Final Report

DE-FG02-05ER64001 Overcoming the hurdles of multi-step targeting (MST) for effective radioimmunotherapy of solid tumors

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There were 4 specific aims of this project and these are listed below.

Specific Aim #1: Optimization of MST to increase tumor uptake

Specific Aim #2: Antigen heterogeneity

Specific Aim #3: Characterization and reduction of renal uptake

Specific Aim #4: Validation in vivo of optimized MST targeted therapy

This proposal focussed upon optimizing multistep immune targeting strategies for the treatment of cancer. Two multi-step targeting constructs were explored during this funding period: (1) anti-Tag-72 and (2) anti-GD2.

(i) The CC49 system avid for the secreted Tag-72 antigen holds great promise for radioimmunodiagnosis and therapy of cancer because this antigen is expressed on a variety of malignancies including colorectal and pancreatic carcinomas. Pretargeting of the tetrameric single-chain using a variable-fragment streptavidin construct of the tetrameric monoclonal antibody CC49 with subsequent administration of radiolabeled 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA)-biotin was explored with an emphasis of overcoming the potential limitation of the MST approach associated with the high and prolonged renal uptake of radioactivity. Studies were conducted aimed at reducing the radiation dose to the kidney from the radiolabeled DOTA-biotin achieved by each of 4 different methods. Experiments were conducted using a human pancreatic adenocarcinoma xenograft model (HPAC) in nude mice. The animals were intravenously injected with the antibody-streptavidin construct and a synthetic clearing agent (biotinylated N-acetyl-galactosamine) 24 and 4 h, respectively, before the administration of ⁶⁷Ga-DOTA-biotin. Four strategies were employed in an attempt to reduce kidney retention of the radiolabeled biotin: (i) streptavidin saturated with biotin, (ii) several administrations of lysine (iii) several administrations of colchicine or (iv) administration of a succinylated antibody-streptavidin construct to decrease the electrical charge on the moiety. Animals were sacrificed at 24 h following ⁶⁷Ga-DOTA-biotin injection for biodistribution and quantitative autoradiography. In addition, selected animals from each study group were also imaged by microSPECT and microCT. Successful targeting of the radiolabeled DOTA-biotin was achieved in tumor bearing animals from in all groups except in antigen-negative tumor control animals. However, only succinylation of the scFv-CC49-streptavidin fusion protein significantly reduced (approximately 30%) kidney uptake without affecting the tumor uptake. QAR corroborated these results and demonstrated that radiolabeled DOTA-biotin localized selectively in the renal cortex. Among the other experimental groups, there was no change in kidney uptake of the

radiolabeled biotin. In contrast to directly labeled antibodies and antibody fragments, administration of the negatively charged amino acid lysine was largely ineffective in pretargeting strategies with a single-chain-immuno-streptavidin fusion protein. Succinylation of the scFv-CC49-streptavidin construct, on the other hand, reduces kidney uptake of subsequently administered radiolabeled biotin, presumably by inhibiting reuptake of the fusion protein in the proximal renal tubules, and, therefore, could significantly reduce renal doses and improve therapeutic indices associated with multistep immune targeting approaches to radioimmunotherapy.

(2) Anti-GD2 binding antibodies exhibit significant potential for the treatment, and are currently in clinical trials of a number of pediatric malignancies including neuroblastoma, medulloblastoma etc. For the purpose of a MST approach, an 5F11 scFv/avidin tetrameric antibody fusion protein specific for the GD2 has been engineered. The 5F11scFv (scFv = single-chain variable fragment) was constructed from the variable regions of the heavy (V_H) and κ -light (V_L) chain complementary DNA (cDNA) of anti-GD2 IgM hybridoma 5F11 and ligated to full-length streptavidin cDNA for expression in *Escherichia coli*. Purified 5F11-scFv-streptavidin (5F11-scFv-SA) was a homotetramer and showed comparable avidity to 5F11 IgM and a 30-fold improvement over monomeric scFv. Biodistribution of 5F11-scFv-SA was studied in nude mice xenografted with neuroblastoma LAN-1. Twenty-four hours after intravenous injection of 300–900 μ g 5F11-scFv-SA, 150–450 μ g of a thiogalactoside-containing clearing agent, (Gal-NAc)_{16- α} -S-C₅H₁₀-NH-LC-N-Me-biotin (molecular weight, 8,652), were administered intravenously, followed by ~ 2.5 μ g (1.85–3.7 MBq) ¹¹¹In-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-biotin (¹¹¹In-DOTA-biotin) intravenously 4 h later and clocked as time 0. Tumor uptake at 2 h was 7 %ID/g and decayed with a half-life of 72 h, whereas blood %ID/g rapidly decreased to <1/500 of that of tumor within the first 24 h. The tumor-to-nontumor (T/NT) ratio at 72 h was high (median, 106; range, 3.4 [kidney] to 1,660 [blood]). When the area under the radioactivity curve was computed, the T/NT organ ratio was favorable (4.8 for kidney and 162 for blood). When human and murine tumors were surveyed, the T/NT ratio of ¹¹¹In-DOTA-biotin uptake correlated with their levels of GD2 expression as assayed by flow cytometry.

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