Cy5.5-Anti-ephrin receptor B4 (EphB4) humanized monoclonal antibody hAb47

Cy5.5-hAb47

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Created: March 18, 2013; Updated: May 9, 2013.

Chemical name:	Cy5.5-Anti-ephrin receptor B4 (EphB4) humanized monoclonal antibody hAb47	
Abbreviated name:	Cy5.5-hAb47	
Synonym:		
Agent category:	Peptide	
Target:	Ephrin receptor B4 (EphB4)	
Target category:	Receptor	
Method of detection:	Optical, near-infrared (NIR) fluorescence imaging	
Source of signal:	Cy5.5	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure is not available in PubChem.

Background

[PubMed]

The ephrin (Eph) receptors constitute the largest group in the receptor tyrosine kinase family (1, 2). The Eph receptors and their ligands (ephrins) mediate numerous biological processes in normal development, particularly in the nervous and cardiovascular systems (3-5). On the basis of their structures and sequence relationships, ephrins are divided into two classes: the ephrin-A class, Ephs that are anchored to the cell membrane by a glycosylphosphatidylinositol linkage, and the ephrin-B class, Ephs that are

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NLM Citation: Leung K. Cy5.5-Anti-ephrin receptor B4 (EphB4) humanized monoclonal antibody hAb47. 2013 Mar 18 [Updated 2013 May 9]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

transmembrane proteins. The Eph family of receptors is divided into two groups, EphA and EphB, on the basis of the similarity of their extracellular domain sequences and their affinities for binding ephrin-A and ephrin-B ligands. The Eph receptors transmit forward signals *via* their kinase domains and reverse signals *via* their transmembrane ephrin ligands (6). EphB–ephrin-B interactions are capable of mediating bidirectional signaling events upon cell–cell contact, either into the receptor-expressing cell as "forward signaling" or into the ligand-expressing cell as "reverse signaling" (7).

Eph-2 is expressed on arterial and activated endothelial cells, whereas EphB4 is normally expressed on venous endothelial cells and various blood cells (8). EphB4 selectively binds to ephrin-2 to promote cell signaling and angiogenesis. EphB4 has been implicated in cancer progression and in pathological forms of angiogenesis. Overexpression of EphB4 has been observed in cancer cells and is associated with tumorigenesis *via* forward signaling and with angiogenesis *via* reverse signaling through Eph-2 interaction (9). EphB4 forward signaling stimulates cellular proliferation. Koolpe et al. (10) identified a 15-mer peptide, Tyr-Asn-Tyr-Leu-Phe-Ser-Pro-Asn-Gly-Pro-Ile-Ala-Arg-Ala-Trp (TNYL-RAW), to be a selective antagonist of EphB4 with the use of phage display screening. Xiong et al. (11) reported the development of ^{64}Cu-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid-TNYL-RAW (^{64}Cu-DOTA-TNYL-RAW) for use with positron emission tomography (PET) imaging of EphB4 in nude mice bearing tumor xenografts. A monoclonal antibody mAb47 was found to have high affinity (0.8 nM) for the fibronectin-like extracellular domain of both human and mouse EphB4. Li et al. (12) conjugated Cy5.5 to the humanized Ab47 (hAb47) antibody to produce Cy5.5-hAb47 for use with near-infrared (NIR) fluorescence imaging of EphB4 expression in tumors.

Related Resource Links:

- Chapters in MICAD (EphB4)
- Gene information in NCBI (EphB4, Eph-B2)
- Articles in Online Mendelian Inheritance in Man (OMIM) (EphB4, Eph-B2)

Synthesis

[PubMed]

A solution of hAb47 (3.3. nmol) and Cy5.5-NHS (3.3 nmol) in borate buffer (pH 8.5) was incubated for 1.5 h at room temperature (12). Cy5.5-hAb47 was isolated with PD-10 column chromatography. Cy5.5-hIgG was prepared similarly as a control. Gel electrophoresis showed that both the heavy and the light chains of the antibodies were labeled with Cy5.5. The Cy5.5 moiety per antibody was not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Li et al. (12) performed binding experiments with Cy5.5-hAb47 immobilized with EphB4-HSA. Fluorescence signal increased with increasing amounts of Cy5.5-hAb47 (0.066–3.3 nmol). The immunoreactivity values for Cy5.5-hAB47 and Cy5.5-hIgG were 81.62 ± 2.08% and 0.024 ± 0.07%, respectively.

Animal Studies

Rodents

[PubMed]

Li et al. (12) performed *in vivo* optical imaging studies of Cy5.5-hAb47 or Cy5.5-hIgG (0.2 nmol/mouse) in nude mice ($n = 6$) bearing HT29 human colorectal tumors. Whole-body optical imaging scans were performed at 6, 24, 48, 72, 96, and 120 h after injection. Cy5.5-hAb47 exhibited a relatively low tumor fluorescence signal of ~1×10^9 photons/s/cm^2/sr at 6 h, and the signal increased to a plateau of ~2×10^9 photons/s/cm^2/sr at 48 h. Tumor accumulation of the control Cy5.5-IgG was low at 6 h (~0.6×10^9 photons/s/cm^2/sr) and reached a peak at 48 h (~0.8×10^9 photons/s/cm^2/sr). The accumulation of Cy5.5-hAb47 was significantly higher than that of Cy5.5-hIgG at all the time points studied ($P < 0.05$). *Ex vivo* imaging studies showed that the tumor fluorescence intensity of mice injected with Cy5.5-hAb47 was 0.8-fold higher than that of Cy5.5-hIgG at 120 h after injection. Of the major normal tissues, only the liver showed a moderate and similar accumulation of both tracers (~0.4×10^9 photons/s/cm^2/sr). No blocking studies were 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.

NIH Support

R21 EB012294-01a1

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