Quenched indocyanine green-anti-prostate-specific membrane antigen antibody J591

ICG-J591

Kam Leung, PhD[1]

Created: December 8, 2011; Updated: March 1, 2012.

Chemical name:	Quenched indocyanine green-anti-prostate-specific membrane antigen antibody J591	
Abbreviated name:	ICG-J591, J591-ICG	
Synonym:		
Agent category:	Antibody	
Target:	Prostate-specific membrane antigen (PSMA), or *N*-acetyl α-linked acidic dipeptidase (NAALADase)	
Target category:	Antigen	
Method of detection:	Optical, near-infrared fluorescence (NIR) imaging	
Source of signal:	Indocyanine green (ICG)	
Activation:	Yes	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about PSMA.

Background

[PubMed]

Optical fluorescence imaging is increasingly used to monitor biological functions of specific targets in small animals (1-3). However, the intrinsic fluorescence of biomolecules poses a problem when fluorophores that absorb visible light (350–700 nm) are used. Near-

[1] National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; Email: micad@ncbi.nlm.nih.gov.

Corresponding author.

NLM Citation: Leung K. Quenched indocyanine green-anti-prostate-specific membrane antigen antibody J591. 2011 Dec 8 [Updated 2012 Mar 1]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

infrared (NIR) fluorescence (700–1,000 nm) detection avoids the natural background fluorescence interference of biomolecules, providing a high contrast between target and background tissues. NIR fluorophores have wider dynamic range and minimal background fluorescence as a result of reduced scattering compared with visible fluorescence detection. They also have high sensitivity, resulting from low background fluorescence, and high extinction coefficients, which provide high quantum yields. The NIR region is also compatible with solid-state optical components, such as diode lasers and silicon detectors. NIR fluorescence imaging is a noninvasive alternative to radionuclide imaging in small animals or with probes in close proximity to the target in humans (4). Among the various optical imaging agents, only indocyanine green (ICG), with NIR fluorescence absorption at 780 nm and emission at 820 nm, is approved by the United States Food and Drug Administration for clinical applications in angiography, blood flow evaluation, and liver function assessment. It is also under evaluation in several clinical trials for other applications, such as optical imaging and mapping of both the lymphatic vessels and lymph nodes in cancer patients for surgical dissection of tumor cells and endoscopic imaging of the pancreas and colon.

Prostate-specific membrane antigen (PSMA) is a cell-surface glycoprotein with a molecular weight of ~100 kDa. It is a unique, type II, transmembrane-bound glycoprotein that is overexpressed on prostate tumor cells and in the neovasculature of most solid prostate tumors but not in the vasculature of normal tissues (5, 6). This unique expression of PSMA makes it an important biomarker as well as a large extracellular target of imaging agents (7, 8). PSMA has also been detected in other tissues such as the kidneys, the proximal small intestine, and the salivary glands (6). PSMA was found to have *N*-acetyl α-linked acidic dipeptidase (NAALADase) or glutamate carboxypeptidase II activity (9). PSMA may play an important role in the progression of prostate cancer and glutamatergic neurotransmission, as well as in the absorption of folate (10). In the central nervous system, PSMA metabolizes *N*-acetyl-aspartyl-glutamate, and in the proximal small intestine it removes γ-linked glutamates from poly-γ-glutamate folate and folate hydrolase (6). PSMA can be used as a marker for the detection of metastatic cancers with imaging agents. Although the commercially available monoclonal antibody ^{111}In-labeled Capromomab pendetide (^{111}In-CYT-356) is in clinical use for the detection of prostate cancer, the results obtained with this antibody are not entirely reliable (11). In addition, this antibody has limited access to tumors and may produce low signal/noise ratios because the target is the intracellular domain of PSMA (12, 13). J591, a humanized monoclonal antibody against the extracellular domain of PSMA (14), was conjugated to QD655 (QD655-J591) and was found to accumulate in a human prostate cancer cell line *in vitro* and in nude mice (15). However, QD655-J591 has suboptimal tissue penetration and autofluorescence from the skin. To improve this approach, Shi et al. (16) used an anti-PSMA antibody J591conjugate with QDs (QD800-J591), which emit light in the NIR 800-nm range.

ICG loses its fluorescence upon conjugation to proteins (17). Ogawa et al. (18) utilized this unique property of ICG to create activatable optical probes with ICG-antibody conjugates. ICG (and thus fluorescence) was released from the targeting antibodies after

cell-binding and internalization. Nakajima et al. (19) prepared quenched ICG-anti-PSMA antibody J591 (ICG-J591) for *in vivo* NIR imaging of PSMA-positive tumors in mice.

Related Resource Links:

- Chapters in MICAD (PSMA, J591)
- Gene information in NCBI (PSMA)
- Articles in Online Mendelian Inheritance in Man (OMIM) (PSMA)
- Clinical trials (J591, ICG)
- Drug information in FDA (J591, ICG)

Synthesis

[PubMed]

J591 (6.2 nmol) was incubated with ICG-sulfo-OSu (50 nmol) in Na_2HPO_4 buffer (pH 8.5) for 30 min at room temperature (19). ICG-J591 was purified with column chromatography. ICG-Trastuzumab (ICG-Tra) was similarly prepared. J591 was also conjugated with IR700DX N-hydroxysuccinimide ester. The number of ICG per antibody molecule was ~1 and ~3 for IR700. The immunoreactivity of ICG-J591 was 69% compared with 76% for J591. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) demonstrated that 70% of ICG was covalently linked to the antibody. Fluorescence intensity was low for both ICG-J591 and ICG-Tra in their native states, whereas exposure to 1% SDS showed an 18-fold and 15-fold increase in fluorescence intensity, respectively.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

LNCaP human prostate cancer cells were reported to have a binding affinity (K_d) of 1.8 nM with ^{131}I-labeled J591 and a B_{max} of 600,000–800,000 sites/cell in a saturation binding assay (14). Using a PSMA-transfected PC3 cell line (PC3-PSMA$^+$) and a control cell line (PC3-PSMA$^-$), saturation binding studies showed that ^{125}I-labeled ICG-J591 and ^{125}I-labeled J951 exhibited a similar K_d value of 2.8 nM with ~2×10^6 binding sites/PC3-PSMA$^+$ cell. Fluorescence microscopy and flow cytometry showed that IR700-J591 binding strongly to PC3-PSMA$^+$ cells and only weakly to PC3-PSMA$^-$ cells (19). The binding of IR700-J591 to PC3-PSMA$^+$ cells was blocked by 100-fold excess of J591, whereas little inhibition was observed in PC3-PSMA$^-$ cells.

Animal Studies

Rodents

[PubMed]

Tumor-imaging studies of ICG-J591 were performed in nude mice (the number of mice was not reported) bearing PC3-PSMA$^+$ and PC3-PSMA$^-$ tumors on opposite dorsum (19). Whole-body fluorescence images were obtained at 0.25, 1, 2, 3, 4, 6, and 10 d after intravenous injection of ICG-J591 (0.04, 0.08, 0.16, and 0.32 nmol/mouse). Maximum NIR fluorescence signals (dose-dependent) in PC3-PSMA$^+$ tumors were obtained at 1 day after injection for the four doses. There were only weak signals in PC3-PSMA$^-$ tumors. The signal intensity ratio of PC3-PSMA$^+$ to PC3-PSMA$^-$ was ~14 at 4 d after injection of 0.04 nmol ICG-J591. PC3-PSMA$^+$ tumors were clearly visualized even at 10 d after injection. No blocking studies (with unconjugated J591) were performed. Injection of 0.08 nmol ICG-Tra exhibited approximately two-fold lower fluorescence signals in 3T3HER2$^+$ tumors as compared with the same dose of ICG-J591 in PC3-PSMA$^+$ tumors. Both cell types exhibited a similar number of their respective receptors. The difference in tumor signals may be due to differences in the antibody delivery to tumors and/or the rates of internalization/activation in the tumor cells.

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.

Human Studies

Intramural Research Program

References

1. Achilefu S. *Lighting up tumors with receptor-specific optical molecular probes.* Technol Cancer Res Treat. 2004;3(4):393–409. PubMed PMID: 15270591.
2. Becker A., Hessenius C., Licha K., Ebert B., Sukowski U., Semmler W., Wiedenmann B., Grotzinger C. *Receptor-targeted optical imaging of tumors with near-infrared fluorescent ligands.* Nat Biotechnol. 2001;19(4):327–31. PubMed PMID: 11283589.
3. Ntziachristos V., Bremer C., Weissleder R. *Fluorescence imaging with near-infrared light: new technological advances that enable in vivo molecular imaging.* Eur Radiol. 2003;13(1):195–208. PubMed PMID: 12541130.

4. Tung C.H. *Fluorescent peptide probes for in vivo diagnostic imaging.* Biopolymers. 2004;76(5):391–403. PubMed PMID: 15389488.
5. Feneley M.R., Jan H., Granowska M., Mather S.J., Ellison D., Glass J., Coptcoat M., Kirby R.S., Ogden C., Oliver R.T., Badenoch D.F., Chinegwundoh F.I., Nargund V.H., Paris A.M., Britton K.E. *Imaging with prostate-specific membrane antigen (PSMA) in prostate cancer.* Prostate Cancer Prostatic Dis. 2000;3(1):47–52. PubMed PMID: 12497162.
6. Ghosh A., Heston W.D. *Tumor target prostate specific membrane antigen (PSMA) and its regulation in prostate cancer.* J Cell Biochem. 2004;91(3):528–39. PubMed PMID: 14755683.
7. Nanus, D.M., M.I. Milowsky, L. Kostakoglu, P.M. Smith-Jones, S. Vallabahajosula, S.J. Goldsmith, and N.H. Bander, *Clinical use of monoclonal antibody HuJ591 therapy: targeting prostate specific membrane antigen.* J Urol, 2003**170**(6 Pt 2): p. S84-8; discussion S88-9.
8. Nargund V., Al Hashmi D., Kumar P., Gordon S., Otitie U., Ellison D., Carroll M., Baithun S., Britton K.E. *Imaging with radiolabelled monoclonal antibody (MUJ591) to prostate-specific membrane antigen in staging of clinically localized prostatic carcinoma: comparison with clinical, surgical and histological staging.* BJU Int. 2005;95(9):1232–6. PubMed PMID: 15892807.
9. Luthi-Carter R., Barczak A.K., Speno H., Coyle J.T. *Molecular characterization of human brain N-acetylated alpha-linked acidic dipeptidase (NAALADase).* J Pharmacol Exp Ther. 1998;286(2):1020–5. PubMed PMID: 9694964.
10. Yao V., Berkman C.E., Choi J.K., O'Keefe D.S., Bacich D.J. *Expression of prostate-specific membrane antigen (PSMA), increases cell folate uptake and proliferation and suggests a novel role for PSMA in the uptake of the non-polyglutamated folate, folic acid.* Prostate. 2010;70(3):305–16. PubMed PMID: 19830782.
11. Ponsky L.E., Cherullo E.E., Starkey R., Nelson D., Neumann D., Zippe C.D. *Evaluation of preoperative ProstaScint scans in the prediction of nodal disease.* Prostate Cancer Prostatic Dis. 2002;5(2):132–5. PubMed PMID: 12497003.
12. Sundaresan G., Yazaki P.J., Shively J.E., Finn R.D., Larson S.M., Raubitschek A.A., Williams L.E., Chatziioannou A.F., Gambhir S.S., Wu A.M. *124I-labeled engineered anti-CEA minibodies and diabodies allow high-contrast, antigen-specific small-animal PET imaging of xenografts in athymic mice.* J Nucl Med. 2003;44(12):1962–9. PubMed PMID: 14660722.
13. Jain R.K. *Transport of molecules, particles, and cells in solid tumors.* Annu Rev Biomed Eng. 1999;1:241–63. PubMed PMID: 11701489.
14. Smith-Jones P.M., Vallabahajosula S., Goldsmith S.J., Navarro V., Hunter C.J., Bastidas D., Bander N.H. *In vitro characterization of radiolabeled monoclonal antibodies specific for the extracellular domain of prostate-specific membrane antigen.* Cancer Res. 2000;60(18):5237–43. PubMed PMID: 11016653.
15. Gao X., Cui Y., Levenson R.M., Chung L.W., Nie S. *In vivo cancer targeting and imaging with semiconductor quantum dots.* Nat Biotechnol. 2004;22(8):969–76. PubMed PMID: 15258594.
16. Shi C., Zhu Y., Xie Z., Qian W., Hsieh C.L., Nie S., Su Y., Zhau H.E., Chung L.W. *Visualizing human prostate cancer cells in mouse skeleton using bioconjugated near-*

infrared fluorescent quantum dots. Urology. 2009;74(2):446–51. PubMed PMID: 19428067.

17. Tadatsu Y., Muguruma N., Ito S., Tadatsu M., Kusaka Y., Okamoto K., Imoto Y., Taue H., Sano S., Nagao Y. *Optimal labeling condition of antibodies available for immunofluorescence endoscopy.* J Med Invest. 2006;53(1-2):52–60. PubMed PMID: 16537996.
18. Ogawa M., Kosaka N., Choyke P.L., Kobayashi H. *In vivo molecular imaging of cancer with a quenching near-infrared fluorescent probe using conjugates of monoclonal antibodies and indocyanine green.* Cancer Res. 2009;69(4):1268–72. PubMed PMID: 19176373.
19. Nakajima T., Mitsunaga M., Bander N.H., Heston W.D., Choyke P.L., Kobayashi H. *Targeted, activatable, in vivo fluorescence imaging of prostate-specific membrane antigen (PSMA) positive tumors using the quenched humanized J591 antibody-indocyanine green (ICG) conjugate.* Bioconjug Chem. 2011;22(8):1700–5. PubMed PMID: 21740058.