^{68}Ga-Labeled anti-EpCAM diabody against epithelial cell adhesion molecule

^{68}Ga-HBED-CC-anti-EpCAM diabody

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Chemical name:	^{68}Ga-Labeled anti-EpCAM diabody against epithelial cell adhesion molecule	
Abbreviated name:	^{68}Ga-HBED-CC-anti-EpCAM diabody	
Synonym:		
Agent category:	Affibody, antibody fragment	
Target:	Epithelial cell adhesion molecule (EpCAM)	
Target category:	Adhesion molecule	
Method of detection:	Positron emission tomography (PET)	
Source of signal:	^{68}Ga	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about EpCAM.

Background

[PubMed]

Epithelial cell adhesion molecules (EpCAM) are found on the cell surface of epithelial cells of many epithelial tissues such as the pancreas, jejunum, colon, kidney, salivary gland, and prostate (1). EpCAM is responsible for intracellular signaling and polarity, and it mediates cell differentiation, proliferation, migration, and adhesion (2, 3). It is a pan-epithelial differentiation antigen that is expressed on almost all carcinomas (4).

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Approximately 70% of human prostate cancers show high levels of EpCAM expression (5, 6).

Single-chain variable fragments (scFvs) of antibodies with a molecular mass of 25 kDa are cleared very rapidly from the circulation, but they exhibit poor tumor retention because they have a lower affinity than the parent antibody (7). On the other hand, bivalent antibody fragments possess more ideal tumor-targeting characteristics, including rapid tissue penetration, high target retention, and rapid blood clearance. The diabody fragment (a dimer of scFvs; molecular mass, 55 kDa) has been evaluated for targeting in several tumor antigen systems and has demonstrated rapid tumor localization and high-contrast imaging (7, 8). In particular, human IgG^{42} anti-EpCAM (clone 42 ($huIgG^{42}$), ~150kDa) was isolated from a human naïve antibody library. The monomeric $scFv^{42}{}_{18}$ (clone 42 with 18 amino acid linker), dimeric $scFv^{42}{}_{9}$ (diabody), and trimeric $scFv^{42}{}_{0}$ (tribody) were genetically constructed. These fragments retain excellent EpCAM-binding properties (dissociation constant (K_d) = 0.24–11.8 nM) and were developed as EpCAM imaging agents. They were conjugated with the bifunctional chelating agent *N,N'*-bis[2-hydroxy-5-(carboxyethyl)benzyl]ethylenediamine-*N,N'*-diacetic acid (HBED-CC) to enable labeling with ^{68}Ga for imaging of EpCAM expression in solid tumors (9).

Related Resource Links:

- Chapters in MICAD
- Gene information in NCBI (EpCAM)
- Articles in OMIM
- Clinical trials (EpCAM)
- Drug information in FDA (EpCAM)

Synthesis

[PubMed]

All antibody variants were conjugated with HBED-CC with ~1 HBED-CC per antibody molecule (9). The conjugates were incubated with $^{68}Ga^{3+}$ for 5 min at pH 4.2 at room temperature. Radiochemical yields were >90% with specific activities of 20–36 GBq/μmol (0.7–1.0 Ci/μmol) for all variants at the end of purification. ^{68}Ga-HBED-CC-$scFv^{42}{}_{18}$, ^{68}Ga-HBED-CC-diabody, ^{68}Ga-HBED-CC-tribody, and ^{68}Ga-HBED-CC-$huIgG^{42}$ were stable in 50% human serum for 4 h at 37°C.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Eder et al. (9) performed binding experiments with the antibody variants using a Biacore sensor chip immobilized with EpCAM-Fc. The K_d values were 11.8, 0.24, and 0.29 for $scFv^{42}{}_{18}$, dimeric $scFv^{42}{}_{9}$ (diabody), and trimeric $scFv^{42}{}_{0}$ (tribody), respectively. Binding specificity tests *in vitro* showed that binding of ^{68}Ga-labeled antibody variants to HT-29

cells expressing EpCAM was receptor-mediated because saturation of receptors by preincubation with a non-labeled antibody variant significantly decreased binding of the corresponding antibody variant. The 50% inhibition concentrations were 58.8, 9.2, 4.8, and 6.7 nM for scFv$^{42}_{18}$, diabody, tribody, and huIgG^{42}, respectively.

Animal Studies

Rodents

[PubMed]

Eder et al. (9) performed *ex vivo* biodistribution studies of ^{68}Ga-HBED-CC-scFv$^{42}_{18}$, ^{68}Ga-HBED-CC-diabody, ^{68}Ga-HBED-CC-tribody, and ^{68}Ga-HBED-CC-huIgG^{42} in nude mice (n = 3/group) bearing HT-29 xenografts after injection of 1–2 MBq (0.027–0.54 mCi) per mouse. The tumor radioactivity was 0.62%, 1.87%, 3.01%, and 3.54% injected dose/gram (ID/g), respectively, at 1 h after injection. There was a rapid renal clearance from blood for the antibody variants. The diabody exhibited the highest kidney radioactivity of 122% ID/g at 1 h. The radioactivity level of the diabody in the blood, lung, heart, intestine, spleen, muscle, and bone was low (<3% ID/g) at 2–3 h after injection. The full-length antibody and tribody revealed high radioactivity levels in the blood, lung, liver, kidney, heart, and spleen at 1-3 h. The diabody showed the best tumor/blood ratio of ~2 at 3 h after injection. In another experiment, tumor accumulation of ^{68}Ga-HBED-CC-diabody was performed in nude mice (n = 3) bearing HT-29 (high EpCAM expression) and LoVo (low EpCAM expression) xenografts. The accumulation in the HT-29 tumor (1.87 ± 0.50% ID/g) was significantly higher (P < 0.05) than the accumulation in the LoVo tumor (0.80 ± 0.40% ID/g). PET imaging was performed in nude mice bearing A-431 tumors. The tumors and kidneys were clearly visualized at 1 h after injection of ^{68}Ga-HBED-CC-diabody. No blocking experiment was performed.

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.

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