^{99m}Tc-Labeled anti-macrophage mannose receptor (MMR; CD206) nanobody

[^{99m}Tc]MMR Nb

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Chemical name:	^{99m}Tc-Labeled anti-macrophage mannose receptor (MMR; CD206) nanobody	
Abbreviated name:	[^{99m}Tc]MMR Nb	
Synonym:		
Agent Category:	Antibody	
Target:	Macrophage mannose receptor (CD206)	
Target Category:	Receptor	
Method of detection:	Single-photon emission computed tomography (SPECT); gamma planar imaging	
Source of signal / contrast:	^{99m}Tc	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure not available in PubChem.

Background

[PubMed]

Cancerous tumors consist of transformed cells that are surrounded by stromal cells (or healthy, normal host cells) consisting of cells such as the fibroblasts, endothelial cells, various myeloid cells, and the extracellular matrix (1). The various cell types in the tumor interact with each other constantly so as to maintain homeostasis in the lesion. In a cancerous tumor, the stromal cells produce ligands that assist in the maintenance and growth of the tumor (e.g., by inducing angiogenesis), and the neoplastic cells reciprocate

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by generating factors that help in the survival, growth, and proliferation of the stromal cells (2). In addition, a large number of macrophages infiltrate and reside in the tumor stroma (known as tumor-associated macrophages; TAMs), and these cells are also known to promote growth and proliferation of the tumor because they produce factors that are inflammatory, pro-angiogenic, and immunosuppressive, and that assist in the metastasis of the cancer (3). On the basis of their gene and protein expression profile, two distinct types of TAMs are found in the tumor stroma. The TAMs either have a low expression of the major histocompatibility complex class II (MHC II) (MHC^{low} TAMs) or express high levels of MHC II (MHC^{hi} TAMs). The MHC^{low} TAMs are present mainly in the hypoxic areas of the tumor, are strong promoters of angiogenesis, and overexpress the macrophage mannose receptor (MMR, CD206), an endocyte C-type lectin receptor (3). The MHC^{hi} TAMs are found primarily in the perivascular regions of a cancerous lesion and exhibit a low angiogenic potential (3). Therefore, MMR could be a valuable target for the noninvasive imaging of MMR^+ stromal cells with single-photon emission computed tomography (SPECT) to visualize tumor stroma, and the stromal cells could be targeted to treat neoplastic lesions.

There is a great deal of interest to develop imaging probes that are based on a nanobody (Nb) scaffold because Nbs are miniature antibodies (Ab) (~15 kDa compared with ~150 kDa for a normal Ab) that are rapidly eliminated through the kidneys and, if labeled with a radionuclide, generate a high signal/noise ratio at the target site (4). To learn about the unique source, structure, properties, and application of Nbs, see Vaneycken et al. (5). Movahedi et al. generated an anti-MMR Nb, labeled it with ^{99m}Tc, and showed that the radiolabeled Nb ([^{99m}Tc]MMR Nb) can be used to detect MMR^+ stromal cells with SPECT in mice bearing either TS/A cell tumors (a mouse mammary adenocarcinoma cell line) or 3LL-R cell tumors (a C57BL/6 Lewis lung carcinoma cell line) (3, 4).

Related Resource Links

Nanobody-related chapters in MICAD

Nanobodies in clinical trials

Synthesis

[PubMed]

Two anti-MMR Nbs, Nb cl1 and Nb cl2 (with a hexahistidine tag on the C-terminus to facilitate labeling with a radionuclide), were generated against the extracellular domain of the MMR. The Nbs were expressed and purified from a bacterial expression system as described elsewhere (4). Bivalent Nbs (biv Nbs), designated Nb biv $(G_4S)_3$, Nb biv IgG2c, and Nb biv IgA, which contained different types of linkers (for details regarding the linkers and the biv Nbs, see Movahedi et al. (4)), were also generated for use in some studies. The various Nbs were radiolabeled with ^{99m}Tc at their H_6 tails, as described by Vaneycken et al. (6). For use as a negative control, another Nb, Bcll10, which is directed against the β-lactamase (7), was also labeled with ^{99m}Tc. The radiochemical yield,

radiochemical purity, specific activity, and stability of the different Nb or biv Nb probes were not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Surface plasmon resonance (SPR) measurements with Nb cl1 and Nb cl2 showed that Nb cl1 had an 8-fold higher affinity ($K_D = 2.31 \times 10{-}^{8}$ mol/L) for MMR compared with Nb cl2 ($K_D = 1.91 \times 10{-}^{7}$ mol/L) (4). In addition, it was shown that the two Nbs did not bind to the same epitope on the MMR (4). The MMR binding affinities of Nb biv $(G_4S)_3$, Nb biv IgG2c, and Nb biv IgA were reported to be $4.22 \times 10{-}^{9}$ mol/L, $4.04 \times 10{-}^{9}$ mol/L, and $4.69 \times 10{-}^{9}$ mol/L, respectively, as determined with SPR.

Flow cytometric analysis showed that fluorescence-labeled Nb cl1 bound only to the $CD11b^{+}$ and not to the $CD11^{-}$ cell fractions of single-cell suspensions derived from either the TS/A tumors or the 3LL-R tumors (4). In addition, the fluorescent Nb bound largely to the MHC^{low} TAMs in the TS/A cells, whereas the binding was restricted to the MHC^{low} TAMs with the 3LL-R cells.

Animal Studies

Rodents

[PubMed]

Biodistribution and SPECT/micro-computed tomography (micro-CT) imaging studies with the different types of ^{99m}Tc-labeled Nbs were performed in naïve wild-type (WT) mice, MMR knockout (MMR-KO) mice, and chemokine receptor 2 knockout (CCR2-KO), mice as described by Movahedi et al. (4). The animals were injected intravenously with 45–155 MBq (1.66–5.73 mCi) of the ^{99m}Tc-labeled Nbs in the presence or absence of an excess amount (quantity not reported) of an unlabeled Nb or a biv Nb. Whole-body SPECT images were acquired from the animals at 60 min or 180 min postinjection (p.i.); at 30 min after acquiring the images, the animals were euthanized to determine the amount of accumulated radioactivity in the various organs of interest. Data were presented as percent of injected activity per gram tissue (% IA/g).

Groups of naïve WT mice (number of animals per group was not reported) were injected with either [^{99m}Tc]Nb cl1, [^{99m}Tc]Nb cl2, or [^{99m}Tc]NbBCII10 (control) to determine if the ^{99m}Tc-labeled Nbs were suitable for the imaging of organs that express MMR under *in vivo* conditions (e.g., the liver and the spleen) (4). Whole-body SPECT/micro-CT images were acquired from the animals at 60 min p.i., and the images showed that the radioactivity from all three probes was present primarily in the kidneys and the urinary bladder of the animals. The images showed that the accumulation of radioactivity with [^{99m}Tc]Nb cl1 and [^{99m}Tc]Nb cl2 was mainly in the liver and spleen of the animals. However, animals injected with [^{99m}Tc]NbBCII10 showed no accumulation of label in

these organs. *Ex vivo* analysis of the various organs obtained from these animals showed that, with [^{99m}Tc]Nb cl1 and [^{99m}Tc]Nb cl2, the amount of radioactivity present in the liver was 15.55 ± 0.54% IA/g and 7.92 ± 0.42% IA/g, respectively, and the amount of radioactivity in the spleen was 6.79 ± 0.08% IA/g and 3.97 ± 0.65% IA/g, respectively. All other organs, such as the heart, lungs, and bone, showed an uptake of 1%–2.5% IA/g. With [^{99m}Tc]NbBCII10, all the organs, including the liver and the spleen, showed an uptake of <1% IA/g. The biodistribution of [^{99m}Tc]Nb cl1, [^{99m}Tc]Nb cl2, and [^{99m}Tc]NbBCII10 in MMR-KO mice was observed to be similar to that of [^{99m}Tc]NbBCII10 in the WT rodents. From this study, the investigators concluded that the cl1 and cl2 Nbs have a high *in vivo* specificity to target organs that express the MMR. The investigators selected Nb cl1 for any further work because it exhibited a higher *in vitro* affinity for the MMR, and the *in vivo* uptake of radioactivity in the various organs was higher with this Nb compared with Nb cl2.

In another study, WT mice (number of animals was not reported) bearing TS/A cell and 3LL-R cell subcutaneous tumors were injected with [^{99m}Tc]Nb cl1 as before. SPECT/micro-CT images of the animals acquired at 3 h p.i. showed that the lesions were clearly visible at this time point (4). In comparison, the tumors showed very little uptake of radioactivity from the radiolabeled control Nb ([^{99m}Tc]BCll10). *Ex vivo* analysis of the TS/A and 3LL-R tumors showed that the uptake of label from [^{99m}Tc]Nb cl1 in the lesions was 3.02 ± 0.10% IA/g and 3.02 ± 0.19% IA/g, respectively, compared with an accumulation of 0.40 ± 0.03% IA/g and 0.74 ± 0.03% IA/g, respectively, with [^{99m}Tc]BCll10 (4). In addition, the accumulation of radioactivity from [^{99m}Tc]Nb cl1 in the 3LL-R tumors in MMR-KO mice was 10-fold less (0.33 ± 0.03% IA/g) compared with the lesions in the WT rodents, indicating that the uptake of radioactivity in the tumors was dependent on the expression of MMR by the host stromal cells (4).

It was shown that low expression of CCR2 in a tumor can significantly reduce the infiltration of TAMs into the lesion; however, CCR2-deficiency does not affect the growth of the neoplasm (4). To ascertain that [^{99m}Tc]Nb cl1 targeted only TAMs in the tumors, WT mice and CCR2-KO mice (number of animals per group was not reported) bearing 3LL-R cell tumors were injected with the ^{99m}Tc-labeled Nb, and the amount of tracer that accumulated in the lesions from the two groups of animals was determined. Tumors from the WT mice showed an accumulation of 2.97 ± 0.22% IA/g, which was significantly higher (*P* value not reported) than the uptake of 1.83 ± 0.1% IA/g observed in the tumors of the CCR2-KO mice (4). This observation suggested that Nb cl1 specifically targeted the TAMs in the cancerous lesions (4).

The biodistribution patterns of the ^{99m}Tc-labeled bivalent Nbs ([^{99m}Tc]Nb biv $(G_4S)_3$, [^{99m}Tc]Nb biv IgG2c, and [^{99m}Tc]Nb biv IgA) were investigated in WT mice bearing TS/A and 3LL-R tumors (4). From SPECT/micro-CT images, it was clear that the label from the bivalent Nbs accumulated mainly the liver and the spleen, but little uptake of the label was observed in the tumors compared with the monovalent cl1 Nb. From this study, the investigators concluded that the bivalent Nbs primarily blocked the extratumoral binding sites in the animals and had little affinity for the intratumoral binding sites. To

test this hypothesis, [^{99m}Tc]Nb cl1 was coinjected with 20-fold excess nonradioactive bivalent Nb (amount not reported) in mice bearing subcutaneous TS/A cell tumors, and SPECT/micro-CT images were acquired at 3 h p.i (4). From the images it was apparent that the incorporation of radioactivity in all the organs was reduced to background levels, i.e., the uptake of label was comparable to that observed with [^{99m}Tc]BCll10, and maximum uptake of radioactivity was observed in the tumors of the rodents.

Imaging studies were performed to evaluate the use of [^{99m}Tc]Nb cl1 for the visualization of microscopic mammary tumors in MMTV-PyVT transgenic mice, which develop the lesions spontaneously (4). For this, mice (n = 3 animals/group) bearing multiple microscopic tumors were injected with [^{99m}Tc]BCll10, [^{99m}Tc]Nb cl1, or a mixture of [^{99m}Tc]Nb cl1 and 20-fold excess nonradioactive biv Nb cl1 at 48-h intervals (to allow for complete decay of the radionuclide). Whole-body SPECT/micro-CT images were acquired from the animals at 3 h p.i. The tumors could not be distinguished easily from the organs in the images obtained from mice injected with [^{99m}Tc]Nb cl1 alone, but the tumors were clearly visible in the images from animals injected with a mixture of [^{99m}Tc]Nb cl1 and the biv Nb cl1. In addition, fluorescence-activated cell-sorting analysis of cells from three select tumors showed the presence of distinct subpopulations of MHC^{low} TAMs that expressed the MMR.

From these studies, the investigators concluded that the ^{99m}Tc-labeled MMR Nbs can be used for the targeted imaging of cancerous tumors in rodents (4).

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

Supplemental Information

[Disclaimers]

No information is currently available.

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