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NLM Citation: Shan L. L36-Cy5 anti-laminin trimerbody. 2009 Jun 11 [Updated 2009 Aug 18]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.
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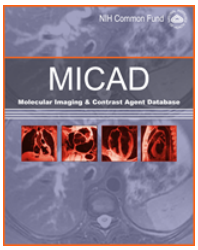

L36-Cy5 anti-laminin trimerbody

L36-Cy5 anti-laminin trimerbody

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Created: June 11, 2009; Updated: August 18, 2009.

Chemical name:	L36-Cy5 anti-laminin trimerbody	
Abbreviated name:		
Synonym:		
Agent Category:	Antibody	
Target:	Laminin	
Target Category:	Antigen	
Method of detection:	Near-infrared fluorescence (NIR) imaging	
Source of signal / contrast:	Cy5	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure available.

Background

[PubMed]

The laminin family of glycoproteins is a major constituent of both epithelial and vascular basement membranes (BMs) (1, 2). L36-Cy5 anti-laminin trimerbody is an anti-laminin single-chain variable fragment (scFv) trimerbody labeled with the fluorescent reporter Cy5, and it has been developed for optical imaging of solid tumors (3, 4).

Monoclonal antibodies (mAbs) and antibody fragments have been used for tumor targeting for many years. An ideal tumor-targeting antibody should fulfill several requirements: rapid tissue penetration, high target retention, and rapid blood clearance (5, 6). The format and molecular weight of an antibody are critical factors that influence its pharmacokinetics and *in vivo* efficacy (5-7). Intact mAbs (~150 kDa) display low blood clearance and incomplete tumor penetration. The production yield of mAbs is limited (5). These disadvantages of mAbs are partially overcome by developing recombinant scFv antibody fragments. Monovalent scFv (25–30 kDa) represents the smallest functional component of an antibody, and it is more efficient in tumor penetration. However, the scFv fragment is cleared from blood too rapidly, and it has poor binding affinity and tumor retention. Bivalent antibodies such as diabodies (55–60 kDa) and minibodies (~80 kDa) possess more ideal

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tumor-targeting characteristics such as compact size and higher binding affinity than monovalent scFv (6). Diabodies are dimeric molecules consisting of two scFv fragments connected with a short linker. Minibodies are formed by the fusion of scFv fragments with the IgG1 CH3 domain. Improved affinity of the bivalent antibodies relies on binding and rebinding, as well as simultaneous binding to different molecules of the antigen (3, 4). An issue is that it will lead to small gains in functional affinity if simultaneous multiple binding is not sterically possible. Taking the advantages of collagen XVIII NC1 domain and scFv fragments, Sanchez-Arévalo Lobo et al. developed a multivalent antibody, termed "trimerbody," for *in vivo* tumor targeting (4). The NC1 domain of collagen XVIII consists of three functionally different subdomains: an N-terminal noncovalent trimerization subdomain implicated in self-assembly of homotrimers, a central protease-sensitive hinge region, and a compact C-terminal endostatin globular subdomain (3, 4, 8). The trimerbody is designed and constructed by connecting scFv fragments to the N-terminal trimerization subdomains of NC1 through flexible linkers. The resulting trimerbody has a tripod-shaped structure with three scFv heads oriented radially outward. The artificial linker between the N-terminal scFv and NC1 domain consisted of 21 amino acids. The maximal length of the linker is 79.8 Å if the conformation is fully extended, which allows the scFv heads to be highly flexible and provides numerous binding geometries (3, 4).

Based on the concept of trimerbody design, Cuesta et al. constructed a fluorescently labeled L36-Cy5 anti-laminin trimerbody (3). The laminin family (15 members and 12 known chain genes to date) plays an important role in cell adhesion, migration, and angiogenesis (1, 9). Laminins interact with endothelial cells through integrin or non-integrin receptor families and modulate their behavior during angiogenesis (10, 11). Knockout of laminin-8 α4 chain is characterized by abnormal blood vessel maturation (11). Deregulation of the cell–laminin interactions is associated with tumor invasion (2, 12). Several laminin members are highly expressed in the tumor blood vessels (13). A switch from β2-containing to β1-containing vascular laminins has been found during the progression of breast and brain tumors, which may constitute a general feature of vascular BM changes in solid tumors (13, 14). The β1 chains of laminin-2, laminin-8, and laminin-10 have been detected in newly formed tumor vessels and are considered to be a potential target for antiangiogenic therapy (13, 15). Sanchez-Arévalo Lobo et al. demonstrated that blocking the cell binding sites of laminins with anti-laminin monovalent scFv fragment (L36) or trimerbody inhibited the tumor angiogenesis and growth *in vivo* (4). The epitope recognized by L36 is located in a highly flexible area in the middle part of the triple coiled-coil laminin domain, which interacts with the $\alpha_2\beta_1$ integrin on the endothelial cell surface (16). With optical imaging, Cuesta et al. further demonstrated that the L36-Cy5 anti-laminin trimerbody was well localized in both fibrosarcomas and adenocarcinomas after systemic administration to the mice bearing the tumor xenografts (3).

Based on the same design concept, the trimerbody targeting the carcinoembryonic antigen (CEA) MFE23-Cy5 anti-CEA trimerbody was also constructed and analyzed for its feasibility in imaging CEA-positive tumors (3).

Synthesis

[PubMed]

The synthesis of the L36-Cy5 anti-laminin trimerbody and B1.8-Cy5 anti-hapten 4-hydroxy-5-iodo-3-nitrophenyl (anti-NIP) trimerbody (used as control) was described in detail by Cuesta et al. and Sanchez-Arévalo Lobo et al., separately (3, 4). Briefly, the NC1 sequence was first amplified from the mouse α1(XVIII) clone mc3b and ligated into the plasmid pCR3.1-L36 (containing the L36 scFv antibody gene) to obtain the plasmid pCR3.1-L36-NC1^{ES+}. A flexible linker was then ligated into the pCR3.1-L36-NC1^{ES+}, resulting in pCR3.1-L36-linker-NC1^{ES+}. To construct the expression plasmid pCR3.1-L36-NC1^{ES-}, a plasmid pCR3.1-NC1 was first constructed by removing the ClaI-BglII fragment from the plasmid pCR3.1-L36-linker-NC1^{ES+}, and by inserting an oligonucleotide containing an NruI site. The NC1 trimerization subdomain was then amplified from the plasmid pCR3.1-NC1 and ligated into the backbone of plasmid pCR3.1-L36, to obtain the pCR3.1-L36-NC1^{ES-}. To construct the B1.8 anti-NIP trimerbody expression plasmid, the NC1 trimerization subdomain (a

252-bp fragment) was generated from the plasmid pCR3.1-L36-NC1^{ES-} and then ligated into the plasmid pCEP4-B1.8, resulting in the expression plasmid pCEP4-B1.8-NC1^{ES-}. The plasmid pCEP4-B1.8 contains the B1.8 scFv gene and the polyhistidine and c-myc epitopes. To express the trimerbodies, HEK-293 (human embryo kidney epithelia) cells were transfected with the expression plasmids pCR3.1-L36-NC1^{ES-} and pCEP4-B1.8-NC1^{ES-}, separately. Stably transfected HEK-293 cell lines were established through G418 or hygromycin B selection. The trimerbodies were isolated in a functionally active form from the serum-free conditioned medium of the transfected HEK-293 cells, and they were purified with immobilized metal affinity chromatography. Purified trimerbodies were labeled with Cy5 *N*-hydroxysuccinimide esters. The labeling ratio of Cy5/trimerbody was close to 1:1. The purity of the yielded trimerbodies was >95%. The trimeric nature of the trimerbodies was confirmed with ultracentrifugation and analytical gel filtration chromatography. The estimated molecular weight of L36 anti-laminin trimerbody was 109.3 kDa (the theoretical mass of the trimer was 111.4 kDa).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Cuesta et al. first analyzed the stability of L36 anti-laminin trimerbody by incubation of the trimerbody with mouse and human serum at 37°C for various lengths of time (3). Their data showed that L36 anti-laminin trimerbody retained 80–90% of its binding activity after 72 h of incubation. They then investigated the functionality of the trimerbodies against plastic immobilized NIP-BSA conjugates (NIP$_{10}$-BSA) and murine laminin with ELISA. Both the anti-laminin and anti-NIP trimerbodies specifically recognized the respective immobilized antigens. The anti-NIP trimerbody had at least a 100-fold increase in functional affinity to NIP$_{10}$-BSA antigen compared to its monovalent counterpart (4). Surface plasmon resonance analysis further demonstrated that the anti-NIP trimerbody had a slower dissociation than the monomeric anti-NIP scFv, consistent with multivalent binding to the antigen molecules. The detailed binding affinity data of the L36-Cy5 anti-laminin trimerbody were not reported.

Animal Studies

Rodents

[PubMed]

The potential of the L36-Cy5 anti-laminin trimerbody for *in vivo* tumor imaging was assessed in nude mice bearing human tumor xenografts (3). MKN45 (human stomach adenocarcinoma), HT1080 (human fibrosarcoma), or HeLa (human cervical adenocarcinoma) cells were implanted subcutaneously into the dorsal space of 6-week-old female Hsd:athymic nude-*Foxn1*^{nu} mice (n = 4/group). Mice were injected with 100 µl of the trimerbody in phosphate-buffered saline (5 mg/kg) through the tail vein. Whole-body fluorescence imaging showed that both anti-laminin and anti-NIP trimerbodies had a rapid renal clearance after intravenous injection, with peak signal intensity at 3 h, and no detectable signal in the bladder at 48 h after injection. Elimination of the L36-Cy5 monovalent scFv (with half-lives <15 min) was more rapid than the trimerbody, and the fluorescence was undetectable in the bladder at 24 h after injection. The L36-Cy5 anti-laminin trimerbody showed localization in all the tumors studied, regardless of tumor type. The maximum tumor uptake was observed at 24 h after injection. The L36-Cy5 monovalent scFv also showed specific tumor accumulation, but at a much lower level than that of the L36-Cy5 trimerbody. The control B1.8-Cy5 anti-NIP trimerbody showed no detectable localization in any of the three tumor types. The detailed biodistribution of the L36-Cy5 anti-laminin trimerbody in blood and other organs was not reported. No blocking experiment was performed.

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.

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