OPTIMIZING THE DELIVERY OF SHORT-LIVED ALPHA PARTICLE-EMITTING ISOTOPES TO SOLID TUMORS

Final Report

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Introduction.
The underlying hypothesis of this project was that optimal alpha emitter-based radioimmunotherapy (RAIT) could be achieved by pairing the physical half-life of the radioisotope to the biological half-life of the targeting vehicle. The project has two specific aims. The first aim was to create and optimize the therapeutic efficacy of ^{211}At-SAPS-C6.5 diabody conjugates. The second aim was to develop bispecific-targeting strategies that increase the specificity and efficacy of alpha-emitter-based RAIT.

Progress.
S.A.1. Dose escalation studies were performed with increasing doses of ^{211}AT-SAPS-C6.5 diabody specific for the HER2 tumor-associated antigen. Mice bearing established s.c. human MDA-MB-361.DYT2 breast cancer xenografts were treated with a single dose ranging from 15 to 45 μCi of ^{211}AT-SAPS-C6.5 diabody. In these studies, a clear dose response was observed with the greatest anti-tumor effect associated with the highest treatment dose (Figure 1). In the 45 μCi dose group, three of five treated animals exhibited durable complete responses, with no sign of tumor regrowth at one year following threatment (Figure 2). In the dose ranges studied, effective treatment was achieved a at below the acute maximum tolerated dose. All of the mice treated with ^{211}AT-SAPS-C6.5 diabody survived the treatment with minimal observed toxicity (minor transient weight loss).

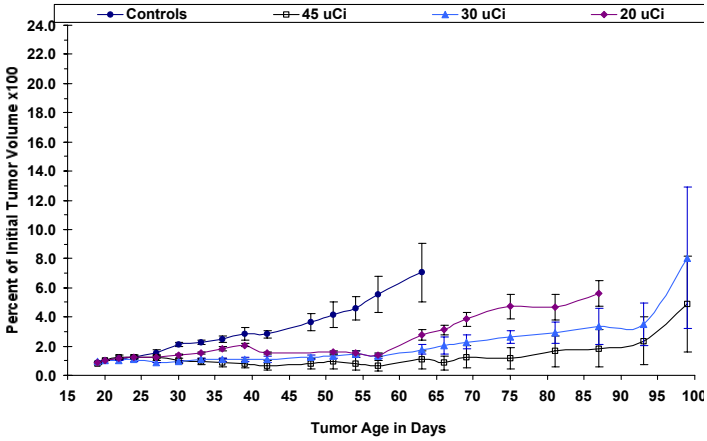
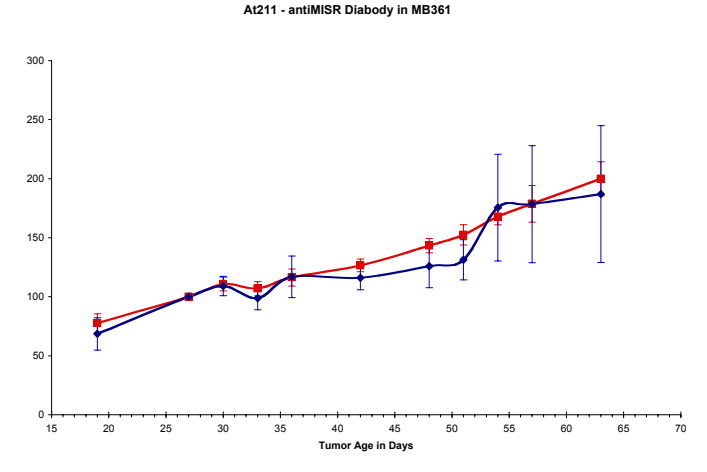


Figure 1. Mice bearing established human MDA-MB-361.DYT2 breast cancer xenografts were treated with a single dose ranging from 15 to 45 μCi of ^{211}AT-SAPS-C6.5 diabody. n = 5-7 per group. Error bars = SEM.

Figure 2. Mice bearing established human MDA-MB-361.DYT2 breast cancer xenografts were treated with a single dose of 20 μCi of ^{211}At-SAPS-control diabody or left untreated. n = 7 per group. Error bars = SEM.



Successful RAIT depends upon specificity of effect. The impact of tumor-targeting specificity on this therapy was studied by performing RAIT using a diabody that did not target the tumor xenografts. Mice bearing established s.c. human MDA-MB-361.DYT2 breast cancer xenografts were treated with a single dose of 20 μCi of ^{211}At-SAPS-control diabody (a dose that caused a significant

growth delay when the anti-HER2 diabody was employed). In these studies, the mice treated with the ^{211}At-SAPS-control diabody displayed the same tumor growth as the untreated mice (**Figure 2**), demonstrating that the anti-tumor effect observed with the anti-HER2 ^{211}At-SAPS C6.5 diabody was specific and targeted.

As outlined in the 2003 report, we observed signs that the treatment with ^{211}At-SAPS C6.5 diabody may be associated with late onset renal toxicity. To examine this, we have started an ambitious project of treating large cohorts of non-tumor bearing nude mice with 20 μCi and 45 μCi of ^{211}At-SAPS C6.5 diabody. These mice are bled every month and samples are analyzed for signs of renal toxicity (increasing BUN and serum creatinine levels), liver toxicity (raising AST and ALT levels) and marrow toxicity (decreasing platelet counts). Every three months, cohorts of three to four treated and untreated mice are euthanized and their kidneys, livers and bone marrow are examined for signs of toxicity. We are currently 9 months past the initial treatment with 20 μCi of ^{211}At-SAPS C6.5 diabody and have so far found no signs of hepatic toxicity in the blood analysis or Pathology studies, however a early signs of renal toxicity were observed in these mice. This study will be continued for three more months.

S.A.2. We have created ALM, an anti-HER2/anti-HER3 bispecific scFv molecule that co-targets both HER2 and HER3. Radiolabeled ALM is capable of specific localization in human tumor xenografts growing in scid mice as detected by biodistribution (**Figure 3**) and ImmunoPET Imaging. As part of this grant, we have successfully demonstrated that we can specifically target ALM to tumor cells that express HER2 and HER3 in vitro in the presence of cells that only express one of the two target antigens (**Table 1**). We have also found that incubation of ALM with MCF7 cells, ZR-75 cells or BT-474 cells decreases phosphorylation of AKT, a member of the PI3-kinase signal transduction pathway that makes tumor cells less likely to undergo apoptosis in response to radiation or chemotherapy. We are currently in the process of combining this bs-scFv with radiation to see if we can achieve additive effects.

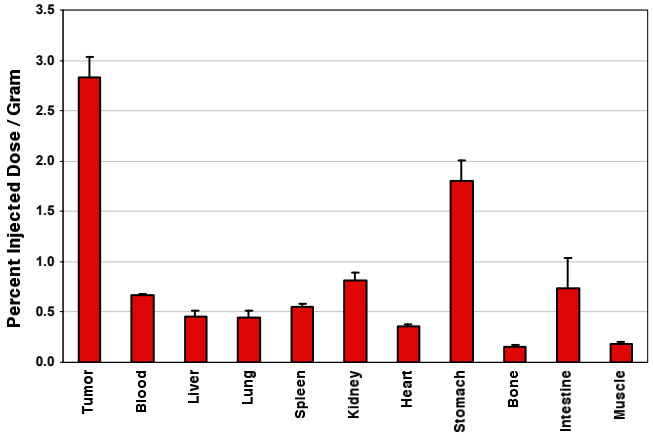


Figure 3. 24 Hr. biodistribution of ^{125}I-ALM in scid mice bearing s.c. BT-474 tumors. N=8, SEM's indicated

Table 1. Selective cellular retention of the ALM bs-scFv on BT-474 cells that express both HER2 and HER3 as compared to MVM2 cells that only express HER2.

Bs-scFv Concentration	**% positive cells**	**% positive cells***
ALM conc	**BT 474 (HER2/3 +)**	**MVM2 (HER2 +)**
1 uM	99.6	99.2
100 nM	98.6	95.5
10 nM	78.2	32.4
1 nM	23.5	3.6
10 pM	17.0	0.8
control	1.26	0.6

* Adjusted for percentage of HER2 positive Cells.

Reportables.

Robinson, M. K. , Weiner, L. M., and Adams, G. P. Improving Monoclonal Antibodies For Cancer Therapy. Drug Development Research, 61:172-187, 2004.

Oral presentation: Adams, G.P.: "Diabody-based ImmunoPET Imaging and Radioimmunotherapy of Cancer." Presented at "AntiBoz2: An International Forum to Predict the Next Wave of Protein-Based Therapies and Immunodiagnostics". (Heron Island, Australia) April 2004.

Oral presentation: Adams, G.P.: "Radioimmunotherapy with Engineered Antibody Molecules – Promise and Problems". Presented at the "Russian/American Workshop on Molecular Imaging and Radionuclide Therapy", (Philadelphia, PA) April 2004.

Oral presentation "Alpha-Emitting Radioisotopes conjugated to anti-HER2/neu diabodies for the radioimmunotherapy of solid tumors". American Chemical Society Meeting, Feb. 2003.

Oral presentation: Adams, G.P., Robinson, M.K., Horak, E., Heitner, T., Garrson, J.L., Shaller, C.C., Tesfaye, A., Simmons, H.H., Marks, J.D., Weiner, L.M. "Anti-HER2/HER3 Bispecific Single-chain Fv Molecules for Cancer Detection and Therapy." (Minisymposia Presentation) AACR. 2003, Washington D.C.

Oral presentation "Radioimmunotherapy of Solid Tumors with anti-HER2/neu Diabodies Conjugated to Alpha and Beta Particle Emitting Radioisotopes". Inaugural Russian American Scientific Conference on Radioimmunoimaging and Radioimmunotherapy. Moscow, Russia. October, 2002.

Oral Presentation. "Effective Radioimmunotherapy of Solid Tumors Using Anti-HER2/*neu* Diabodies Conjugated to Alpha Particle and Beta Particle Emitting Radioisotopes". G. P. Adams, C. Shaller, K. Garmestani, A. Tesfaye, E.M. Horak, H.H. Simmons, K. Dadachova, L.L. Chappell, C. Wu, J.D. Marks, T. Waldmann, L.M.

Weiner, and M.W. Brechbiel. Ninth Conference on Cancer Therapy with Antibodies and Immunoconjugates (Princeton, N.J.) Cancer Biotherapy & Radiopharmaceuticals, 17:481, 2002.

Oral presentation "Targeting Members of the Epidermal Growth Factor Receptor Family" at "AntibOZ", An International Forum: Predicting the Next Wave of Protein-Based Therapies and Immunodiagnostics. Heron Island, Queensland Australia. April 8-12th, 2002.

Oral presentation "Cancer Radioimmunotherapy with Engineered Antibody Fragments" at IBC's 12th Annual International Conference on Antibody Engineering (San Diego, CA), December 2001.