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Progress Report:
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SINGLE CHAIN FV CONSTRUCTS OF ANTI-GANGLIOSIDE GD2 ANTIBODIES FOR RADIOIMAGING AND RADIOIMMUNOTHERAPY

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Introduction:

OVERALL OBJECTIVE/RELATIONSHIP TO DOE'S PROGRAM/GENERAL PLAN AND APPROACH: This new application pursues our continuing long term goal of promoting nuclear medicine applications by **improving the scientific basis for tumor diagnosis, treatment and treatment follow-up based on the use of cyclotron produced radiotracers in oncology.** This proposal complements the current DOE grant (# DE-FG02-86ER60407) entitled "Improving cancer treatment with cyclotron produced radionuclides" under the direction of Steven M. Larson M.D. The nuclear medicine component of the DOE mission is aimed at enhancing the beneficial applications of radiation, radionuclides, and stable isotopes in the diagnosis, study and treatment of human diseases. This program is administered within the Medical Applications and Biophysical Component of the Office of Health and Environmental Research, Office of Energy Research, DOE.

This grant includes 3 interactive components: Molecular biology, Immunology, and Nuclear Medicine. It responds to the RFA for molecular biology applications in nuclear medicine. For the past several years, we have studied the anti-G_{D2} murine monoclonal antibody, 3F8, in radiolabeled form, for diagnosis and therapy of neuroblastoma. The targeting properties of this antibody/antigen system are exceptional, with uptakes (%dose/gram) consistently in the highest range of reported results for in vivo human studies. At MSKCC, the radioiodinated antibody 3F8 is now used by us as our criteria for diagnosis and staging of advanced neuroblastoma. This antibody is showing considerable promise also in our Phase I trials in Stage 4 neuroblastoma, and major responses are being seen at current dose level (24 mCi/kg), with manageable marrow toxicity, but no limiting organ toxicity (supported by NCI grant PO1 CA 33049). Other human tumors which express G_{D2} antigen may also be amenable to similar approaches. Recent experience in pre-phase I biodistribution and imaging trial of human small cell lung cancer, sarcoma, and brain tumor has been particularly promising in this regard.

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Main research accomplishments of the grant period from 8/1/93 to present:

1.1 Construction of ScFv Derivatives of Monoclonal Antibodies

a. Cloning of V_H and V_L Segments of Rearranged Immunoglobulin Genes

In order to construct a scFv gene, the two variable regions (V_H and V_L) cDNAs are cloned by amplification from first-strand cDNA by polymerase chain reaction (PCR). Using a panel of light chain and heavy chain primers (table 1), the variable regions of the heavy and light chains of the antibodies 3A7, and 3F8 have been amplified.

The preliminary cDNA sequence of the variable regions of the heavy and light chains of 3F8 and 3A7 have been determined.

b. Assembly and scFv

scFv genes can be efficiently made by direct assembly of the scFv gene using PCR. To assemble the gene by PCR, the linker segment is generated by overlap in two of the primers used to amplify the two variable regions. Hence, when the first two PCR fractions are mixed, one of the two possible cross-annealing products will provide 3 hydroxyl groups that will be extended by the polymerase to give a full-length scFv gene with the linker sequence joining the V_L and V_H .

1.2 Alternative metallic ligands for radiolabeling

NOTA-3F8 Using 3F8 as the prototype, the macrocyclic chelator NOTA (see diagram) was conjugated under high salt conditions to the anti-GD2 monoclonal antibody. Immunoreactivity as measured by ELISA showed minimal loss of activity (10-20%). Using ^{14}C as trace label of the chelator, the number of chelator per protein molecule was found to be 1.2. Indium-111 was then conjugated to the NOTA-3F8 at a specific activity of 0.96 mCi/mg. In vitro binding assay using solid phase GD2 showed an immunoreactivity comparable to that of ^{131}I -3F8. The immunoreactivity was unchanged after storing the labeled antibody at 4°C over a 5-day period. Nude mice xenografted with human neuroblastoma were injected with 20 uCi of the labeled antibody and used for immunoscintigraphy as well as biodistribution studies. Percent injected dose per gm tumor averaged 9% ID/gm at 2 h, 26% at 24 h, 30% at 44 h (peak), and declined to 25% at 96 h and 20% at 120 h, all corrected for physical decay of ^{111}In . Tumor to blood ratio was 10:1 at 120 h. Rest of the tissue distribution was comparable to those of ^{131}I -3F8, with the only exception of kidney which showed 6.3 %ID/gm at 120 h. Although the peak uptake was comparable to that of ^{131}I -3F8, the biological decay appeared to be slower with ^{111}In -3F8, supporting the hypothesis that in vivo dehalogenation is adversely affecting the biodistribution of ^{131}I -3F8, but not ^{111}In -3F8. Three mice injected with ^{111}In -3F8 (retroorbitally) were scanned at 2, 24, 44, and 96 hours afterwards. Intense uptake in the tumors were noted.

Table 1.

<u>Primer</u>	<u>Primer sequence</u>	<u>PCR</u>	
		<u>3F8</u>	<u>3A7</u>
MuIgV _H 5'-A	GGGAATTCATGRASTTSKGGYTMARCTKGRTTT	+	-
MuIgV _H 5'-B	GGGAATTCATGRAATGSASCTGGGYWYCTCTT	-	-
MuIgV _H 5'-C	ACTAGTCGACATGGACTCCAGGCTCAATTTAGTTTTCTT ACTAGTCGACATGGCTGTCTYTRGBGCTGYTCYTCTG ACTAGTCGACATGGVTTGGSTGTGGAMCTTGCTATTCCT	+	+
MuIgV _H 5'-D	ACTAGTCGACATGAAATGCAGCTGGRTYATSTTCTT ACTAGTCGACATGGRCAGRCTTACWYTYTCATTCTT ACTAGTCGACATGATGGTGTTAAGTCTTCTGTACCT	+	-
MuIgV _H 5'-E	ACTAGTCGACATGGGATGGAGCTRTATCATSYTCTT ACTAGTCGACATGAAGWTGTGGBTRAAGTGGRT ACTAGTCGACATGGRATGGASCKKIRTCTTTMTCT	+	+
MuIgV _H 5'-F	ACTAGTCGACATGAACTTYGGGYTSAGMTTGRTTT ACTAGTCGACATGTACTTGGGACTGAGCTGTGTAT ACTAGTCGACATGAGAGTGCTGATTCTTTTGTG ACTAGTCGACATGGATTTTGGGCTGATTTTTTTTATTG	-	-
MuIgMV _H 3'-1	CCCAAGCTTACGAGGGGGAAGACATTTGGGGAA		+
MuIgGV _H 3'-2	CCCAAGCTTCCAGGGGCCARKGGATARACIGRTGG	+	
MuIgkV _L 5'-A	GGGAATTCATGRAGWCACAKWCYCAGGTCTTT	+	+
MuIgkV _L 5'-B	GGGAATTCATGGAGACAGACACACTCCTGCTAT	+	+
MuIgkV _L 5'-C	ACTAGTCGACATGGAGWCAGACACACTSCTGYTATGGGT	+	+
MuIgkV _L 5'-D	ACTAGTCGACATGAGGRCCCTGCTCAGWTTYTTGGIWTCTT ACTAGTCGACATGGGCWTCAGATGRAGTCACAKWYYCWGG	+	+
MuIgkV _L 5'-E	ACTAGTCGACATGAGTGTGCYCACTCAGGTCCTGGSCTT ACTAGTCGACATGTGGGGAYCGKTTTYAMMCTTTTCAATTG ACTAGTCGACATGGAAGCCCCAGCTCAGCTTCTCTTCC	+	-
MuIgkV _L 5'-F	ACTAGTCGACATGAGIMMKTCIMTTCAITTCYTGGG ACTAGTCGACATGAKGTHCYCIGCTCAGYTYCTIRG ACTAGTCGACATGGTRTCCWCASCTCAGTTCCTTG ACTAGTCGACATGTATATATGTTTGTGTCTATTTCT	+	-
MuIgkV _L 5'-G	ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCT ACTAGTCGACATGGATTTWCARGTGCAGATTWTCAGCTT ACTAGTCGACATGGTYCTYATVTCCTTGCTGTTCTGG ACTAGTCGACATGGTYCTYATVTTRCTGCTGCTATGG	+	-
MuIgkV _L 3'-1	CCCAAGCTTACTGGATGGTGGGAAGATGGA	+	+

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