EXTENDED REPORT

Scintigraphic detection of tumour necrosis factor in patients with rheumatoid arthritis

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Objectives: To investigate the biodistribution and specific targeting for tumour necrosis factor (TNF) of a fully human, radiolabelled anti-TNF monoclonal antibody (anti-TNF mAb) in patients with active rheumatoid arthritis (RA). To assess whether this agent is suitable for visualisation of synovitis.

Methods: Ten patients with RA underwent whole body scintigraphy after administration of a tracer—subtherapeutic dose of 100 µg ^{99m}Tc human anti-TNF mAb. After two weeks, the procedure was repeated to assess the specificity of the radiolabelled antibody for TNF and its sensitivity for changes in inflammation. Therefore, a competition study was performed in five patients, who received excess unlabelled anti-TNF mAb before the tracer dose of ^{99m}Tc-anti-TNF. Another five patients received 120 mg methylprednisolone two days before the second scintigraphy.

Results: Radiolabelled anti-TNF mAb allowed clear visualisation of inflamed joints in patients with active RA with a high specificity. Concomitant administration of excess unlabelled anti-TNF reduced the joint uptake of ^{99m}Tc-anti-TNF mAb by a median of 25% as a percentage of the injected dose after 24 hours, whereas uptake in liver and spleen remained unchanged. Systemic corticosteroids reduced the disease activity, which was mirrored by a decreased joint uptake of the tracer. The anti-TNF mAb retained its high affinity for TNFα after labelling and was cleared from the circulation with an elimination half life of 48 hours. The procedure was well tolerated.

Conclusions: Radiolabelled human anti-TNF mAb allows visualisation of synovitis in patients with RA. Joint accumulation of this agent is partly due to specific TNF targeting and is highly predictive for inflammation.

Rheumatoid arthritis (RA) is characterised by a chronic and destructive polyarthritis. The role of proinflammatory cytokines such as tumour necrosis factor alpha (TNFα) in the pathogenesis of the disease is nowadays beyond question. Treatments aimed at neutralising TNF now used in clinical practice encompass chimeric monoclonal antibodies (mAbs) such as infliximab and TNF receptor-fusion proteins such as etanercept.

TNFα is produced as a soluble mediator and as a membrane bound molecule, and immunohistological studies in RA have shown that this cytokine and its receptors are present in inflamed synovial tissue.[1][2] Nevertheless, there is a large inter- and intraindividual variability in the level of TNF expression,[3][4] with the latter possibly having biological consequences. For instance, it is well known that a subset of patients with RA do not respond to TNF neutralisation. This might be due to lower levels of TNF expression or to a major involvement of other proinflammatory cytokines in the disease, or both. Accurate information on TNF expression in the joints might therefore be helpful to optimise and/or monitor the effect of TNF antagonism. Because RA is a polyarticular disease, immunohistological assessments cannot routinely be used to assess the degree and extent of TNF expression. Imaging studies aimed at targeting and visualising TNF in vivo would be an attractive alternative to other more invasive techniques.

Recent animal studies have shown that certain cytokines such as interleukin 1 and interleukin 8 specifically target their receptors in inflammatory foci and can be used for imaging studies.[5][6] However, their use in humans is hampered by adverse events. In contrast, radiolabelled mAbs and cytokine antagonists can be used for specific targeting of membrane markers such as adhesion molecules[7][8] or tumour antigens.[9] Such agents can provide accurate information about the disease activity and localisation and some of them are nowa-days used for imaging and for therapeutic purposes. To date, no imaging studies with TNF antagonists have been performed in humans.

Adalimumab (Humira, Abbott Laboratories) is a fully human, IgG1, anti-TNF mAb developed using phage display technologies. This compound has a high specificity and affinity for TNF (Kd 6×10^{-10} M) and specificity for TNF.[10][11] Phase I to III studies have shown that repeated administration of this agent results in rapid and sustained clinical improvement and slows radiological progression in patients with RA.[12–15]

In this report we assess the scintigraphic imaging characteristics and the tissue biodistribution of systemically administered ^{99m}Tc-anti-TNF mAb in patients with active RA. Additionally, we assess whether imaging with this agent is sensitive to changes in disease activity and whether it targets TNF specifically.

PATIENTS AND METHODS

Patients

Enrolled patients had RA according to the American College of Rheumatology criteria,[16] were aged 18 years or older, had active disease defined by a disease activity score (DAS[17]) >3.2, and had given informed consent. All patients received concomitant treatment with non-steroidal anti-inflammatory drugs and antirheumatic drugs at doses that were kept constant for at least four weeks before and during the study. Exclusion criteria were lactation or pregnancy, systemic or intra-articular corticosteroid administration within three

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Abbreviations: DAS, disease activity score; HPLC, high performance liquid chromatography; HYNIC, hydrazinicotinamide; ID, injected dose; mAb, monoclonal antibody; RA, rheumatoid arthritis; TNFα, tumour necrosis factor α

months before the study, major surgery or infection in the past four weeks, history of tuberculosis, white cell counts $<3.5\times10^9/l$, concomitant serious diseases, and previous treatment with TNF blockers or any other biological agent in the past.

Study protocol

Each patient underwent two scintigraphic sessions separated by an interval of two weeks. Clinical and laboratory assessments of disease activity were obtained immediately before, one day and two weeks after each scintigraphy. Just before imaging, patients received a single intravenous subtherapeutic dose of 100 µg (740 MBq) ^{99m}Tc labelled human anti-TNF mAb. The purpose of the first scintigraphy was to assess the biodistribution of anti-TNF in vivo. The purpose of the second scintigraphy was to assess (*a*) the specificity for TNF targeting by means of an excess anti-TNF mAb competition study and (*b*) the sensitivity to reflect decreased inflammation after administration of systemic corticosteroids. For the competition study, a subset of five patients received a therapeutic dose (10 mg/kg) of unlabelled human anti-TNF mAb immediately before the tracer dose of 100 µg ^{99m}Tc labelled anti-TNF. Another patient subset was treated with a single intramuscular dose of methylprednisolone 120 mg two days before the second scintigraphy. The study was approved by the institutional review board of the University Medical Centre, Nijmegen.

Imaging procedures

Whole body images and spot views of inflamed joints were obtained with a dual head gammacamera (MultiSPECT2, Siemens, Hoffmann Estates, IL) connected to a Scintiview image processor and ICON computer system at 5 minutes, 4 and 24 hours after injection. The scintigraphic images were collected in digital format (1024×256 and 256×256 matrix for whole body and spot views, respectively) and scored for joint uptake (0=no uptake, 1=uptake) without knowledge of the clinical scores. Analysis was performed by calculation of whole body clearance and ratios of uptake in regions of interest drawn over the positive joints and periarticular normal tissue and other organs showing uptake, normalised for the area of the region. Uptake data are expressed as a percentage of the injected dose (%ID).

Blood clearance

Radioactivity in blood samples, obtained at 1 minute, 1, 2, 4, and 24 hours after injection, was measured in the gamma-counter and expressed as percentage of the injected dose per gram of blood (%ID/g) after correction for radioactive decay.

Radiolabelling and characterisation of the anti-TNF mAb

The human anti-TNF mAb was labelled using the indirect approach described by Abrams *et al*.[18] Briefly, succinimyl-hydrazinicotinamide (S-HYNIC), a bifunctional chelator, was conjugated to the anti-TNF antibody at a 1:3 molar ratio in 0.1 M $NaHCO_3$, pH 8.2 during 30 minutes at room temperature. Unconjugated HYNIC was removed by extensive dialysis against sterile acetate buffer, pH 5.0. Subsequently, 100 µg aliquots of mAb-HYNIC conjugated antibody were stored at $-20°C$ until use. The anti-TNF mAb-HYNIC conjugate was radiolabelled with ^{99m}Tc just before each imaging session by mixing an aliquot of 100 µg conjugate with 10 µg $SnSO_4$, 10 mg tricine, and 750 MBq (20 mCi) TcO_4^- and incubation for 15 minutes at room temperature. Labelling efficiency was determined by instant thin layer chromatography on silica gel strips. The stability of the radiolabelled antibody was tested by high performance liquid chromatography (HPLC; Phenomenex 2000 size exclusion column, 300×4 mm) and by transchelation assays,[19] in which the ^{99m}Tc-anti-TNF mAb was

Table 1 Characteristics of the patients studied. Data are given as mean (SD) unless indicated otherwise

Characteristic	Human anti-TNFα mAb (n = 5)	Steroids (n = 5)
Age (years)	57 (5)	56 (3)
Female sex (n)	3	3
Rheumatoid factor positive (n)	4	4
Shared epitope positive (n)	5	5
Disease activity score	5.17 (0.630)	5.63 (0.40)
No of swollen joints (44 joint score)	21 (6.30)	20 (5.5)
Ritchie score, median (IQR) (53 joint score)	20 (19 –22)	24 (22 – 27)
ESR (mm/1st h)	45 (24)	41 (20)
Haemoglobin (mmol/l)	7.4 (0.96)	7.5 (0.65)
Platelet count (×10⁹/l)	471 (164)	307 (98)
HAQ	1.195 (0.31)	1.369 (0.79)

incubated for four hours at 37°C in the presence of increasing concentrations of glutathione and cysteine. The choice of ^{99m}Tc was based on its ideal physical characteristics (140 keV γ emission, $t_\frac{1}{2}$ six hours) and on its wide use for antibody mediated imaging.[9 21 21]

RESULTS

### ^{99m}Tc-anti-TNF mAb allows visualisation of affected joints in patients with RA

Ten patients with active RA were enrolled in this study and all underwent a first imaging with a tracer dose of 100 µg ^{99m}Tc-anti-TNF mAb alone. Thereafter, patients were subdivided into two groups—the first examined in a competition study and the second in repeated scintigraphy after steroid administration. Table 1 shows that the patients' characteristics in both groups were similar.

Figure 1 shows the biodistribution of ^{99m}Tc-anti-TNF mAb illustrated by the anterior whole body images. In all 10 patients ^{99m}Tc-anti-TNF mAb localised in inflamed joints within minutes after injection. Joints were clearly visualised at 4 and 24 hours after injection and the median percentage increase in uptake between these two times was 30%. The uptake of ^{99m}Tc-anti-TNF mAb in inflamed joints (fig 1, hand joints detail) was at least two- to threefold higher than in periarticular normal tissue and reached peak values of around fourfold. Uptake of radiolabelled anti-TNF mAb was also noticed in the liver and spleen (mean (SD) 13.8 (4.7) %ID and 2.1 (1.2) %ID, respectively). The ^{99m}Tc labelled anti-TNF mAb cleared from blood with a median elimination $t\frac{1}{2}\alpha$ of 48 hours.

No uptake of ^{99m}Tc-anti-TNF mAb was seen in clinically unaffected joints of any of the patients examined. Conversely, not all clinically affected joints showed ^{99m}Tc-anti-TNF mAb uptake and this was especially true for the small joints in the hands and feet. Systemic administration of ^{99m}Tc anti-TNF mAb was well tolerated and no adverse events occurred. As expected, the tracer subtherapeutic dose of ^{99m}Tc labelled anti-TNF mAb used in the first imaging sessions did not yield significant changes in disease activity parameters. This was reflected by similar DAS values at baseline and before the second scintigraphy in the anti-TNF group (fig 2).

TNF mediated uptake of anti-TNF mAb in RA

Two weeks after the first scintigraphic procedure a subset of five patients underwent a competition study to assess to what extent the uptake and retention of ^{99m}Tc labelled anti-TNF mAb in inflamed joints was mediated by specific binding to TNF. To this aim, an excess of unlabelled anti-TNF mAb (at a therapeutic dose of 10 mg/kg) was given immediately before the second injection of a tracer dose of 100 µg ^{99m}Tc-anti-TNF mAb.
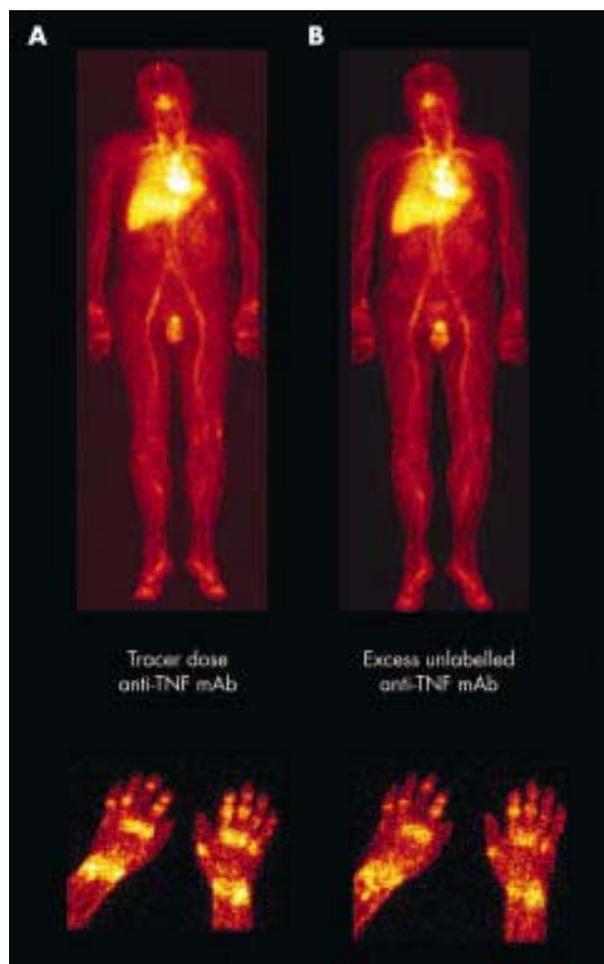
Figure 1 Scintigraphic images of the whole body and hands of a patient with active RA after injection of ^{99m}Tc human anti-TNF mAb. (A) The first imaging session. (B) Decreased uptake in joints after administration of an excess unlabelled anti-TNF mAb (hands detail), whereas the uptake in the reticuloendothelial organs remains unchanged (whole body).

As shown in fig 1B, the joint uptake of radiolabelled anti-TNF decreased after excess of unlabelled anti-TNF. Quantitative analysis of the uptake in positive joints showed a decrease by a median of 16% and 25% (expressed as %ID) at 4 and 24 hours after injection, respectively, whereas the pharmacokinetics and the uptake in liver and spleen did not change when the label was given with a saturating dose of anti-TNF (data not shown). This rapid decrease in joint uptake could not be explained by changes in disease activity or in pharmacokinetics: the DAS (fig 2) and the number of swollen joints (median 20 and 19 before and 1 day after the second scintigraphy, respectively) remained unchanged within this period and the elimination t½α was similar in both imaging sessions.

Scintigraphy with ^{99m}Tc-anti-TNF mAb is sensitive to changes in disease activity
To assess whether imaging with ^{99m}Tc-anti-TNF mAb could detect clinically significant changes in disease activity, a single dose of systemic corticosteroids was given to another subset of five patients two days before the second scintigraphy. As expected in this group, a decrease in DAS (fig 2) and its individual components was seen at the time of the second scintigraphy. This was also reflected by a lower uptake of ^{99m}Tc-anti-TNF mAb in inflamed joints (median decrease in %ID 25% and 8% at 4 and 24 hours after the second imaging respectively).
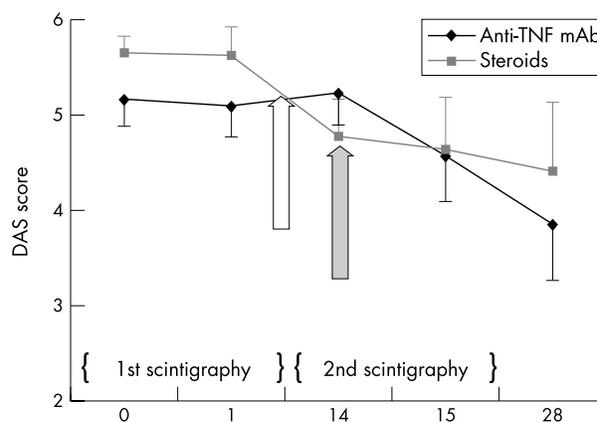
Figure 2 Course of the DAS during the study. Patients with active RA underwent a first scintigraphy with a tracer dose of a fully human anti-TNF mAb radiolabelled with ^{99m}Tc. Two weeks thereafter, a subset of patients (n=5, rhomboids) received a therapeutic dose of the same antibody (grey arrow) before the second imaging session to assess the TNF targeting in the joints. Another patient subset (n=5, squares) was treated with systemic steroids two days before the second scintigraphy (white arrow).

The uptake in liver and spleen remained unchanged after corticosteroid administration.

Comparison of disease activity parameters seen two weeks after the second scintigraphy with those at study baseline in the whole group of 10 patients showed significant decreases in swollen joint counts (p=0.002), DAS, and erythrocyte sedimentation rate (p<0.02). Although the samples were too small for statistical comparisons, the clinical response to a single therapeutic dose of anti-TNF mAb and to systemic steroids was roughly similar at study termination (fig 2). The efficacy of the procedures, assessed by the patients on a visual analogue scale, was also similar (median of 67 and 64 for the anti-TNF and steroid group, respectively).

Radiolabelling and characterisation of the ^{99m}Tc human anti-TNF mAb
The radiochemical purity of each batch of ^{99m}Tc-labelled anti-TNF mAb, determined by thin layer chromatography, was always higher than 95%. The radiolabelled antibody was highly stable in serum. HPLC analysis showed only a minimal release (<3%) of the radiolabel after 24 hours' incubation at 37°C. In the transchelation assay the ^{99m}Tc label remained associated with the anti-TNF mAb and a 10 000-fold molar excess of glutathione was needed to detect some evidence of ^{99m}Tc release.

DISCUSSION
In this study we show that radioimmunoscintigraphy with a ^{99m}Tc labelled, human anti-TNF mAb is feasible, can be used to image affected joints in patients with active RA, and is highly predictive for inflammation. Additionally, this procedure can detect clinical relevant changes in disease activity as reflected by the DAS.

Such results were not totally unexpected because adalimumab, the anti-TNF antibody used in this study, is an IgG1 molecule. Non-specific IgG labelled with the radioisotope ^{99m}Tc has been used now for years to image inflammation.[20][21] The localisation of the latter to inflammatory foci is mainly due to non-specific mechanisms such as increased vascular permeability.[21][22] None the less, the selectivity of IgG and other radiopharmaceutical agents can be increased by targeting them at certain molecules.[23] The choice to target TNF in arthritis is attractive because the safety and efficacy of the human antibody used in our study and of other TNF blocking agents is now widely accepted.

In this study we investigated whether the localisation of ^{99m}Tc labelled anti-TNF mAb in inflammatory foci might be due to interaction with TNF present in the target tissue. Our results show that the uptake and retention of this radiolabelled agent in arthritis is to a large extent due to targeting TNF in the joint. This is supported by the fact that the uptake of ^{99m}Tc labelled anti-TNF in inflamed joints, expressed as %ID, was reduced by 25% when excess unlabelled anti-TNF was coadministered, whereas the uptake in the reticuloendothelial organs, such as liver and spleen, and the pharmacokinetics remained unchanged. Furthermore, the imaging could detect changes in inflammation because the joint uptake decreased markedly after administration of systemic corticosteroids, reflecting a decrease in disease activity. We also show that imaging with ^{99m}Tc human anti-TNF mAb is highly predictive for inflammation because, in all patients studied, uptake of this agent occurred exclusively in inflamed joints. The reason why not all clinically affected joints showed uptake of radiolabelled anti-TNF remains speculative but might be explained by the absence of TNF because this cytokine is not always seen in inflamed synovial tissue.[3] [4] [24]

Taken together, our results show that anti-TNF mAb does target TNF in inflamed joints of patients with RA. It seems very likely that this is also the case in other inflammatory conditions. The use of this agent as radiopharmaceutical agent to monitor inflammation may therefore offer advantages over non-specific IgG, especially in diseases where TNF is involved, including RA, juvenile chronic arthritis, seronegative spondyloarthropathies, and Crohn's disease.

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REFERENCES

1 **Deleuran BW**, Chu CQ, Field M, Brennan FM, Mitchell T, Feldmann M, et al. Localization of tumor necrosis factor receptors in the synovial tissue and cartilage-pannus junction in patients with rheumatoid arthritis. Implications for local actions of tumor necrosis factor alpha. Arthritis Rheum 1992;35:1170–8.
2 **Chu CQ**, Field M, Feldmann M, Maini RN. Localization of tumor necrosis factor alpha in synovial tissues and at the cartilage-pannus junction in patients with rheumatoid arthritis. Arthritis Rheum 1991;34:1125–32.
3 **Barrera P**, Joosten LA, den Broeder AA, van de Putte LB, van Riel PL, van den Berg WB. Effects of treatment with a fully human anti-tumour necrosis factor alpha monoclonal antibody on the local and systemic homeostasis of interleukin 1 and TNFα in patients with rheumatoid arthritis. Ann Rheum Dis 2001;60:660–9.
4 **Ulfgren AK**, Grondal L, Lindblad S, Khademi M, Johnell O, Klareskog L, et al. Interindividual and intra-articular variation of proinflammatory cytokines in patients with rheumatoid arthritis: potential implications for treatment. Ann Rheum Dis 2000;59:439–47.
5 **van der Laken CJ**, Boerman OC, Oyen WJ, van de Ven MT, van der Meer JW, Corstens FH. Imaging of infection in rabbits with radioiodinated interleukin-1 (alpha and beta), its receptor antagonist and a chemotactic peptide: a comparative study. Eur J Nucl Med 1998;25:347–52.
6 **van der Laken CJ**, Boerman OC, Oyen WJ, van de Ven MT, Ven der Meer JW, Corstens FH. The kinetics of radiolabelled interleukin-8 in infection and sterile inflammation. Nucl Med Commun 1998;19:271–81.
7 **Chapman PT**, Jamar F, Keelan ET, Peters AM, Haskard DO. Use of a radiolabeled monoclonal antibody against E-selectin for imaging of endothelial activation in rheumatoid arthritis. Arthritis Rheum 1996;39:1371–5.
8 **Jamar F**, Chapman PT, Manicourt DH, Glass DM, Haskard DO, Peters AM. A comparison between 111In-anti-E-selectin mAb and 99mTc-labelled human non-specific immunoglobulin in radionuclide imaging of rheumatoid arthritis. Br J Radiol 1997;70:473–81.
9 **Steffens MG**, Oosterwijk E, Kranenborg MH, Manders JM, Debruyne FM, Corstens FH, et al. In vivo and in vitro characterizations of three 99mTc-labeled monoclonal antibody G250 preparations. J Nucl Med 1999;40:829–36.
10 **Salfeld J**, Kaymakcalan Z, Tracey D, Roberts A, Kamen R. Generation of fully human anti-TNF antibody D2E7 [abstract]. Arthritis Rheum 1998;41(suppl):S57.
11 **Santora LC**, Krull IS, Grant K. Characterization of recombinant human monoclonal tissue necrosis factor-alpha antibody using cation-exchange HPLC and capillary isoelectric focusing. Anal Biochem 1999;275:98–108.
12 **Rau R**. Adalimumab (a fully human anti-tumour necrosis factor α monoclonal antibody) in the treatment of active rheumatoid arthritis: the initial results of five trials. Ann Rheum Dis 2002;61(suppl II):ii70–3.
13 **Kempeni J**. Update on D2E7: a fully human anti-tumour necrosis factor α monoclonal antibody. Ann Rheum Dis 2000;59(suppl I):i44–5.
14 **den Broeder AA**, Joosten LA, Saxne T, Heinegard D, Fenner H, Miltenburg AM, et al. Long term anti-tumour necrosis factor α monotherapy in rheumatoid arthritis: effect on radiological course and prognostic value of markers of cartilage turnover and endothelial activation. Ann Rheum Dis 2002;61:311–18.
15 **Keystone E**, Kavanaugh AF, Sharp J, Hua Y, Teoh L, Fischkoff S, et al. Adalimumab (D2E7), a fully human anti-TNF-α monoclonal antibody, inhibits the progression of structural joint damage in patients with active A despite concomitant methotrexate therapy [abstract]. Arthritis Rheum 2002;46(suppl):S205.
16 **Arnett FC**, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
17 **van der Heijde DM**, van 't Hof MA, van Riel PL, Theunisse LA, Lubberts EW, van Leeuwen MA, et al. Judging disease activity in clinical practice in rheumatoid arthritis: first step in the development of a disease activity score. Ann Rheum Dis 1990;49:916–20.
18 **Abrams MJ**, Juweid M, tenKate CI, Schwartz DA, Hauser MM, Gaul FE, et al. Technetium-99m-human polyclonal IgG radiolabeled via the hydrazino nicotinamide derivative for imaging focal sites of infection in rats. J Nucl Med 1990;31:2022–8.
19 **Hnatowich DJ**, Qu T, Chang F, Ley AC, Ladner RC, Rusckowski M. Labeling peptides with technetium-99m using a bifunctional chelator of a N-hydroxysuccinimide ester of mercaptoacetyltriglycine. J Nucl Med 1998;39:56–64.
20 **Dams ET**, Oyen WJ, Boerman OC, Claessens RA, Wymenga AB, van der Meer JW, et al. Technetium-99m labeled to human immunoglobulin G through the nicotinyl hydrazine derivative: a clinical study. J Nucl Med 1998;39:119–24.
21 **Buijs WC**, Oyen WJ, Dams ET, Boerman OC, Siegel JA, Claessens RA, et al. Dynamic distribution and dosimetric evaluation of human nonspecific immunoglobulin G labelled with In-111 or Tc-99m. Nucl Med Commun 1998;19:743–51.
22 **Oyen WJ**, Boerman OC, van der Laken CJ, Claessens RA, van der Meer JW, Corstens FH. The uptake mechanisms of inflammation- and infection-localizing agents. Eur J Nucl Med 1996;23:459–65.
23 **van der Laken CJ**, Boerman OC, Oyen WJ, van de Ven MT, van der Meer JW, Corstens FH. Scintigraphic detection of infection and inflammation: new developments with special emphasis on receptor interaction. Eur J Nucl Med 1998;25:535–46.
24 **Joosten LA**, Radstake TR, Lubberts E, van Lent PL, den Bersselaar LA, van Riel PL, et al. IL-18 expression in synovial biopsies of patients with active rheumatoid arthritis is associated with enhanced levels of both IL-1 and TNF [abstract]. Arthritis Rheum 2002;46(suppl):S550.