubMed: 18491958]
- Swijnenburg RJ, Tanaka M, Vogel H, Baker J, Kofidis T, Gunawan F, ... Robbins RC (2005). Embryonic stem cell immunogenicity increases upon differentiation after transplantation into ischemic myocardium. *Circulation*, 112(9 Suppl), I166–172. doi:10.1161/CIRCULATIONAHA.104.525824 [PubMed: 16159810]
- Swijnenburg RJ, van der Bogt KE, Sheikh AY, Cao F, & Wu JC (2007). Clinical hurdles for the transplantation of cardiomyocytes derived from human embryonic stem cells: role of molecular imaging. *Curr Opin Biotechnol*, 18(1), 38–45. doi:10.1016/j.copbio.2006.12.003 [PubMed: 17196814]
- Theruvath AJ, Nejadnik H, Muehe AM, Gassert F, Lacayo NJ, Goodman SB, & Daldrup-Link HE (2018). Tracking Cell Transplants in Femoral Osteonecrosis with Magnetic Resonance Imaging: A Proof of Concept Study in Patients. *Clin Cancer Res* doi:10.1158/1078-0432.CCR-18-1687
- Thompson HL, & Manilay JO (2011). Embryonic stem cell-derived hematopoietic stem cells: challenges in development, differentiation, and immunogenicity. *Curr Top Med Chem*, 11(13), 1621–1637. [PubMed: 21446908]
- Thorek DL, Ulmert D, Diop NF, Lupu ME, Doran MG, Huang R, ... Grimm J (2014). Non-invasive mapping of deep-tissue lymph nodes in live animals using a multimodal PET/MRI nanoparticle. *Nat Commun*, 5, 3097. doi:10.1038/ncomms4097 [PubMed: 24445347]
- Thu MS, Bryant LH, Coppola T, Jordan EK, Budde MD, Lewis BK, ... Frank JA (2012). Self-assembling nanocomplexes by combining ferumoxytol, heparin and protamine for cell tracking by magnetic resonance imaging. *Nat Med*, 18(3), 463–467. doi:10.1038/nm.2666 [PubMed: 22366951]
- Toma C, Wagner WR, Bowry S, Schwartz A, & Villanueva F (2009). Fate of culture-expanded mesenchymal stem cells in the microvasculature: in vivo observations of cell kinetics. *Circ Res*, 104(3), 398–402. doi:10.1161/CIRCRESAHA.108.187724 [PubMed: 19096027]
- Trattinig S, Winalski CS, Marlovits S, Jurvelin JS, Welsch GH, & Potter HG (2011). Magnetic Resonance Imaging of Cartilage Repair: A Review. *Cartilage*, 2(1), 5–26. doi:10.1177/1947603509360209 [PubMed: 26069565]
- van Buul GM, Farrell E, Kops N, van Tiel ST, Bos PK, Weinans H, ... Bernsen MR (2009). Ferumoxides-protamine sulfate is more effective than ferucarbotran for cell labeling: implications for clinically applicable cell tracking using MRI. *Contrast Media Mol Imaging*, 4(5), 230–236. doi:10.1002/cmml.289 [PubMed: 19839030]
- van der Bogt KE, Swijnenburg RJ, Cao F, & Wu JC (2006). Molecular imaging of human embryonic stem cells: keeping an eye on differentiation, tumorigenicity and immunogenicity. *Cell Cycle*, 5(23), 2748–2752. [PubMed: 17172859]
- von Bahr L, Batsis I, Moll G, Hagg M, Szakos A, Sundberg B, ... Le Blanc K (2012). Analysis of tissues following mesenchymal stromal cell therapy in humans indicates limited long-term engraftment and no ectopic tissue formation. *Stem Cells*, 30(7), 1575–1578. doi:10.1002/stem.1118 [PubMed: 22553154]
- Wynn TA, & Vannella KM (2016). Macrophages in Tissue Repair, Regeneration, and Fibrosis. *Immunity*, 44(3), 450–462. doi:10.1016/j.immuni.2016.02.015 [PubMed: 26982353]

- Yang XF (2007). Immunology of stem cells and cancer stem cells. *Cell Mol Immunol*, 4(3), 161–171. [PubMed: 17601370]
- Zanganeh S, Hutter G, Spitler R, Lenkov O, Mahmoudi M, Shaw A, ... Daldrup-Link HE (2016). Iron oxide nanoparticles inhibit tumour growth by inducing pro-inflammatory macrophage polarization in tumour tissues. *Nat Nanotechnol*, 11(11), 986–994. doi:10.1038/nnano.2016.168 [PubMed: 27668795]
- Zangi L, Margalit R, Reich-Zeliger S, Bachar-Lustig E, Beilhack A, Negrin R, & Reisner Y (2009). Direct imaging of immune rejection and memory induction by allogeneic mesenchymal stromal cells. *Stem Cells*, 27(11), 2865–2874. doi:10.1002/stem.217 [PubMed: 19750539]
- Zhang C, Yu X, Gao L, Zhao Y, Lai J, Lu D, ... Liu Z (2017). Noninvasive Imaging of CD206-Positive M2 Macrophages as an Early Biomarker for Post-Chemotherapy Tumor Relapse and Lymph Node Metastasis. *Theranostics*, 7(17), 4276–4288. doi:10.7150/thno.20999 [PubMed: 29158825]
- Zhang SJ, & Wu JC (2007). Comparison of imaging techniques for tracking cardiac stem cell therapy. *J Nucl Med*, 48(12), 1916–1919. doi:10.2967/jnumed.107.043299 [PubMed: 18056330]
- Zhang Y, Dodd SJ, Hendrich KS, Williams M, & Ho C (2000). Magnetic resonance imaging detection of rat renal transplant rejection by monitoring macrophage infiltration. *Kidney Int*, 58(3), 1300–1310. doi:10.1046/j.1523-1755.2000.00286.x [PubMed: 10972694]
- Zhang ZY, Teoh SH, Hui JH, Fisk NM, Choolani M, & Chan JK (2012). The potential of human fetal mesenchymal stem cells for off-the-shelf bone tissue engineering application. *Biomaterials*, 33(9), 2656–2672. doi:10.1016/j.biomaterials.2011.12.025 [PubMed: 22217806]
- Zhao T, Zhang ZN, Rong Z, & Xu Y (2011). Immunogenicity of induced pluripotent stem cells. *Nature*, 474(7350), 212–215. doi:10.1038/nature10135 [PubMed: 21572395]
- Zhou R, Acton PD, & Ferrari VA (2006). Imaging stem cells implanted in infarcted myocardium. *J Am Coll Cardiol*, 48(10), 2094–2106. doi:10.1016/j.jacc.2006.08.026 [PubMed: 17112999]

Pro-inflammatory environment

Figure 1).

Concept of *in vivo* stem cell – immune system interaction in a pro-inflammatory environment.

Figure 2: The *in vivo* ferumoxytol labelled MSCs could be tracked with MR imaging in patients.

(A) An overview of the study: patients received an intravenous injection of ferumoxytol, which is taken up by bone marrow cells. Then, the ferumoxytol-labelled cells were aspirated from the iliac crest. The osteonecrotic bone was decompressed by drilling a channel to the osteonecrosis area, and the harvested ferumoxytol-labelled cells were injected into the channel. (B) Iron-labelled cell transplants can be detected in the decompression channel: Coronal T2-weighted MRI scan of the left hip joint after decompression surgery and transplantation of iron labeled cells shows areas of hypointense signal in the decompression track. Superimposed color encoded T2 signal map shows iron-labeled cells displayed by blue color. T2-weighted MRI scan of the left hip joint after decompression surgery and transplantation of unlabeled cells does not show hypointense signal areas in the decompression track. Superimposed color encoded T2 signal map does not show iron signal in the track. Signal-to-noise ratios (SNR) and T2* relaxation time of unlabeled cell transplants were significantly higher compared to labeled cell transplants. Data are displayed

as mean SNR and T2* relaxation time data of nine patients and standard errors. * $P < 0.05$ and ** $P < 0.01$ (Reprinted with permission from (Theruvath et al., 2018) Copyright rights 2018 Managed by American Association for Cancer Research).

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Figure 3: Concept of indirect detection of apoptosis of stem cell *in vivo* by MR imaging: (A) Mesenchymal stromal cells (MSC) were labeled *in vitro* with ferumoxytol. Viable or apoptotic stem cell were seeded in scaffold and implanted into osteochondral defects. (B) Iron labelled viable MSC showed hypointense T2-signal directly after implantation and moderate T2-signal at 2 weeks after implantation, apparently due to slow iron metabolism. (C) Iron labelled apoptotic MSC showed hypointense T2-signal directly after implantation and loss of T2-signal at 2 weeks after implantation, apparently due to loss of iron labeled cells and faster iron metabolism. Histopathologies (not shown) revealed an increased number

of macrophages in apoptotic implants. (D) T2 relaxation times of iron labelled viable, and apoptotic MASIs demonstrated a significant difference at day 14. Data are displayed as mean T2 relaxation times of five cell transplants per group and standard errors. * $P < 0.05$ (Reprinted with permission from (Nejadnik et al., 2016) Copyright rights 2016 Managed by Nature Publishing Group).

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Figure 4: Concept of *in vivo* tracking of macrophages:

(A) Experimental rats received intravenous ferumoxytol injections before stem cell implantation. At 48 hours after ferumoxytol injection, unlabeled viable and apoptotic stem cells were implanted into osteochondral defects of bilateral knee joints. Iron labeled bone marrow cells migrated into apoptotic cell transplants. (B) Sagittal T2-weighted fast spin-echo MR images show hyperintense T2-signal of unlabeled cells directly after implantation and moderate T2-signal at 4 weeks after implantation, apparently due to minor migration of iron labeled cells into the transplant. (C) Sagittal T2-weighted fast spin-echo MR images show

hyperintense T2-signal of unlabeled cells directly after implantation and marked hypointense (dark) T2-signal at 4 weeks after implantation, apparently due to migration of iron labeled cells into the transplant. (D) T2 relaxation times of the apoptotic implants showed a significant decrease compared with viable implants at week 4. Data are displayed as mean SNR data of six cell transplants and standard errors. (Reprinted with permission from (Khurana et al., 2012) Copyright rights 2012 Managed by RSNA journals).

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Figure 5: Concept of indirect tracking of macrophages responses via *ex vivo* labelling of stem cells with an activatable probe:

(A) Mesenchymal stromal cells (MSC) were labeled with caspase-3-cleavable ferumoxytol-AFC nanoparticles. Immune matched murine MSC or immune mismatched pig MSC were implanted into calvarial defects of experimental mice. Both implants could be localized based on the iron label of the cells. Caspase expression in immune mismatched cells cleaved the fluorophore AFC and caused green fluorescent signal.. (B) Intravital microscopy (IVM) scan of viable MSC implant does not show any fluorescent signal on day 1 and 6 after implantation. By contrast, apoptosis of mismatched MSC activated the Feru-AFC NPs as

indicated by activated fluorescence signal on day 1. Corresponding quantitative FITC signal was significantly higher in apoptotic cell transplants compared to viable cell transplants. Data are displayed as mean FITC sum intensity of six cell transplants and standard errors. * $P < 0.05$ (C) Coronal T2-weighted MRI scan of the same immune-matched and immune-matched MSC implants shows significant T2-signal on day 1 and 7 after implantation. Corresponding T2 relaxation times did not show a significant difference between immune-matched and immune-matched MSC during the early postotransplant phase of 7 days. Data are displayed as mean T2 relaxation time of six cell transplants and standard deviations. * $P < 0.05$ (Reprinted with permission from (K. Li et al., 2018) Copyright rights 2018 Managed by Ivyspring International Publisher).