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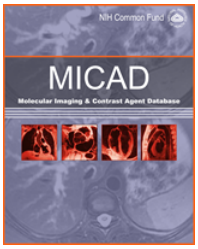

^{131}I-Labeled recombinant anti-glycoprotein A33 antibody single chain variable fragment fused to cytosine deaminase

[^{131}I]A33scFv::CDy

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Chemical name:	^{131}I-Labeled recombinant anti-glycoprotein A33 antibody single chain variable fragment fused to cytosine deaminase	
Abbreviated name:	[^{131}I]A33scFv::CDy	
Synonym:		
Agent Category:	Antibody fusion protein	
Target:	Glycoprotein A33	
Target Category:	Antigen	
Method of detection:	Single-photon emission computed tomography (SPECT); gamma planar imaging	
Source of signal / contrast:	^{131}I	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click here for protein and nucleotide sequence of human glycoprotein A33.

Background

[PubMed]

The palmotylated glycoprotein A33 (gpA33) is a cell-surface differentiation antigen that belongs to the immunoglobulin superfamily (1, 2). This antigen is expressed primarily in the normal intestine and in >95% of colon tumors, and it has not been detected in any other tissue (1, 2). Because of its presence only in the intestine or in tumors in the intestine, the gpA33 has been evaluated as a target for the detection and radioimmunotherapy of colon cancer tumors (2). The biodistribution of a radioiodinated humanized monoclonal antibody (Ab) directed against the human gpA33 antigen (huA33) is discussed in a separate MICAD chapter (3). It was reported that the labeled Ab used to treat the colon cancers bound to the entire intestinal tissue, including the tumors; however, although the radioactivity persisted in the tumors for at least 6 weeks, the label was lost from the normal gut tissue within 1 week after administration (1-3). The more rapid

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clearance of a radioiodinated huA33 from the normal intestinal tissue was observed to correspond to the turnover period of basal colonocytes in this tissue (4).

Despite the extensive use of immunotherapy to treat cancers, the efficacy of this regimen is limited by the fact that not all individuals are responsive to this therapy. This is either due to the lack of or irresponsiveness of the receptors (or antigens) to which the Abs are directed. Also, because of its large size, the Ab may not be able to penetrate solid cancerous tumors completely, remain in blood circulation for extended periods, or may not be effective due to other factors as described by von Mehren et al. (5). As an alternative to Abs, investigators have developed Ab fragments such as the single chain variable fragments (scFv), which are much smaller (~30 kDa) than intact Abs (~150 kDa), contain the antigen-binding site, exhibit high tumor penetration, generate excellent tumor/normal tissue concentration ratios, and show rapid clearance through the kidneys (6, 7). Several scFv fragments targeted toward different antigens are under evaluation in clinical trials approved by the United States Food and Drug Administration and by similar regulatory agencies around the world (8).

To further enhance scFv efficacy for preclinical or clinical application, these protein chains have been linked, either by synthesis or recombinant methods, to a variety of molecules such as enzymes (for prodrug therapy), toxins (for treatment of cancer), etc., to generate bifunctional molecules (7, 9, 10). The scFv chain directs the enzyme or toxin to a predetermined cellular antigen where the enzyme converts a prodrug into a cytotoxic molecule or the toxin is activated and has a lethal effect on the cell. Therefore, an Ab fused to an enzyme could be used for an Ab-directed enzyme-prodrug therapy for the treatment of cancers or other diseases. On the basis of this principle, Coelho et al. fused a recombinant scFv directed against the A33 antigen (A33scFv) with the yeast cytosine deaminase (CDy) enzyme to generate A33scFv::CDy as a possible colon cancer treatment (6). The CDy part of the recombinant fusion protein deaminates 5-fluorocytosine (5-FC), the prodrug, to produce cytotoxic 5-fluorouracil after the A33scFv part binds to the A33 antigen on the cell surface. Therefore, the A33scFv::CDy fusion protein is selectively lethal to only those cells that overexpress the A33 antigen, i.e., colon cancer cells. Panjideh et al. investigated the binding specificity of ^{131}I-labeled A33scFv::CDy under *in vitro* conditions and also studied its biodistribution in athymic mice bearing xenograft tumors derived either from LIM 1215 cells, a gpA33-positive human colon carcinoma cell line, or the negative control HT29 cells, a gpA33-negative human colon carcinoma cell line (11).

Synthesis

[PubMed]

The production and purification of A33scFv::CDy were described by Coelho et al. (6). Purity of the recombinant fusion protein was reported to be >95% as determined with sodium dodecylsulfate polyacrylamide gel electrophoresis. The ^{131}I labeling of purified A33scFv::CDy, to obtain [^{131}I]A33scFv::CDy, was done according to the chloramine-T method as detailed by Panjideh et al. (11). The specific activity of [^{131}I]A33scFv::CDy was reported to be ~400 kBq/mg (~10.8 μCi/mg). The radiochemical yield, purity, and stability of the labeled fusion protein were not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The binding activity and specificity of A33scFv::CDy was investigated with the use of flow cytometry using LIM 1215 cells (6). Using a secondary polyclonal Ab directed toward the CDy part of the fusion protein, both the crude production supernatant and the purified fusion protein were shown to bind to the LIM 1215 cells. In another assay, the binding of A33scFv labeled with the green fluorescent protein (A33scFv::GF) to LIM 1215 cells was shown to be inhibited quantitatively by A33scFv::CDy, which suggested that the fusion protein bound specifically to the A33 antigen.

A cytotoxicity assay was performed to confirm the dual activity of A33scFv::CDy (6). LIM 1215 cells treated with A33scFv::CDy were exposed to different concentrations of 5-FC, and, with the use of appropriate controls, it was shown that 1 ng/ml of the fusion protein was sufficient for the maximum cytotoxic effect (25% cell survival) under these experimental conditions (6). Prior exposure of the cells to A33scFv::GF followed by treatment with A33scFv::CDy and 5-FC, as described above, was reported to eliminate the cytotoxic effects of the prodrug.

Using a saturation assay by exposing LIM 1215 cells to [^{131}I] A33scFv::CDy, the equilibrium dissociation constant and the binding capacity of A33scFv::CDy were determined to be 15.8 nM and 5.02 nM/2 × 10^6 cells, respectively (11).

Animal Studies

Rodents

[PubMed]

To show the specificity of [^{131}I]A33scFv::CDy binding to the A33 antigens in tumors, athymic mice (n = 4 animals/group) bearing either LIM 1215 cell (gpA33-positive) or HT29 cell (gpA33-negative) tumors were injected through the tail vein with the radiolabeled fusion protein (11). The animals were euthanized 47 h after treatment, and the tumors were removed to determine the amount of accumulated radioactivity. The LIM 1215 tumors were reported to accumulate ~90% of the injected dose/gram of tissue (% ID/g) compared with ~10% ID/g accumulated by the HT29 cell tumors. This indicated that the labeled fusion protein had a high binding specificity for cells expressing the A33 antigen.

The biodistribution of [^{131}I]A33scFv::CDy was studied in athymic mice bearing LIM 1215 cell tumors (11). The animals were injected with [^{131}I]A33scFv::CDy through the tail vein and euthanized at different time points (n = 4 animals/ time point) between 6 and 96 h after treatment. The various organs, including the tumors, were harvested and counted for accumulated radioactivity. By 47 h after injection the tumors were reported to accumulate almost 90% ID/g of the label, which decreased to ~20% ID/g by 96 h after treatment. In comparison, the other organs (liver, heart, and lungs) accumulated between ~2% ID/g and 10% ID/g during the entire period of the study. The kidneys were reported to accumulate between ~15% ID/g (at 67 and 96 h after treatment) and 70% ID/g (at 29 h after treatment), indicating that this was the label's primary route of excretion.

In another study, mice bearing either LIM 1215 or HT29 cell xenograft tumors were treated with A33scFv::CDy followed by an injection of 5-FC 47 h later (11). The tumors were monitored daily, and by day 12 the LIM 1215 tumors were reported to shrink significantly (P = 0.0096) compared with the HT29 tumors, which were observed to have grown bigger during the observation period.

With results obtained from the various studies, the investigators concluded that A33scFv::CDy could probably be used for radioimmunotherapy, and its use for Ab-directed enzyme-prodrug therapy had therapeutic effects in a rodent model (11).

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.

Supplemental Information

[Disclaimer]

No information is currently available.

References

1. Sakamoto J., Kojima H., Kato J., Hamashima H., Suzuki H. *Organ-specific expression of the intestinal epithelium-related antigen A33, a cell surface target for antibody-based imaging and treatment in gastrointestinal cancer.* . Cancer Chemother Pharmacol. 2000;46 Suppl:S27–32. PubMed PMID: 10950144.
2. Ackerman M.E., Chalouni C., Schmidt M.M., Raman V.V., Ritter G., Old L.J., Mellman I., Wittrup K.D. *A33 antigen displays persistent surface expression.* . Cancer Immunol Immunother. 2008;57(7):1017–27. PubMed PMID: 18236042.
3. Chopra, A., *Radioiodinated humanized monoclonal antibody A33 [Radioiodinated-huA33 mAb].* Molecular Imaging and Contrast agent Database (MICAD) [database online]. National Library of Medicine, NCBI, Bethesda, MD, USA. Available from www.micad.nih.gov, 2004 -2009.
4. Scott A.M., Lee F.T., Jones R., Hopkins W., MacGregor D., Cebon J.S., Hannah A., Chong G. P. U, A. Papenfuss, A. Rigopoulos, S. Sturrock, R. Murphy, V. Wirth, C. Murone, F.E. Smyth, S. Knight, S. Welt, G. Ritter, E. Richards, E.C. Nice, A.W. Burgess, and L.J. Old, *A phase I trial of humanized monoclonal antibody A33 in patients with colorectal carcinoma: biodistribution, pharmacokinetics, and quantitative tumor uptake.* Clin Cancer Res. 2005;11(13):4810–7. PubMed PMID: 16000578.
5. von Mehren M., Adams G.P., Weiner L.M. *Monoclonal antibody therapy for cancer.* . Annu Rev Med. 2003;54:343–69. PubMed PMID: 12525678.
6. Coelho V., Dernedde J., Petrausch U., Panjideh H., Fuchs H., Menzel C., Dubel S., Keilholz U., Thiel E., Deckert P.M. *Design, construction, and in vitro analysis of A33scFv::CDy, a recombinant fusion protein for antibody-directed enzyme prodrug therapy in colon cancer.* . Int J Oncol. 2007;31(4):951–7. PubMed PMID: 17786329.
7. Holliger P., Hudson P.J. *Engineered antibody fragments and the rise of single domains.* . Nat Biotechnol. 2005;23(9):1126–36. PubMed PMID: 16151406.
8. Hudson P.J., Souriau C. *Engineered antibodies.* . Nat Med. 2003;9(1):129–34. PubMed PMID: 12514726.
9. Delves P.J., Roitt I.M. *The immune system. First of two parts.* . N Engl J Med. 2000;343(1):37–49. PubMed PMID: 10882768.
10. Delves P.J., Roitt I.M. *The immune system. Second of two parts.* . N Engl J Med. 2000;343(2):108–17. PubMed PMID: 10891520.
11. Panjideh H., Da Silva Coelho V.C., Dernedde J., Bachran C., Forster G.J., Franke J., Fasold P., Fuchs H., Thiel E., Deckert P.M. *Biodistribution and efficacy of [131I]A33scFv::CDy, a recombinant antibody-enzyme protein for colon cancer.* . Int J Oncol. 2008;32(4):925–30. PubMed PMID: 18360720.