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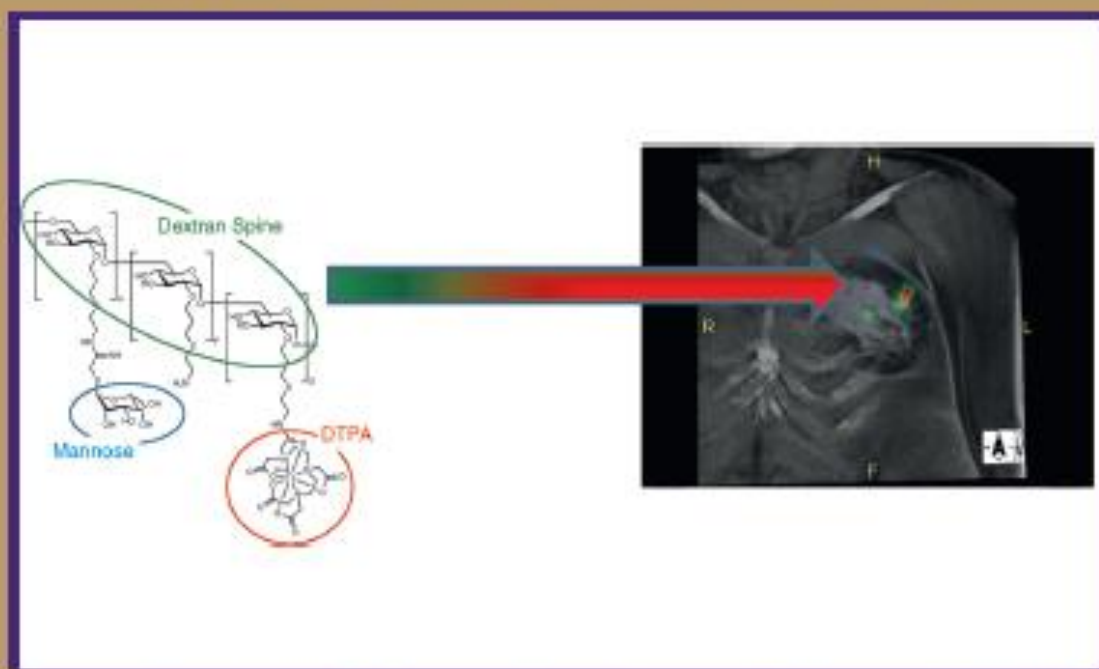
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The inextricable axis of targeted diagnostic imaging and therapy: An immunological natural history approach[☆]



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SUMMARY

In considering the challenges of approaches to clinical imaging, we are faced with choices that sometimes are impacted by rather dogmatic notions about what is a better or worse technology to achieve the most useful diagnostic image for the patient. For example, is PET or SPECT most useful in imaging any particular disease dissemination? The dictatorial approach would be to choose PET, all other matters being equal. But is such a totalitarian attitude toward imaging selection still valid? In the face of new receptor targeted SPECT agents one must consider the remarkable specificity and sensitivity of these agents. ^{99m}Tc-Tilmanocept is one of the newest of these agents, now approved for guiding sentinel node biopsy (SLNB) in several solid tumors. Tilmanocept has a K_d of 3×10^{-11} M, and its specificity for the CD206 receptor is unlike any other agent to date. This coupled with a number of facts, that specific disease-associated macrophages express this receptor (100 to 150 thousand receptors), that the receptor has multiple binding sites for tilmanocept (>2 sites per receptor) and that these receptors are recycled every 15 min to bind more tilmanocept (acting as intracellular “drug compilers” of tilmanocept into non-degraded vesicles), gives serious pause as to how we select our approaches to diagnostic imaging. Clinically, the size of SLNs varies greatly, some, anatomically, below the machine resolution of SPECT. Yet, with tilmanocept targeting, the SLNs are highly visible with macrophages stably accruing adequate ^{99m}Tc-tilmanocept counting statistics, as high target-to-background ratios can compensate for spatial resolution blurring. Importantly, it may be targeted imaging agents per se, again such as tilmanocept, which may significantly shrink any perceived chasm between the imaging technologies and anchor the diagnostic considerations in the targeting and specificity of the agent rather than any lingering dogma about the hardware as the basis for imaging approaches. Beyond the elements of imaging applications of these agents is their evolution to therapeutic agents as well, and even in the neo-logical realm of *theranostics*. Characteristics of agents such as tilmanocept that exploit the natural history of diseases with remarkably high specificity are the expectations for the future of patient- and disease-centered diagnosis and therapy.

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1. Diagnosis and imaging: setting up the therapeutic continuum

In the preceding 25 years there has evolved a confluence of tumor biology ideology, nuclear medicine and surgical oncology that has led

to the development of the theory of the “sentinel node” [1–5]. Data accrued over the intervening years have provided a confirmation of the sentinel node theory as it relates to the incorporation of sentinel lymph node detection/biopsy in breast cancer and melanoma patient outcome in surgical practice [6–14]. Sentinel node theory holds that there is a predictable anatomical relationship between the immediate tumor environment and the proximate lymphatic system such that assessment of this nexus can provide a reliable appraisal of the nodal disease stage and reduce or eliminate the need for expanded surgery as this relates to lymphadenectomy, and be equally predictive of nodal

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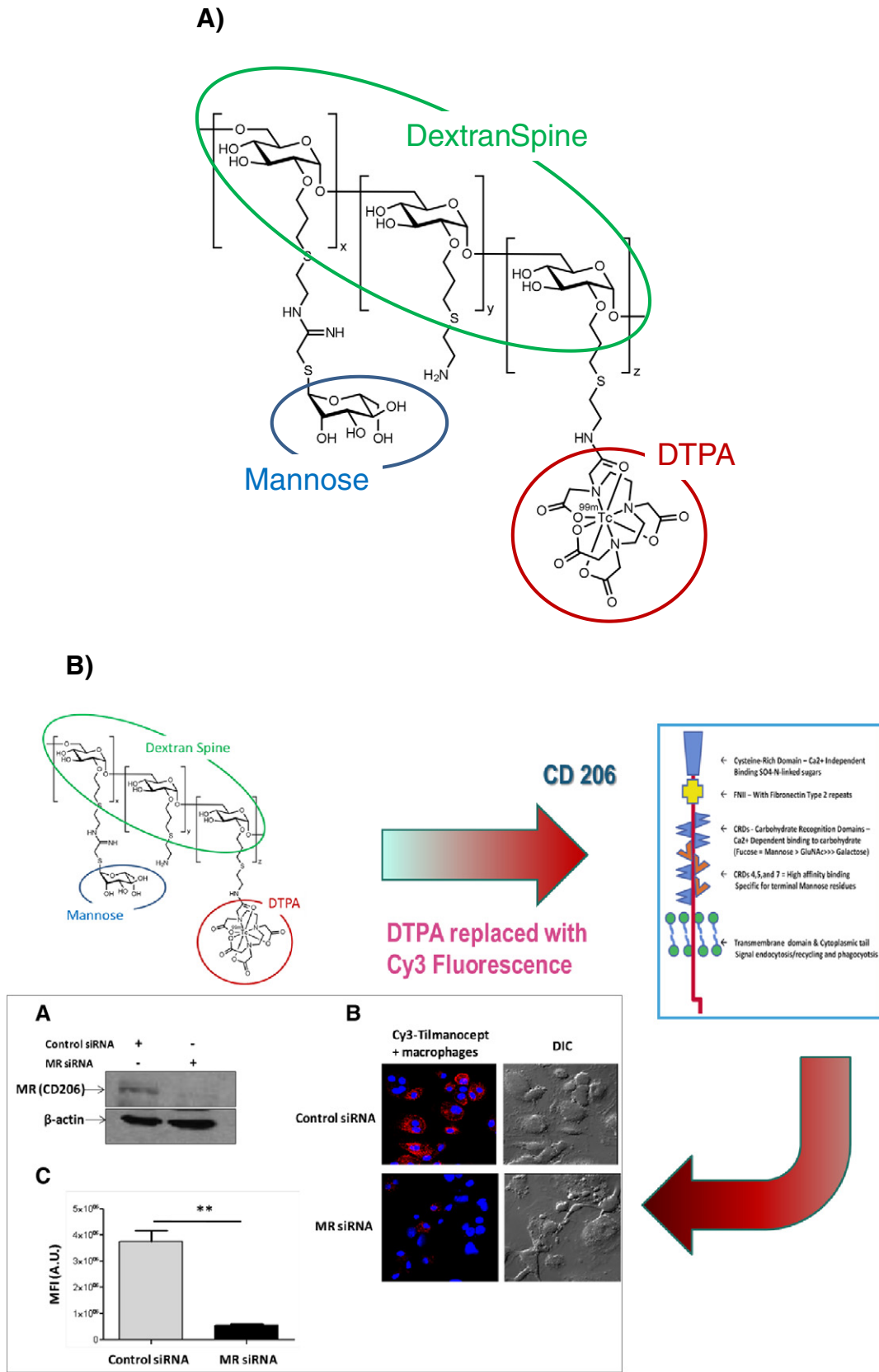


Fig. 1. A. ^{99m}Tc-tilmanocept (Lymphoseek®; Navidea). B. Tilmanocept specifically binds to CD206.

status with similar outcomes with regard to any such expatiated surgery [15–24]. The initial sentinel lymph node biopsy (SLNB) forays relied on the application of dyes injected into or around the tumor

area, with visual tracing of these dyes, or “chasing” the drainage of the dyes into the lymphatic ducts and nodes. The flow and adsorption of the dyes into proximal nodes (and in many cases distal nodes) were

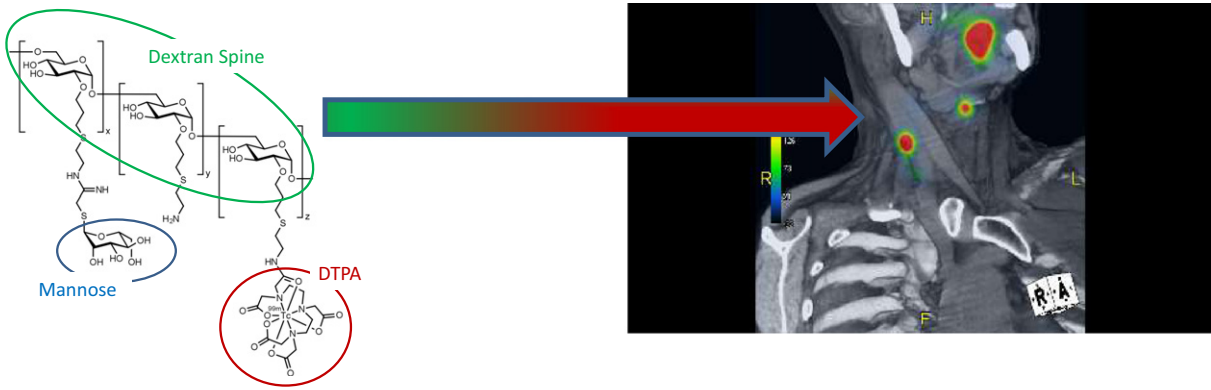


Fig. 2. Sentinel node discrimination in patient with oral squamous cell carcinoma of the tongue; multiple sentinel nodes are visible 15 min after injection.

implicative of a node’s anatomic or biological linkage to the tumor bed and increased potential for the residence of tumor cells whose derivation was from the primary tumor [25–31]. This procedure of SLNB was tested repeatedly in clinical studies and these studies provided validation of the concept and its positive impact on patient outcome [15–24].

However, other factors altered the clinical landscape of SLNB, in particular, the adoption of radiolabeled particulates used in other diagnostic procedures [32–39]. Although neither blue dyes nor particulate colloids provided any real specificity, the combined use of these two agents has improved SLNB detection reliability to singly employed dyes or colloids [40–42]. The results of this unlikely amalgam lead to their use in numerous clinical trials and the expanded adoption of the SLNB procedure, primarily for breast cancer followed by its use in melanoma surgery.

In the face of this seeming remedy for mitigating some unnecessarily extensive surgery, the adoption of SLNB in solid tumors other than melanoma and breast cancer seemed highly likely. However, out of the evolving SLNB clinical data sets for breast cancer and melanoma, and especially the experiential data of SLNB in other solid tumors failed to realize the efficacy found in breast and melanoma surgery, came the observation that there was a need for an SLN-discriminating agent in order to thoroughly potentiate the reliability and positive patient outcome of SLNB. These data strongly suggested that an SLN detection agent would have to provide true target specificity such that the tumor-node axis was more reliably mapped.

The agent that has risen to fill this void is ^{99m}Tc-tilmanocept (Lymphoseek®, Navidea Biopharmaceuticals, Inc.) (Fig. 1A and B). Tilmanocept functions through a mechanism of action that relies on its mannose moieties, which bind strongly (as multivalent ligands) and specifically to C-type lectin receptors (CD206), which are present

on the surface of macrophages and dendritic cells residing in SLNs. It is this mechanism of action that gives the tilmanocept platform its tremendous capability as a diagnostic and SLN detection agent (Figs. 2 and 3). It is now clear from the clinical studies of tilmanocept not only in breast cancer and melanoma, but in head/neck (oral) squamous cell carcinoma that such targeting significantly reduces the false negative rate for SLNB (Table 1).

2. Recognition of the imaging-therapeutic axis

As noted, ^{99m}Tc-tilmanocept is currently used as a mapping agent for the identification of sentinel lymph nodes in multiple solid tumors. The approved indications for ^{99m}Tc-tilmanocept include guiding SLNB in clinically node negative breast cancer, melanoma, and oral cavity squamous cell carcinoma.

In the context of studying and developing ^{99m}Tc-tilmanocept, the cellular biology underlying the diagnostic targeting specificity is ineluctably linked to the potential for ^{99m}Tc-tilmanocept to be used as a targeting agent against those diseases studied. The basis for this eureka moment was the understanding of the pleotropic impact of the CD206-expressing macrophages not only in the oncology realm (the tumorigenesis process) but in diseases in general.

Tumor evolution and the necessity of tumor-associated macrophages (TAMs) even in the early carcinogenesis process are well established. It is no coincidence that such macrophages express CD206, the explicit receptor for Tilmanocept, and that this coincident convenience is an element of the efficacy of ^{99m}Tc-tilmanocept in SLNB. But the matter is more than this. Macrophages are an integral process element in a host of other diseases. We now realize that their integration into disease processes is remarkably complex, with many

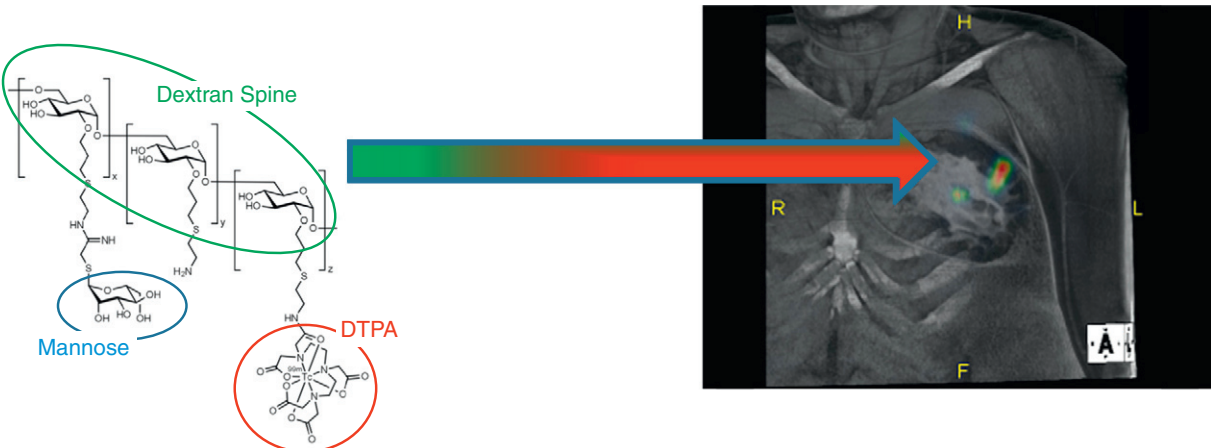


Fig. 3. Sentinel node discrimination in patient with breast cancer; multiple sentinel nodes are visible 15 min after injection.

Table 1
Sentinel Lymph Node False Negative Rate (FNR) in Head and Neck Squamous Cell Carcinoma.

Lymphoseek Detection	Pathology	
	+	-
	≥ 1 Lymph Nodes (SLN or non-SLN) ¹ Are Pathology Positive	All Lymph Nodes (SLN and non-SLN) Are Pathology Negative
+ ≥ 1 Lymphoseek Detected Lymph Node(s) (SLNs) Are Pathology Positive	38 True Positive	N/A ² (0)
- Lymphoseek Detected Lymph Nodes (SLNs) Are ALL Pathology Negative (or no SLNs exist)	1 False Negative ^{3,4}	44 True Negative

1 SLN=sentinel lymph node; non-SLN=non-sentinel lymph node.

2 N/A=Not applicable. False positives were not applicable to the analysis as lymph nodes did not fit into this category (Nodes cannot both be pathology positive and pathology negative).

3 The FNR was 0.0256 and the exact binomial test of this result against the null hypothesis H0: FNR ≥ 0.14 was statistically significant at p = 0.0205. Of the 39 patients with at least 1 pathology-positive lymph node, Lymphoseek detected nodes with positive pathology in all but 1 patient.

4 Due to a positive (low) FNR of an interim-analysis significance level for a 1-sided exact test of binomial proportion being ≤ 0.02486 after ≥ 38 patients with a pathology-positive lymph node, the DSMC recommended stopping the trial for efficacy (which ended in approximately 40 months).

highly integrated macrophage and disease elements, testifying to the biologically synchronized transduction of macrophage roles in disease natural history.

In many maladies, including autoimmune diseases (such as multiple sclerosis, diabetes, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and Crohn's disease), infectious diseases (HIV and tuberculosis), tumorigenesis, neurodegenerative disorders (dementias) and cardiovascular disease (vulnerable plaque and atherosclerosis), the presence of CD206-macrophages becomes an autogenous progression of the disease state. Table 2 lists a number of these diseases and macrophage involvement. This table effectively defines not only the potential for diagnostic use of ^{99m}Tc-tilmanocept, but also the opportunity for therapeutic targeting using tilmanocept as the targeting agent.

3. Selected opportunities — exploiting the diagnostic/therapeutic axis: tilmanocept to Manocepttm

An adaptation of tilmanocept from imaging to therapy is supported by both in vivo and ex vivo data; we have adapted the nomenclature to segregate these initiatives: “Manocept” agents that target the same

Table 2
Macrophages in the Natural History of Disease.

Innate Immunity
Allergy
Asthma
Atherosclerosis
Cancer
COPD
Diabetes
Infection/HIV
Rheumatoid Arthritis
Sepsis
Transplant Rejection

CD206-macrophage mannose receptor and are derived from Tilmanocept now represent a therapeutic enterprise. In this vane, we outline below selected clinical sorties in which there is a validated transition of tilmanocept diagnostic imaging to Manocept therapeutic targeting.

3.1. Kaposi's sarcoma (KS)

AIDS-related KS is an aggressive, multifocal, angioproliferative neoplasm associated with Kaposi's sarcoma herpes virus (HHV8/KSHV) infection. It involves cutaneous and visceral tissues, with later forms of disease associated with widespread organ involvement. It is the most common cancer in patients infected with HIV. Effective antiviral therapy has produced a decline in the incidence of AIDS related KS, but HIV-infected individuals still have a 3640-fold greater risk of developing KS than the uninfected population. In general, no imaging studies have been able to identify specific KS-involved tissues, apart from standard ultrasound and CT imaging, in which therapy-associated changes are implied to be associated with KS lesion shrinkage.

Inflammation appears to play a critical role in tumor development of HIV-associated KS. Specifically, emerging data show that KS tumor cells that co-express various macrophage antigens, especially CD206, the tilmanocept receptor, become resistant to current anti-viral therapies used to treat KS and AIDS. Macrophages also are a known source of KS tumor cell growth factors and substantial evidence suggests that TAMs represent a reservoir for HIV and its evolved retroviral variants. The macrophage pool driving these two pathological pathways share a common element rooted in the macrophages, the CD206 human macrophage mannose receptor. Manocept, as a molecular targeting agent, binds and enters macrophages via pinocytosis of holo-CD206, providing a cell portal for the evaluation of Manocept as a macrophage and KS targeting agent.

Kaposi's sarcoma lesions are comprised of KS spindle cells infected with HHV8/KSHV, as well as numerous macrophage antigen-expressing cells. In a study of over 100 KS lesions we demonstrated that both skin and visceral forms of KS express the CD206 molecule; CD206 was found on both KS tumor cells and TAMs, allowing the potential for use of tilmanocept as a tumor-specific imaging agent capable of identifying both tumor cells and TAMs in patients with KS (Figs. 4 and 5). The results of imaging in vivo in both HIV+ and HIV- KS patients suggest the therapeutic potential value of Manocept. The supra-specificity of tilmanocept for the KS lesions and the virtual lack of off-target (non-CD206+) background in vivo are strong evidences for employing Manocept congeners in KS and other solid tumors as tumor specific therapies.

3.2. Rheumatoid arthritis (RA)

Rheumatoid arthritis is a common, chronic, systemic, and progressive autoimmune disease associated with inflammation and pathology throughout the body, but perhaps most noticeably in the peripheral joints of the skeleton (i.e., hands, feet, knees, hips, etc.). In the affected joints, RA is characterized by macrophage and lymphocyte infiltration, proliferation of synovial fibroblastic tissue (pannus), and joint destruction. RA causes joint pain, stiffness and reduced mobility. If not successfully treated, joint inflammation and destruction in RA patients can lead to crippling loss of function, severe chronic pain, and disfigurement of the joints. Also, RA patients have significantly higher risk of coronary heart disease including acute myocardial infarction (odds ratio = 3.17). Patients with uncontrolled RA may also suffer a reduction of 3–10 years in their life expectancies. RA can strike anyone at any age, but is diagnosed most frequently in women in their 40s and 50s. Worldwide, about one adult in every 200 has RA. In the United States, approximately 1.3 million adults have RA. RA is a chronic disease and the prevalence of RA increases with age. Because of aging demographics in the United States and elsewhere, the number of patients with RA and the burden of RA on society are expected to

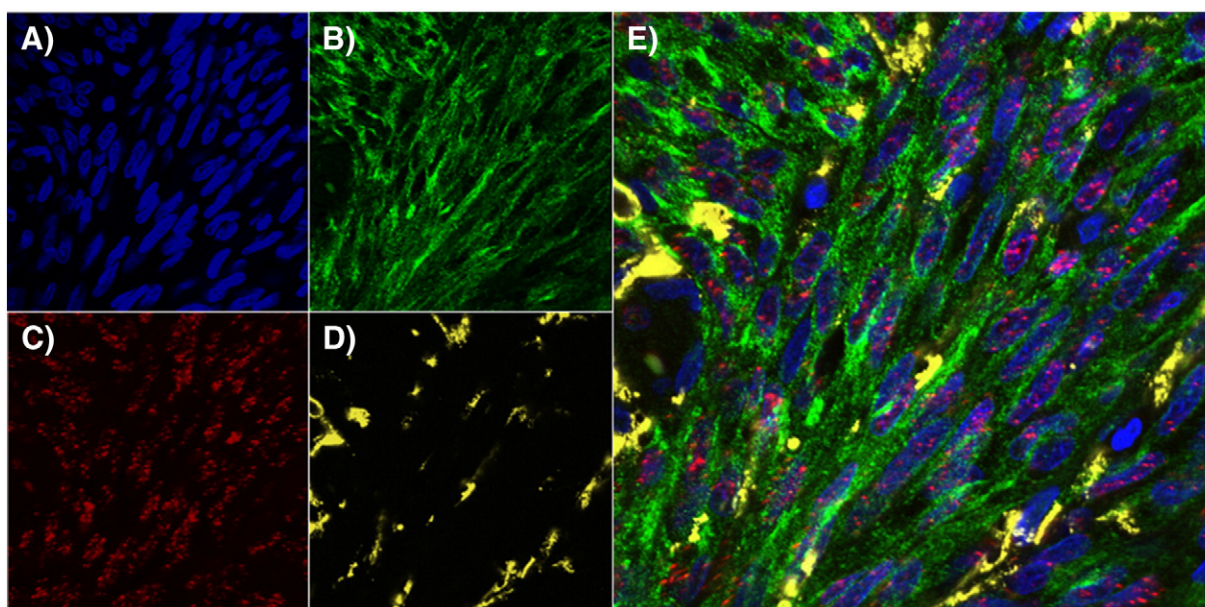


Fig. 4. CD206 expresses on both Kaposi's sarcoma spindle cells and associated macrophages (A, Nuclear stain DAPI; B, Anti-CD206; C, Anti-LANA/HHV8; D, Anti-CD68 macrophage marker; E, merged images).

increase in the coming decades. Therefore, there is a significant current and growing need to manage RA patients more effectively to limit the morbidity and mortality caused by RA.

While many types of cells, including T-cells, B-cells, dendritic cells, and activated synovial fibroblasts, contribute significantly to the establishment and maintenance of RA, macrophages play a critical role in RA pathogenesis. They produce most of the TNF α that drives and perpetuates the inflammatory cycle in RA. In the synovial sub-lining of a joint affected by RA, macrophages are the dominant cell type. In the inflamed joint as a whole, macrophages in RA patients make up at least 30%–40% of all cells. Furthermore, macrophages participate directly in the destruction of bone and cartilage. Activated macrophage populations and synoviocytes are the predominant cell types at the interface between pannus and cartilage and secrete destructive proteases in abundance. As a result, it may not be surprising that synovial macrophage numbers – but not the numbers of other immune cell types – correlate with radiographically determined joint destruction in RA. While macrophages may play a role in other pathologies that cause joint pain and inflammation, the degree to which macrophages are involved in the pathological process of RA and the sheer mass or volume of macrophages that infiltrate the joints inflamed due to RA differentiate RA from other rheumatic diseases. Therefore, detection of the density or numbers of macrophages in inflamed joints may permit differentiation of patients with RA from those with other causes of arthritis. In addition, it is known that the RA pathology begins significantly before, perhaps years before, the onset of symptoms (i.e., joint pain and inflammation) and well before the beginning of bone destruction. Macrophage infiltration of synovial tissues precedes development of RA clinical signs in animal studies. In humans, macrophage infiltrates in synovial tissues are present when RA patients first develop clinical symptoms. Therefore, detection of the density or numbers of macrophages in inflamed joints may facilitate more sensitive and specific identification of RA patients as soon as they present with symptoms and early in the course of their illnesses when disease-modifying anti-rheumatic drugs (DMARDs) are likely to be most effective.

Below are select data from studies using tilmanocept as an imaging agent for synovial macrophages in the inflamed joints of RA patients and in animal models. We investigated CD206 expression and tilmanocept binding to synovial macrophages in an anti-type II collagen monoclonal antibody induced mouse model of RA. Male 8-week-old

Dbal mice ($n = 8$) were injected intraperitoneally (IP) with 1.5 mg Athrogen-CIA arthrogenic five monoclonal antibody cocktail to Type II collagen followed in three days by an IP injection with *E. coli* 0111:B4 lipopolysaccharide. Control mice ($n = 4$) were injected with phosphate-buffered saline. Evidence of arthritis (joint swelling and redness) developed in 5–6 days, and the severity of arthritis was scored for each limb daily. On days 9 or 11, mice were imaged 1–2 h after they had received an intravenous injection of fluorescent Cy3-tilmanocept. The mice were then euthanized followed by limb dissection and reimaging. The primary result of this experiment was that Cy3-tilmanocept administered intravenously localized to synovial macrophages in the affected joints of arthritic mice but not control mice (Fig. 6).

Ex vivo experiments showed specific binding of Cy3-tilmanocept to human synovial macrophages obtained in samples from patients with active RA undergoing therapeutic surgical procedures (Fig. 3). For these studies, RA patients were recruited through approved institutional review board protocols. Flash-frozen synovial tissue was sectioned to 4 μm onto glass slides for immunohistochemistry. Slides were incubated with DAPI nuclear stain (blue), an anti-CD206 antibody and/or Cy3-tilmanocept (red). Bound anti-CD206 antibody was visualized with a secondary antibody conjugated with Alexa Fluor 647 (green). Images were obtained using a Zeiss fluorescent microscope and merged to show co-localization. It is important to note that, first, human synovial macrophages from human RA patients abundantly express CD206, and (second) Cy3-tilmanocept binds to human synovial macrophages and co-localizes with CD206. Finally (third), CD206-expressing macrophages are a highly abundant cell type in RA synovia. The specificity of Cy3-tilmanocept binding to synovial macrophages was further demonstrated by pre-incubating with a tenfold excess of tilmanocept that had not been conjugated with Cy3. Pre-incubation with unconjugated tilmanocept completely abolished Cy3-tilmanocept binding (Fig. 7).

In order to provide perspective around the specificity of tilmanocept binding/uptake into RA synovial macrophages, additional experiments performed with synovial tissues obtained from joints of patients with osteoarthritis and from healthy controls (obtained from a tissue bank), provided results showing that Cy3-tilmanocept binding was much greater in synovial tissues from RA patients than it was in similar tissues obtained from patients with

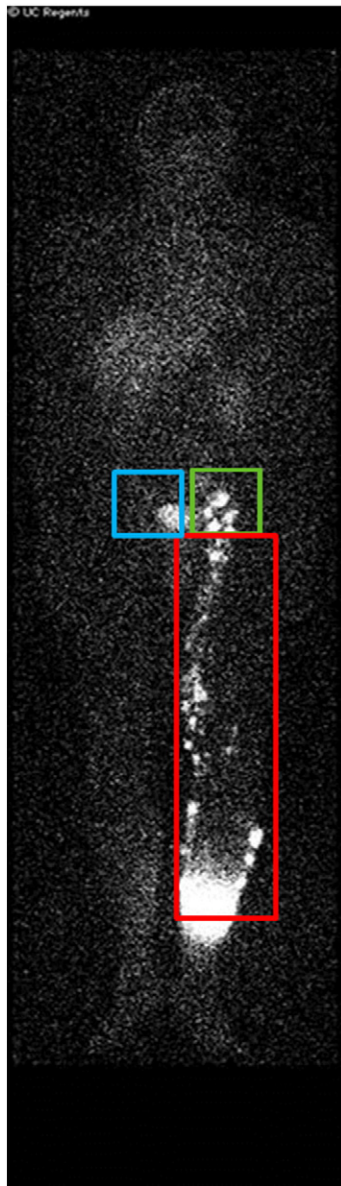


Fig. 5. Patient injected subcutaneously with Tc99m-Tilmanocept (50 μ g; 2.0 mCi) and imaged 4 h post-injection; SPECT WHOLE BODY SCAN: Injection site lower left leg; KS lesions match tracings of leg lesions; TC99m-tilmanocept localizes in KS chain linked by lymphatic ducts; localizes in (KS+?) inguinal nodes; left of nodes in image is bladder.

osteoarthritis or healthy controls. It is well known that osteoarthritic joints contain macrophage infiltrates; however, in osteoarthritis, synovial macrophages are less numerous than in RA and as this experiment shows, synovial macrophages in osteoarthritis produce much less CD206 (Fig. 8).

Similar to KS, the specificity of tilmanocept for the RA loci and the virtual lack of off-target (non-CD206+) background reactivity provide strong supportive evidence for employing Manocept congeners in RA therapies.

3.3. Tuberculosis (TB)

Mycobacterium tuberculosis (Mtb) is an airborne pathogen that infects the lungs and then can disseminate throughout the body. Mtb is spread when an individual with symptomatic or active TB coughs, creating an Mtb-rich aerosol that can be inhaled into the lungs of previously uninfected persons. Alveolar macrophages recognize Mtb by various means but importantly through interactions between the mannose caps on the Mtb

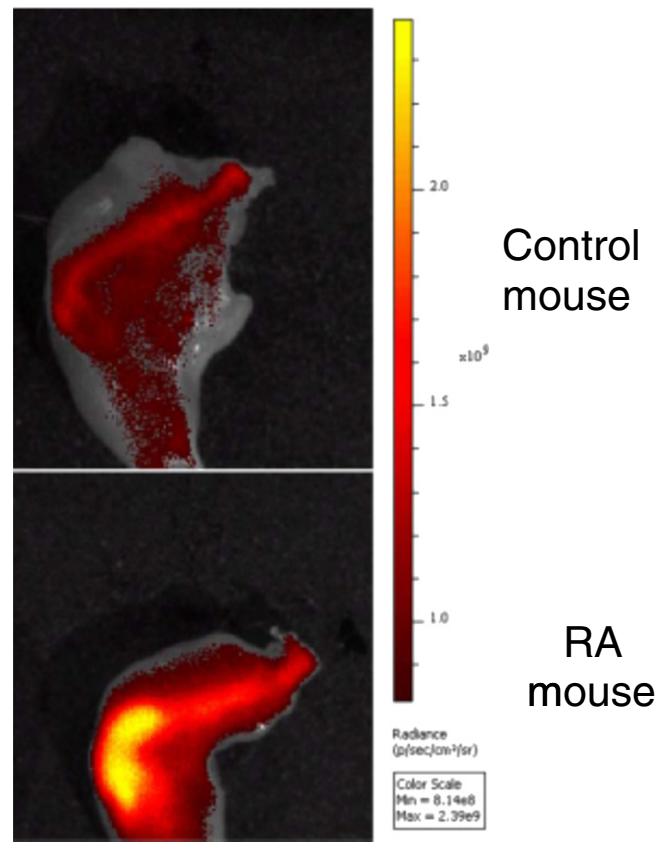


Fig. 6. Mice were imaged in the IVIS (in vivo imaging system) to measure Cy-3 levels. In these ex vivo images, the limbs were detached and the skin was removed before imaging. On the bottom you can see the enhanced signal in the RA mice knees. The values are statistically significant when quantitated.

coat molecule, mannosylated lipoarabinomannan (ManLAM), and CD206. The interaction between ManLAM and CD206 is important because it alters phagosome trafficking, inhibiting fusion of the Mtb-containing phagosome with lysosomes, and allowing Mtb to survive and replicate in these cells. The infected alveolar macrophage then secretes various cytokines that attract additional macrophages and various other immune cells. Mtb replication and dissemination combined with the accumulation of various immune cells lead to a robust systemic immune response and the early formation of a granuloma. The granuloma (granulomatous pulmonary Mtb) is comprised of T and B lymphocytes surrounding a fibrous cuff. Within the fibrous cuff there are macrophages (both infected and uninfected) and some

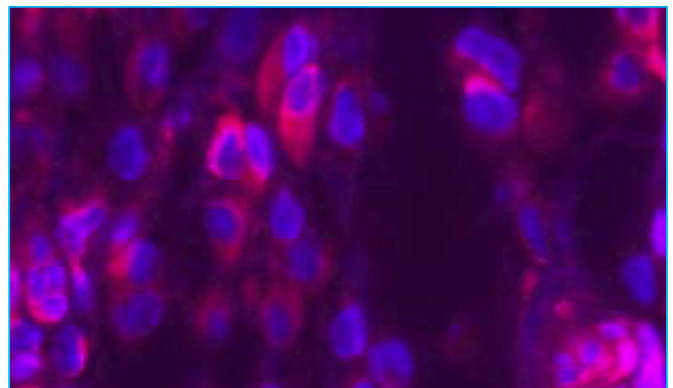


Fig. 7. Staining of synovial tissue frozen sections from a patient with Rheumatoid arthritis. Sections were stained with 4',6-diamidino-2-phenylindole, BLUE (DAPI = DNA) and Cy3-tilmanocept-RED/PINK (CD206) and examined under fluorescence microscopy.

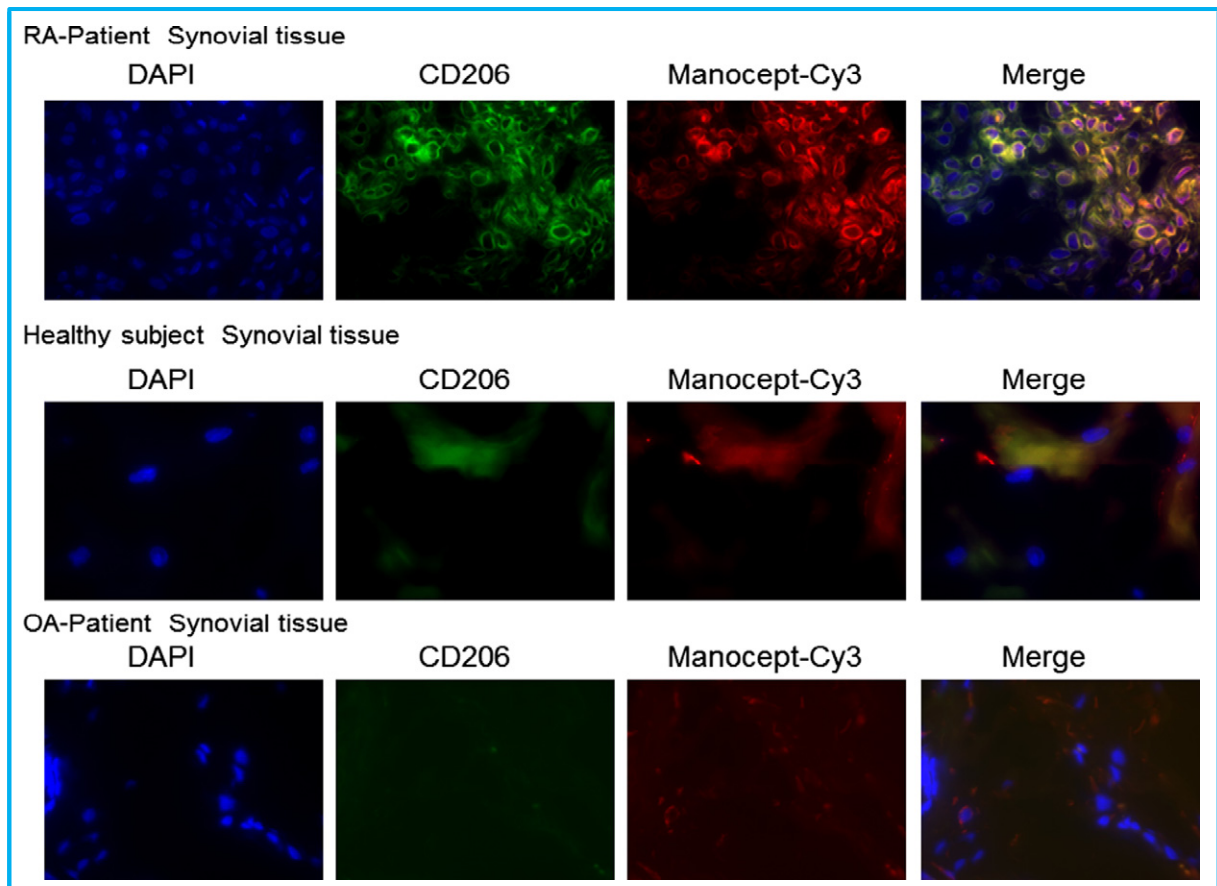


Fig. 8. We then examined CD206 and Manoecept in synovial tissue. In the merged image, you can clearly see the co-localization of CD206 and Manoecept. Healthy and osteoarthritis synovial tissue samples were negative for both.

neutrophils. Lipid metabolism in many of the macrophages becomes perturbed, perhaps in response to stimuli from the Mtb, causing them to further differentiate into foam cells containing lipid micro-droplets. The granuloma sequesters Mtb and prevents it from spreading to other parts of the body. However, Mtb can persist in a metabolically quiescent state for years or decades in granulomas. The large majority of individuals who become infected with Mtb do not exhibit symptoms, having their Mtb sequestered in granulomas. Such asymptomatic persons are said to have latent TB infections (LTBI). Unfortunately over time, there is risk that a granuloma can progress, with the patient developing active TB. In active TB, the granuloma enlarges with a necrotic center comprised of Mtb cells and a lipid rich material derived from the cellular debris of dead foam cells, which is called caseum due to an appearance resembling

milky cheese. In time, the granuloma may rupture releasing Mtb and necrotic debris into the patient's airway, causing coughing that spreads the infection to other people. If a person with active TB is not treated, on average they will transmit Mtb to 10–15 people per year. A latently infected person without co-morbidities such as AIDS or diabetes has a 5%–10% chance of developing active TB over their lifetime. It is estimated that one third of the world's population is latently infected with Mtb.

Our recent data show (Fig. 9) that the infection of macrophages with Mtb does not downregulate the synthesis and expression of CD206, nor does infection abrogate the uptake of tilmanocept via CD206 and its accrual intracellularly.

These data are, again, notable evidence for the use of a Manoecept therapeutic congener for the treatment of Mtb and drug resistant

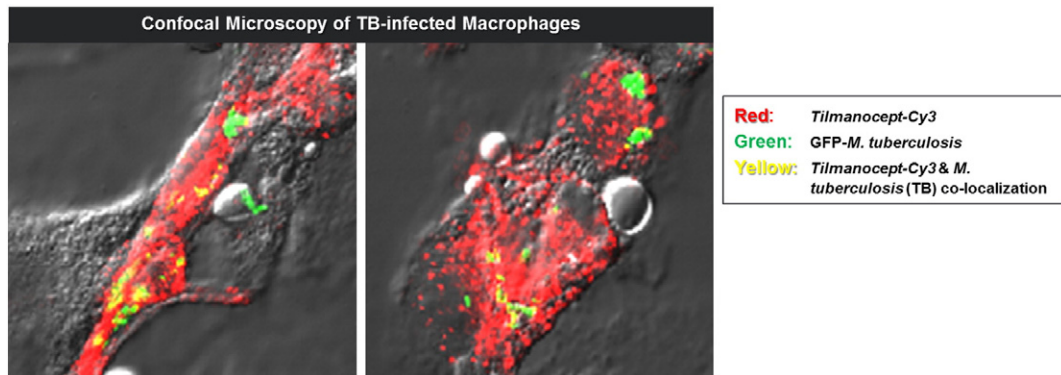


Fig. 9. Human monocyte-derived macrophages in monolayer culture that make up the components of the TB granulomas are infected with a GFP-expressing *M. tuberculosis* which is internalized by macrophages. The infected cells were exposed to tilmanocept-Cy3 and then analyzed by confocal microscopy.

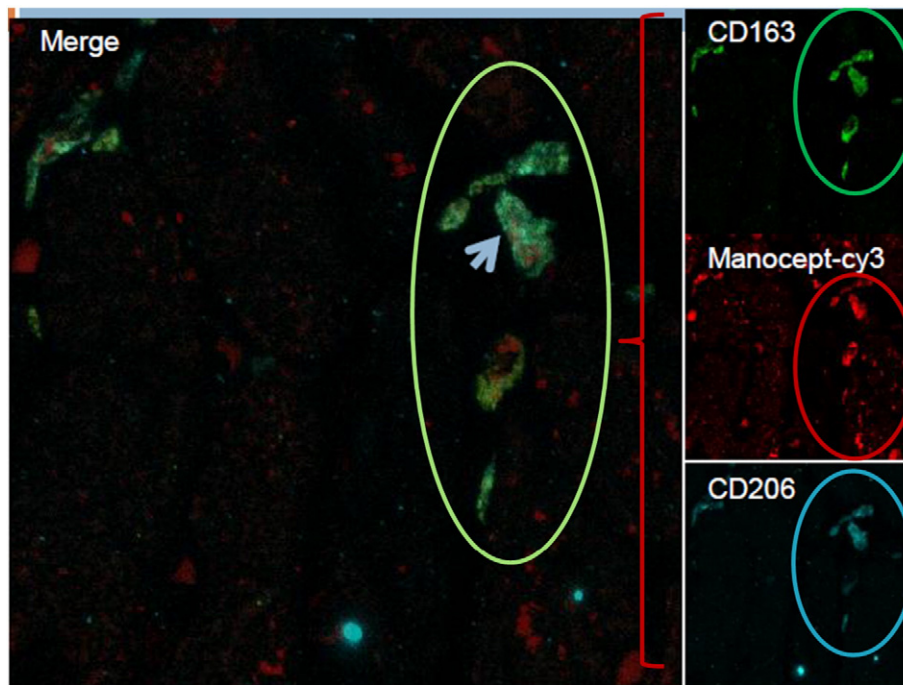


Fig. 10. Cy3-Tilmanocept targets macrophages in arteries with atherosclerosis; figure shows co-localization of CD206 antibodies (blue), Cy-3-tilmanocept (red) and CD163 (green) macrophage markers in arteries of SIV-infected macaque monkeys.

Mtb to the extent that we believe these data suggest that the biology of the granuloma is now made vulnerable to attack using many congener strategies.

3.4. Cardiovascular disease

Atherosclerosis is a chronic inflammatory syndrome that develops slowly in the walls of arteries over the course of many years or decades. The initiation and progression of atherosclerosis involve interactions between plasma lipoproteins, cytokines extracellular matrix, inflammatory signaling molecules and several cell types including macrophages. The primary lesion of atherosclerosis is the atherosclerotic plaque. Atherosclerotic plaques can expand into the lumen of an artery, eventually impacting and diminishing the flow of blood. This impairment of blood flow can result in angina pectoris, which is painful and debilitating. Alternatively, atherosclerosis can progress to an advanced state without producing symptoms. Eventually, an atherosclerotic plaque can rupture causing the internal contents of the plaque to come into contact with the blood, thereby initiating thrombus (i.e., clot) formation. These clots can lead to ischemic events that manifest as myocardial infarctions, sudden cardiac deaths or strokes. For a tragically large proportion of patients, experiencing one of these potentially catastrophic and/or lethal events is the first observed symptom experienced by a patient with advanced atherosclerosis. To prevent these potentially catastrophic plaque rupture associated events, it is necessary to identify patients with atherosclerotic plaques that are at high risk of near term rupture so that appropriate intensive rupture preventing therapeutic interventions can be administered.

Autopsies performed on people who have died as a result of infarctions caused by ruptures of atherosclerotic plaques have identified the relevant ruptured plaques, which are termed the “culprit lesions.” To identify individuals at high-risk of impending plaque rupture, atherosclerotic plaques that most closely resemble culprit plaques but which have not yet ruptured have been identified. These culprit-resembling plaques are termed “vulnerable plaques.” Vulnerable plaques have morphological features and internal compositions that differentiate them from other atherosclerotic plaques (i.e., stable plaques). These differentiating features include the presence of large necrotic cores

associated with a lipid pool and a thin (<65 micron) fibrous cap. This type of vulnerable plaque is also referred to as a thin cap fibroatheroma (TCFA). TCFA and culprit plaques are not typically highly calcified, but may display evidence of nascent calcification that is not yet extensive. Interestingly, despite their relatively large sizes, TCFA most commonly does not cause severe narrowing of the lumen of the arteries in which they occur. Instead TCFA is frequently associated with remodeling of their arterial walls that expands their arterial lumens to accommodate their relatively large volumes.

Macrophages contribute a key and evolving role at each stage of the pathological development of atherosclerosis. Following an injury to the arterial endothelium, low density lipoproteins (LDLs) invade the endothelium and become oxidized, initiating an inflammatory response that attracts monocytes. These monocytes ingest the oxidized LDLs and become macrophage “foam cells” that further propagate the inflammatory response by secreting pro-inflammatory cytokines. Eventually, the foam cells die or undergo apoptosis creating the lipid rich necrotic core of vulnerable plaque. The necrotic core further attracts macrophages. Narula et al. examined atherosclerotic plaques from patients who died suddenly [132]. They observed that an increase in the number of infiltrating macrophages was a key discriminator between TCFA and stable plaques. Importantly, Tahara et al. also examined atherosclerotic plaques from patients that had suffered sudden cardiac death [133]. They confirmed Narula’s findings of increased macrophages in TCFA. In addition, immunohistochemical analyses on the plaques found that large proportions of macrophages in TCFA, but not in stable thick capped plaques, confirmed positive expression of CD206, the macrophage mannose receptor and the specific target for ^{99m}Tc-tilmanocept binding, and CD163, both markers for alternative, M2, macrophage activation [29–32]. These results suggest that Tc-tilmanocept will bind to abundant macrophage targets in TCFA and that CD206 imaging results can differentiate TCFA from stable atherosclerotic plaques.

Macrophages can be activated to differentiate into various gene expression phenotypes when stimulated by various combinations of cytokines and their local environment. Macrophages can be activated to a pro-inflammatory, M1, phenotype, or anti-inflammatory, M2a, M2b, and M2c, phenotypes. Early in the development of atherosclerosis, M1 macrophages predominate in atherosclerotic plaques, with foam cells

expressing many pro-inflammatory cytokines characteristic of an M1 phenotype. M2a and M2c express high levels of CD206. M2c macrophages are particularly interesting because they accumulate in areas where apoptotic cells are present. The necrotic cores of vulnerable atherosclerotic plaques result largely from the apoptosis of foam cells and as such, would be expected to attract M2c macrophages. M2c macrophages abundantly express CD163 in addition to CD206.

These observations are consistent with our approach to exploiting this cardiovascular disease natural history with Manocept congeners for both imaging and therapy. Our ex vivo data showing both the expression of CD206 and the localization of fluorescent Manocept in the coronary arteries on this CD206 are strong evidence for the axis initiative in this disease (Fig. 10).

4. Conclusions

In the wake of innovation, tilmanocept (and as Manocept) is self-transforming from a powerful diagnostic/imaging agent in cancer staging procedures into a potential multi-application molecule for diagnosis and treatment of an array of diseases. It has displayed its effectiveness in applications with regards to SLNB, much due to its unique structure and targeting. As a non-particulate, small molecular size, receptor targeted (CD206) molecule with the ability to interchange not only radionuclides but also lethal or biological modifier molecules, tilmanocept/Manocept has great potential for targeting macrophage mediated diseases and delivering an effective, concentrated dose for purposes of diagnosis or treatment. As Manocept continues to generate clinically relevant data for future applications in immunotherapies for KS (and other solid tumors), RA, TB, and cardiovascular disease, one thing remains clear: the selection of this approach, targeting CD206, appears to provide encouraging results with inter-disease consistency and reliability on the this immuno-biological strategy [43–139].

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