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TITLE: Development of a novel platform for in vivo delivery of antagomirs to study cetuximab resistance in colorectal cancer

PRINCIPAL INVESTIGATOR: Jacob Houghton, PhD

CONTRACTING ORGANIZATION: Vanderbilt University Medical Center

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INTRODUCTION: *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The subject of the research is colorectal cancer treatment, and more specifically, overcoming epigenetic mechanisms of acquired cetuximab resistance in colorectal cancer. We have identified the epigenetic mechanisms that lead to cetuximab resistance in many patients, but the targets are microRNA molecules that are not able to be targeted by traditional drugs. The purpose of our research is to develop a novel delivery mechanism for so-called antagomirs, which could reverse cetuximab resistance. The scope of the proposed work will develop a novel delivery platform and to ultimately test in animal models cetuximab-resistant colorectal cancer to determine if it is efficacious.

1. KEYWORDS: *Provide a brief list of keywords (limit to 20 words).*

Colorectal cancer, PET imaging, molecular imaging, microRNA, cetuximab, cetuximab resistance, antagomir, precision medicine, epigenetics

2. ACCOMPLISHMENTS: *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

This project began in August 2019 and work in our laboratory ceased due to the COVID-19 pandemic in March 2020. The overall goal of this project is to develop a platform for delivering labeled antagomirs to human colorectal cancer (CRC) cells utilizing an affibody targeting vector and a linear polyethylenimine (LPEI) delivery vector. Before in vitro and in vivo experiments can take place, we first have to produce the triconjugate polyplex. The high cationic charge density of the LPEI forms a non-covalent complex with the antagomir known as a polyplex, which is then internalized by cells resulting in delivery of the nucleic acid inside the cell. Specificity in this delivery is obtained by covalently linking the polyplex to an affibody targeting vector via a polyethylene glycol (PEG) phenyloxadiazole (PODS) based linker. The PEG part of this linker reacts with one of the secondary amines on the LPEI via a terminal N-hydroxysuccinimide (NHS) ester moiety at the end of the PEG to form the diconjugate (Fig 1, step 1). The PODS is then attached via dibenzocyclooctyne (DBCO) based copper free click chemistry (Fig 1, step 2). The PODS then reacts specifically with a terminal cystine engineered into the affibody to form the triconjugate (Fig 1, step 3). The major goals of our project through this time frame were the “Synthesis of 1:1 and 1:3 triconjugates” as outlined in our SOW. To date, we have completed ~75% of this work.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major Activities and Specific Objectives

We proposed to improve upon previous iterations of polyplexes by utilizing a new linker strategy that is based on work recently reported by the Zeglis group in Bioconjugate Chemistry. The previous syntheses of similar polyplexes utilized an orthopyridyl disulfide (OPSS) moiety to directly conjugate with the cystine tag on the affibody. However, the disulfide bond formed in this conjugation is not stable in vivo, so we sought to utilize a phenyloxadiazoly methylsulfone (PODS) based conjugation because it has been shown to exhibit remarkable stability in vivo. Our main objective at this point in our research was to develop synthesis, purification, and characterization methods for the “triconjugates” based on our novel approach, which would be the result of step 3 in Figure 1.

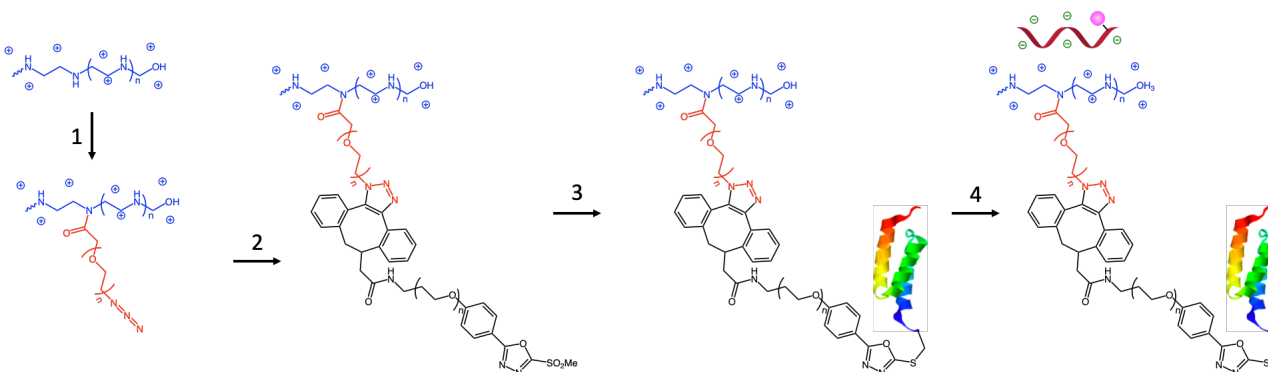


Figure 1: Proposed Synthesis. First, the PEG-N₃ conjugated to the LPEI via NHS ester reaction with one of the secondary amines on the LPEI. Then the PODS is attached via DBCO copper free click chemistry. Then the affibody is attached by a reaction between the PODS and a terminal cystine engineered into the affibody. Finally, the antagomir is added via a non-covalent complexation with the LPEI. The final result is an affibody-LPEI-antagomir triconjugate polyplex.

Before the first step in the synthesis could be completed, it was necessary to setup a system for analysis and purification of the products. This proved to be more challenging than initially anticipated. The previous work in the literature utilized an advanced fast protein liquid chromatography (FPLC) unit and methodology based on ion exchange chromatography (IEC). Despite Dr. Houghton’s start-up contract stating that all of his equipment needs would be met by the outfitting of a new floor and lab spaces, we were not allocated any funds to purchase the equipment necessary to achieve these research goals. Therefore, paid access time on an existing ÄKTA Start FPLC was provided by the Radiochemistry Core to perform these experiments. However, the ÄKTA Start was an inferior instrument and not capable of generating the same methods used in the literature, so a new methods had to be developed by Drs. Houghton and Carney. This method required many iterations to fully optimize so that it would be capable of separating LPEI conjugates from less densely charged precursors. Eventually, due to Dr. Carney’s tireless efforts, we were able to achieve separation of our reaction components and products using the ÄKTA Start FPLC.

In the first step of the triconjugate synthesis, we sought to incorporate an azide (N_3) functionalized PEG into the linear polyethylenimine (LPEI) backbone, rather than previously utilized OPSS functionalized PEG in the first step. Surprisingly, the N_3 -PEG has not exhibited the same reactivity as reported for the OPSS-PEG. Both PEG groups conjugate to the LPEI via NHS ester chemistry, and there is no reason to believe that the N_3 functional group should interfere in this reaction. However, under the same reaction conditions reported for the OPSS-PEG (5 equivalents PEG stirring for 3 hours at room temperature in ethanol with the free base form of the LPEI), we observed no product (**Figure 2**). For that reaction, LPEI in the free base form was obtained by dissolving the hydrochloride salt obtained from Sigma into H₂O (100mg in 5mL), then adding 6M NaOH until the final hydroxide concentration of the reaction mixture was 3M NaOH (5mL 6M NaOH added). The resulting precipitate was separated from the supernatant by centrifugation, washed 3 times with 1M NaOH, and then washed several times with deionized H₂O until the wash tested neutral on pH paper. The resulting free base LPEI was dried overnight under vacuum, aliquoted and stored at -20°C for future experiments. In an example of the reaction based on literature precedence, 2mg of base form LPEI (LPEI_B) was dissolved in 100μL of EtOH. 0.5mg PEG- N_3 (5eq) in 100uL EtOH was added. The reaction was stirred for 3 hours at room temperature. The resulting LPEI was then converted back into its hydrochloride for by adding 400uL 1M HCl. The reaction was then air dried and redissolved in neutral H₂O (pH of the solution was 7) before injection onto the FPLC.

The chromatogram in Figure 2 represents the UV absorbance of the components of the reaction mixture. The LPEI has no UV active functional groups, so passes through the detector without a signal. The only UV active group in the reaction mixture is the N_3 group, so the entire chromatogram represents the state of that group. The position of the arrow indicating the expected location LPEI-PEG- N_3 product is that of the product from subsequent experiments. This position also matches that of the LPEI-fluorescein conjugates used to optimize the FPLC method.

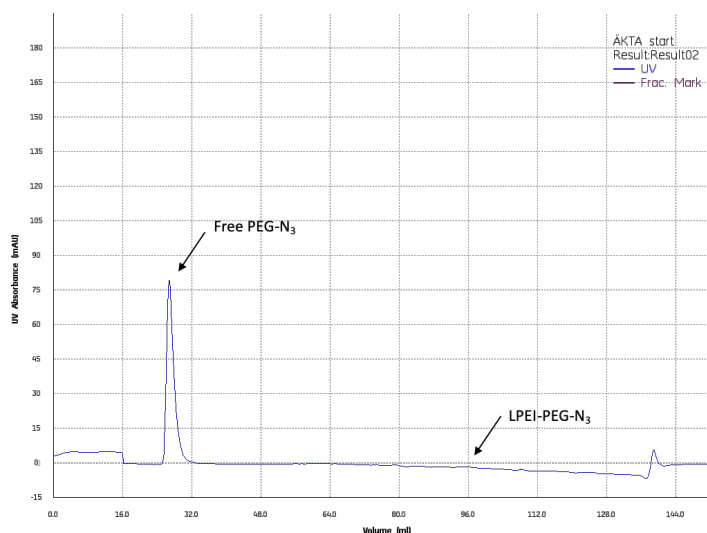


Figure 2: Literature Reaction. The results from the reaction using the procedure from the literature (at a smaller scale) showed no yield. This FPLC chromatogram shows the results of a reaction attempted using the procedure from the literature (at a smaller scale).

Further experiments showed a 5% yield utilizing N-butanol as a solvent and stirring for 3h at 100°C (**Figure 3**), however, the N₃ moiety is unstable at high temperatures for long periods of time. 2mg of base form LPEI (LPEI_B) was dissolve in 100μL of N-BuOH. 0.5mg PEG-N₃ (5eq) in 100uL N-BuOH was added. The reaction was stirred for 3 hours at 100°C. The resulting LPEI was then converted back into its hydrochloride for by adding 400uL 1M HCl. The reaction stirred for 30 minutes at room temperature and then air dried and redissolved in neutral H₂O (pH of the solution was 7) before injection onto the FPLC. Efforts leading up to the shutdown of all laboratory activities were focused upon scaling up this synthesis to acquire suitable amounts of material to proceed as no further increase in yield has been observed at longer time points.

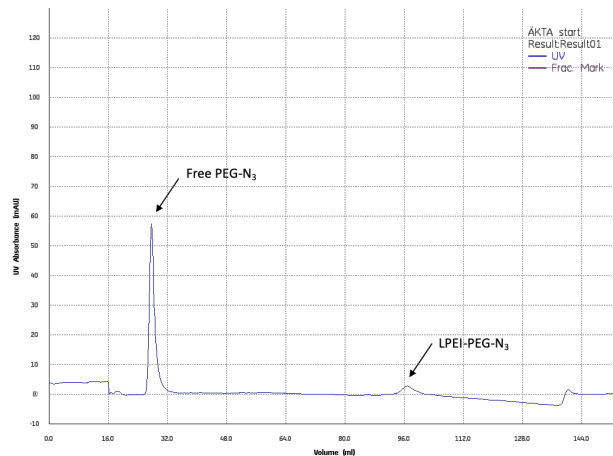


Figure 3: High Temperature Reaction. This FPLC chromatogram shows the results of a reaction attempted at much higher temperature using N-BuOH as a solvent to accommodate the higher temperature. 5% yield was determined by dividing the area of the LPEI-PEG-N₃ peak by that of the sum of the free PEG-N₃ and the LPEI-PEG-N₃ peaks adjusted for baseline.

Simultaneously to work on the LPEI-PEG-N₃, Dr. Carney has been developing the synthesis of the novel PODS-DBCO (**Figure 4**) in anticipation of a successful resolution to the LPEI-PEG conjugation. We have completed the multistep synthesis, purification, and characterization of the PODS molecule. Prior to be restricted from the labs due to the pandemic, we scaled up the reaction in preparation for the DBCO modification. This step is anticipated to be straightforward, and upon completion (estimated to occur prior to award transfer) we will begin testing conjugation strategies to the LPEI-PEG substrates and affibody.

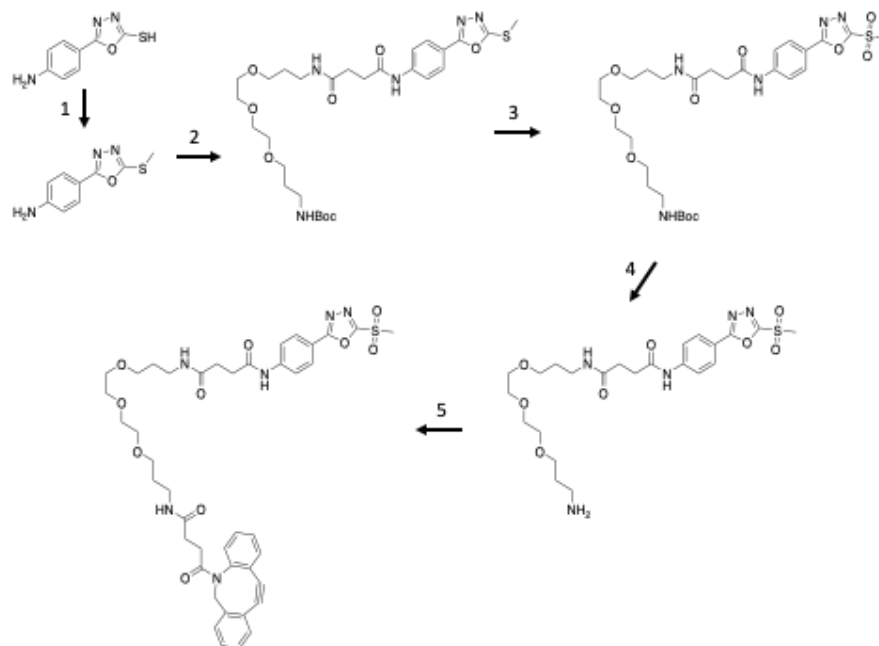


Figure 4: PODS-DBCO Synthetic Scheme. First the thiol group is methylated with iodomethane (1), then the PEG group is added with NHS ester chemistry (2). The sulfur is then oxidized with mCPBA (3) and the primary amine is deprotected (4). Finally, the DBCO is added with NHS ester chemistry to yield the final product (5).

Unfortunately, we have not yet met the goals stated in the first 6 months of our SOW at this time. However, we have made significant progress, and we are happy to report that this was achieved despite significant and unexpected difficulties.

Significant results

- Developed and optimized synthesis conditions for LPEI-PEG-N₃
 - Dr. Carney trained in bioconjugate chemistry by Dr. Houghton
- Developed and optimized purification techniques LPEI-PEG-N₃
 - Dr. Carney trained in FPLC method development
- Completed all but the final step of novel bioconjugate linker, PODS-DBCO

What opportunities for training and professional development has the project provided?

If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. “Training” activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. “Professional development” activities result in increased knowledge or skill in one’s area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Training

Dr. Carney began training in polymer and bioconjugation chemistry with Dr. Houghton in August 2019 and began working on this project in January 2020. In addition to learning chemical synthesis techniques, he also learned how to perform numerous purification techniques on poly ionic compounds using ion exchange chromatography. Dr. Carney is an accomplished radiochemist but these techniques and principles were new to him at the time and he has exceeded expectations. Originally, Dr. Houghton planned to hire a postdoctoral fellow with experience in these fields, but VUMC refused to allow him to hire the personnel (a second postdoc) guaranteed in his start up contract. As a result, Dr. Carney switched projects at that time and has become an invaluable resource and productive researcher in a very short period of time. The training he received will allow him to carry on the project at Stony Brook University in a seamless manner, allowing for a much more rapid progression than we have previously been able to achieve.

Dr. Houghton has gained didactic training in colorectal cancer biology and imaging by attending the weekly Coffey Group Meeting, weekly GI SPORE meeting, the Epithelial Biology Center monthly seminars, and Vanderbilt Digestive Disease Resource Center (VDDRC) seminar series. Dr. Coffey’s research as well as that of the researchers in the EBC and VDDRC are largely focused on colorectal cancer. Attending these meetings and seminars have allowed Dr. Houghton to expand upon his knowledge base in colorectal cancer. These training opportunities are in line with the proposed Career Development tasks. Additionally, though not originally planned until month 12-24 of the Career Development plan, Dr. Houghton and Dr. Coffey have begun planning for potential grant applications (e.g. Impact Award) for research that we anticipate we result directly from this project. During the pandemic-related work from home order, we turned our efforts into an exhaustive analysis of the literature and plans for future grants.

Professional Development

Dr. Houghton has worked with Dr. Coffey to expand his network of GI and colorectal cancer experts during this period of the award. The Epithelial Biology Center at VU has a number of outstanding researchers in the field and I have become well acquainted with a number of them including Drs. Goldenring, Lau, Crow, and others. Importantly, under Dr. Coffey's guidance, I have developed my laboratory and personnel management skills. Due to the issues I faced with VUMC regarding staffing, lab space, and other issues, I have learned many lessons regarding personnel management and budget management under unusually difficult circumstances. Having Dr. Coffey as a Career Guide was invaluable. Furthermore, when it became obvious that my situation at VUMC was untenable in the long term, I began applying for new positions. In doing so I made many more connections in the field and I was offered each of the three academic positions for which I applied and interviewed. Dr. Coffey was again crucial in helping me to prepare for interviews and research presentations. While it is unfortunate that our laboratory needed to change institutions in order to succeed in our research, I fully believe that the process has contributed significantly to my professional development.

Shortly prior to arriving at SBU, I also became a member of the ad-hoc group of GI Cancer researchers. Our administration was aware of my DoD Career Development Award, and asked me to take part in this group that is working to develop program level grants in GI cancer. My project was identified as one that may be included in future grant applications, which is leading to great networking opportunities within my new institutions. In sum, I would summarize my professional development as having benefitted from the recent challenging circumstances, including research delays due to COVID-19 and moving our laboratory.

Additionally, Dr. Carney has great professional development opportunities as well. He has spent a majority of his time outside of the lab learning about colorectal cancer biology. He has done so by reading primary literature as well as attending Dr. Coffey's laboratory meetings and the Epithelial Biology Center seminars. During his short involvement in this project, he has made great strides in his understanding of colorectal cancer, microRNA signaling, and strategies for targeting microRNA-related epigenetic mechanisms of drug resistance. He has also networked extensively with the GI Cancer researchers at VUMC and will continue to do so at Stony Brook.

How were the results disseminated to communities of interest?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

If this is the final report, state “Nothing to Report.”

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

The non-covalent complexation of the antagomir to form polyplexes is performed after we have achieved the scale up of the triconjugates. This process is shown in Figure 1 (step 4) affibody-LPEI-antigomir triconjugate polyplex (Fig 1, step 4). A possible secondary strategy is to form the LPEI-PEG-N₃ and DBCO-PODS-Affibody constructs separately and then combine those two components. Based on further examination of literature precedent, we believe this strategy may be more feasible. While awaiting award transfer, we will investigate this alternative strategy.

4. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report.

What was the impact on other disciplines?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

- 5. CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, “Nothing to Report,” if applicable:*

Changes in approach and reasons for change

Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

No significant changes to the approach were required. We did find that published methodology for synthesizing and purifying intermediates for the polyplexes were not reproducible using the equipment available to us. Unfortunately, we were not provided with the equipment budget guaranteed in Dr. Houghton’s start up contract and thus we were not able to acquire the equipment necessary to reproduce the methods, as planned. Thus, we have attempted different approaches, including gel filtration chromatography based on size exclusion. This was not fruitful as the polycationic nature of the linear polyethylenimine was not amendable to commercially available gel filtration resins. Thus, we choose to troubleshoot the reported methods extensively and were able to identify alternative methods. It is worth noting that as of submitting this report, the Houghton Laboratory is purchasing the appropriate equipment for these studies at Stony Brook University using provided start up funding for equipment.

Actual or anticipated problems or delays and actions or plans to resolve them

Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

The biggest delay has been a result of COVID-19. Starting the first week of March, we were not able to access the laboratory on a regular basis and this persisted through the time we left VUMC on June 3rd, 2020. During that time, I persisted in my career development by attending Dr. Coffey’s virtual lab meetings and other colorectal cancer seminars. We also began preparing for grant submissions that we believe will be possible as a direct result of our proposed research. As of the preparation of this document, the Houghton Laboratory has received full clearance to resume research activities at Stony Brook University and no such further delays are anticipated.

There was a delay in research due to an unanticipated lack of personnel for the project. My graduate student, Vilma Jallinoja, was to be the primary trainee to perform the work on this project. However, she was awarded a fellowship to pursue another project in interim time between submission and funding. As part of my start up package, I was guaranteed salary support for two postdocs, but I was refused the hiring of a second postdoc, even after award notification. Thus, I had Dr. Carney switch to this project upon completion of his T32 training in January 2020. I was able to train Dr. Carney during the August – December 2019 period, but his primary effort was required on T32-related project. Due to VUMC not meeting the obligations in my start up contract, we have moved to SBU and anticipate no such further issues.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

The restrictions on travel will lead to decreased expenditures on travel. Currently, we have not charged any travel to the award, and it seems unlikely any travel will be advisable in the coming year. Rather, we will utilize the travel funds to register for virtual meetings at a reduced price. Additionally, we have not spent as much as anticipated on radioactive isotopes or consumables during the first year. This is predominantly due to the change in institution as well as the reduced research performed due to COVID-related restrictions on laboratory access. Due to delays in getting personnel involved in the project, no trainee was paid during the first 4 months of the award period, but to catch up on the research progress Dr. Carney was assigned more dedicated time in the following months to catch up. This should have little impact on total expenditure.

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Does not apply.

Significant changes in use or care of vertebrate animals

We have not reached the point of animal studies at this point. Currently, we are working to obtain IACUC approval using our previous approvals from VUMC as a template. We expect to submit our IACUC documents on August 18, 2020. We have not previously submitted as Stony Brook University is currently transitioning to a new online system which they expect to be available in August. We anticipate approval and submission to ACURO well in advance of grant transfer and certainly before we reach the proposed animal studies.

Significant changes in use of biohazards and/or select agents

No changes. We anticipate completing the appropriate trainings and submitting for approval by the end of July 2020 at Stony Brook University.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume; year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

The work has yet to advance to the point of publishing.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

None.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year*

(international, national, local societies, military meetings, etc.). Use an asterisk () if presentation produced a manuscript.*

None.

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

None.

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

We have developed novel synthesis and purification strategies which will be published in a peer reviewed journal along with the rest of our study results.

- **Inventions, patent applications, and/or licenses**

Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

None

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*

- *new business creation; and*
- *other.*

None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate “no change”.

Name: *Jacob Houghton, PhD*

Project Role: *PI*

Nearest person month worked: *5.5*

Contribution to Project: *Dr. Houghton is the PI. His laboratory work to date has involved training Dr. Carney in the areas of polymer synthesis, purification, and characterization. Additionally, his work has made significant strides in his career development under the mentorship of Dr. Coffey (Career Guide). Dr. Houghton has regularly attended lab meetings in person and virtually when in person attendance was not possible as well as the Epithelial Biology Center monthly meetings. Dr. Houghton has also advanced his network significantly by pursuing a better environment for his laboratory to perform the proposed studies. During the pandemic-related work from home mandate, Dr. Coffey recommended that, as part of his career development efforts, Dr. Houghton work with Dr. Carney to thoroughly review relevant research literature with the aim of preparing applications for additional funding for research that will directly derive from the funded project.*

Funding Support: *All work on this project was supported by this award.*

Name: *Brandon Carney, PhD*

Project Role: *Postdoctoral Fellow*

Nearest person month worked: *4.5*

Contribution to Project: *Dr. Carney’s laboratory work to date has involved being trained in the areas of polymer synthesis, purification, and characterization. Dr. Carney is an excellent radiochemist but he has limited experience in polymer chemistry and characterization. He has been responsible for troubleshooting the methodology for purification and characterization of the polyplexes. During January – early March, Dr. Carney worked out new methodology for our equipment to successfully isolate the polyplex intermediates. During the work from home mandate and up to our departure from VUMC, Dr. Carney worked diligently to support Dr. Houghton’s efforts to plan for future funding applications that are anticipated to derive directly from this research.*

Funding Support: *All work on this project was supported by this award.*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Upon moving to Stony Brook University, Dr. Houghton was provided with \$1,000,000 in start up funds. These funds are being utilized to outfit new laboratory space and to support staff and trainee salaries. This support will ensure that the Houghton Laboratory has all of the equipment and personnel required to complete the proposed studies. These funds are also being used to bridge the gap in funding while awaiting for award transfer. The new equipment and lab space will meet or exceed all requirements to complete the proposed studies.

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner’s contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner’s facilities for project activities);*
- *Collaboration (e.g., partner’s staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner’s staff use each other’s facilities, work at each other’s site); and*
- *Other.*

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to <https://ers.amedd.army.mil> for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on <https://www.usamraa.army.mil>) should be updated and submitted with attachments.*

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*