Final Report

for the project entitled:

Development of Reagents for Application of At-211 and Bi-213 to Targeted Radiotherapy of Cancer
Award #: DE-FG02-04ER63789

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December 22, 2011

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FINAL REPORT FOR DE-FGO2-04ER63789

"Development of Reagents for Application of At-211 and Bi-213 to Targeted Radiotherapy of Cancer.

Principal Investigator: D. Scott Wilbur, Ph.D.

I. Introduction

This grant was a one-year extension of another grant with the same title (DE-FG03-98ER62572). The objective of the studies was to continue in vivo evaluation of reagents to determine which changes in structure were most favorable for in vivo use. The specific aims were the same as in the previous research effort. Rather than provide the results from the final year of research on the continuing project, the results from the extension year are incorporated into the full funding period so that the rationale for the work is understood. The results are outlined below.

II. Research Summary.

The focus of our studies was development and optimization of reagents for pretargeting α-emitting radionuclides ^{211}At or ^{213}Bi to cancer cells. Testing of the reagents was conducted in vitro and in animal model systems. During the funding period, all three specific aims set out in the proposed studies were worked on, and some additional studies directed at development of a method for direct labeling of proteins with ^{211}At were investigated.

We evaluated reagents in two different approaches in "two step" pretargeting protocols. These approaches are: (1) delivery of the radionuclide on recombinant streptavidin to bind with pretargeted biotinylated monoclonal antibody (mAb), and alternatively, (2) delivery of the radionuclide on a biotin derivative to bind with pretargeted antibody-streptavidin conjugates. The two approaches were investigated as it was unclear which will be superior for the short half-lived α-emitting radionuclides. The results obtained from the research conducted are outlined under each of the Specific Aims set out in the previous proposal.

Specific Aim 1: Reagents to Optimize Pretargeting of ^{211}At and ^{213}Bi.

A. Optimization of reagents used in pretargeting approach that employs a monoclonal antibody-streptavidin conjugate and a biotin derivative.

Antibody-Streptavidin Conjugates: We have evaluated monoclonal antibody (mAb)-streptavidin (SAv) conjugates in optimization studies. We have successfully prepared a number of intact antibody-streptavidin conjugates, including 107-1A4 (anti-PSMA) antibody-streptavidin conjugate, at high levels (e.g. up to 400 mg of conjugate) for in vivo studies. In optimization studies, we have prepared and evaluated antibody Fab´-SAv conjugates for tumor targeting. The Fab´-SAv conjugates are much smaller than the intact mAbs (Figure 2), which may permit using them without clearing agents. In vivo tumor targeting with the 107-1A4 Fab´-SAv was equivalent to the corresponding Fab´-SAv-Fab´ conjugate, and much better than either the equivalent sized F(ab´)$_2$ or Fab´ by itself. The distribution data for 107-1A4-SAv, Fab´-SAv-Fab´ and Fab´-SAv is provided in Figure 3, panels A, B & C.

Figure 2: Graphic Depiction of intact antibody (IgG) and Fab´ conjugates of Streptavidin

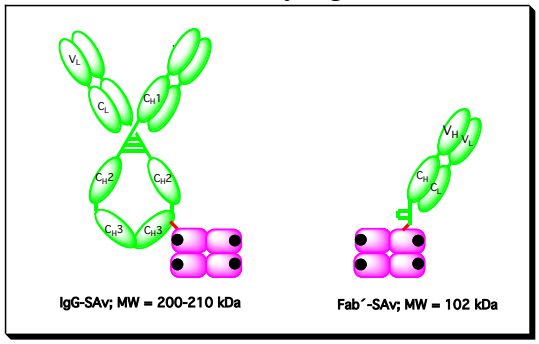
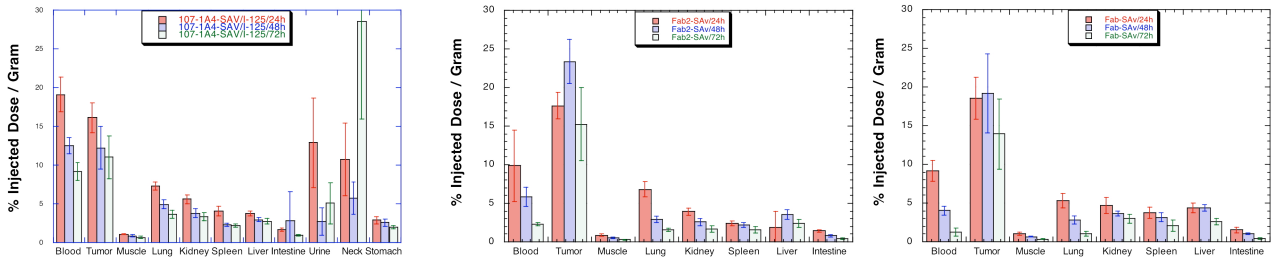


Figure 3: Biodistributions of intact 107-1A4-SAv, Fab´-SAv-Fab´, and Fab´-SAv



Additional studies have been conducted with another mAb, A6H, an anti-renal cell carcinoma antibody. In those studies, it was found that the Fab´-SAv-Fab´ conjugate had slightly better tumor targeting and retention than the Fab´-SAv conjugate. The Fab´-SAv studies are complete and a manuscript is being written on that research.

We believe that the smaller Fab´-SAv conjugates will be the conjugate of choice for some pretargeting applications. However, alternatives to the chemically cross-linked mAb-SAv conjugates are fusion proteins made of mAb single chain fragments (scFv) and recombinant SAv, i.e. $scFv_4$-SAv. We are investigating their application in collaborative studies with Dr. Press and Dr. Yukang Lin at the Fred Hutchinson Cancer Research Center. While excellent results have been obtained with the fusion proteins, the difficulty of their preparation will assure that chemical conjugates remain important in the development of pretargeting with some mAb-SAv.

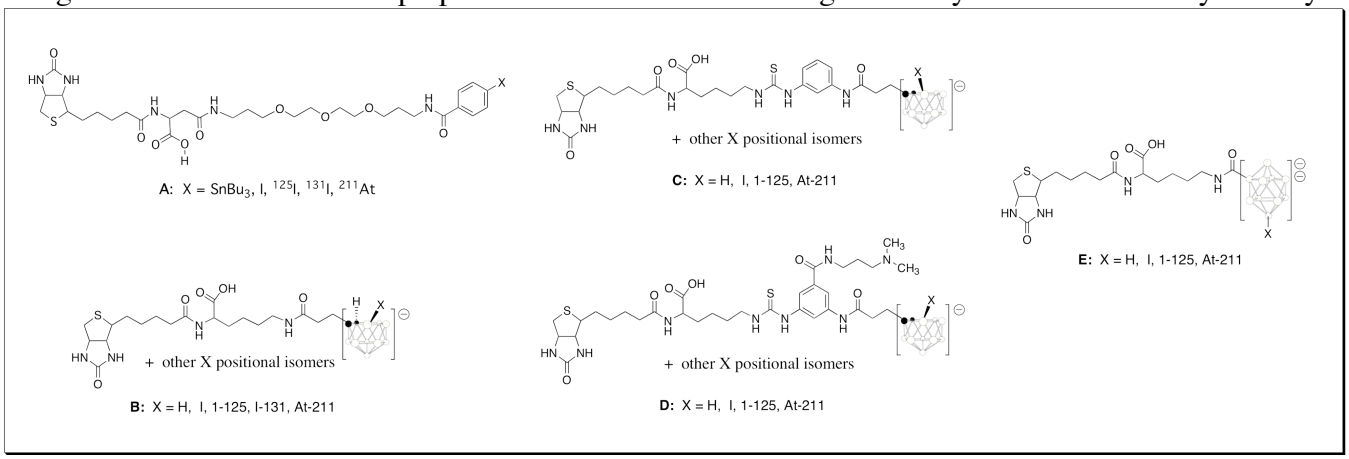
Reagents that alleviate the problem of endogenous biotin. One of the problems with using the pretargeting approach with the biotin/streptavidin binding pair is the fact that the body has biotin present in blood and tissues (endogenous). The endogenous biotin can bind with the biotin binding sites of the administered agent to block the binding of radiolabeled biotin. In practice, this problem is successfully circumvented by administering a large quantity of the mAb-SAv conjugate so that the endogenous biotin only occupies a small percentage of the biotin binding sites. The requirement to administer large quantities of mAb-SAv conjugates is problematic, however, as this is a very expensive reagent and can cause higher levels of circulating conjugate which is difficult to remove. We have been investigating an alternative pretargeting approach, which was based on developing a new biotin/SAv binding pair, to circumvent the problem of endogenous biotin. The new biotin/SAv binding pair is composed of a streptavidin mutant (with decreased binding to biotin) and radiolabeled biotin dimer. By decreasing the biotin binding affinity for streptavidin, a more rapid dissociation rate is obtained. If a biotin dimer is used, the dissociation rate is decreased to the point where its binding is favored over that of endogenous biotin. In collaborative studies with Dr. Oliver Press (Fred Hutchinson Cancer Research Center) and Pat Stayton (University of Washington), we prepared a radiolabeled biotin dimer, which contained aspartate groups adjacent to the biotin to protect it from cleavage by biotinidase. The in vitro evaluation of this

compound and several other biotin dimers has been published [66], and a second manuscript on the in vivo targeting has been submitted for publication [70]. Additional studies are continuing using a new bis-biotin clearing agent developed for use with the lower affinity rSAv mutants.

Optimization of biotin structure. A number of studies have been conducted to optimize the structure of biotin derivatives for in vivo use. In the studies, the effect of structural variations of biotin derivatives on binding with streptavidin and on blocking degradation by biotinidase was examined. Most of those studies are published [64, 71, 72]. Additional studies on the use of an ethyl group alpha to the biotin carboxylate functionality have not been published, but will be part of a publication on biotin multimers that is being written. The reason studies were conducted with that derivative is that the t-butyl ester used with the biotin-aspartate can come off under some reaction conditions, complicating the purification and isolation of multi-biotin derivatives containing this biotinidase blocking agent. Testing the alpha-ethyl derivative for biotinidase cleavage demonstrated that it did not completely block biotinidase activity. This was a surprise as the ethyl group is fairly close in steric encumbrance to the hydroxymethylene group of serine, which fully blocks biotinidase. Another study involving biotin structure optimization was conducted with charged biotin derivatives (ammonium and sulfonate vs. carboxylate derivatives). Intestinal concentrations were decreased by approximately one third for both the sulfonate and ammonium derivatives. A manuscript will be written on these data.

Radiolabeled Biotin Derivatives: In vivo studies with biotin derivatives that contain the benzyl-DTPA-CHX-A´´ derivative were found to be quite stable to demetallation when labeled with ^{213}Bi, and was successful in pretargeting studies. Therefore, no additional studies have been conducted in that area. However, this has not been the case for ^{211}At labeled biotin derivatives. One of our major goals has been to prepare biotin derivatives that can carry ^{211}At without undergoing in vivo deastatination. Our initial studies in this area focused on increasing the aqueous solubility and biotinidase stability of the radiohalogenated biotins. A large number of biotin derivatives that contained aryl halides were prepared and evaluated. Much of the data from those studies are published in two papers on biotinidase evaluations [73, 74]. The optimized biotin structure for ^{211}At labeling appeared to be the biotin-aspartate derivative **A** in Figure 4. Unfortunately, when the astatinated version of that compound was evaluated in vivo, it was found to deastatinate very quickly. Biodistributions of astatide and iodide are provided

Figure 4: Biotin derivatives prepared that contain a radiohalogenated aryl or *nido*-carboranyl moiety



with the biodistribution of biotin derivative **A** in Figure 5 to show the correlation. The difference in localization of ^{211}At and ^{125}I in lung, spleen and neck are indicators of how much free astatide is present. To improve the in vivo stability, we studied *nido*-carboranyl derivatives of biotin **B**, **C**, and **D** (Figure 4). These biotin derivatives had *higher stability towards in vivo deastatination*, but also had much higher blood concentrations than observed for the aryl derivatives. The higher blood concentrations are

believed to be caused by the *nido*-carborane's propensity to bind with amines (on serum proteins). This appears to result in a low availability for diffusing into tumors. Therefore, we have begun to study other borane compounds to replace the *nido*-dicarbon carboranes in biotin derivatives. Our first studies have involved the *closo*-borane, decaborate(2-) ($B_{10}H_{10}^{2-}$). The biotin derivative **E** has been prepared and studied. It has been found to have high in vivo stability, but has a high concentration in the kidney. The localization and slow release from kidney for biotin derivative **E** will be addressed in the proposed studies.

Figure 5: Biodistributions of $Na^{211}At/Na^{125}I$ (left) and $^{211}At/^{125}I$-Labeled Biotin Derivative **A** (right) at 4 hours post injection

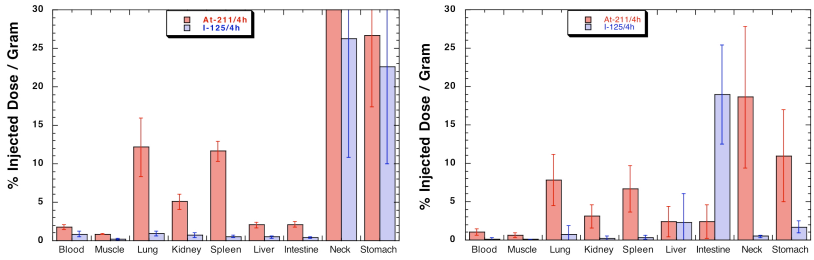
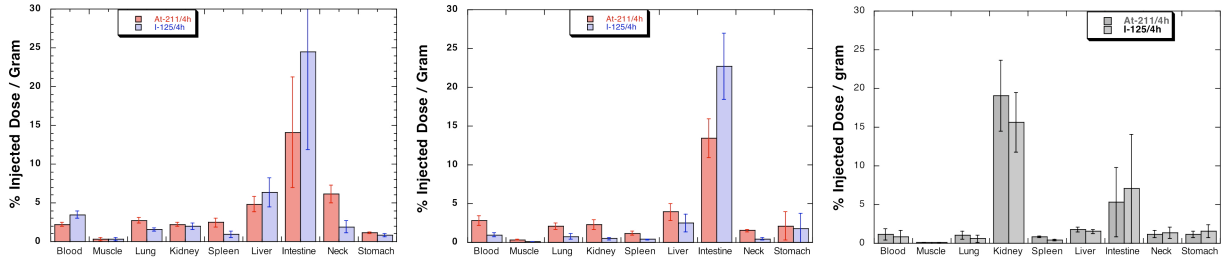
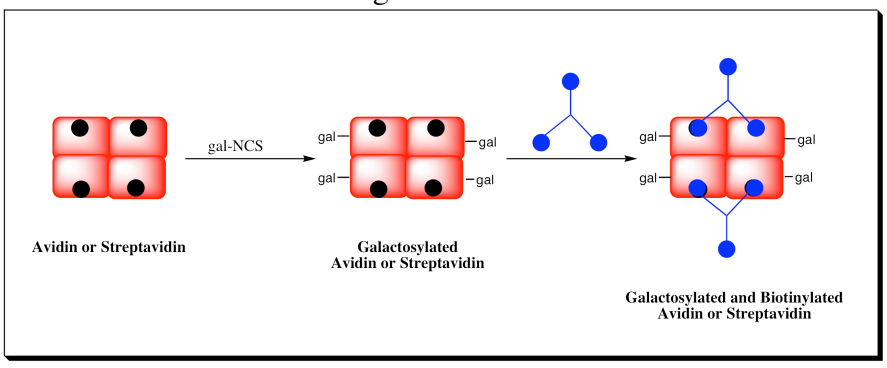


Figure 6: Biodistributions of Biotin derivatives **C**(left), **D**(middle), **E**(right) at 4h post injection



Development of new Biotinylated Blood Clearing Agents. Although we would like to conduct the pretargeting protocols without clearing agents, this may not be possible. The clearing agent that NeoRx Corporation supplied to Dr. Oliver Press for our successful collaborative studies was not made available for our α-emitter studies. Therefore, we have prepared a number of new clearing agents during the funding period. One of the new clearing agents is composed of galactosylated avidin or streptavidin that has two biotin trimers bound. A depiction of the method for preparing this conjugate is shown in Figure 7. We found efficient clearing with the streptavidin conjugate but not with the avidin conjugate. The studies of an alternative binding pair using biotin dimer and a lower affinity streptavidin mutant also

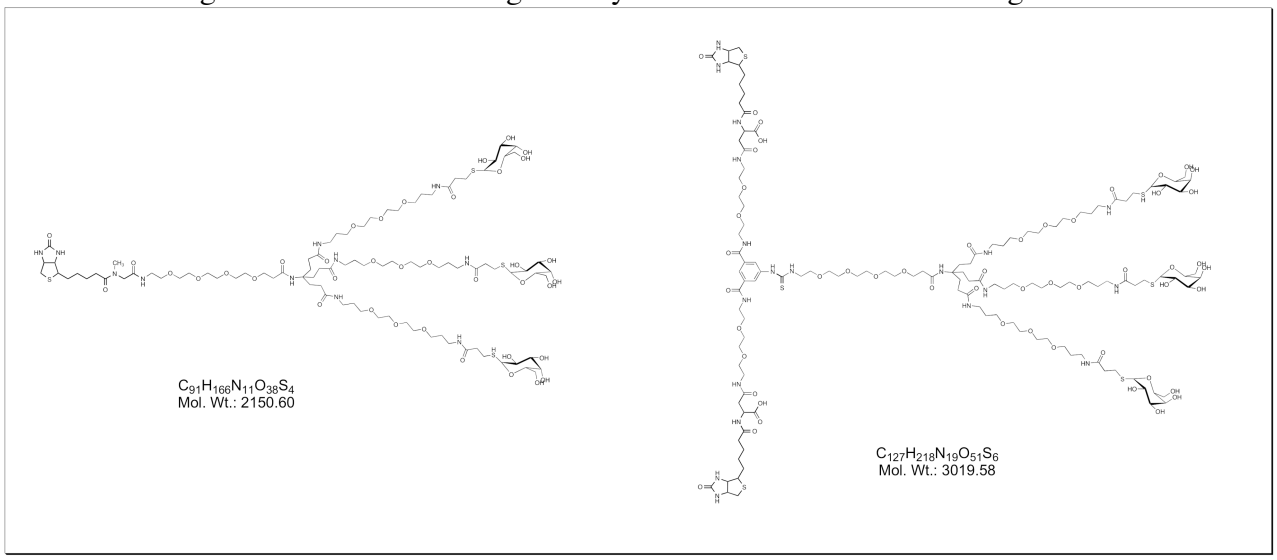
Figure 7: Preparation of Galactosylated and Biotinylated Avidin or SAv using Biotin Trimer



required a clearing agent, however, good clearance could not be attained with normal biotin clearing agents. Therefore, a biotin dimer conjugate of human serum albumin (HSA) was prepared. Studies with

the biotin dimer clearing agent were encouraging, however, it appeared that biotin was released from the clearing agent which caused a large decrease in targeting of a radiolabeled biotin molecule. To improve the clearance of biotin-binding reagents, we researched the literature. What we found was that the a trigalactose is required (at a minimum) because the asialoglycoprotein receptors (Ashwell receptors) are "arranged in space as vertices of a triangle whose sides are 15, 22, and 25Å" [75, 76]. With the appropriate spacers in the structures, it has been found that affinities for galactosyl groups may be increased by a factor of 2000x [77]. The structure of the clearing agent that NeoRx Corporation designed has 16 galactosyl groups. However, that many galactosyl moieties may not be required. We have recently prepared two new clearing agents based on the dendrimer backbones that are being studied for biotin multimers. Those structures are show in Figure 8. Studies with these reagents are only beginning. The bis-biotin clearing agent will be investigated with the lower affinity SAv mutants.

Figure 8: Structures of trigalactosyl biotin derivatives for clearing blood



B. Optimization of reagents used in pretargeting approach that employs a biotinylated monoclonal antibody and radiolabeled streptavidin.

Streptavidin Derivatives. Due to their short half-lives, we believe that it may be better to have the α-emitting radionuclides, ^{211}At and ^{213}Bi decay in the blood rather than excreted through organs. Thus, it may be of advantage to use radiolabeled streptavidin as a carrier in pretargeting, where biotinylated antibody is prebound with tumor cells. Unfortunately, native and recombinant streptavidin has a high propensity to localize and be retained in kidneys. We have previously shown that succinylation of streptavidin greatly diminishes its concentration in kidneys [78]. As a continuation of those studies, we investigated whether modification of arginine moieties on streptavidin would also work to diminish the kidney localization. The arginine modified streptavidin decreased kidney localization, but it was not an improvement over the succinylated streptavidin. A manuscript on those studies has been published [65]. Because this is such an important issue, we continue to evaluate methods of decreasing kidney localization of streptavidin. In a collaboration with investigators at NeoRx Corporation, we have studied the kidney localization of streptavidin mutants where specific lysines have been replaced by an alanine (K80A, K121A, K132A, K134A) or an arginine has been replaced by histidine (R59H). The studies are not yet complete, but we have found that all kidney binding appears to be absent in K134A, which makes it a more attractive candidate for this use than the succinylated SAv. Additional studies are being conducted to determine if other mutants are similar. The information that we have obtained thus far is being written in a manuscript for publication.

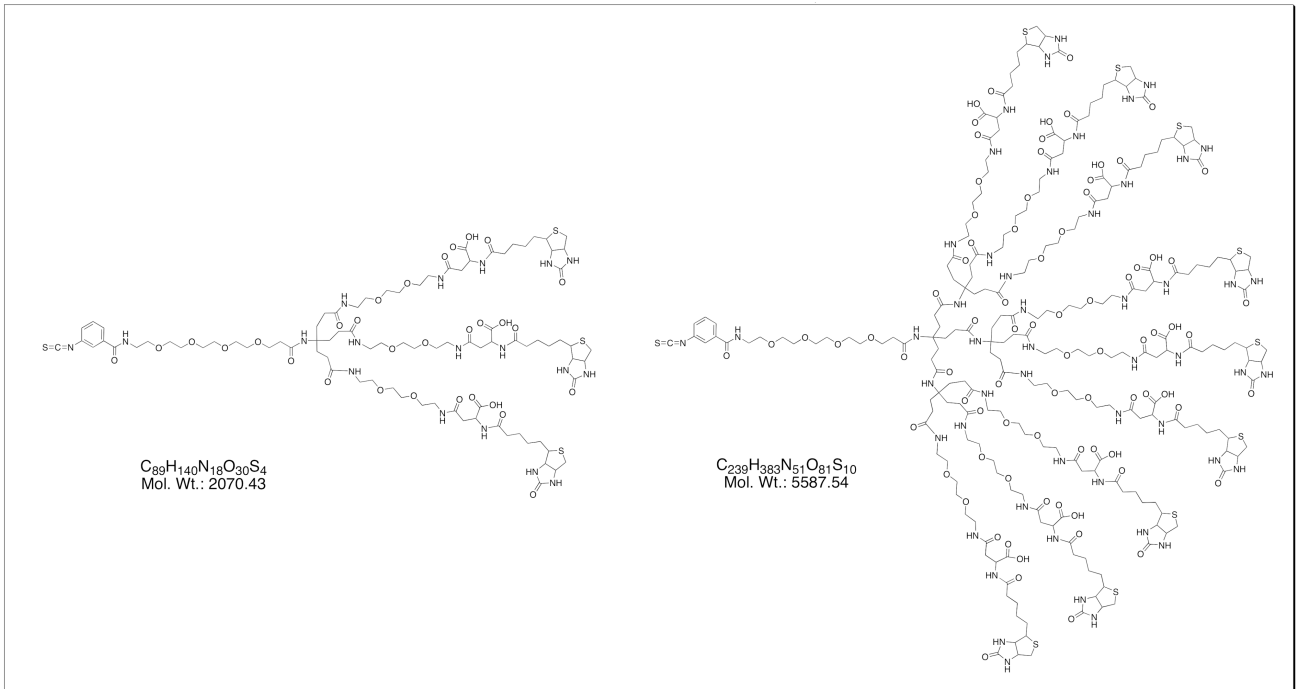
Development of new Biotin-Binding Blood Clearing Agents. Although we would like to conduct the pretargeting protocols without clearing agents, this may not be possible. Therefore, we have prepared a new clearing agent composed of galactosylated avidin or streptavidin. These conjugates did not greatly improve the clearance of biotinylated antibodies, so we are interested in conjugating the galactosyl trimer to see if an improvement can be made.

Specific Aim 2: Reagents to Optimize the Quantity of ^{211}At and ^{213}Bi in tumors.

A. Background. The rationale for developing methods for increasing the amount of radioactivity on/in cancer cells is based on the fact that it: (1) would provide the potential for using the same antigenic sites for targeting multiple doses (fractionation of doses) and (2) would increase the probability of attaining ample numbers of radionuclides on a cancer cell to kill that cell (even with low specific activity). Our previous studies demonstrated that biotin dimers do not cross-link rSAv (using SAv in solution and surface bound), but biotin trimers [79] and biotin multimers having Starburst™ dendrimer cores [80] are efficient in cross-linking rSAv. These structures did not have functional groups for conjugation to mAbs, and the core is highly charged in aqueous media due to the amine branching. Therefore, studies to obtain new biotin multimers, which have a "cascade" structure were carried out.

B. Optimization of Biotin Multimer Cross-linking. We are continuing to prepare and evaluate compounds that have three or more biotin moieties attached for the purpose of increasing the amount of radionuclide attached to, or internalized in, cancer cells. These studies have proven to be very difficult and complex, with several approaches leading to non-viable synthetic routes. In the studies, many different biotin multimers have been prepared and in vitro evaluations of the streptavidin cross-linking ability of some of the biotin multimers prepared have been conducted. Two of the many different derivatives that have been prepared are shown in Figure 9. Compounds similar to these have been evaluated for in vitro amplification and the nonamer (9 biotin moieties) has been found to provide the same level of amplification (multiple SAv binding) as the starburst dendrimers with 16 or more biotin moieties. Thus, we believe that the structures have been optimized. Both biotin multimers in Figure 9 have been conjugated with mAbs. Two manuscripts are being written based on these studies.

Figure 9: Biotin multimers that can be conjugated with mAbs



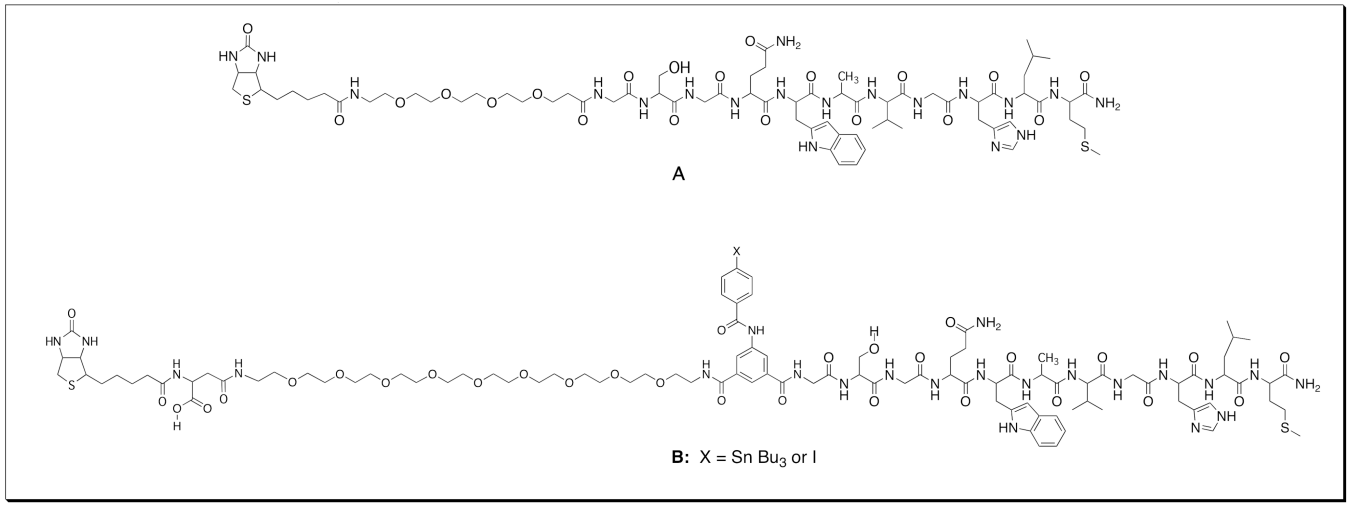
Specific Aim 3: Develop Reagents Composed of Peptide-SAv Conjugates.

Note: The reviewers of the previous proposal indicated that they felt this Specific Aim did not warrant much effort, so we conducted only a few studies relating to it.

Rapidly growing cancer cells often have increased numbers of receptors for peptide growth factors and hormones. Those peptides that have large increases in receptor molecule numbers on cancer cells make particularly attractive targets for Targeted Radionuclide Therapy. However, there are inherent problems with peptides that place barriers to their application in Targeted Radiotherapy using ^{213}Bi and ^{211}At. We hypothesized that the most serious barriers can be circumvented by conjugation of cancer cell targeting peptides to radiolabeled succinylated rSAv. Some advantages that SAv conjugation of peptides may provide are: (1) residence time of the peptide in blood will be increased dramatically, (2) four peptides can bind with SAv, perhaps increasing the affinity (avidity) for the receptor, (3) very high specific activities can be obtained on derivatized rSAv making it possible to use small quantities of that material (note that endogenous biotin is not a problem in this use), (4) minimal kidney localization is expected if succinylated or mutant rSAv is used, and (5) internalization of the receptor bound peptide-SAv complex will not result in a release of free radionuclide due to the SAv resistance to proteases. Although all of these points are important, the last point is particularly important as selective targeting is not sufficient for therapy, rather, there is a requirement that the radionuclide stay associated with the targeted cell during its decay period. Radiohalogenated peptides suffer from rapid loss of activity from the cell after internalization. As a model for peptides, bombesin derivatives have been prepared. We chose bombesin as it binds with the gastrin-releasing peptide receptor (GRP-R), which is found on some prostate cancer cell lines, and is present on cells from a number of other types of cancers.

The studies conducted have focused on obtaining biotinylated derivatives of the 11 amino acid derivative of bombesin. The concept was to simply combine bombesin with biotin so that it could be bound with SAv, and its tumor targeting could be evaluated in the PC-3 prostate tumor line. Two bombesin derivatives have been prepared and fully characterized. Those derivatives are shown in Figure 10. The first derivative did not have a moiety for radiolabeling, but the second one did. We felt that it would be best to be able to follow both the labeled SAv and labeled bombesin. Both of these derivatives have been coupled with SAv, but no additional studies have been done. A long linker was used between the biotin and the bombesin so that it could readily bind with the receptor.

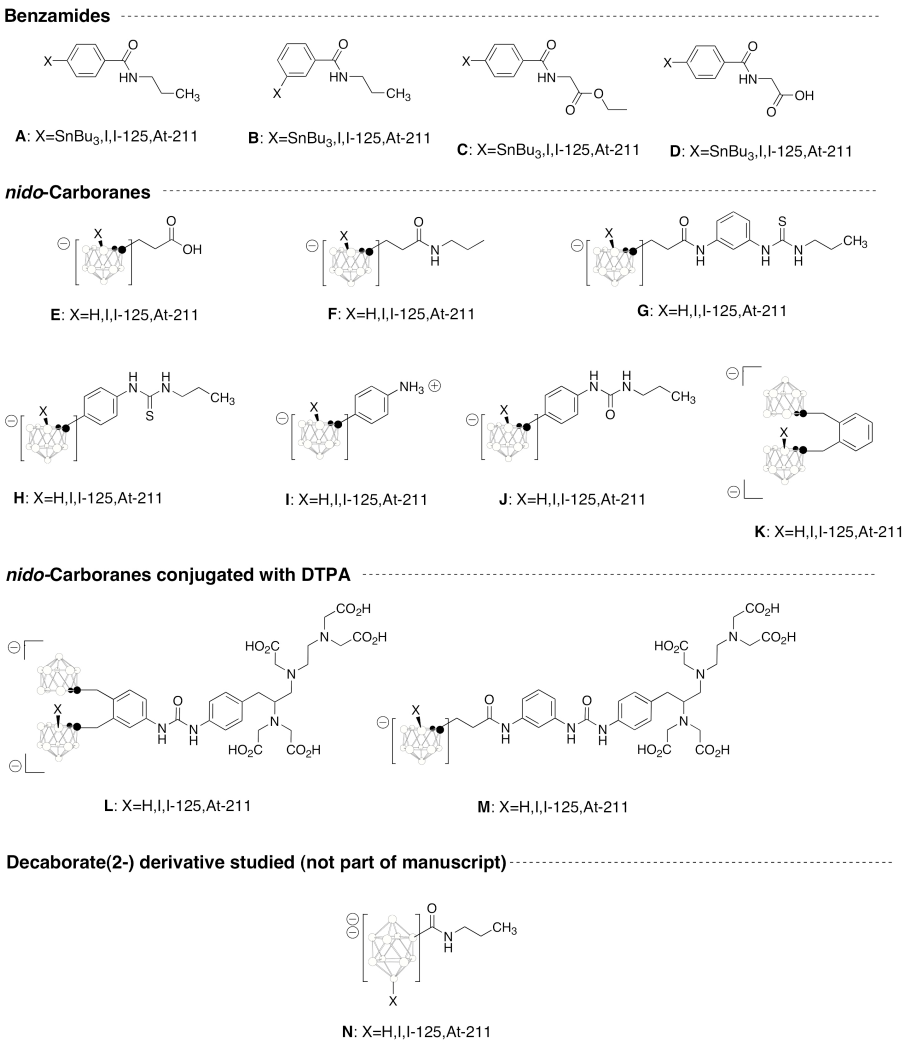
Figure 10: Biotinylated bombesin derivatives synthesized



III. ASTATINE-LABELED COMPOUND STABILITY STUDIES

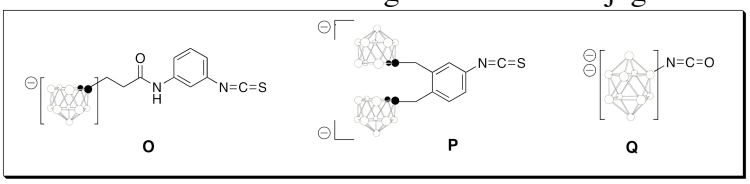
Based on our studies of astatinated mAbs and astatinated biotin derivatives, it became clear that there was a problem with in vivo stability of some astatinated compounds. We sought and obtained additional funding to investigate the stability of astatine on a series of model compounds, and as Fab´ derivatives. The compounds studied are shown in Figure 11. Aryl astatine derivatives were included in the study, because they are the ones that most people planning to astatinate biomolecules would use. The studies demonstrated very conclusively that some small molecules with astatinated aryl moieties (i.e. **A**-**D**) are rapidly deastatinated. We also studied some *nido*-carborane derivatives and found that in the compounds studied (i.e. **E**-**J**), they too were quite unstable to in vivo deastatination. Interestingly, small molecules containing two *nido*-carborane moieties in close proximity did not undergo deastatination in vivo. Additionally, incorporation of the bulky benzyl-DTPA moiety into compounds having a *nido*-carborane moiety appeared to decrease the in vivo deastatination. The syntheses, radiohalogenation, and in vivo data for all of the compounds studied are included in a manuscript submitted for publication. A copy of that manuscript is in the Appendix Items. An additional compound to those listed in the manuscript has been studied in vivo. That is the carbonyl-dodecaborate(2-) adduct with propylamine, **N**. This compound was found to be quite stable to in vivo deastatination, suggesting that compounds other than bis-*nido*-carboranes might be found to be stable in vivo.

Figure 11: Table of compounds that were astatinated and evaluated in vivo



In the same funded studies, 107-1A4 Fab´ was conjugated with some amine reactive intermediates used in the preparation of compounds in Figure 11. Those compounds are shown in Figure 12. Without the

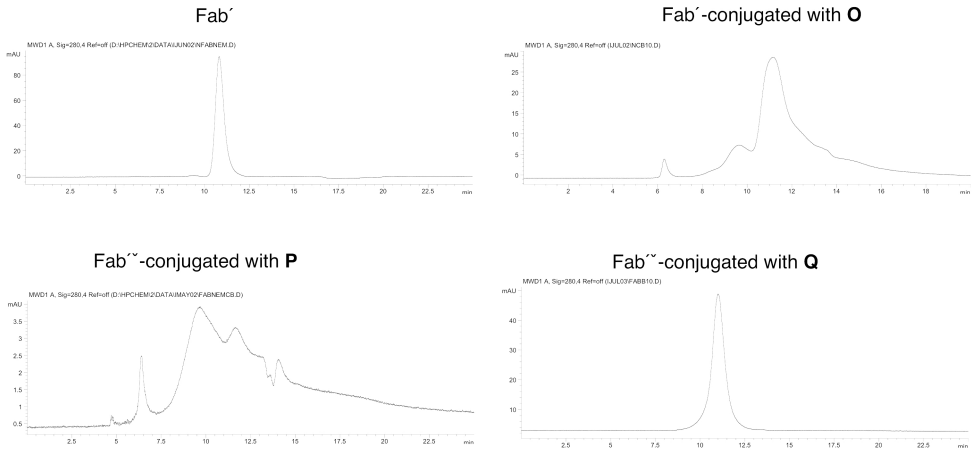
Figure 12: Amine reactive boron cage molecules conjugated with Fab´



conjugates, direct astatination of Fab´ gave less than 1% yield. With compounds **O**, **P**, or **Q** conjugated to Fab´, direct astatination yields ranged from 28-32%. The most important factor was how the conjugates affected the in vitro and in vivo behavior of Fab´. Size-exclusion HPLC chromatograms of Fab´ and its conjugates with **O**-**Q** are shown in Figure 13. Of the borane cage molecules studied, only the dodecaborate conjugate **Q** has minimal affect on the Fab´ characteristics on HPLC.

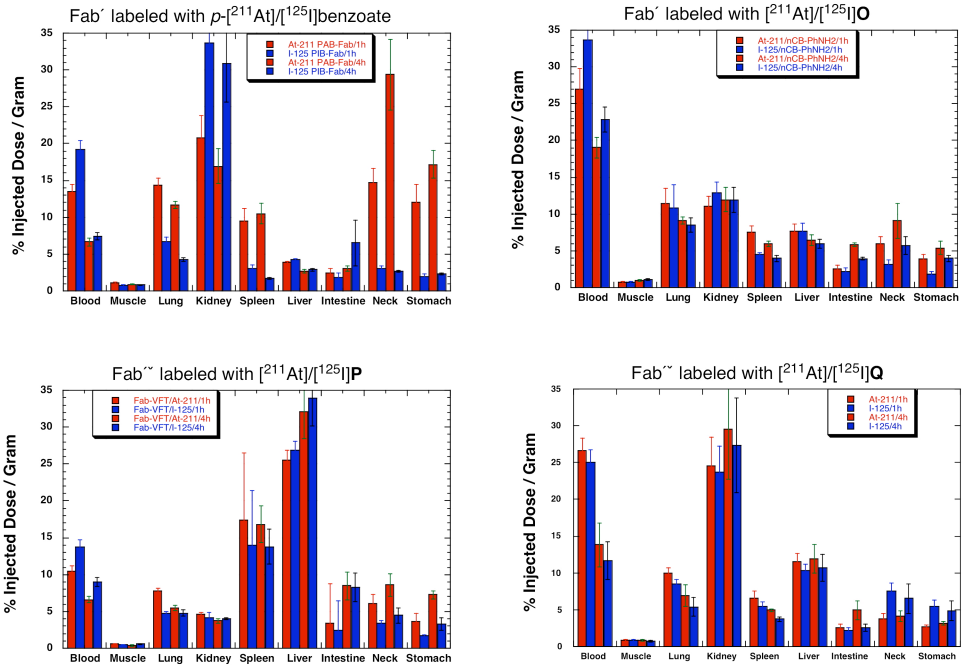
Biodistribution data for the ^{211}At- and ^{125}I-labeled Fab´ conjugated **O**, **P** or **Q**, or labeled with *para*-[^{211}At/^{125}I]halobenzoate are shown in Figure 14. The biodistribution data show the instability of the astatinated benzoate (note difference of At and I in lung, spleen, neck). The *p*-[^{125}I]benzoate (PIB)-labeled Fab´ is probably the best indicator of the natural distribution of the Fab´. Importantly, high kidney concentrations are seen for that labeled species, as is expected. The *nido*-carboranyl conjugate **O** appears to be fairly stable to in vivo deastatination over the short time period (4h), but high blood concentration and only about half of the expected kidney concentration is observed. The bis-*nido*-carborane conjugate **P** appears quite stable to in vivo deastatination, but most of the activity is localized

Figure 13: Size-exclusion HPLC chromatograms of Fab´ and its conjugates



in the spleen and liver. The dodecaborate(2-) conjugate **Q** has a biodistribution similar to that of the PIB radioiodinated Fab, except the blood concentration is slightly higher and the liver concentration is twice the level observed for PIB-labeled Fab´. This data demonstrates the favorable properties of the dodecaborate(2-) conjugate, but also points out that additional studies are required to improve the dodecaborate conjugates before they can be generally used for protein labeling.

Figure 14: Biodistribution data for ^{211}At/^{125}I-labeled Fab´ conjugates



IV. COMMENTS

We have prepared and evaluated a large number of new reagents in our optimization studies. Many of the new reagents do not require additional studies, but rather are, or will be made available, through the literature for application to Targeted Radionuclide Therapy by other investigators. In our own studies, we will apply the best of the new reagents and conduct additional studies to improve them. Those studies will be conducted under other funding in collaboration with Dr. Oliver Press, and additional funding that will be sought at a later time. The data obtained in the prior studies involving in vivo stability of astatinated small molecules and Fab´ fragments is important as it is suggestive that we can obtain astatine bonding moieties (pendant groups) that will have stable attachment of astatine to biomolecules, and will also have a minimal affect on the biomolecules. Additional optimization studies need to be conducted to find the appropriate molecules to serve as astatination pendant groups. Those optimization studies are the basis for the studies outlined in this proposal.

V. PUBLICATIONS AND PRESENTATIONS

From the funding, we have 12 papers published, and have made 22 presentations on our studies at professional meetings. The authors and titles for the manuscripts and presentations are listed below.

A. Manuscripts from funded research during grant period : (Published / In Press / Submitted)

1. Wilbur D.S., Hamlin D.K., Chyan M.-K., Kegley B.B., and Pathare P.M. (2001) Biotin Reagents for Antibody Pretargeting. 5. Additional Studies of Biotin Conjugate Design to Provide Biotinidase Stability. *Bioconjugate Chem. 12*, 616-623.
2. Wilbur D.S. (2001) Overcoming the Obstacles to Clinical Evaluation of Astatine-211 Radiopharmaceuticals. [Invited Commentary] *J. Nucl. Med. 42*, 1516-1518.
3. Hamblett K.J., Kegley B.B., Hamlin D.K., Chyan M.-K., Hyre D.E., Press O.W., Wilbur D.S., and Stayton P.S. (2002) A Streptavidin-Biotin Binding System that Minimizes Blocking by Endogenous Biotin. *Bioconjugate Chem. 13,* 588-598.
4. Wilbur D.S., Hamlin D.K., Meyer D.L., Mallett R.W., Quinn J., Vessella R.L., and Press, O.W. (2002) Streptavidin in Antibody Pretargeting. 3. Comparison of Biotin Binding and Tissue Localization of 1,2-Cyclohexanedione and Succinic Anhydride Modified Recombinant Streptavidin. *Bioconjugate Chem. 13*, 611-620.
5. Wilbur D.S., Chyan M.-K., Hamlin D.K., Kegley B.B., Nilsson R., Sandberg B.E.B., and Brechbiel M. (2002) Trifunctional Conjugation Reagents. Reagents that Contain a Biotin and a Radiometal Chelation Moiety for Application to Extracorporeal Affinity-Adsorption of Radiolabeled Antibodies. *Bioconjugate Chem. 13*, 1079-1092.
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12. Wilbur D.S., Hamlin D.K., Sanderson J.A., Lin Y., Quinn J., and Vessella R.L. (2004) Streptavidin in Antibody Pretargeting. 5. Streptavidin Mutants Engineered to Decrease Kidney Localization. *Bioconjugate Chem. 15,*1454-1463.

B. Presentations of funded research at Professional Meetings during grant period:

1. Wilbur D.S., Chyan M.-K., Hamlin D.K., and B.E.B. Sandberg (2001) Synthesis and Evaluation of Protein Biotinylation Reagents that also Contain UV and/or Fluorescence Absorbing Moieties. Presented at the 221st American Chemical Society Meeting, San Diego, CA, April 1-5, 2001.

2. Wilbur D.S., Hamlin D.K., Kegley B.B., Chyan M.-K., Pathare P.M., and Wan F. (2001) Studies Which Indicate that Thiourea Bonds Interfere with Radioiodination Reactions. Presented at the 14th International Symposium on Radiopharmaceutical Chemistry held in Interlaken, Switzerland, June 10-15, 2001.
3. Wilbur D.S., Kegley B.B., Hamlin D.K., Chyan M.-K., Quinn J., and Vessella R.L. (2001) Preparation and Evaluation of Radioiodinated Biotin Derivatives which Contain Ionic Charges. Presented at the 14th International Symposium on Radiopharmaceutical Chemistry held in Interlaken, Switzerland, June 10-15, 2001.
4. Wilbur D.S., Chyan M.-K., Hamlin D.K., Brechbiel M., Nilsson R., and Sandberg B.E.B. (2001) Trifunctional Biotinylation Reagents which contain a Radiometal Binding Chelate and an Isothiocyanate Group for Conjugation with Biomolecules. Presented at the 14th International Symposium on Radiopharmaceutical Chemistry held in Interlaken, Switzerland, June 10-15, 2001.
5. Wilbur D.S., Hamlin D.K., Quinn J. and Vessella R.L. (2001) Development of an Improved Biotinylated Clearing Agent for Pretargeting Applications. Presented at the 48th Annual Society of Nuclear Medicine meeting held in Toronto, Ontario, Canada, June 23-27, 2001.
6. Hamlin D.K., Wilbur D.S., Quinn J. and Vessella R.L. (2001) Evaluation of galactosylated avidin / streptavidin as a blood clearing agent in cancer therapy. Presented at the Northwest Regional meeting of the American Chemical Society, Seattle University, Seattle, WA, June 14-17, 2001.
7. Hamlin D.K., Kegley B.B., Wilbur D.S., Quinn J. and Vessella R.L. (2001) *nido*-Carborane pendent groups for direct labeling of proteins with astatine-211. Presented at the Northwest Regional meeting of the American Chemical Society, Seattle University, Seattle, WA, June 14-17.
8. Wan F., Kegley B.B., and Wilbur D.S. (2001) Synthesis of Protein Reactive Polybiotin containing Dendrimers. Presented at the Northwest Regional meeting of the American Chemical Society, Seattle University, Seattle, WA, June 14-17, 2001.
9. Kegley B.B., Hamlin D.K., Wilbur D.S., Quinn J. and Vessella R.L. (2001) Synthesis and Radiohalogen Labeling of Biotin Derivatives Containing nido-Carboranes. Presented at the Northwest Regional meeting of the American Chemical Society, Seattle University, Seattle, WA, June 14-17, 2001.
10. Wilbur D.S. (2002) Addressing the Issue of Stability in At-211 Labeled Radiopharmaceuticals. Invited Presentation at: Workshop on Radioisotope Therapy with Alpha-Emitters: Present Status and Future Directions. Held at TRIUMF, Vancouver, B.C., April 29.
11. Hamlin D.K., Wilbur D.S., Chyan M.-K., Quinn J., and Vessella R.L. (2002) Assessment of the In Vivo Stability of Some Simple Astatinated Aryl- and *nido*-Carboranyl- Compounds. Presented at the 49th Annual Meeting of the Society of Nuclear Medicine, Los Angeles, CA June 15-19; *J. Nucl. Med. 43*, 370P.
12. Wilbur D.S., Hamlin D.K., Chyan M.-K., Quinn J., and Vessella R.L. (2002) Development of Astatinated Biotin Derivatives that are Stable to In Vivo Deastatination. Presented at the 49th Annual Meeting of the Society of Nuclear Medicine, Los Angeles, CA June 15-19; *J. Nucl. Med. 43*, 134P-135P.
13. Wilbur D.S., Hamlin D.K., Smelser H., Quinn J. and Vessella R.L. (2002) Comparison of Tumor Targeting and Pharmacokinetics of 107-1A4 Fab´-Streptavidin and Fab´$_2$-Streptavidin Conjugates in a Mouse Model. Presented at the 49th Annual Meeting of the Society of Nuclear Medicine, Los Angeles, CA June 15-19. *J. Nucl. Med. 43*, 135P.
14. Wan F., Kegley B.B., and Wilbur D.S. (2002) Synthesis of a biotinylation reagent containing three biotin moieties. Presented at the 224th ACS National Meeting, Boston, MA, Aug. 18-22.
15. Wilbur D.S., Hamlin D.K., Foulon C., Zalutsky M.R., Wedge T., and Hawthorne M.F. (2003) Optimizing methods for labeling biomolecules with astatine-211. Presented at Symposium on the Development of High LET Radiopharmaceuticals, American Chemical Society meeting, New Orleans, LA, March 23-27.

16. Wilbur D.S., Sanderson J., Lin Y.K., Quinn J., and Vessella R.L. (2003) Comparison of Kidney Localization of Streptavidin Mutants with Wild Type and Succinylated Recombinant Streptavidin. Presented at the 50th annual meeting of the Society of Nuclear Medicine, New Orleans, LA, June 21-25.
17. Wilbur D.S. (2003) Therapeutic Radiopharmaceuticals-Present and Future Directions. [Invited Presentation] Presented at the 50th annual meeting of the Society of Nuclear Medicine, New Orleans, LA, June 21-25.
18. Wilbur D.S., Hamlin D.K., Chyan M.-K., Quinn J., Vessella R.L., Wedge T., and Hawthorne M.F. (2003) Comparison of the in vivo distributions of an antibody Fab´ labeled by conjugation of N-succinimidyl *para*-[^{211}At]astatobenzoate and by direct ^{211}At labeling using preconjugated *nido*-carboranes, and a *closo*-decaborane. Presented at the 15th International Symposium on Radiopharmaceutical Chemistry, Sydney, Australia, Aug. 10-14.
19. Wilbur D.S., Hamlin D.K., Quinn J., and Vessella R.L. (2003) A Method for Efficient Preparation of an Anti-PSMA Antibody Fab´-Streptavidin Conjugate to be used as a Prostate Cancer Targeting Agent. Presented at the 15th International Symposium on Radiopharmaceutical Chemistry, Sydney, Australia, Aug. 10-14.
20. Wan F., Hamlin D.K. and Wilbur D.S. (2003) Synthesis and In Vitro Evaluation of Biotin Nonamers for Cross-Linking of Multiple Molecules of Radiolabeled Avidin or Streptavidin. Presented at the 15th International Symposium on Radiopharmaceutical Chemistry, Sydney, Australia, Aug. 10-14.
21. Wilbur D.S., Hamlin D.K., Quinn J., and Vessella R.L. (2003) In Vivo Evaluatioin of an Anti-PSMA Antibody Conjugated with Varying Numbers of Biotin Molecules in a Pretargeting Protocol. Presented at the 12th International Congress on Radiation Research, Brisbane, Australia, August 17-22.
22. Wilbur D.S., Hamlin D.K., Sanderson J.A., Lin Y., Quinn J., and Vessella R.L. (2003) Comparison of Succinylated Recombinant Streptavidin and a Streptavidin Mutant (K134A) as Carriers for Astatine-211 in Pretargeting Protocols. Presented at the 12th International Congress on Radiation Research, Brisbane, Australia, August 17-22.