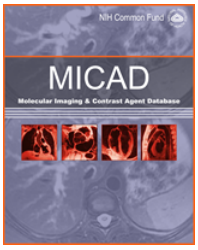
U.S. National Library of Medicine
National Center for Biotechnology Information

NLM Citation: Shan L. Biotinylated anti-Tn MLS128 monoclonal antibody-streptavidin-^{111}In-DTPA-biotin. 2009 Jul 21 [Updated 2009 Sep 17]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.
Bookshelf URL: https://www.ncbi.nlm.nih.gov/books/



Biotinylated anti-Tn MLS128 monoclonal antibody-streptavidin-^{111}In-DTPA-biotin

Bt-MLS128-SA-^{111}In-biotin

Liang Shan, PhD[1]

Created: July 21, 2009; Updated: September 17, 2009.

Chemical name:	Biotinylated anti-Tn MLS128 monoclonal antibody-streptavidin-^{111}In-DTPA-biotin	
Abbreviated name:	Bt-MLS128-SA-^{111}In-biotin	
Synonym:		
Agent Category:	Antibody	
Target:	Tumor-associated carbohydrate Tn antigen	
Target Category:	Antigen	
Method of detection:	Single-photon emission computed tomography (SPECT); gamma planar imaging	
Source of signal / contrast:	^{111}In	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure available.

Background

[PubMed]

Tn antigen is a tumor-associated carbohydrate epitope (*N*-acetyl-galactosamine (GalNAc)-*O*-Ser/Thr (GalNAca-*O*-Ser/Thr)) (1-3). Three-step targeting with biotinylated MLS128 monoclonal antibody (Bt-MLS128 mAb), streptavidin (SA), and ^{111}In-diethylenetriamine pentaacetic acid (DTPA)-biotin (^{111}In-biotin) was developed for *in vivo* imaging of Tn antigen-expressing tumors (4). It was designed on the basis of avidin's extraordinarily high binding affinity for biotin.

Avidin's high affinity for biotin was first exploited in histochemical applications in the mid-1970s (5, 6). This affinity is more than one million times higher than that of most antibodies for most antigens. Avidin has four binding sites for biotin, and most proteins, including enzymes, can be conjugated with several molecules of biotin. The avidin-biotin binding is essentially irreversible. These properties allow molecular complexes to be formed between avidin and biotinylated antibodies. In addition, small molecular sizes of avidin and biotin allow improved tumor uptake and rapid intratumoral spatial distribution.

Author Affiliation: 1 National Center for Biotechnology Information, NLM, NIH; Email: micad@ncbi.nlm.nih.gov.

Corresponding author.

Altered glycosylation on the cell surface is a hallmark of malignant transformation and tumor progression. Incomplete synthesis of the carbohydrate chains and precursor accumulation result in loss of the normal carbohydrate antigens and high expression of the tumor-associated carbohydrate antigens (7-9). Lewis Y, TF, Globo H, GM2, polysialic acid, sialyl Lewis A, Tn, and sialyl Tn are some of the antigens investigated intensively as diagnostic markers or as vaccine antigens (8-11). Tn antigen was first reported as a tumor-associated antigen nearly 40 years ago (12). It is composed of a single GalNAc glycan residue attached *via* an α-linkage to either the serine (Ser) or the threonine (Thr) of a polypeptide chain (9, 11). In normal tissues, Tn antigen is masked by covalently bound terminal carbohydrate moieties, but in tumors it is unmasked because of defective *O*-glycosylation. Accordingly, Tn antigen is rarely expressed in normal tissues, but it is widely expressed in human carcinomas or hematological cancers. It has been reported that the Tn antigen is expressed in 70–90% of breast, colon, lung, bladder, cervical, ovarian, stomach, and prostate tumors (1, 3, 7). The expression levels of the Tn antigen are closely associated with tumor aggressiveness and poor survival of patients (8, 10). In addition, Tn antigen is recognized by the human immune system as a novel epitope, provoking immune responses in patients. There is a strong correlation among the expression of the Tn antigen, the development of the spontaneous antibodies against Tn, and the prognosis for patients with carcinomas. Clinical trials are under way to deliberately provoke or enhance human immune responses by injecting patients with synthetic peptide antigens bearing Tn structure (3, 8, 13-15). Tn antigen has attracted significant interest as a target for tumor diagnosis and immunotherapy. A number of anti-Tn IgG and IgM antibodies have been generated and investigated for their imaging feasibilities and anti-tumor activities (2, 4, 16-22). The results are generally inconsistent. There are still some issues to be resolved, such as immunogenicity, reduced effectiveness *in vivo*, and cross-reactivity against type-A blood antigen. In addition, directly radiolabeled antibodies usually show a slow and low uptake by tumors, and their blood clearance is also slow. Nakamoto et al. tested the imaging feasibility of three-step targeting with Bt-MLS128, SA, and ^{111}In-biotin in mice bearing LS180 human colon cancer xenografts (4).

Note: Investigators from the same research group as Nakamoto et al. also labeled the anti-Tn MLS128 mAb directly with ^{125}I/^{131}I (^{125}I/^{131}I-MLS128) and ^{111}In (^{111}In-MLS128), separately, and investigated their biodistribution and the feasibility of imaging tumors in mice bearing LS180 tumor xenografts. They also tested the imaging feasibility of two-step targeting with Bt-MLS128 and ^{125}I-SA (Bt-MLS128-^{125}I-SA) in mice with LS180 tumor xenografts (17, 19, 20, 23, 24).

Synthesis

[PubMed]

Imaging with three-step targeting included three agents: Bt-MLS128, SA, and ^{111}In-biotin (4, 20). The anti-Tn MLS128 is a mouse IgG3 antibody with κ light chain, and it was produced by immunizing mice with LS180 human colorectal cancer cells (21). The antibody was purified from the ascitic fluid of the hybridoma-bearing mice with protein A affinity chromatography. The murine OST6 mAb against an alkaline phosphatase-related substance was used as the control. The antibodies were biotinylated by mixing them with sulfosuccinimidyl-6-(biotinamido)hexanoate, and they were then purified with chromatography on PD-10 gel. The average numbers of biotin molecules coupled to the MLS128 and OST6 were 1.2 and 1.7, respectively. Bt-MLS128, Bt-OST6, and SA were also labeled with ^{125}I with the chloramine-T method. The labeling efficiencies of radioiodinated Bt-MLS128, Bt-OST6, and SA were 63.2, 58.3, and 81.3%, respectively, and their specific activities were 23.4, 21.6, and 30.1 GBq/μg (0.63, 0.58, and 0.81 kCi/μg), respectively. The ^{111}In-DTPA-biotin was developed by mixing DTPA-biotin with ^{111}In-chloride. The labeling efficiency of the ^{111}In-biotin was >99%, and its specific activity was 296.0 GBq/μg (8 kCi/μg).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Nakamoto et al. analyzed the binding abilities of ^{125}I-Bt-MLS128 and ^{125}I-SA to the immobilized avidin, biotin, and LS180 tumor cells by incubating them together for 1 h at 4°C (4). More than 95% of ^{125}I-Bt-MLS128 bound to immobilized avidin. The immunoreactive fraction of ^{125}I-Bt-MLS128 that bound to the LS180 tumor cells was 47.2%, which was slightly lower than that of the unmodified MLS128 (data not shown). ^{125}I-SA showed >90% binding to the biotin-coated beads. There was no significant binding between the control ^{125}I-Bt-OST6 and the LS180 cells.

Animal Studies

Rodents

[PubMed]

Nakamoto et al. analyzed the biodistribution of the Bt-MLS128, ^{125}I-SA, and ^{111}In-biotin system in female BALB/c *nu/nu* mice bearing subcutaneous LS180 tumor xenografts (4). The mice were pretreated with intravenous (i.v.) administration of Bt-MLS128, followed by i.v. injection of ^{125}I-SA 2 days later. ^{111}In-biotin was administered *via* i.v. injection on day 1, 4, or 7, separately, after injection of ^{125}I-SA (n = 4–5 mice/time point). The mice were euthanized 2 h later, and the radioactivity levels of ^{125}I and ^{111}In were simultaneously counted with dual-channel counting. For the control mice, Bt-OST6 was given to the mice instead of Bt-MLS128.

The biodistribution data collected by counting the ^{125}I-SA accumulation showed that the tumor had a significantly higher uptake of ^{125}I-SA in the Bt-MLS128–pretreated mice than in control mice ($P < 0.05$). The ^{125}I-SA radioactivity in the tumors decreased gradually over time: 6.39 ± 1.23, 4.66 ± 0.52, and 3.24 ± 0.92% injected dose per gram of tissue (ID/g) on days 1, 4, and 7, respectively. The radioactivity levels of ^{125}I-SA decreased much faster in blood than in tumors: 1.75 ± 0.32, 0.5 ± 0.17, and 0.28 ± 0.03% ID/g in blood on days 1, 4, and 7, respectively. The difference in ^{125}I-SA clearance between the tumor and blood resulted in increased tumor/blood ratios over time: 3.69, 9.90, and 11.62, on days 1, 4, and 7, respectively ($P < 0.05$). Among the organs examined, the kidney had the highest level of ^{125}I-SA radioactivity: 44.67 ± 6.58, 44.04 ± 7.12, and 26.36 ± 2.08% ID/g on days 1, 4, and 7, respectively. The biodistribution data collected by counting the ^{111}In-biotin accumulation also showed that the tumor had a significantly higher level of ^{111}In-biotin radioactivity in the Bt-MLS128–pretreated mice than in control mice (0.51 ± 0.07 *versus* 0.14 ± 0.01% ID/g on day 4, $P < 0.05$). The radioactivity levels of ^{111}In-biotin in the tumors decreased with the prolonged interval between injections of ^{125}I-SA and ^{111}In-biotin, but the facilitated blood clearance of ^{111}In-biotin compensated for the decreased tumor uptake, providing higher tumor/blood ratios. The radioactivity levels in the tumors were 1.41 ± 0.24, 0.51 ± 0.07, and 0.56 ± 0.14% ID/g, respectively, and the tumor/blood ratios were 1.51, 2.61, and 4.01, on day 1, 4, or 7, respectively ($P < 0.05$). Again, among the organs examined, the kidney had the highest level of ^{111}In-biotin radioactivity: 8.83 ± 1.81, 6.97 ± 0.70, and 5.88 ± 1.29% ID/g on days 1, 4, and 7, respectively. Scintigraphic images were consistent with the results of biodistribution data. Clear tumor images were obtained as early as 2 h after injection of ^{111}In-biotin.

Zhang et al. from the same research group as Nakamoto et al. analyzed the biodistribution of the Bt-MLS128, SA, and ^{111}In-biotin system in female BALB/c *nu/nu* mice bearing LS180 intraperitoneal tumor xenografts (20). The study involved i.v. pretreatment with Bt-MLS128 for 48 h, followed by intraperitoneal (i.p.) injection of SA. At 24 h after SA injection, ^{111}In-biotin was administered *via* i.v. or i.p. injection, and the mice were euthanized 2 h later. The data showed that i.p. injection of ^{111}In-biotin resulted in significantly higher tumor uptake (9.54 ± 3.51 *versus* 3.60 ± 0.77% ID/g, $P < 0.05$) and tumor/blood ratio (2.80 ± 1.24 *versus* 1.03 ± 0.21, $P < 0.05$) than i.v. injection. With increasing doses from 0.3 μg to 10 μg of ^{111}In-biotin, the tumor uptake of ^{111}In-biotin decreased (9.54 ± 3.51 *versus* 1.47 ± 0.30% ID/g, $P = 0.007$), but the tumor/blood ratio was not different (2.80 ± 1.24 *versus* 2.97 ± 0.44, $P > 0.5$). It is worth noting that the relative doses of the various reagents in multistep targeting are

more complicated than that of antibody in one-step targeting. Tumor uptake of the ^{125}I-SA improved with an increased dose of Bt-MLS128. However, this also provided more antibodies in circulation and slowed down the clearance of radioactivity, thus affecting the tumor/blood ratio. On the other hand, the higher dose of radiolabeled SA or biotin decreased the tumor uptake of radioactivity, which may be related to relative limited number of the binding site in tumor. At the same time, the less complex formed in circulation, the faster the clearance of radioactivity, leading to less change of the tumor/blood ratio.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

References

1. Springer G.F. *Immunoreactive T and Tn epitopes in cancer diagnosis, prognosis, and immunotherapy.* . J Mol Med. 1997;75(8):594–602. PubMed PMID: 9297627.
2. Ando H., Matsushita T., Wakitani M., Sato T., Kodama-Nishida S., Shibata K., Shitara K., Ohta S. *Mouse-human chimeric anti-Tn IgG1 induced anti-tumor activity against Jurkat cells in vitro and in vivo.* . Biol Pharm Bull. 2008;31(9):1739–44. PubMed PMID: 18758069.
3. Li Q., Anver M.R., Butcher D.O., Gildersleeve J.C. *Resolving conflicting data on expression of the Tn antigen and implications for clinical trials with cancer vaccines.* . Mol Cancer Ther. 2009;8(4):971–9. PubMed PMID: 19372570.
4. Nakamoto Y., Saga T., Sakahara H., Yao Z., Zhang M., Sato N., Zhao S., Nakada H., Yamashina I., Konishi J. *Three-step tumor imaging with biotinylated monoclonal antibody, streptavidin and 111In-DTPA-biotin.* . Nucl Med Biol. 1998;25(2):95–9. PubMed PMID: 9468022.
5. Becker J.M., Wilchek M. *Inactivation by avidin of biotin-modified bacteriophage.* . Biochim Biophys Acta. 1972;264(1):165–70. PubMed PMID: 4553809.
6. Hsu S.M., Raine L., Fanger H. *Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures.* . J Histochem Cytochem. 1981;29(4):577–80. PubMed PMID: 6166661.
7. Babino A., Oppezzo P., Bianco S., Barrios E., Berois N., Navarrete H., Osinaga E. *Tn antigen is a pre-cancerous biomarker in breast tissue and serum in n-nitrosomethylurea-induced rat mammary carcinogenesis.* . Int J Cancer. 2000;86(6):753–9. PubMed PMID: 10842187.
8. Hakomori S. *Tumor-associated carbohydrate antigens defining tumor malignancy: basis for development of anti-cancer vaccines.* . Adv Exp Med Biol. 2001;491:369–402. PubMed PMID: 14533809.
9. Kulkarni K.A., Sinha S., Katiyar S., Surolia A., Vijayan M., Suguna K. *Structural basis for the specificity of basic winged bean lectin for the Tn-antigen: a crystallographic, thermodynamic and modelling study.* . FEBS Lett. 2005;579(30):6775–80. PubMed PMID: 16310781.

10. Kurtenkov O., Klaamas K., Rittenhouse-Olson K., Vahter L., Sergejev B., Miljukhina L., Shljapnikova L. *IgG immune response to tumor-associated carbohydrate antigens (TF, Tn, alphaGal) in patients with breast cancer: impact of neoadjuvant chemotherapy and relation to the survival.* . Exp Oncol. 2005;27(2):136–40. PubMed PMID: 15995632.
11. Lescar J., Sanchez J.F., Audfray A., Coll J.L., Breton C., Mitchell E.P., Imberty A. *Structural basis for recognition of breast and colon cancer epitopes Tn antigen and Forssman disaccharide by Helix pomatia lectin.* . Glycobiology. 2007;17(10):1077–83. PubMed PMID: 17652409.
12. Dausset J., Moullec J., Bernard J. *Acquired hemolytic anemia with polyagglutinability of red blood cells due to a new factor present in normal human serum (Anti-Tn).* . Blood. 1959;14:1079–93. PubMed PMID: 13814231.
13. Springer G.F., Desai P.R., Spencer B.D., Tegtmeyer H., Carlstedt S.C., Scanlon E.F. *T/Tn antigen vaccine is effective and safe in preventing recurrence of advanced breast carcinoma.* . Cancer Detect Prev. 1995;19(4):374–80. PubMed PMID: 7553680.
14. Slovin S.F., Ragupathi G., Musselli C., Olkiewicz K., Verbel D., Kuduk S.D., Schwarz J.B., Sames D., Danishefsky S., Livingston P.O., Scher H.I. *Fully synthetic carbohydrate-based vaccines in biochemically relapsed prostate cancer: clinical trial results with alpha-N-acetylgalactosamine-O-serine/threonine conjugate vaccine.* . J Clin Oncol. 2003;21(23):4292–8. PubMed PMID: 14645418.
15. Cipolla L., Rescigno M., Leone A., Peri F., La Ferla B., Nicotra F. *Novel Tn antigen-containing neoglycopeptides: synthesis and evaluation as anti tumor vaccines.* . Bioorg Med Chem. 2002;10(5):1639–46. PubMed PMID: 11886825.
16. Takahashi H.K., Metoki R., Hakomori S. *Immunoglobulin G3 monoclonal antibody directed to Tn antigen (tumor-associated alpha-N-acetylgalactosaminyl epitope) that does not cross-react with blood group A antigen.* . Cancer Res. 1988;48(15):4361–7. PubMed PMID: 3390832.
17. Yao Z., Sakahara H., Zhang M., Kobayashi H., Nakada H., Yamashina I., Konishi J. *Radioimmunoimaging of colon cancer xenografts with anti-Tn monoclonal antibody.* . Nucl Med Biol. 1995;22(2):199–203. PubMed PMID: 7767313.
18. Yao Z., Zhang M., Sakahara H., Saga T., Kobayashi H., Nakamoto Y., Toyama S., Konishi J. *Increased streptavidin uptake in tumors pretargeted with biotinylated antibody using a conjugate of streptavidin-fab fragment.* . Nucl Med Biol. 1998;25(6):557–60. PubMed PMID: 9751423.
19. Zhang M., Yao Z., Saga T., Sakahara H., Nakamoto Y., Sato N., Nakada H., Yamashina I., Konishi J. *Improved intratumoral penetration of radiolabeled streptavidin in intraperitoneal tumors pretargeted with biotinylated antibody.* . J Nucl Med. 1998;39(1):30–3. PubMed PMID: 9443734.
20. Zhang M., Yao Z., Sakahara H., Saga T., Nakamoto Y., Sato N., Zhao S., Nakada H., Yamashina I., Konishi J. *Effect of administration route and dose of streptavidin or biotin on the tumor uptake of radioactivity in intraperitoneal tumor with multistep targeting.* . Nucl Med Biol. 1998;25(2):101–5. PubMed PMID: 9468023.
21. Numata Y., Nakada H., Fukui S., Kitagawa H., Ozaki K., Inoue M., Kawasaki T., Funakoshi I., Yamashina I. *A monoclonal antibody directed to Tn antigen.* . Biochem Biophys Res Commun. 1990;170(3):981–5. PubMed PMID: 2390097.
22. Oppezzo P., Osinaga E., Tello D., Bay S., Cantacuzene D., Irigoin F., Ferreira A., Roseto A., Cayota A., Alzari P., Pritsch O. *Production and functional characterization of two mouse/human chimeric antibodies with specificity for the tumor-associated Tn-antigen.* . Hybridoma. 2000;19(3):229–39. PubMed PMID: 10952411.
23. Yao Z., Zhang M., Kobayashi H., Sakahara H., Nakada H., Yamashina I., Konishi J. *Improved targeting of radiolabeled streptavidin in tumors pretargeted with biotinylated monoclonal antibodies through an avidin chase.* . J Nucl Med. 1995;36(5):837–41. PubMed PMID: 7738661.
24. Zhang M., Sakahara H., Yao Z., Saga T., Nakamoto Y., Sato N., Nakada H., Yamashina I., Konishi J. *Intravenous avidin chase improved localization of radiolabeled streptavidin in intraperitoneal xenograft pretargeted with biotinylated antibody.* . Nucl Med Biol. 1997;24(1):61–4. PubMed PMID: 9080476.