Citation for published version (APA):
Monitoring therapy response of experimental arthritis with radiolabeled tracers targeting fibroblasts, macrophages or integrin $\alpha_\beta_3$

Samantha Y.A. Terry$^{1,2*}$, Marije I. Koenders$^3$, Gerben M. Franssen,$^1$, Tapan K. Nayak$^4$, Anne Freimoser-Grundschober$^5$, Christian Klein$^5$, Wim J. Oyen, MD$^1$, Otto C. Boerman$^1$, Peter Laverman$^1$

$^1$Department of Radiology and Nuclear Medicine, Radboud University Medical Center, Nijmegen, the Netherlands; $^2$Department of Imaging Chemistry and Biology, King’s College London, London, UK; $^3$Department of Experimental Rheumatology, Radboud University Medical Center, Nijmegen, the Netherlands; $^4$Roche Pharmaceutical Research and Early Development, Roche Innovation Center Basel, Switzerland; $^5$Roche Innovation Center Zurich, Zurich, Switzerland

* Correspondence to:
Samantha Terry, Ph.D.

Address: Division of Imaging Sciences and Biomedical Engineering, King's College London, St. Thomas' Hospital, London, UK SE1 7EH

T: +44 20 71888370, Fax: +44 20 71885442, E-Mail: samantha.terry@kcl.ac.uk

Running title: Imaging experimental arthritis

Financial support: S. Terry was funded by the Roche Postdoc Fellowship (RPF) Program.

Word count: 4945
ABSTRACT
Rheumatoid arthritis is an autoimmune disease resulting in chronic synovial inflammation. Molecular imaging could be used to monitor therapy response, thus enabling tailored therapy regimens and enhancing therapeutic outcome. Here, we hypothesized that response to etanercept could be monitored by radionuclide imaging in arthritic mice. We tested three different targets namely fibroblast activation protein (FAP), macrophages, and integrin $\alpha_v\beta_3$.

METHODS
Male DBA/1J mice with collagen-induced arthritis were treated with etanercept. SPECT/CT (Single photon emission computed tomography/ X-ray computed tomography) scans were acquired at 1, 24 and 48 h after injecting $^{111}$In-RGD$_2$ (integrin $\alpha_v\beta_3$), $^{111}$In-anti-F4/80-A3-1 (anti-murine macrophage antibody), or $^{111}$In-28H1 (anti-FAP antibody), respectively, with nonspecific controls included. Mice were dissected after the last scan and scans were analyzed quantitatively and were correlated with macroscopic scoring.

RESULTS
Experimental arthritis was imaged with $^{111}$In-28H1 (anti-FAP), $^{111}$In-anti-F4/80-A3-1, and $^{111}$In-RGD$_2$. Tracer uptake in joints correlated with arthritis score. Treatment decreased joint uptake of tracers; it decreased from 23±15 %ID/g, 8±4 %ID/g, 2±1 %ID/g to 11±11 %ID/g (p<0.001), 4±4 %ID/g (p<0.001), 1±0.2 %ID/g (p<0.01) for $^{111}$In-28H1, $^{111}$In-anti-F4/80-A3-1, and $^{111}$In-RGD$_2$, respectively. Arthritis-to-blood ratios (in mice with arthritis score 2 per joint) were higher for $^{111}$In-28H1 (5.5±1; excluding values over 25), $^{111}$In-anti-F4/80-A3-1 (10.4±4), and $^{111}$In-RGD$_2$ (7.2±1) than for control $^{111}$In-DP47GS (0.7±0.5; p=0.002), $^{111}$In-rat IgG2b (0.5±0.2; p=0.002), or coinjection of excess RGD$_2$ (3.5), indicating specific uptake of all tracers in arthritic joints.

CONCLUSION
$^{111}$In-28H1, $^{111}$In-anti-F4/80-A3-1, and $^{111}$In-RGD$_2$ can be used to specifically monitor the response to therapy in experimental arthritis at the molecular level. Further studies however still need to be carried out.
KEYWORDS

experimental arthritis, fibroblast activation protein, macrophages, RGD peptide, therapy response
INTRODUCTION

Rheumatoid arthritis is an autoimmune disease with a global prevalence of 0.24% in 2010 (1). Arthritis is marked by symptoms including joint pain and swelling caused by chronic inflammation in synovial joints and can be treated systemically or intra-articularly by either non-steroidal anti-inflammatory drugs, corticosteroids or disease-modifying anti-rheumatic drugs (DMARDs) (2, 3). In addition, treatment with biologicals has been shown to be quite effective in patients.

Because arthritis is a chronic disease characterized by disease exacerbations (4), it has become crucial to develop tools to effectively monitor disease progression, prevent progressive destruction, and predict or monitor response to therapy, which are as important for a positive outcome as early diagnosis. Monitoring response to therapy could allow for the therapeutic schedules- aimed to reduce inflammation, relieve pain, and reduce disabilities - to be altered if deemed necessary. This could thus minimize unwarranted side effects and irreversible joint damage. This is especially pertinent for therapies such as DMARDs, where it can take several weeks to months for clinical improvements to show (5).

Many cell types that play a role in joint inflammation and destruction are present in the synovial lining. One of the most prominent cell populations is activated fibroblast-like synoviocytes (6), which express fibroblast activation protein (FAP) (7) and could be imaged by microSPECT using radiolabeled antibodies against FAP, as shown by us recently (8). Other cell types include osteoclasts (9), which express integrin αvβ3 (10). Integrin αvβ3 is a much-used target for imaging tumour cells and angiogenesis in oncology using radiolabeled RGD peptides. Preclinically, F4/80 receptor-positive macrophages also play a role in arthritis pathogenesis (11). These targets have previously been successfully imaged by either radiolabeled RGD-based peptides (12), and an anti-F4/80-A3-1 labeled with In-111 (13), respectively, albeit in an oncological setting.

Non-invasive imaging could be a great tool in determining early on whether therapies are successful (14, 15), yet few approaches have been aimed at a molecular level specific for arthritis. Here, we focused on imaging these three different targets, namely FAP, macrophages, and integrin...
αβ₃, and determined whether they could be useful tools to monitor response to the Tumour Necrosis Factor (TNF) receptor fusion protein etanercept therapy in arthritic mice. These targets were also chosen because the treatment used here, etanercept, targets TNF, which is produced mainly by monocyte-macrophages, activated endothelial cells, which also express integrin αβ₃ (16), and synovial fibroblasts (17).

**MATERIALS AND METHODS**

**Antibody Conjugation And RGD Peptide**

Rat anti-mouse anti-F4/80-A3-1 antibody (AbD Serotec, Kidlington, UK) and rat IgG2b (R&D Systems, Abingdon, UK) were dialyzed against 1:1 phosphate buffered saline (PBS, pH 7.4):water (1:1) to remove sodium azide. Antibodies 28H1 (anti-FAP), DP47GS (8), anti-F4/80-A3-1 and rat IgG2b (13), were conjugated with isothiocyanatobenzyl–diethylenetriaminepentaacetic acid (p-SCN-Bz-DTPA) (Macrocyclics, Inc., Dallas, TX) in 0.1 M NaHCO₃, pH 9.5, using a 5-fold (28H1, DP47GS) or 10-fold (anti-F4/80-A3-1, IgG2b) molar excess of p-SCN-Bz-DTPA for 1 h at room temperature. Unconjugated p-SCN-Bz-DTPA was removed by dialysis against 0.25 M ammonium acetate buffer, pH 5.5 (28H1, DP47GS) or 0.1 M 2-(N-morpholino)ethanesulfonic acid (MES), pH 5.5 (anti-F4/80-A3-1, IgG2b). DP47GS and rat IgG2b antibodies served as isotype controls for 28H1 and anti-F4/80-A3-1, respectively.

DOTA-E-[c(RGDFK)]₂ (RGD₂) was acquired from Peptides International, KY, USA. Here we used the 28H1 and DP47GS antibodies, which are non-internalizing antibodies bearing a mutation in the Fc effector part, preventing binding to the Fc receptor. All buffers used were metal-free.

The affinity of all three tracers and the immunoreactive fraction of anti-F4/80-A3-1 (75%) have been determined previously (8, 13, 18). The immunoreactive fraction for ¹¹¹In-28H1 was 91.4% (Figure S1).

**Radiolabeling**
For radiolabeling, 750 µg of DTPA-28H1 or DTPA-DP47GS was incubated with 250-320 MBq $^{111}$InCl$_3$ (Mallinckrodt BV, Petten, The Netherlands) in 0.1 M 2-(N-morpholino)ethanesulfonic acid buffer pH 5.4 (MES buffer) for 30 min at room temperature. For studies involving anti-F4/80-A3-1 or rat IgG2b, 280-320 MBq $^{111}$InCl$_3$ (Mallinckrodt, Petten, the Netherlands) was added to 140 µg DTPA-anti-F4/80-A3-1 or 125 µg rat IgG2b for 1 h at room temperature in 0.1 MES buffer pH 5.4. For studies involving RGD$_2$, 8 µg DOTA-conjugated peptide, dissolved in 0.1 M MES buffer, pH 5.4, was incubated with 200 MBq $^{111}$InCl$_3$ for 20 minutes at 95 °C. Unincorporated $^{111}$In was complexed by adding ethylenediaminetetraacetic acid (final concentration of 5 mM) after the labeling.

Labeling efficiencies of all antibodies and the RGD$_2$ peptide were determined by instant thin-layer chromatography on instant thin-layer chromatography-silica gel strips (Agilent Technologies, Santa Clara, CA), with 0.1 M acetate buffer, pH 5.4, as the mobile phase. Labeling efficiencies were 93%, 99%, 91%, 95%, and 99%, for $^{111}$In-28H1, $^{111}$In-DP47GS, $^{111}$In-anti-F4/80-A3-1, $^{111}$In-rat IgG2b, and $^{111}$In-RGD$_2$, respectively. Tracers were diluted with PBS (with 0.5% bovine serum albumin (BSA)) and unlabeled antibody or peptide was added to obtain the required antibody or peptide dose.

**Animal Model**

Mice (22-25g) were housed in IVC cages with 5 mice/cage in a temperature- and humidity-controlled room with 12/12-hour light/dark cycle. Animals had unlimited access to water and food. Arthritis was induced in male DBA/1J mice by intradermal immunization at the tail base with 100 µg of bovine type II collagen (CII) in Freund’s complete adjuvant followed by an intraperitoneal (i.p.) booster injection of 100 µg of CII in PBS three weeks later (collagen-induced arthritis; CIA). Arthritic scores per paw ranged from mild to severe (Score 0 = no sign of arthritis, 0.25 = 1-2 toes red or swollen; 0.5 = 3-5 toes red or swollen; 1 = swollen ankle; 1.5 = swollen footpad; 2 = severe swelling and ankylosis). Etanercept treatment (10 mg/kg, 3x per week, i.p.) started when mice showed first signs of the onset of arthritis (Score ≥0.25 in one joint). Untreated arthritic mice were used as
Biodistribution Studies

In dose finding studies, arthritic male DBA/1J mice were injected intravenously (i.v.) with either 10 or 40 µg $^{111}$In-anti-F4/80-A3-1 or $^{111}$In-IgG2b, as done previously for $^{111}$In-28H1, and the biodistribution was measured by ex vivo analysis of the accumulation of the tracer in different organs and joints after dissection of the mice. In other biodistribution studies, treated and untreated male mice were injected i.v with 50 µg $^{111}$In-DTPA-28H1 or $^{111}$In-DTPA-DP47GS, 10 µg $^{111}$In-anti-F4/80-A3-1 or $^{111}$In-rat IgG2b, or 1 µg (radiolabeled) or 50 µg (1 µg + 49 µg unlabeled) $^{111}$In-RGD$_2$ (3-4 MBq). At 48 h ($^{111}$In-DTPA-28H1 and $^{111}$In-DTPA-DP47GS), 24 h ($^{111}$In-anti-F4/80-A3-1 and $^{111}$In-rat IgG2b) and 1 h ($^{111}$In-RGD$_2$) after tracer injection, mice were euthanized by CO$_2$/O$_2$ asphyxiation. In total ten mice were used per group where blood, arthritic joints and major organs and tissues were dissected, weighed and counted in a shielded well-type gamma counter (Perkin-Elmer, Boston, MA, USA) to provide the %ID/g.

Micro-SPECT/CT Imaging

In imaging studies, treated and untreated male mice were injected i.v. with 50 µg $^{111}$In-DTPA-28H1 or $^{111}$In-DTPA-DP47GS, 10 µg $^{111}$In-anti-F4/80-A3-1 or $^{111}$In-rat IgG2b, or 1 µg (radiolabeled) or 50 µg (1 µg + 49 µg unlabeled) $^{111}$In-RGD$_2$ (15-21 MBq). At 48 h ($^{111}$In-DTPA-28H1 and $^{111}$In-DTPA-DP47GS), 24 h ($^{111}$In-anti-F4/80-A3-1 and $^{111}$In-rat IgG2b) and 1 h ($^{111}$In-RGD$_2$) after tracer injection, mice were euthanized by CO$_2$/O$_2$ asphyxiation for ex vivo biodistribution studies (3/group). Mice were then scanned with the U-SPECT-II/CT scanner (MILabs, Utrecht, The Netherlands) in prone position. Micro-SPECT scans were acquired as 3 frames of 15 min using a 1.0 mm pinhole ultra high sensitivity mouse collimator, followed by a CT scan for anatomic reference (65 kV, 615 µA). Scans were reconstructed and all frames combined using software from MILabs, using
an ordered-subset expectation maximization algorithm, with a voxel size of 0.4 mm. For high-resolution micro-SPECT imaging of an arthritic joint, a focal image was acquired using a 0.35 mm ultra-high resolution pinhole collimator at 9 frames of 18 min. Images were reconstructed to a voxel size of 0.125 mm without attenuation correction.

Quantitative SPECT Analysis

Reconstructed micro-SPECT scans were coregistered with CT images using Inveon Research Workplace software (version 3.0; Siemens Preclinical Solutions, LLC). The regions of interest were drawn using CT images and transferred to the SPECT data to obtain mean voxel intensities of the SPECT data corresponding to those regions of interest. Mean voxel intensity values were converted to %ID using decay correction and a standard curve (mean voxel intensity vs. kBq) acquired by scanning and reconstructing known In-111 activities from 15 to 310 kBq under the same conditions as the animal scans.

Statistical Analysis

Statistical analysis was performed using Wilcoxon signed-rank test or 2-way ANOVA with GraphPad Prism (version 5.03; GraphPad Software). Each paw is analyzed as an independent event. Spearman r correlation values between arthritis:blood values and arthritis score were calculated for Figure 1. Statistical significance was represented as *(p≤0.05), ***(p≤0.001).

RESULTS

Dosing Study Of ¹¹¹In-anti-F4/80-A3-1

The optimal dose of ¹¹¹In-anti-F4/80-A3-1 in mice was determined to be 10 µg; accumulation in most tissues remained largely unaltered compared to 40 µg ¹¹¹In-anti-F4/80-A3-1, yet the arthritis:blood ratio was increased (Figure S2). For instance, uptake of 10 or 40 µg ¹¹¹In-anti-F4/80-A3-1 in the blood equaled 1.4 ± 0.4 %ID/g or 2.3 ± 0.6 %ID/g, respectively. Notably, splenic uptake
was higher at 10 µg $^{111}$In-anti-F4/80-A3-1 (103.9 ± 6.4 %ID/g) than at 40 µg $^{111}$In-anti-F4/80-A3-1 (34.6 ± 4.4 %ID/g). The arthritis:blood ratios were higher for 10 µg $^{111}$In-anti-F4/80-A3-1 (10.1 ± 5.2) compared to 40 µg $^{111}$In-anti-F4/80-A3-1 (4.6 ± 3.2) and both values were higher than for $^{111}$In-IgG2b, which equaled 0.3 ± 0.2 and 0.4 ± 0.3 at 10 and 40 µg, respectively (Figure S2C).

**Etanercept Therapy**

Treatment with etanercept was successful as macroscopic scores of arthritis were lower in treated mice than in untreated mice (Figure S3). At day 6 after start of therapy, average arthritis scores were 4.0 ± 1.7 in untreated mice and 1.8 ± 1.1 in treated mice, as measured on a scale of 0-8 per mouse (i.e. 0-2 per paw).

**Biodistribution Studies**

Biodistribution studies after dissection showed that uptake of $^{111}$In-28H1 (Spearman r 0.4069; p<0.001), $^{111}$In-anti-F4/80-A3-1 (Spearman r 0.7542; p<0.0001), and $^{111}$In-RGD2 (Spearman r 0.6527; p<0.01) was decreased in arthritic joints in mice treated with etanercept and that tracer uptake correlated with arthritic score (Figure 1). For instance, joint uptake of $^{111}$In-28H1 in treated and untreated mice averaged 11 ± 11 %ID/g (CI: 7.0-15.1) and 23 ± 15 %ID/g (CI: 17.6-29.1), respectively (p<0.001). Joint uptake of $^{111}$In-anti-F4/80-A3-1 decreased from 8 ± 4 %ID/g (CI: 6.8-9.3) to 4 ± 4 %ID/g (CI: 2.5-4.9) after treatment (p<0.001 for both tracers between treated and untreated) as did $^{111}$In-RGD2 uptake (from 2 ± 1 %ID/g (CI: 1.5-2.8) to 1 ± 0.2 %ID/g (CI: 0.8-1.2)) (p=0.0078). Uptake of all three tracers when normalized to blood (arthritis:blood values), was higher at each arthritic score (0.25-2) for $^{111}$In-28H1, $^{111}$In-anti-F4/80-A3-1, and $^{111}$In-RGD2 than for controls $^{111}$In-DP47GS, $^{111}$In-anti-ratIgG2b, and $^{111}$In-RGD2 plus unlabeled excess (Figures 1B, D, F). P values at arthritis score 2 (per joint) were 0.002 comparing $^{111}$In-28H1 and $^{111}$In-anti-F4/80-A3-1 to their controls.
In untreated mice, blood levels were higher, whereas femur uptake values were lower, for $^{111}$In-DP47GS compared to $^{111}$In-28H1 (Figure S4A). Etanercept therapy resulted in lower blood values and higher liver values for $^{111}$In-28H1 and $^{111}$In-DP47GS compared to untreated mice (Figure S4A), most likely due to a mouse-anti-human IgG response. Whole body biodistribution varied between $^{111}$In-anti-F4/80-A3-1 and its isotype-matched control $^{111}$In-ratIgG2b with lower blood circulating levels and higher uptake in the spleen, liver and femur compared to blood values for $^{111}$In-anti-F4/80-A3-1 (Figure S4B). Etanercept treatment had no effect on general biodistribution of $^{111}$In-anti-F4/80-A3-1, $^{111}$In-ratIgG2b, $^{111}$In-RGD2 or $^{111}$In-RGD2 plus excess unlabeled (Figures S4B, C).

Table 1 shows a summary of the arthritis:blood values obtained in biodistribution studies.

Quantitative SPECT Analysis

Arthritic joints were specifically visualized with $^{111}$In-28H1 (Figures 2, S5), $^{111}$In-anti-F4/80-A3-1 (Figures 3 and S6), and $^{111}$In-RGD2 (Figures 4, S7). Quantitative SPECT analysis showed that tracer uptake within joints significantly decreased in treated animals compared to untreated controls (Figures 2-4). For instance, joint uptake equaled 2.0 ± 0.6 %ID, 0.6 ± 0.2 %ID and 0.2 ± 0.1 %ID in untreated animals for $^{111}$In-28H1, $^{111}$In-anti-F4/80-A3-1, and $^{111}$In-RGD2, respectively. These values are higher than in treated animals (0.7 ± 0.8 %ID, 0.3 ± 0.2 %ID, and 0.08 ± 0.02 %ID). Uptake of $^{111}$In-28H1 in arthritic, untreated joints was far higher (2.0 ± 0.6 %ID) than uptake of $^{111}$In-DP47GS (0.8 ± 0.4 %ID) and joint uptake of $^{111}$In-anti-ratIgG2b was not significantly decreased in untreated versus treated mice (0.7 ± 0.4 %ID and 0.5 ± 0.4 %ID, respectively).

Values obtained for $^{111}$In-28H1, $^{111}$In-anti-F4/80-A3-1, and $^{111}$In-RGD2 by quantitative SPECT analysis correlated well with macroscopic arthritis scores (Figure S8).

DISCUSSION

The data acquired in the dosing study is consistent with the idea of the spleen and liver acting as an antigen sink for $^{111}$In-anti-F4/80-A3-1 due to the presence of a large number of...
macrophages (13). Due to its short circulation time, studies involving $^{111}$In-anti-F4/80-A3-1 were completed at 24 hours post injection.

Etanercept, the drug of choice in these studies, is a soluble fusion protein consisting of human p75 TNF-α receptor and the Fc portion of human IgG (19). It is a biological DMARD that inhibits binding of inflammatory cytokine TNF-α to its receptor and is often given to arthritis patients if other treatments are contra-indicated or withdrawn due to adverse events.

Here, we show that the effect of etanercept on macroscopic/clinical arthritis score can not only be visualized non-invasively using imaging tracers that target either FAP, macrophages or integrins, but can also be measured quantitatively in either ex vivo biodistribution studies or quantitative SPECT. In previous studies, the authors did not see accumulation of any of the tracers in the joints of non-arthritic mice (8, 12, 13, 18). The three tracers are very different with regard to their targets, mechanism and pharmacokinetics; they all measure something different, so a straight comparison was not performed here.

The uptake of $^{111}$In-28H1, $^{111}$In-anti-F4/80-A3-1, and $^{111}$In-RGD$_2$ is antigen/receptor-mediated as joint:blood values decreased for antibody controls or for $^{111}$In-RGD$_2$ when competed with excess peptide (Figure 1). For all tracers, including the RGD peptide, the enhanced permeability and retention (EPR) effect did add to total tracer uptake as the nonspecific uptake of the tracer, displayed by total joint uptake values of $^{111}$In-DP47GS, $^{111}$In-ratIgG2b, and $^{111}$In-RGD$_2$ plus excess, was altered in treated mice (Figure 1).

Decreased antigen-mediated uptake of $^{111}$In-28H1 in arthritic joints of treated CIA mice could be explained by diminished expression of FAP at the site, which fits the notion of etanercept inducing apoptosis in activated fibroblast-like synoviocytes (20). The potential presence of anti-drug antibodies, as seen in arthritis cases for anti-TNF drugs, such as etanercept, (21, 22), human anti-murine antibodies (23), or anti-human murine antibodies (24), could explain the enhanced liver uptake of $^{111}$In-28H1 after etanercept treatment (Figure S4A). In turn this enhanced liver uptake might also explain the lower uptake in joints of treated mice.
Decreased uptake of $^{111}$In-anti-F4/80-A3-1 in arthritic joints after treatment may also be explained by lowered targeted availability as etanercept has been shown to induce apoptosis in macrophages resident in the synovium of arthritis patients 8 weeks as well as in mononuclear cells from synovial fluid in vitro (25). Similar targeted-mediated responses are also thought to be responsible for changes in $^{111}$In-RGD$_2$ uptake in arthritic joints after etanercept therapy by affected osteoclasts (26, 27) and vasculature (28). The latter option is possible, as endothelial cells have previously been targeted for use in imaging of inflammation (29).

It is important to note that quantitative SPECT values (%ID) and biodistribution data (%ID/g) were not directly comparable in these studies, as quantitative SPECT values were derived from the whole paw (toe/finger to above ankle/wrist) and biodistribution data were derived from the areas directly surrounding the ankle/wrist. Interestingly, total non-antigen-mediated uptake of $^{111}$In-ratIgG2b in the joint significantly decreased in treated compared to untreated CIA mice in biodistribution studies (Figure 1C), yet not in the quantitative SPECT studies (Figure 3C); likely due to a limited sensitivity of quantitative SPECT.

Due to the large variation in arthritis scores induced in the CIA mice and different responses to etanercept in individual mice, many animals had to be used per group. Despite this due the correlative figures show that these tracers can be used to objectively and quantitatively determine arthritis score in individual paws.

For a review on general tracers and radiolabeled biologicals used to image inflammatory processes see Put et al. (30). Previous studies have focused mostly on targeting macrophages, either with radiolabeled folate (31-33), (R)-$^{11}$C-PK11195 or translocator protein TSPO (34-36), which has also been used clinically, radiolabeled nanobodies (37), or by targeting CD163 receptors (38), or mannose receptors (39). Unfortunately, images acquired using radiolabeled folate led to poor quality images (31), most probably due to general low uptake of the tracers in the joints (32), a feature also seen with the anti-CRIg nanobody $^{99m}$Tc-NbV4m119 (37). So far, the only tool to image
F4/80 receptors specifically has been near infrared-labeled antibodies \((40)\), making our radiolabeled anti-F4/80-A3-1 antibody a unique nuclear tool.

Other, more generalized ways of imaging arthritis include \(^{18}\text{F}\)-FLT \((41)\), which showed high background levels due to uptake in bone marrow, and \(^{18}\text{F}\)-FDG. Despite the fact that \(^{18}\text{F}\)-FDG was suggested as a good tool to develop the therapeutic effect of novel therapies of inflammatory arthritis with \((42)\), in a preclinical model Laverman et al. showed that \(^{18}\text{F}\)-FDG was inferior to other nuclear imaging tracers when correlating uptake of \(^{18}\text{F}\)-FDG with arthritis score \((8)\).

Monitoring response to therapy in arthritis is usually carried out by clinical macroscopic scoring, ultrasonography, conventional radiography or MRI. Not only does this highlight the originality of the studies carried out here, by targeting integrins, macrophages or FAP in preclinical models of arthritis, but also the novelty of assessing these tracers as tools to monitor therapy response. Radionuclide imaging targeting specific molecular mechanisms could therefore be a great tool alongside the standard techniques, although cost:benefit analysis will need to be performed to determine whether any potential risk from radiation dose outweighs the benefits of early and quick therapy response monitoring.

CONCLUSION

In conclusion, \(^{111}\text{In}\)-28H1, \(^{111}\text{In}\)-anti-F4/80-A3-1, and \(^{111}\text{In}\)-RGD2 can be used to specifically monitor therapy response in experimental arthritis at the molecular level. Further studies however still need to be carried out to not only confirm that altered target expression is the cause of the diminished uptake of all three tracers, but also to determine whether these tracers could be used for early diagnosis of arthritis as well as whether they can be used to monitor response to other types of therapies.
ACKNOWLEDGMENTS

The authors would also like to thank Tessa van der Geest, Danny Gerrits, Janneke Molkenboer-Kuenen for help with the biodistribution studies.
REFERENCES


DISCLOSURE

Financial support: S. Terry was funded by the Roche Postdoc Fellowship (RPF) Program.

Tapan K. Nayak, Anne Freimoser-Grundschober, and Christian Klein are all employed by Roche, who provided the 28H1 and DP47GS antibodies.
FIGURE LEGENDS

FIGURE 1

Joint uptake of tracers in untreated or etanercept-treated CIA mice at 48 h ($^{111}$In-28H1, $^{111}$In-DP47GS; A, B), 24 h ($^{111}$In-anti-F4/80, $^{111}$In-ratIgG2b; C, D) and 1 h p.i. ($^{111}$In-RGD2 with/without excess unlabeled; E, F). Data are average ± standard deviation (n=7-10 mice/group (A-D) or 2 mice/group (E-F), 4 joints per mouse (A, C, E)) or individual joints (n=14-20 mice/group, 4 joints per mouse; B, D, F). *(p≤0.05), **(p≤0.01), and ***(p≤0.001).
FIGURE 2

3D SPECT/CT scans of etanercept-treated CIA mice 48 h p.i. of $^{111}$In-28H1 (A) and $^{111}$In-DP47GS (B). All images are scaled equally. Panel C represents the quantitative analysis of the SPECT images. Data are average ± standard deviation (n=3 mice/group, 4 joints/mouse). **(p≤0.01).
FIGURE 3

3D SPECT/CT scans of etanercept-treated CIA mice 24 h p.i. of $^{111}$In-anti-F4/80 (A) and $^{111}$In-ratIgG2b (B). All images are scaled equally. Panel C represents the quantitative analysis of the SPECT images.

Data are average ± standard deviation (n=3 mice/group, 4 joints/mouse). ***($p\leq0.001$).
FIGURE 4

3D SPECT/CT scans of untreated CIA mice 1 h p.i. of $^{111}$In-RGD$_2$ (A). Panel B represents the quantitative analysis of the SPECT images of joint uptake after injection of $^{111}$In-RGD$_2$ in untreated or treated CIA mice. Data are average ± standard deviation (n=1-2 mice/group, 4 joints/mouse).
### TABLE 1

Mean and 95% coincidence intervals (CI) of arthritis:blood uptake ratios from Figures 1B, D, F to provide. Where no CIs are shown, data is from n=1. All values over 25 were excluded.

<table>
<thead>
<tr>
<th>Arthritis score</th>
<th>In-111-28H1</th>
<th>In-111-DP47GS</th>
<th>In-111-anti-F4/80-A3-1</th>
<th>In-111-anti-ratIgG2b</th>
<th>In-111-RGD2</th>
<th>cold excess RGD2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5.0</td>
<td>0.3</td>
<td>1.8</td>
<td>0.2</td>
<td>3.5</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>(2.7-7.3)</td>
<td>(0.3-0.4)</td>
<td>(0.6-2.9)</td>
<td>(0.2-0.2)</td>
<td>(2.9-4.1)</td>
<td>(1.2-2.8)</td>
</tr>
<tr>
<td>0.25</td>
<td>4.8</td>
<td>0.5</td>
<td>1.7</td>
<td>0.2</td>
<td>3.9</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>(1.8-7.7)</td>
<td>(0.1-0.9)</td>
<td>(0.9-2.6)</td>
<td>(0.1-0.3)</td>
<td>(0.1-7.7)</td>
<td>(-3.2-7.3)</td>
</tr>
<tr>
<td>0.5</td>
<td>8.9</td>
<td>5.8</td>
<td>1.6</td>
<td>no data</td>
<td>no data</td>
<td>no data</td>
</tr>
<tr>
<td></td>
<td>(-65.7-77.3)</td>
<td>(-0.2-1.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.75</td>
<td>1.2</td>
<td>0.4</td>
<td>no data</td>
<td>no data</td>
<td>no data</td>
<td>no data</td>
</tr>
<tr>
<td></td>
<td>(-0.2-1.0)</td>
<td>(-0.2-1.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>no data</td>
<td>0.6</td>
<td>6.6</td>
<td>no data</td>
<td>no data</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(-29.4-42.5)</td>
<td>(1.4-129.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.25</td>
<td>11.8</td>
<td>0.3</td>
<td>7.1</td>
<td>0.4</td>
<td>no data</td>
<td>no data</td>
</tr>
<tr>
<td></td>
<td>(-2.9-26.6)</td>
<td>(-0.2-0.5)</td>
<td>(3.2-11.1)</td>
<td>(0.1-0.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5</td>
<td>8.3</td>
<td>2.2</td>
<td>6.4</td>
<td>0.5</td>
<td>8.2</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>(1.9-14.7)</td>
<td>(-1.7-6.0)</td>
<td>(5.3-7.5)</td>
<td>(0.4-0.7)</td>
<td>(1.8-5.8)</td>
<td></td>
</tr>
<tr>
<td>1.75</td>
<td>8.4</td>
<td>0.6</td>
<td>7.6</td>
<td>0.5</td>
<td>8.1</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>(5.5-11.2)</td>
<td>(0.5-0.8)</td>
<td>(6.4-8.9)</td>
<td>(0.4-0.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5.5</td>
<td>0.7</td>
<td>10.4</td>
<td>0.5</td>
<td>7.2</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>(4.7-6.4)</td>
<td>(0.3-1.2)</td>
<td>(7.9-13.0)</td>
<td>(0.4-0.6)</td>
<td>(1.8-5.8)</td>
<td></td>
</tr>
</tbody>
</table>