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TITLE: Development of a Novel Platform for In Vivo Delivery of Antagomirs to Study Cetuximab Resistance in Colorectal Cancer

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14. ABSTRACT

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. To date we have made significant progress on the synthesis of our antagomir delivery molecules, though that progress has been hampered in part by COVID-related absence from the laboratory.

15. SUBJECT TERMS

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

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1. INTRODUCTION:

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.

2. KEYWORDS:

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

3. ACCOMPLISHMENTS:

What were the major goals of the project?

This project began in August 2019 and work in our laboratory ceased due to the COVID-19 pandemic in March 2020. After our laboratory moved to Stony Brook University (SBU), the grant was transferred and work resumed in November of 2020, albeit at a reduced pace due to COVID-based restrictions on lab personnel lasting through April 2021.

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 were the “Synthesis of 1:1 and 1:3 triconjugates”, the development of a transiently transfected luciferase cell line to provide a read out of the biological effects of the antagomirs, and to test their ability to reverse cetuximab resistance in vitro and using in vivo mouse models of CRC.

Major Activities and Specific Objectives

Major Task 1 (partially completed)

We have developed the methodology for the synthesis, purification, and characterization methods for the "triconjugates" based on our novel approach, which would be the result of step 2 in Figure 1. Unfortunately, Dr. Carney left the laboratory in December 2021 after developing these techniques and we then had to train a new lab member to replicate his work and scale up the previously reported conjugates. Ms. Abbriano, a research support specialist in the Houghton Laboratory, worked with Dr. Carney during his final weeks in the lab and thereafter with Dr. Houghton to train in the preparation, purification, and characterization of the LPEI-PEG conjugates. Dr. Kaur, a new postdoctoral fellow in the Houghton Laboratory, was also hired and has been training to take over as the primary "hands on" researcher on this project along with Ms. Abbriano and Ms. Bhatt, who is performing the cell line and related *in vitro* work.

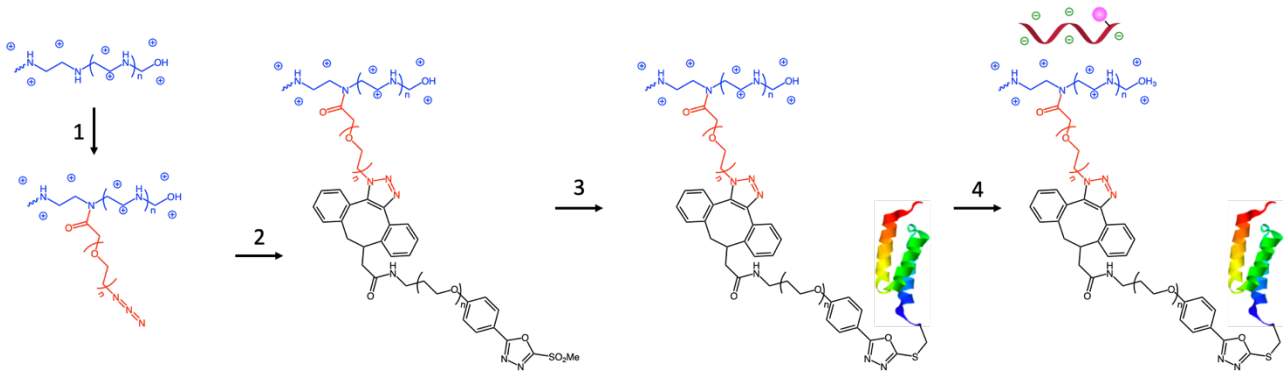

Figure 1: Proposed Synthesis. First, the PEG-N_3 conjugated to the LPEI via NHS ester reaction with one of the secondary amines on the LPEI. Then the PODS is attached via DBCO copper free click chemistry. Then the affibody is attached by a reaction between the PODS and a terminal cystine engineered into the affibody. Finally, the antagomir is added via a non-covalent complexation with the LPEI. The final result is an affibody-LPEI-antigomir triconjugate polyplex.

To synthesize the triconjugates, we had followed a previous literature report as proposed in our application. However, those methods, which reported a very specific ratio of PEG to LPEI, were not reproducible despite extensive efforts to optimize the reported procedure. We discussed this synthetic route with several nanomaterial experts at Vanderbilt University and Stony Brook University and concluded that the data reported was likely not reproducible as reported. Thus, we developed our own procedure and we successfully achieved formation of the triconjugates.

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). Previous work was done in the Houghton Lab on a ÄKTA Start FPLC at VUMC, however a new

system was purchased from BioRad to carry out the remainder of the work. Some work developing methods for the new system was required. This work was done with good results. The lab is now able to use the new system to separate the LPEI products after synthesis. In addition to the ion exchange method in reported in the literature, the Houghton lab has developed a method using size exclusion chromatography (SEC) to perform the separations without the need to vary the salt content of the eluent (**Figure 2**). This has eliminated the need for a dialysis step in the purification to remove excess salt.

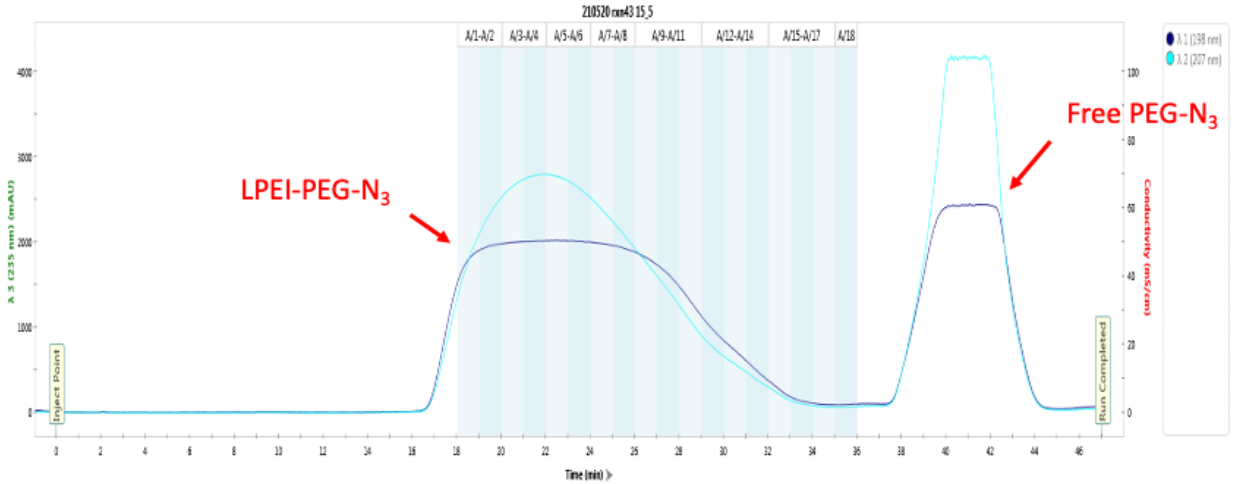

Figure 2: SEC Method Development. This SEC chromatogram shows the results typical purification of the first step of a reaction. Here, the LPEI hydrochloride salt that is commercially available was first converted to the base, then reacted with 5eqs of PEG at room temperature for 3 hours, then converted back into the hydrochloride salt before being separated from the unreacted PEG on a Cytiva Superdex 75 GL SEC column.

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. 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 dissolve 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.

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. We worked to optimize this synthesis with good results. 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. By collecting fractions of the large peak associated with the expected product, we were able to obtain LPEI-PEG-N_3 constructs (diconjugates) with varying LPEI:PEG ratios. We are now able to tune the ratio of the ethylene glycol moieties (EG) to the ethylenimine (EI) moieties. In addition to tuning the EG:EI ratio, we also developed a method to use SEC chromatography to separate different length LPEI polymers. We found that using different length LPEI chains could affect the EG:EI ratio up to 40% (**Figure 3**). These were suitable for formation of the triconjugates and for the remainder of the experiments we are utilizing the diconjugates with a 2:1 ratio.

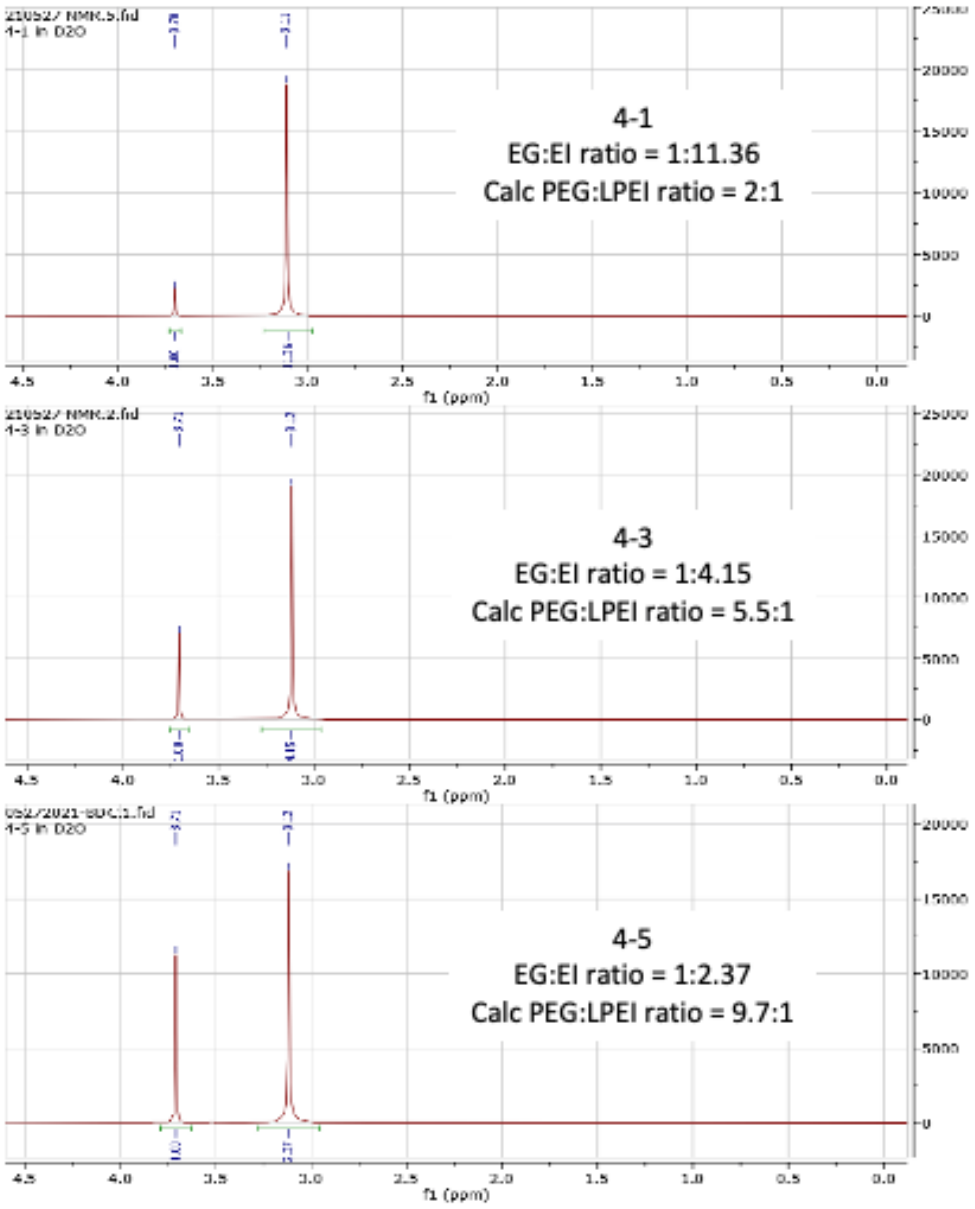

Figure 3: Tuning the EG:EI ratio. These NMRs show differing EG:EI ratios based solely on the amount of PEG added (1, 3, and 5 eqs). All syntheses were done at similar scales (~20mg) and with similar reaction conditions (3 hours at room temp).

We have also produced and validated the novel site specific linker PODS-DBCO (**Figure 4, next page**). We have developed the synthesis and characterized the molecule and scaled up the synthesis of the linker to be adequate for the remainder of the study. This linker is an exciting addition to our tool kit for producing LPEI based conjugates because it is widely applicable to numerous other applications. With the individual components fully synthesized and characterized, the next step was to determine appropriate conditions for completion of the polyplexes.

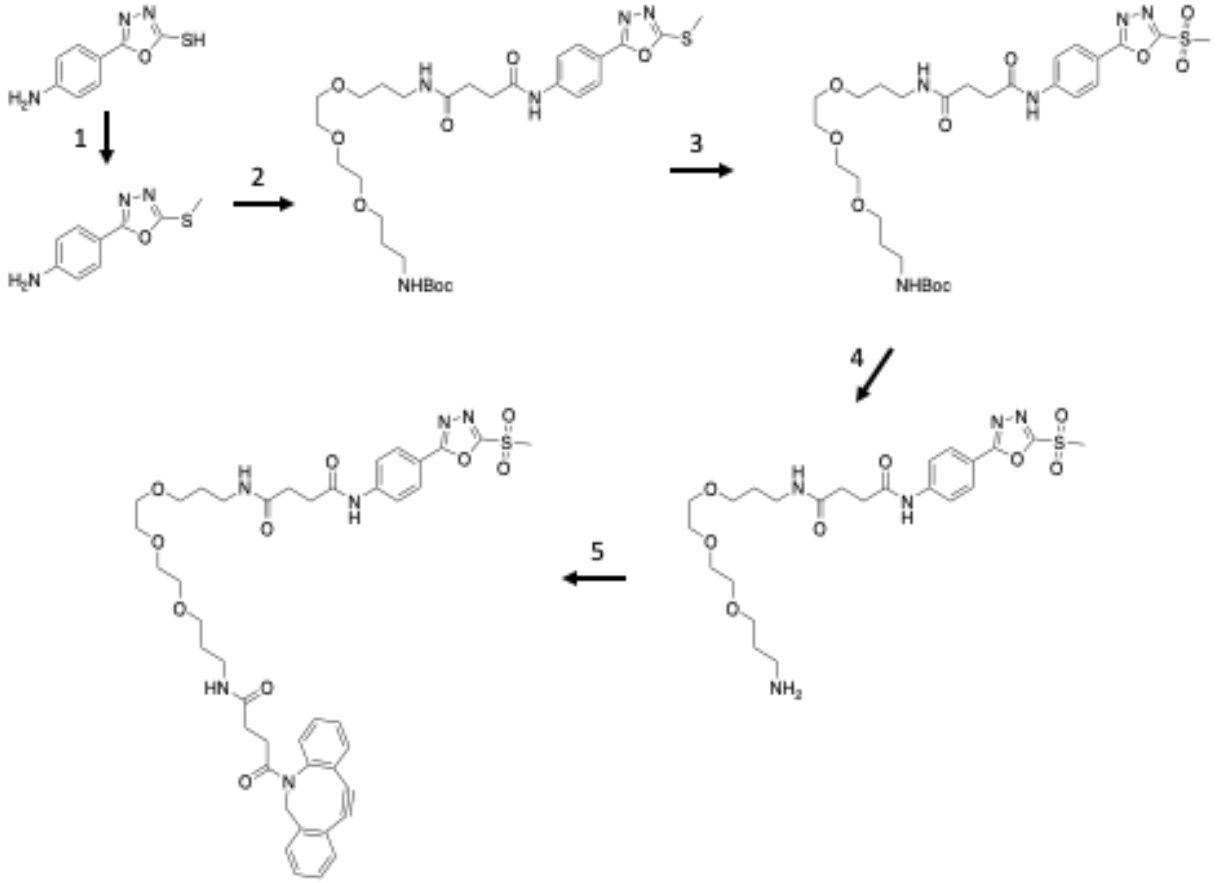

Figure 4: PODS-DBCO Synthetic Scheme. First the thiol group is methylated with iodomethane (1), then the PEG group is added with NHS ester chemistry (2). The sulfur is then oxidized with mCPBA (3) and the primary amine is deprotected (4). Finally, the DBCO is added with NHS ester chemistry to yield the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

The next steps in the synthesis of our triconjugates was the attachment of the affibody to form the completed triconjugates. Towards that goal, we attempted to screen reaction conditions for the directly conjugating the affibody to the diconjugate under basic conditions. However, this step proved impossible due to low solubility, and we were not able to observe the formation of the triconjugates. For that reason, we decided to first conjugate the PODS-DBCO to the the affibody. This was achieved under mild reducing conditions as reported for other antibody-PODS bioconjugations and confirmed by then reacting a fluoroscein-azide. To test the effects of the conjugation on the binding of the affibody, we performed cell binding assays using the EGFR expressing cell line BxPC3 but we found that the affibody was not demonstrating specific binding. We hypothesized that it may have been due to the long-term storage of the affibody and shipment from our previous institution. We requested that our collaborator send us new aliquots of the affibody to test as it is not commercially available. *However, the laboratory of our collaborator did not have any additional affibody to send us, which led us to consider an alternate path forward to achieve EGFR targeting with our polyplexes.*

As an alternative, we have been pursuing the development of cetuximab and cetuximab fragments that can be directly substituted for the affibody that we had originally proposed to use. To achieve this we have used a commercially available kit (FragIT; Genovis) to generate the f(ab')2 fragments. The kits utilize a proprietary cysteine protease to cleave the antibody below the hinge site yielding a fragment with a conveniently exposed free cysteine to modify with our PODS-DBCO. To date, we have produced the fragments from cetuximab, labeled then with a fluorescent tag, and determined that they do bind to EGFR expressing cells. We have recently optimized the conjugation of the PODS-DBCO to the cetuximab f(ab')2 fragment and characterization of that bioconjugate. We have found that we can tune the number of PODS per cetuximab fragment by simply altering the equivalents of PODS in the reaction which takes place in PBS with a pH of 7.4 over 2 hours. T

Very recently we have worked out the conditions for the next step, which is the formation of the triconjugates by incorporating the PODS-conjugated fragment to the LPEI-PEG-N_3 and then using the fluorescently labeled antagomirs to form the final polyplexes to use for in vitro validation. This is achieved in a "single pot" reaction. The antagomir, LPEI-PEG-N_3, and cetuximab-PODS are mixed in a 0.1:1:2 (antagomir to cetuximab to LPEI-PEG-N_3) ratio in PBS pH 7.4 for two hours. Our spectra from the size exclusion chromatography of the reaction shows formation of the final polyplexes, which is an incredibly exciting results given the wholly new process we had to develop. However, these show that there is likely a mixture of two different products. Based on the size difference, we suspect that there are some PODS-cetuximab with 1 LPEI or 2 LPEI conjugated. We are currently working on conditions for separation of the two species via size exclusion chromatography by reducing the flow rate to more slowly elute and separate the two species. While we expect that a 1:1 LPEI to cetuximab will be best, we will evaluate both via in vitro cell assays to determine which is best for future in vivo experiments.

The size exclusion chromatograms of the full size cetuximab starting material and the fully formed polyplex product are shown in Figure 5. The reduced size (longer retention time) of the polyplex (~125kDa) relative to the full size cetuximab (~155 kDa) is apparent and the small peak arising from the fluorescent antagomir (560nm) aligns with the expected molecular weight of the polyplex. The peak is relatively small due to the small amount of antagomir we used in this pilot study. We are optimizing the conditions to obtain a fully 1:1:1 construct currently. We expect that the separation process will take a few weeks to optimize and that once that is completed, we will be able to rapidly complete Subtask 3 and 5 to wrap up Major Task 1.

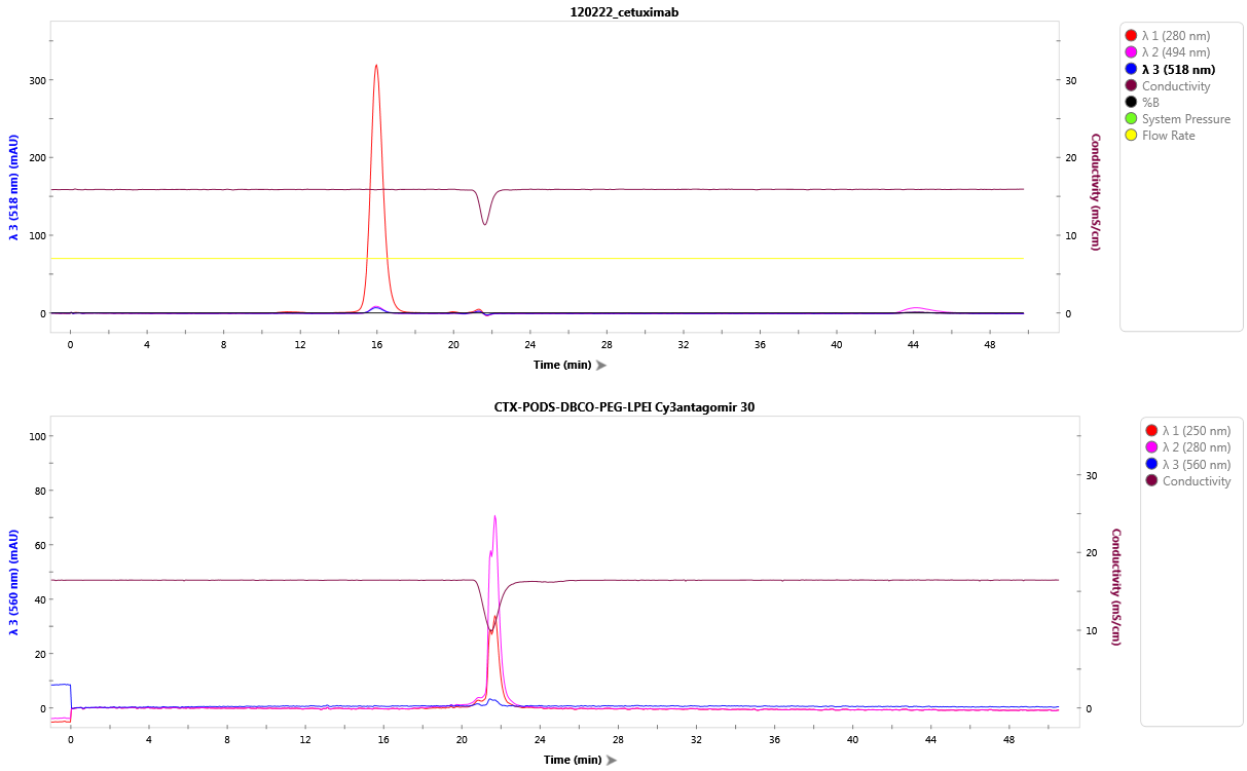

Figure 5. Size exclusion chromatogram of full size cetuximab (top panel) compared to fully formed polyplex (bottom panel).

One caveat to the completion of Subtask 2 is that the supplier who agreed to prepare the antagomirs with DFO for radiolabeling with ^{89}Zr will no longer prepare them citing that it could affect the binding. While we disputed this with them for months, arguing that it is no different than the fluorophore-modified versions, they will not attempt it. We do not have the equipment or expertise to synthesize antagomirs in our own lab and we will use the fluorophore-modified versions for all our in vitro work as planned. So that we can use PET imaging to determine the fate of the polyplexes in vivo, we will use standard conjugation techniques that Dr. Houghton has used for more than a decade to directly label the polyplex and label with ^{89}Zr.

Major Task 2 (complete)

During the current award period, we have also focused substantial efforts on transient transfection of our established cell lines with TOPFlash reporting system. Dr. Houghton and Ms. Bhatt have used a commercially available kit, screening a number of conditions for transfection and detection of luciferase activity in the cell lines. The BPS Bioscience TCF/LEF Reporter kit (Catalog # 60500) was used for transfection of TCF/LEF into the CC-CR cells according to the manufacturer's protocol. The BPS Bioscience Dual Luciferase (Firefly-Renilla) Assay system (Catalog # 60683-1) was used for detection of the Firefly and Renilla luciferase activity in the CC-CR cells transfected with the TCF/LEF reporter or a Non-Inducible luciferase reporter vector according to the manufacturer's protocols. After initial failure of the transfections using the precise instructions from the manufacturer, we also screened additional concentrations of the TCF/LEF luciferase reported vector DNA, amounts of lipofectamine 2000, incubation times, and amounts of recombinant human Wnt3a in order to determine if we could successfully transfect and detect signal in the cell lines. Unfortunately, none of the conditions that were screened led to any indication of successful transfection. As a result, we worked with Dr. Coffey's laboratory at VUMC to develop a stably

transfected version of these cell lines to use for the in vitro and eventually the in vivo work outlined in our SOW. The transfection using a lentiviral vector have proven to be a more suitable route and the development of these stably transfected cell lines was recently completed in collaboration with Dr. Coffey. We will soon verify their suitability for these experiments using the same BPS Bioscience Dual Luciferase (Firefly-Renilla) Assay system for detection of the Firefly and Renilla luciferase activity. While this was a slight variation of the proposed work in Major Task 2, it was actually a major comment by our reviewer in the initial application that turned out to be very pertinent. We believe that these cell lines will be much more suitable for the in vivo work we have planned in the coming months due to their stable transfection rather than transient transfection.

Major Task 3 (underway)

We now have the materials to pursue this aim and plan to begin them in the coming month once the full polyplex purification has been optimized and we have collected sufficient material for the studies. This was delayed because the cell lines proposed were not suitable for transfection with the proposed commercial kit and the alternative of producing a stably transfected cell line took substantially longer than the development of a transiently transfected line. Once the cell lines have been expanded and enough to place vials in cryostorage, we will complete this aim with an estimated time frame of 1-2 months.

Major Task 4 (incomplete)

These studies will utilize the stably transfected cell lines that were recently developed. Our first step will be a pilot xenografting study to determine the appropriate number of cells to grow suitable xenografts. These xenograft models will then be utilized to test the polyplexes that we have recently prepared using our novel methodology. Once the polyplexes have been validated in vitro, we will bring up the xenograft model for testing in vivo. We expect the xenografts to take 4-6 weeks to establish and we will then utilize them for imaging and ex vivo experiments as proposed. We anticipate this Task will take approximately 3-4 months to complete but it will begin concurrently with Major Task 3, so we believe that overall this will take approximately 5 months in total.

Major Task 5 (incomplete)

This task will be possible only after the conclusion of our in vivo studies. We plan to publish the results in a single journal article and to present the findings at a scientific meeting in late 2023 (*Subtasks 1 and 2*). With the polyplexes and cell line complete, we have enough preliminary data to support future grant applications and we are working to identify appropriate award mechanisms that will allow us to continue this exciting work in the future and to assess its suitability for studying other mRNA mechanisms of resistance. We expect to submit a proposal to NIH and/or DoD in late 2023 (*Subtask 3*). We expect to have a manuscript ready for submission in approximately 6 months.

Significant results to date

- Polyplex synthesis
    - Optimized synthesis conditions for LPEI-PEG-N_3
    - Developed and optimized purification techniques LPEI-PEG-N_3
    - Developed techniques for tuning EG:EI ratios
    - Produced and validated PODS-DBCO
    - PODS-DBCO conjugated to affibody
        - Affibody does not show binding to EGFR-expressing cells
    - Developed a new approach to utilize cetuximab antibody fragments which will provide an easily accessible commercial source of targeting molecules for the polyplexes
    - Completed formation of final polyplexes
        - Currently optimizing purification and scale up
    - New fluorescent antagomirs delivered
        - DFO-conjugated antagomirs will *not* be available
- Cell lines
    - All cell lines obtained, cultured, and stocks frozen
    - Plating density and growth conditions completed for each cell line
    - CC-CR transfection with TOPFlash attempted
        - Despite screening conditions, it was found that the cells are not amenable to transient transfection using commercial kits
    - Worked with Dr. Coffey's laboratory to develop a stably transfect TOPFlash CC and CC-CR cell line for use in these studies
        - Clones selected and being cultured
        - Ready for use in coming weeks
- IACUC and ACURO approval obtained at SBU
- New laboratory staff have been trained to take over in the absence of Dr. Carney who was responsible for developing the polyplex chemistry and related methodology

    - *FTE levels were actively adjusted (reduced) during training periods to reflect actual work on the project so support would remain available for staff for completion of all the experiments in the SoW*

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

Training

Dr. Kaur and Ms. Abbriano began training in polymer and bioconjugation chemistry with Dr. Houghton. In addition to learning chemical synthesis techniques, he also learned how to perform numerous purification techniques on poly ionic compounds using ion exchange chromatography, which are new to them. They are also working to expand their knowledge of colorectal cancer biology. Dr. Houghton has continued to gain didactic training in colorectal cancer biology and imaging as previously reported.

Professional Development

Dr. Houghton has continued to work with Dr. Coffey to expand his network of GI and colorectal cancer experts during this period of the award. Additional opportunities have been afforded to Dr. Houghton since the move to SBU including participation in numerous groups that focus on imaging and cancer biology including, becoming a member and regularly presenting to the Imaging, Biomarker Discovery, and Engineering Sciences program, participating in the pancreatic and colorectal CRTs, and presenting for Pathology Grand Rounds. Additionally, Dr. Houghton has recently (August 2022) become a full affiliate member of the Chemistry Department at SBU where he will be able to recruit students to continue this project long term.

Dr. Houghton has also been 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 continues to lead to great networking opportunities within my new institution. 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.

Due to the departure of Dr. Carney shortly after the final project period, I have been training a new laboratory staff member to continue his work while efforts to recruit a new postdoctoral fellow are ongoing. Ms. Abbriano and Dr. Kaur have taken over for Dr. Carney as the primary "hands on" researchers carrying out the development of the polyplexes. They will continue professional development activities suited to trainees.

Based on the Major Tasks in the Career Development SoW, Dr. Houghton has achieved all of the stated goals in terms of didactic, grantsmanship, and personnel management training. Furthermore, Dr. Houghton has developed a strong professional network in the field of colorectal cancer research.

How were the results disseminated to communities of interest?

Nothing to report.

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

We are currently awaiting the outcome of our rerquest for an NCE owing to the delays experienced with COVID, moving our laboratory to a new institution, and the unexpected staff turnover we experienced last year. We will then study the ability of our polyplexes to deliver the antagomirs in vitro with the newly developed, stably transfected cell lines. Simultaneously, we will develop the method to conjugate DFO to the polyplexes and radiolabel them for PET imaging applications as described in our SOW. Once these methods are established, we will proceed to the animal studies under our approved protocol. *We now have fully trained staff to proceed as well as the materials and methodology developed to carry the project forward.* With all of these pieces in place, we expect to complete the studies outlined in the SoW within the coming 6 months (before August 2023).

4. IMPACT:

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

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

There were 4 minor changes to our approach that did not change our objectives or the scope.

1. We have confirmed that the published methodology for synthesizing and purifying intermediates for the polyplexes were not reproducible. We conferred with numerous experts in polymer chemistry, and they confirmed that the published methods were ambiguous. We sought to identify alternative methods, and Dr. Carney developed a novel approach to prepare well-characterized polyplexes, which is reported above.

2. We had to change our approach to the development of the cell lines for testing the polyplexes. The cell lines were not amenable to transient transfection after months of screening conditions and testing commercial kits. Working with Dr. Coffey are developing a stably transfected version of the CC and CC-CR cell lines with TOPFlash. We are currently selecting clones after successful lentiviral transfection and expect to have the cell lines fully available within 1-2 months. This approach was actually suggested by one of the reviewers on our initial application and will provide a more robust model for testing our polyplexes.

3. A third minor change in the approach was necessary due to our loss of access to the affibody. This was previously provided by a collaborator who has since retired, and his laboratory is no longer able to provide material for these studies. We are currently working to develop antibody fragments that will be amenable to our application. We anticipate not major changes in the performances of the polyplex, but this has added an additional challenge to their development.

4. A final change has been made necessary due to the companies that originally agreed to prepare and provide functionalized antagomirs. The two companies that provide this service will no longer provide the functionalized versions and we are now planning to attach our PET isotopes directly to the triconjugates.

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

The TOPFlash reported kit required for our experiments was backordered for nearly 9 months due to the company being out of the lab due to the pandemic. We finally received the kit during this project period and we prioritized the development of our TOPFlash harboring CC-CR cell lines. Unfortunately these cell lines have proven unsuitable for the transient transfection, despite our best efforts over 6 months in screening conditions with the support from Dr. Coffey's laboratory. For this reason, we are collaborating with Dr. Coffey to develop a stably transfected version of these cell lines which has been successful so far. We are now selecting clones and expect to have the cell lines established in the coming months.

Additionally, the company from which we originally planned to source the antagomirs was acquired and *drastically* increased their prices. We have identified a new vendor however both companies (the only two of which we are aware that supply these items) will no longer provide chelator conjugated versions of the antagomirs as previously promised, citing the potential loss of function. We have continued to seek a vendor to provide these but we have no found one to date. This has caused us to rework our approach and we are now working on methodology to append the chelator and radionuclides to the LPEI portion of our polyplexes.

A final delay was caused by the departure of Dr. Carney from the laboratory. He spent two years developing the methodology for the polyplex synthesis and we have only recently been able to hire a replacement postdoc. This required that Dr. Houghton train laboratory support staff to fill his role in the interim which significantly delayed the progress of the project as Dr. Carney's expertise was critical to the project.

Changes that had a significant impact on expenditures

The restrictions on travel led to decreased expenditures on travel. Currently, we have not charged any travel to the award. Additionally, we have not spent as much as anticipated on radioactive isotopes or consumables. Lastly, we have had personnel turnover in the lab and only recently hired a replacement postdoc so personnel costs were lower than anticipated to date. We do not anticipate any impact on total expenditure.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Does not apply.

Significant changes in use or care of vertebrate animals

No changes

Significant changes in use of biohazards and/or select agents

No changes. All trainings and approvals at SBU are now completed.

6. PRODUCTS:

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

Journal publications.

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

Books or other non-periodical, one-time publications.

None.

Other publications, conference papers and presentations.

None.

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

None.

- **Technologies or techniques**

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. Our methodologies, unlike currently published methodologies will be easily reproducible from our published protocols which will allow any researchers to take advantage of these advances.

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

None

- **Other Products**

None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Jacob Houghton, PhD (PI) – No change

Name: Brandon Carney, PhD
Project Role: Postdoctoral Fellow
Nearest person month worked: 3
Contribution to Project: Dr. Carney's laboratory work 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.
Funding Support: All work on this project was supported by this award.

Name: Courtney Abbriano
Project Role: Research Support Specialist
Nearest person month worked: 2
Contribution to Project: Ms. Abbriano's laboratory work to date has involved being trained in the areas of polymer synthesis, purification, and characterization. She has also worked with Ms. Bhatt to develop the cell lines.
Funding Support: All work on this project was supported by this award.

Name: Kavita Bhatt
Project Role: Research Support Specialist
Nearest person month worked: 1
Contribution to Project: Ms. Bhatt's laboratory work to date has involved the development of the transiently transfected TOPFlash cell lines and bringing up all cell lines for the project.
Funding Support: All work on this project was supported by this award.

Name: Amritjyot Kaur
Project Role: Postdoctoral Fellow
Nearest person month worked: 1
Contribution to Project: Dr. Kaur's laboratory work has focused on training for the development of the polyplex synthesis, picking up where Dr. Carney left off as well as training in antibody fragment preparation.
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?

Upon moving to Stony Brook University, Dr. Houghton was provided with start up funds. These funds were utilized to outfit new laboratory space and to support staff and trainee salaries and those funds were also 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?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS
COLLABORATIVE AWARDS: QUAD CHARTS:

9. APPENDICES: