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NLM Citation: Shan L. MFE23-Cy5 anti-carcinoembryonic antigen 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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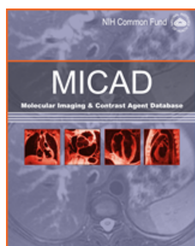

MFE23-Cy5 anti-carcinoembryonic antigen trimerbody

MFE23-Cy5 anti-CEA trimerbody

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

Chemical name:	MFE23-Cy5 anti-carcinoembryonic antigen trimerbody	
Abbreviated name:	MFE23-Cy5 anti-CEA trimerbody	
Synonym:		
Agent Category:	Antibody	
Target:	Carcinoembryonic antigen (CEA)	
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]

Carcinoembryonic antigen (CEA) is a marker of tumor prognosis, staging, recurrence, metastasis, and treatment response. MFE23-Cy5 anti-CEA trimerbody is an anti-CEA single-chain variable fragment (scFv) trimerbody labeled with the fluorescent reporter Cy5, and it has been developed for optical imaging of CEA-positive tumors (1).

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 (2, 3). The format and molecular weight of an antibody are critical factors that influence its pharmacokinetics and *in vivo* efficacy (2-4). Intact mAbs (~150 kDa) display low blood clearance and incomplete tumor penetration. The production yield of mAbs is limited (2). 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

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retention. Bivalent antibodies such as diabodies (55–60 kDa) and minibodies (~80 kDa) possess more ideal tumor-targeting characteristics such as compact size and higher binding affinity than monovalent scFv (3). 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 (1, 5). 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 (5). 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 (1, 5, 6). 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 (1, 5). Based on the concept of the trimerbody construction, Cuesta et al. constructed a fluorescently labeled MFE23-Cy5 anti-CEA trimerbody (1). CEA is a 180-kDa glycoprotein, and it is involved in cell adhesion and is localized in the apical surface of mature enterocytes (7). In a majority of cancers from the colorectum, stomach, pancreas, breast, and lung, as well as several other carcinomas of epithelial origin, CEA is overexpressed and has been used as a marker of tumor prognosis, staging, recurrence, metastasis, and treatment response (8-12). With optical imaging, Cuesta et al. demonstrated that the MFE23-Cy5 anti-CEA trimerbody was well localized the tumors after systemic administration to the mice bearing CEA-positive tumor xenografts (1).

Based on the same design concept, the trimerbody targeting the laminin epitope (L36-Cy5 anti-laminin trimerbody) was also constructed and analyzed for its feasibility in imaging fibrosarcomas and adenocarcinomas (1, 5).

Synthesis

[PubMed]

The synthesis of the MFE23-Cy5 anti-CEA 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 (1, 5). The plasmids of pCEP4-B1.8, pCR3.1-L36-NC1^{ES-}, and pVOM1.C23 were used to construct the trimerbody expression vectors. Plasmid pCEP4-B1.8 contains the B1.8 anti-NIP scFv gene and the polyhistidine and c-Myc epitopes. pCR3.1-L36-NC1^{ES-} contains the L36 anti-laminin scFv gene and the NC1 trimerization subdomain. The plasmid pVOM1.C23 contains the MFE23 anti-human CEA scFv gene. The MFE23 expression cassette from the plasmid pVOM1.C23 was subcloned into the vector pCEP4.6xHis-Myc, resulting in the plasmid of pCEP4-MFE23. The NC1 trimerization domain (a 252-bp fragment) was first amplified from the plasmid pCR3.1-L36-NC1^{ES-} and then ligated into the plasmids pCEP4-MFE23 and pCEP4-B1.8, resulting in the expression plasmids pCEP4-MFE23-NC1^{ES-} and pCEP4-B1.8-NC1^{ES-}, respectively. To express the trimerbodies, HEK-293 (human embryo kidney epithelia) cells were transfected with the expression plasmids pCEP4-MFE23-NC1^{ES-} and pCEP4-B1.8-NC1^{ES-}, separately. Stably transfected HEK-293 cell lines were established through hygromycin B selection. The trimerbodies were isolated in a functionally active form from the serum-free conditioned medium of the transfected HEK293 cells, and they were purified with immobilized metal affinity chromatography. Purified trimerbodies were labeled with Cy5 *N*-hydroxysuccinimde 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 molecular weights of MFE23-Cy5 anti-CEA trimerbody and B1.8-Cy5 anti-NIP trimerbody were not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The functionality of the trimerbodies was investigated against plastic immobilized NIP-BSA conjugates (NIP_{10}-BSA), human CEA, and the CEA antigen in a cellular context with ELISA and flow cytometry (1, 5). The results demonstrated that the trimerbodies recognized not only immobilized antigen but also native antigen on the surface of tumor cells. Positive staining with MFE-23 trimerbody was observed for the CEA-positive $HeLa^{CEA}$ (human cervix carcinoma) cells but not for CEA-negative HeLa cells. Staining with B1.8 trimerbody revealed no specific signal for either CEA-positive or CEA-negative cells. Surface plasmon resonance analysis demonstrated that the anti-NIP trimerbody had a higher binding signal and slower dissociation than the corresponding monomeric antibody, consistent with multivalent binding to the antigen. The anti-NIP trimerbody had at least a 100-fold increase in functional affinity to NIP_{10}-BSA antigen compared with its monovalent counterpart. The detailed binding affinity and *in vitro* stability of the anti-CEA trimerbody were not reported.

Animal Studies

Rodents

[PubMed]

The potential of the MFE23-Cy5 anti-CEA trimerbody for *in vivo* targeting was assessed in nude mice bearing human tumor xenografts (1). MKN45 (human stomach adenocarcinoma), HT1080 (human fibrosarcoma), or HeLa 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 Cy5-labeled trimerbody in phosphate-buffered saline (5 mg/kg) through the tail vein. Optical imaging showed that the MFE23-Cy5 anti-CEA trimerbody localized rapidly and specifically to CEA-positive MKN45 tumor xenografts. The tumor uptake reached a maximum at 3 h after injection and slowly washed out over time. The fluorescence was still detectable in the tumors at 48 h after injection. The control B1.8-Cy5 anti-NIP trimerbody showed no detectable localization in any of the three tumor types. The biodistribution of the MFE23-Cy5 anti-CEA trimerbody 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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