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14. ABSTRACT

Prostate cancer is not only heterogeneous among individuals, but tumors are heterogeneous on the molecular level within a given individual. One well-documented phenomenon is the presence of castrate-resistant prostate cancer with neuroendocrine features (CRPC-NE) that evolves over the course of anti-androgen therapy. Our group has discovered that CRPC-NE have a unique cell surface glycan composition enriched with fucose sugars. Therefore, the ability to image glycan fucosylation could be used to predict the emergence of lethal prostate cancer in men. This proposal tests the feasibility of a previously developed PET agent, [^{18}F] fluorofucose (Fuc-PET) as a functional method to quantify PCa glycan fucosylation, and thus, the burden of lethal disease using animal models. Our **hypothesis** is that Fuc-PET can quantify the amount of glycan fucosylation in tumors noninvasively and thus predict aggressive pathology in vivo. In the first year, we have made advancements in the characterization of fucose modifications to glycans through core glycan modifications and the synthesis of the Lewis-y antigen that are associated with more aggressive disease and shorter survival. We have also discovered that the ketogenic diet can inhibit the synthesis of these fucosylated glycans, suggesting that dietary modifications could enhance therapeutic efficacy in men with advanced prostate cancer.

15. SUBJECT TERMS
Prostate cancer, CRPC-NE, glycans, fucose

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INTRODUCTION

Prostate cancer is not only heterogeneous among individuals, but tumors are heterogeneous on the molecular level within a given individual. One well-documented phenomenon is the presence of castrate-resistant prostate cancer with neuroendocrine features (CRPC-NE) that evolves over the course of anti-androgen therapy. Our group has discovered that CRPC-NE have a unique cell surface glycan composition enriched with fucose sugars. Moreover, this enriched glycan fucosylation is not only present in CRPC-NE, but also a subset of prostate adenocarcinomas at initial diagnosis and are associated with adverse clinical outcomes. Therefore, the ability to image glycan fucosylation could be used to stratify men and initial diagnosis as well as predict the emergence of lethal prostate cancer in men. This proposal tests the feasibility of a previously developed PET agent, [^{18}F] fluorofucose (Fuc-PET) as a functional method to quantify PCa glycan fucosylation. The degree of fucosylation will be proportional to the burden of lethal disease using animal models. Our hypothesis is that Fuc-PET can quantify the amount of glycan fucosylation in tumors noninvasively and thus predict aggressive pathology in vivo. Aim 1 will correlate Fuc-PET activity with tumor glycan fucosylation. Aim 2 will identify the effects of key fucosyltransferases (FUT's) on tumor glycan synthesis. Aim 3 will assess the effect of pharmacologic inhibition of tumor fucosylation on tumor growth and tumor immunity.

KEYWORDS

Prostate Cancer
Neuroendocrine Prostate Cancer
Castrate Resistant Prostate Cancer
Glycans
Fucose
Positron Emission Tomography
Fucosyltransferases
Fluorofucose
Immunotherapy

ACCOMPLISHMENTS

What were the major goals of the project?

Aim 1. To correlate fucose-PET (Fuc-PET) activity with tumor glycan fucosylation. (80% complete)
1a. To characterize fucosylation patters across prostate cancer models.
1b. To perform Fuc-PET imaging in models.

Aim 2. To identify the effects of key fucosyltransferases (FUT's) on tumor glycan synthesis. (20% complete)
2a. To knock out key FUT in neuroendocrine prostate cancer cell lines.
2b. To identify effects of knockouts with MALDI and PET imaging of mice with tumors.

Aim 3: To assess the effect of pharmacologic inhibition of tumor fucosylation on tumor growth and tumor immunity. (80% complete)
3a. To determine the effect of inhibition of tumor fucosylation on tumor growth.
3b. To determine the effect of co-inhibition of tumor fucosylation in combination with immunotherapy.

What was accomplished under these goals?
We have made substantial progress despite the COVID delays from last year. The details are provided below as part of each Aim.

Identification of fucosylated glycans as markers for small cell neuroendocrine prostate cancer. Because the overall goal is to identify glycomic signatures of lethal prostate adenocarcinoma, we started with a panel of primary and metastatic prostate cancers with phenotypic small cell neuroendocrine histology. These included 5 transurethral prostate resections, 2 lymph node metastases, and a liver metastasis. Two additional control specimens included 1 primary small cell lung carcinoma tumor, and 1 lymph node metastasis from a urothelial carcinoma. Conserved among all of these specimens were a panel of glycans (**Figure 1**). There are two key components of the structures of these glycans that are relevant to this proposal. First, there is a core fucose (red triangle within the red circle) on the bases of all of these glycans. This core fucosylation is performed by fucosyltransferase 8 (discussed later) and has been implicated in as a regulator of castration resistance in prostate cancers [4]. Another intriguing feature of this glycan panel is the presence of the lewis-y antigen (red box, **Figure 1**) composed of two fucose sugars attached to a galactose (yellow circle) and a N-acetylglucosamine (blue square). Lewis-y antigen is implicated in tumorigenesis in numerous cancers including prostate and ovary and expression is associated with poor outcomes [5]. This is a significant finding because Lewis-y is a target for clinical monoclonal antibody trials and could be repurposed for imaging and therapy (see below).

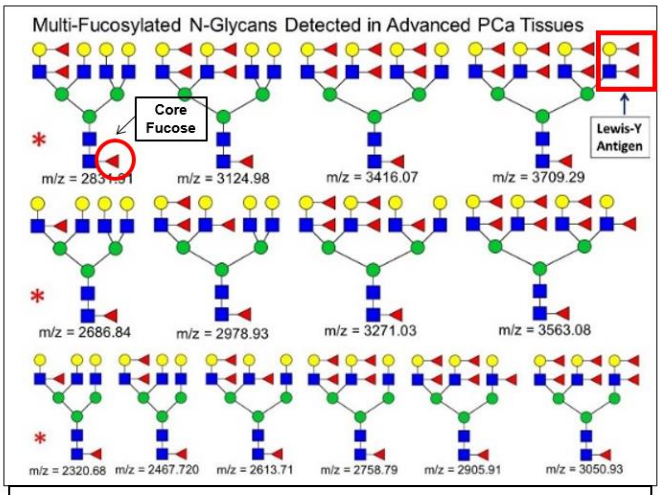

Figure 1. A panel of multi-fucosylated glycans as markers for lethal prostate cancer. Glycan structures composed of carbohydrate monomers (shapes) are listed along with their masses. The Lewis-y antigen (red box) and core fucosylation (red circle) are common features to this glycan signature. Fucose=red triangle.

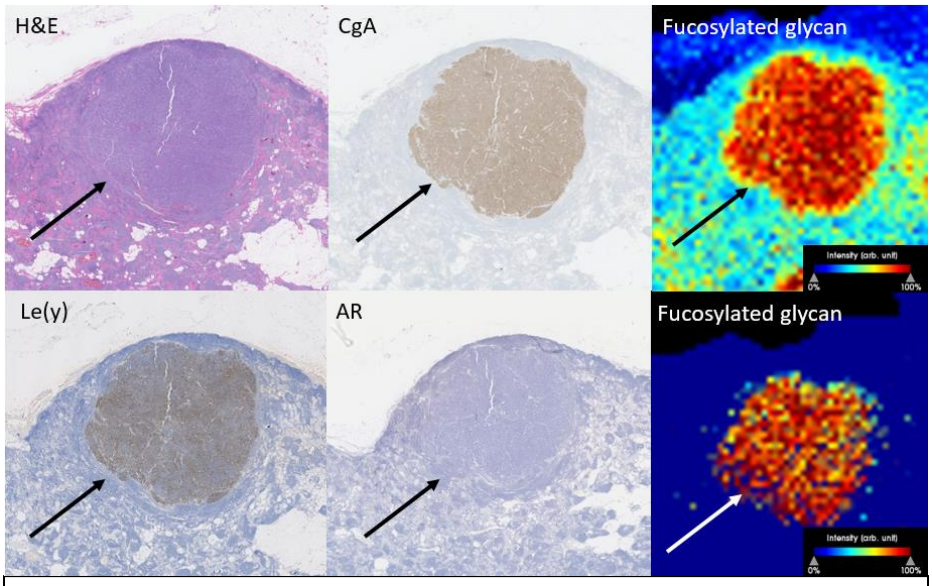

Figure 2. Lewis-y (Le(y)) expression in small cell prostate cancer. A lymph node metastasis (arrow) is positive for the neuroendocrine marker chromogranin A (CgA) and negative for the androgen receptor (AR) and positive for Le(y), further validating the presence of fucosylated glycans in lethal prostate cancer**.** This tumor is also positive high fucosylated glycans detected with MALDI.

To provide orthogonal validation of the presence of lewis-y in these specimens, we stained a small cell prostate cancer metastasis to a lymph node (**Figure 2**). As expected, this metastasis was positive for the conventional neuroendocrine marker chromogranin A (CgA) and negative for the androgen receptor (AR). This metastasis was also positive for Lewis-y that was superimposable with MALDI-detected fucosylated glycans (**identified in Figure 1**).

Expression of fucosylated glycans in prostate adenocarcinomas with adverse histologic features. Next, we wanted to identify if these fucosylated glycan biomarkers, expressed in lethal small cell carcinomas, were also present in adenocarcinomas and associated with poor outcomes and adverse histologic features. A series of 33 prostatectomy specimens diagnosed as adenocarcinoma were tested that ranged from Gleason scores 6-10. We identified that the presence of fucosylated glycans were enriched in the higher grade adenocarcinomas. An example of an invasive, metastatic (T4N1) adenocarcinoma with Gleason score 10 is provided in **Figure 3**. Expression of conventional neuroendocrine immunohistochemical markers chromogranin A (CgA) and synaptophysin (SYN) was positive, but among scattered cancer cells in the tumor. However, MALDI imaging focused on the glycan signature in Figure 1 identified multiple fucosylated glycans (three represented in Figure 3) that were spatially heterogeneous I their expression. These data further validate

MALDI imaging as a means to better molecularly characterize histologic samples that cannot be done with conventional immunohistochemistry. It further suggests that these fucosylated glycans could be better biomarkers than conventional CgA and SYN immunohistochemistry for neuroendocrine markers.

Because of the expression of potential neuroendocrine markers in aggressive prostate adenocarcinomas, we wanted to determine if there were specific neuroendocrine fucosylated glycans expressed in adenocarcinomas and if those glycans were associated with aggressive features. We profiled fucosylated glycan composition across all of our small cell neuroendocrine and adenocarcinoma prostatectomy specimens.

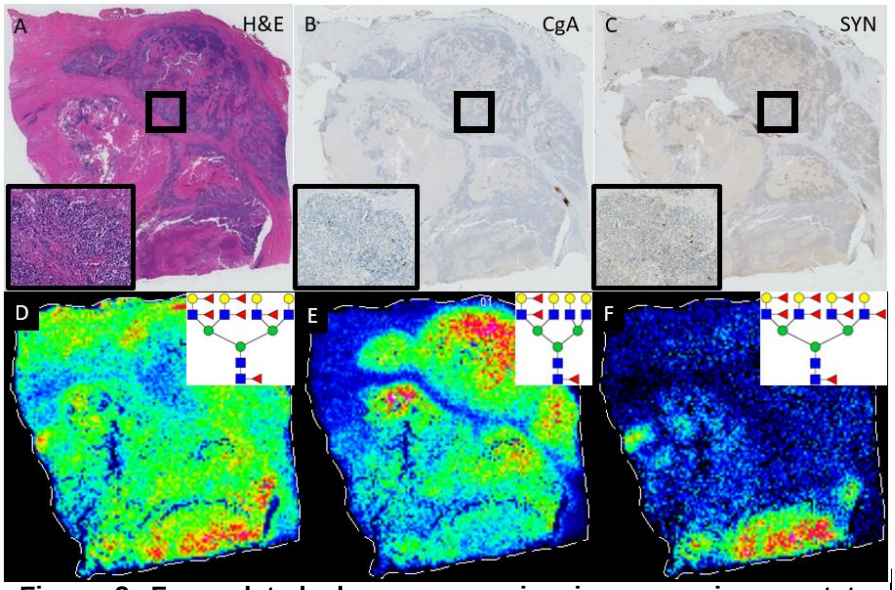

Figure 3. Fucosylated glycan expression in aggressive prostate adenocarcinomas is spatially heterogeneous. A. H&E demonstrating a poorly differentiated adenocarcinoma. B-C: Weak, but positive expression of the neuroendocrine markers chromogranin A (CgA) and synaptophysin (SYN). D-F. MALDI imaging of three selected fucosylated glycans showing spatial heterogeneity of glycan expression. Note the overall extent in expression of these glycans relative to conventional immunohistochemistry for neuroendocrine markers. Boxes in A-C represent the site for the magnified inset.

We plotted each sample as a function of each fucosylated glycan that was detected with MALDI mass spectrometry in that specimen. We discovered an intriguing dichotomy in the fucosylation patterns of small cell versus adenocarcinoma specimens (**Figure 4A**). Specifically, the small cell samples expressed more fucosylated glycans than the conventional adenocarcinomas. That gave us the idea to express the data in terms of a "fucose score", i.e., the sum of the expressed fucosylated glycans in each specimen. When the fucose score was plotted as a function of tissue histology, our initial observations were confirmed that the fucose score was higher in small cell specimens (**Figure 4B**). We also compared the fucose score to conventional staining methods for small cell and neuroendocrine features. As expected, higher fucose scores had higher levels of chromogranin A and synaptophysin staining (**Figure 4C-D**). In addition, the samples with higher fucose scores also had the lowest androgen

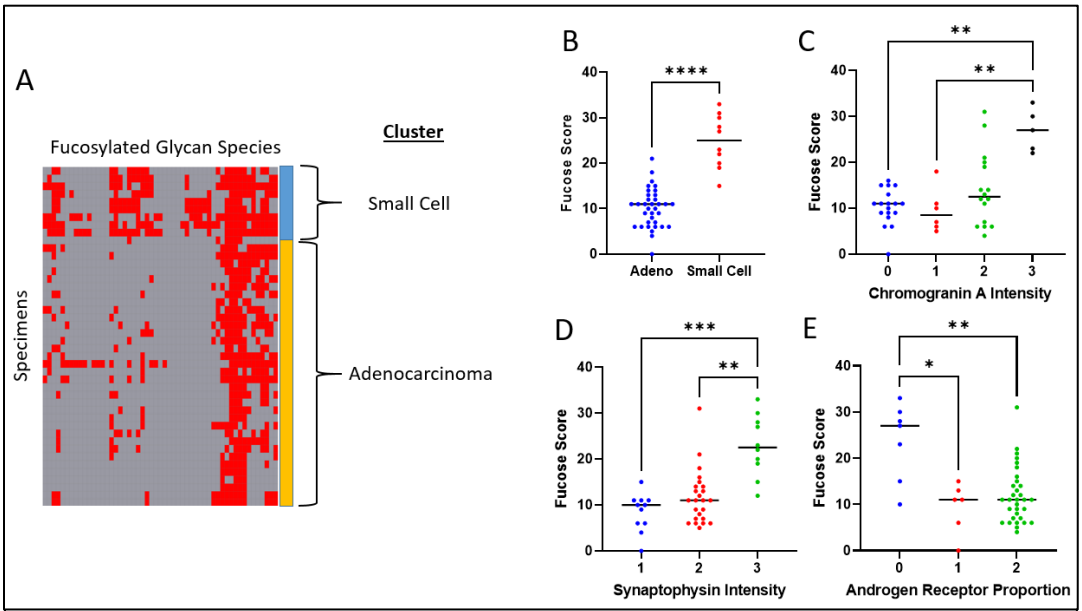

Figure 4. The Fucose Scoring System – A new method for quantifying neuroendocrine characteristics. A. Heatmap of fucosylated glycans (x-axis) and prostatectomy specimens (y-axis). Colored cells indicate the presence (red) or absence (gray) of the glycan in that specific specimen using MALDI. K means clustering identifies that the cluster with the most glycan hits is the small cell cluster. B. Plotting the fucose score (the sum of the glycan hits in each sample) as a function of histology confirms that small cell samples have the highest number of expressed fucosylated glycans. C. Chromogranin A, a small cell neuroendocrine immunohistochemical marker is highest in the samples with highest expression as is D. Synaptophysin. E. Fucose scoring has an opposite trend in androgen receptor staining, with the highest scores seen in androgen receptor negative samples.

receptor stains (**Figure 4E**). This supports the current literature, as small cell tumors do not express androgen receptors.

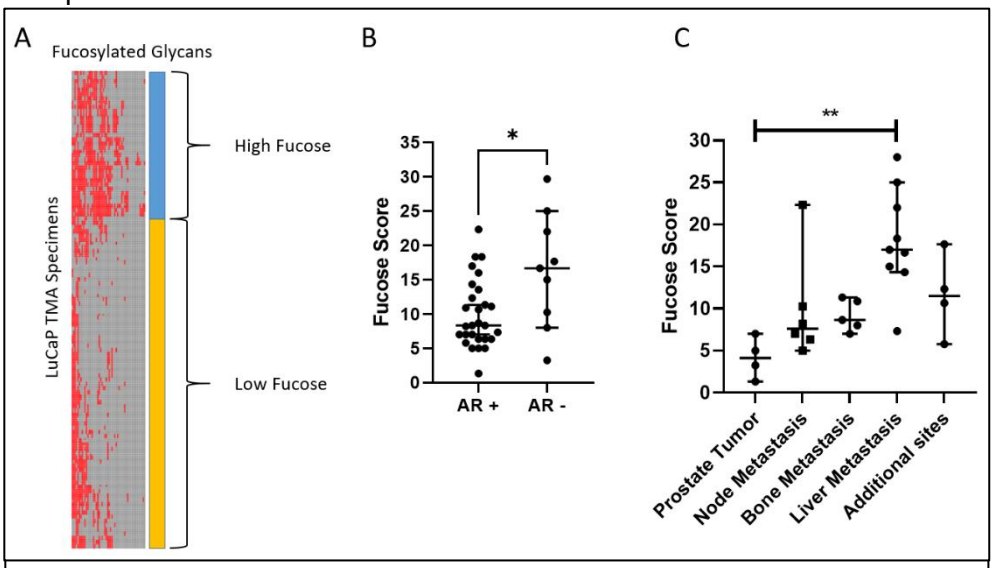

Figure 5. Confirmation of the fucose score in the LuCaP PDX models. A. Heatmap of fucosylated glycans across all samples in the LuCaP TMA identify fucose high and fucose low specimens. B. The fucose score is highest in the AR negative specimens. C. The fucose score is highest in liver metastases.

The fucose score is relevant to PDX models of prostate cancer. We wanted to confirm this finding as well as to expand this into translational laboratory research. Rather than focusing on established cell lines such as LNCaP, PC3, etc, as specified initially in the grant, we expanded our profiling to a very robust panel of patient-derived xenografts that have been extensively studied with patient-level data. We contacted Dr. Eva Corey at University of Washington in Seattle to use their LuCaP prostate models that are robust in the evaluation of prostate cancer therapeutics in the laboratory setting [6]. We profiled their LuCaP tissue microarray (TMA) of prostate cancer specimens. Similar to the previous dataset, we identified that there were a cluster of prostate cancer specimens with a different pattern of "high-fucosylation" (**Figure 5A**). We confirmed that in those specimens, the fucose score was associated with androgen receptor (AR) negative disease (**Figure 5B**). These data further support the relevance of the fucose score in assessing prostate cancer that is androgen unresponsive. However, we made an unexpected discovery – the fucose score was significantly enriched in liver metastases (**Figure 5C**) compared to primary tumor.

Despite the higher expression of fucosylated glycans in liver metastases versus primary tumors, it was unclear if this was a direct effect of the liver microenvironment on tumor glycan biology or could just be related to dataset heterogeneity. We accessed a small cohort (n=4) of primary ileal neuroendocrine tumors (NET) with matched metastases to liver (n=3) and ovary (n=1). We identified that indeed, fucose scores increased in metastases versus primary tumors in the same patient (**Figure 6**). These findings highly suggest the possibility of direct effects of the microenvironment on extracellular glycosylation of metastatic deposits. This has clear implications for immunotherapy as will be discussed below.

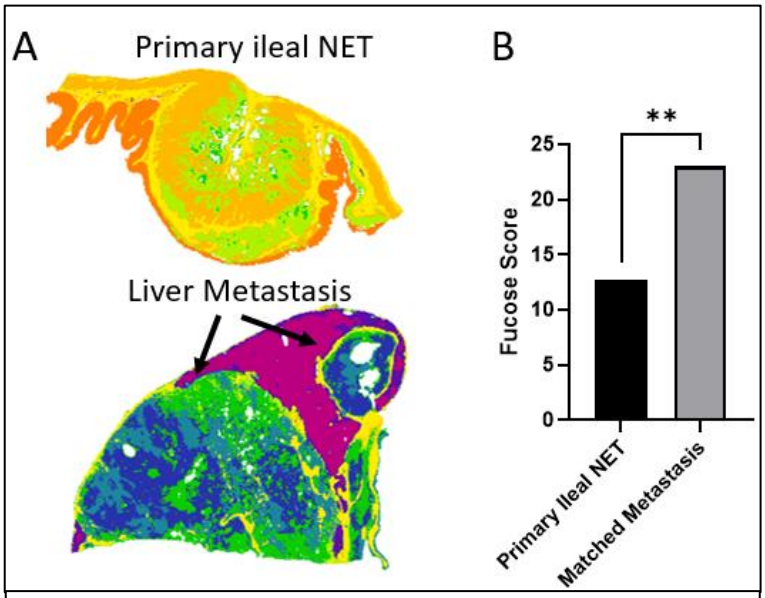

Figure 6. Fucose scores of primary neuroendocrine tumors (NET) of the small bowel increase in matched metastases. A. Molecular segmentation map based upon fucosylated glycans in primary tumor are distinctly different compared to liver metastases. B. Fucose scoring of n=4 matched tumors demonstrate increase in fucosylation in metastasis.

Fucose scoring predicts visceral metastatic disease in prostate adenocarcinoma. Because most of our data thus far has been focused on neuroendocrine small cell and AR negative prostate cancer, we wanted to identify if the fucose score was associated with adverse histologic features in prostatectomy specimens. Through a collaboration with University of Texas, San Antonio, we acquired a prostatectomy TMA where we correlated fucose scores to various clinical features. We identified that once again, there was a cluster of tumors with high fucosylation (**Figure 7A**). Interestingly, the most significant clinical variable with fucose scoring

analyses was the presence of visceral metastasis (Figure 7B). This further substantiated our previous analyses demonstrating the potential for the fucose score to predict metastatic disease. We also identified potential trends in nodal disease and survival (**Figure 7C-D**), also supporting the fucose score as a potentially prognostic tool for patients with prostate cancer.

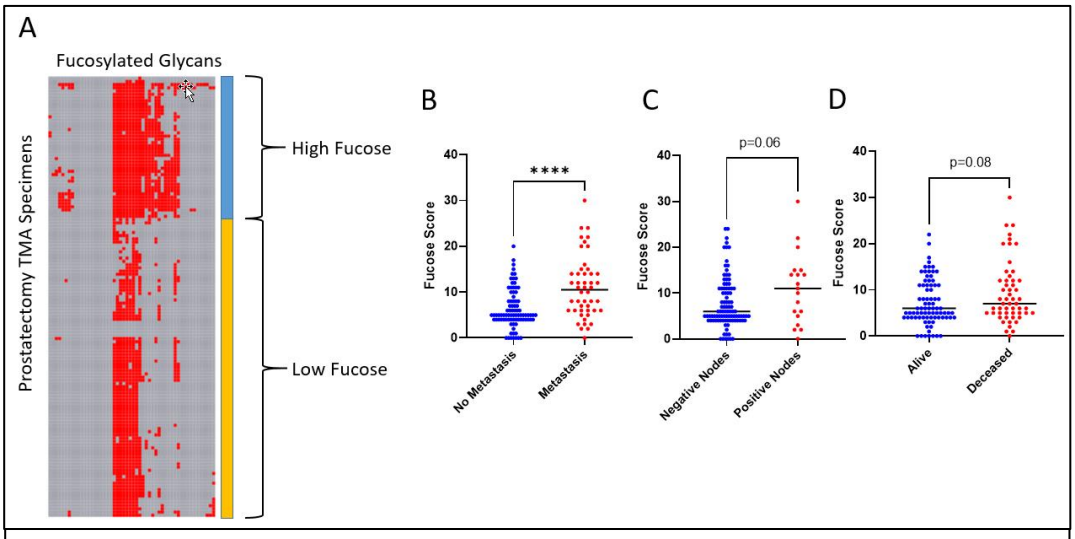

Figure 7. Fucose scoring of prostate adenocarcinomas are predictive of visceral metastasis A. Heatmap of prostatectomy specimens again identifies a high vs low fucose group as before. B. Visceral metastases are more likely from prostate tumors with higher fucose scores. C-D fucose scores of tumors with nodal metastasis and worse survival trend toward significance.

Glycan fucosylation gene expression is prognostic in castrate-resistant and hormone-naïve prostate cancer. Because the short observation period for this institutional cohort was not amenable to overall and progression free survival analyses, RNA expression of enzymes involved in (i) the de novo synthesis of fucose from other carbohydrates, (ii) the salvage synthesis pathway using imported fucose and (iii) the fucosylation of glycans (i.e. fucosyltransferases) were analyzed from The Cancer Genome Atlas (TCGA). First, the effects of these pathways on overall survival (OS) in metastatic CRPC patients were assessed [3]. A total of 7 genes were identified whose overexpression were associated with decreased OS. Two of these genes were associated with the salvage pathway (FUCA2, FUOM), four of these genes were associated with the de novo pathway (MPI, PMM1, GMDS, and TSTA3), and one of the genes was a fucosyltransferase (FUT8). Interestingly, there were 3 fucosyltransferases whose overexpression was associated with better OS (FUT3, FUT6, FUT7).

Next, the same methods were applied to OS in hormone-naïve prostate cancer at the time of prostatectomy. Similarly, overexpression of FUOM from the salvage pathway was associated with worse OS. PMM1 and GMPPB overexpression from the de novo pathway was associated with poor OS. Multiple fucosyltransferases (FUT2, FUT5, FUT6, FUT8, FUT9, POFUT2) including the GDP-fucose transporter SLC35C2 were also associated with worse OS. Overexpression of 1 gene in the salvage pathway (FUCA1) and 2 fucosyltransferases (FUT1,

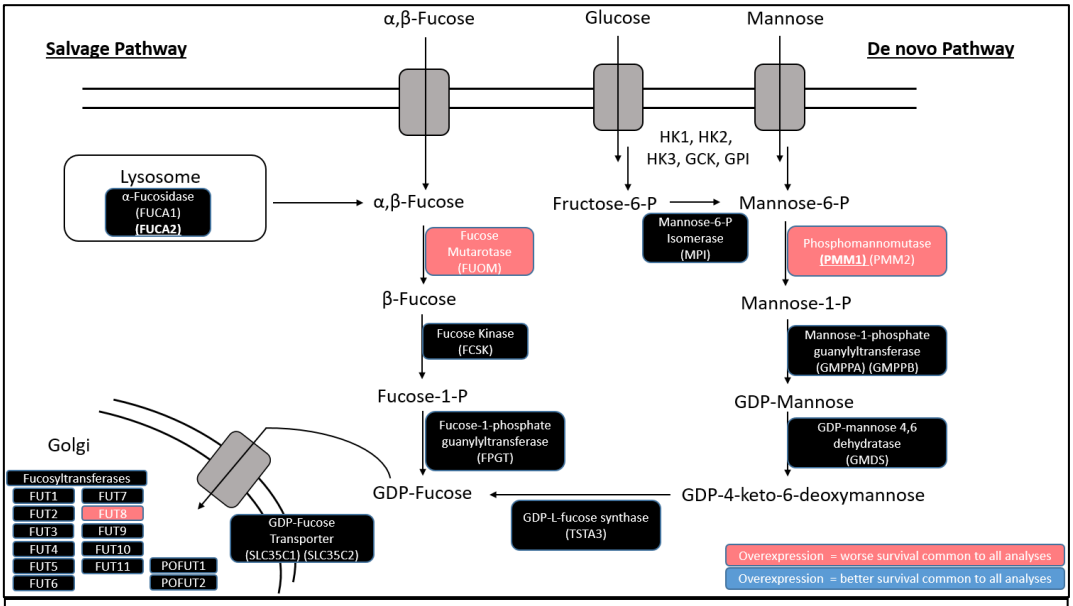

Figure 8. Overexpression of three genes involved fucose synthesis and glycan fucosylation are associated with worse OS in castrate resistant prostate cancer and hormone-naïve prostate cancer. OS analysis of the salvage and de novo pathways for fucose synthesis as well as glycan fucosylation were performed using a threshold biomarker optimization algorithm [1, 2]. Three enzymes in red (FUOM, PMM1 and FUT8) were identified in both hormone-naïve prostate cancer and castrate-resistant prostate cancer whose overexpression was associated with significantly worse OS ($p<0.05$). Expression and outcomes data obtained from [3].

FUT10) were associated with better OS.

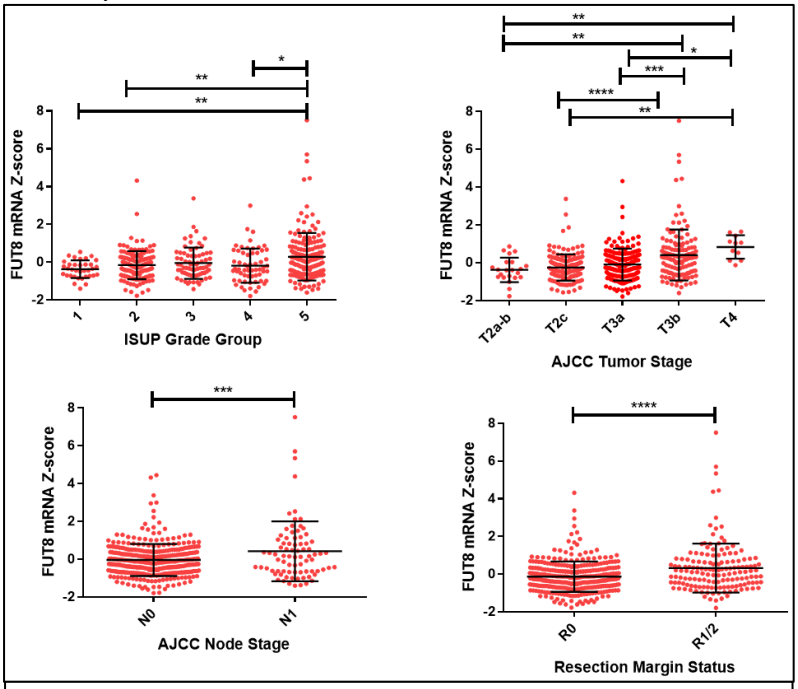

Figure 9. Overexpression of fucosyltransferase 8 (FUT8) is associated with adverse histologic features in hormone-naïve prostatectomy specimens. A. FUT8 expression increases with ISUP Gleason Group classification of the adenocarcinoma. B. FUT8 expression singificnatly increases with stage. C. FUT8 expression significantly increases with nodal metastasis. D. FUT8 expression is significantly higher with prostatectomies with positive margins. Data obtained from [3].

However, three enzymes were identified that were common to both the hormone-naïve prostatectomy and castrate-resistant prostate cancer datasets: fucose mutarotase (FUOM), phosphomannomutase 1 (PMM1), and fucosyltransferase 8 (FUT8), all of which whose overexpression was associated with worse OS (**Figure 8**). All three genes were tested for associations with adverse histopathologic features in light of the proposed fucose scoring system. Intriguingly, only FUT8 emerged of the three genes with significance. FUT8 mRNA expression increased with the ISUP grade group, tumor stage, was significantly increased in node positive cases, and increased with positive surgical margins (**Figure 9**). In contrast to other fucosyltransferases that target peripheral glycan fucosylation, FUT8 catalyzes core fucosylation [7, 8]. Upon correlation with the 14 glycans displayed in **Figure 1**, core fucosylation of the innermost N-acetylglucosamine by FUT8 activity is the common denominator to all glycans, further supporting the potential for the fucose score as potential marker for poor outcomes and aggressive disease.

Successful synthesis and imaging of mouse models using [^{18}F] fluorofucose. As proposed in our grant, fluorofucose has been previously synthesized as a potential positron emission tomographic (PET) tumor imaging agent [9, 10]. We used the same synthetic scheme and tested it on sMIC expressing TRAMP-C2 (sMIC-TC2) tumors in C57/BL6 syngeneic mice that are known to express high levels of fucosylated glycans. Regions of interest were placed on tumor as well as critical organs including muscle, heart, blood pool, etc, to compare uptake characteristics. We put regions of interest (ROI) over tumor as well as major organs/structures such as heart, blood pool, muscle, liver. We noticed that uptake in tumors was weak overall, although was greater than muscle (**Figure 10A**). Following PET imaging, we performed a post-PET biodistribution of fucose activity in the organs and confirmed that tumor had one of the lowest activities in the body. In fact, there was extensive activity in the urine (**Figure 10B**), which could be problematic for a prostate imaging agent. We also investigated the effects of fasting on fucose tumor uptake and identified at least in increase in 50% tumor activity (Figure 10C). Although these differences are small, we will continue to investigate new areas for imaging. There is the potential to

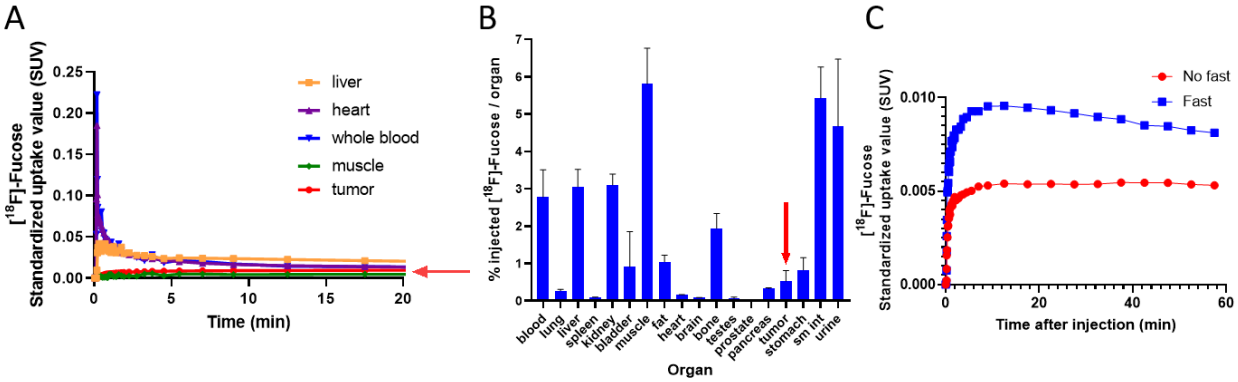

Figure 10. Fluorofucose imaging of sMIC-TC2 tumors in mice demonstrate weak activity in tumors compared to surrounding organs. A. Kinetics of fluorofucose uptake in tumors (red) compared to other organs is greater than muscle, but overall low. B. Post-PET biodistribution of fucose activity in harvested tissues identify low signal in tumors. Arrow signifies tumor data. Data in B represents averaging of 3 mice. Data in C represents averaging of 4 mice per group.

re-purpose a previously used imaging agent to target Lewis-y antigen (mentioned above) that has the ability to produce much better signal to noise [11].

Aim 2. To identify the effects of key fucosyltransferases (FUT's) on tumor glycan synthesis.
2a. To knock out key FUT in neuroendocrine prostate cancer cell lines.
2b. To identify effects of knockouts with MALDI and PET imaging of mice with tumors.

We are working in our final year to complete this Aim. We have two options: First, we could focus on knockout of FUT8 as highlighted above. There may be come criticisms to this approach as of this point. First, this aim was the most critically reviewed at the initial finding stage. FUT has been investigated in several cell culture models including prostate and been linked to poor outcomes [4, 8, 12, 13]. Thus, this may result in diminishing returns. Second, we have uncovered potentially significant data in Aim 1 with regard to liver-specific modulation of tumor fucosylation and Aim 3 with regard to the effects of fucosylation inhibition on immunotherapy. We have uncovered an immediately translatable method for reducing tumor fucosylation through the ketogenic diet as will be described below.

Carbohydrate depletion from the ketogenic diet (KD) reduces fucosylated glycan content. The dependence of aggressive tumors on glucose is well known. Dietary carbohydrate restriction in the form of calorically-limited ketogenic diets (KD) has been investigated as a means to reduce tumor growth and enhance therapeutic efficacy in PCa through reduction in circulating carbohydrates [14-19].

Using MALDI imaging, the molecular effects of the KD on lethal PCa was assessed. The KD was fed to mice with *de novo* small cell prostate carcinoma (PNEC model; [20-24]). As expected, the KD can modulate the glycomes of small cell prostate carcinoma as noted by depletion of multiple fucosylated glycans that are part of the 14 fucosylated glycan signature (**Figure 11**). However, we *unexpectedly discovered* a cohort of glycans that were enriched from the KD. These glycans are characterized by the expansion of mannose sugars (green circles, **Figure 11**). This is significant because high mannose glycans are typically secreted [25, 26]. This further advances our rationale for noninvasive serum profiling of glycans, which will be described below. This is also significant, as high mannose glycans can be associated with activated macrophages [27] and has implications for using metabolism to modulate immune recognition of tumors. Therefore, we will be able to integrate this discovery into Aim 3 as a clinically-relevant strategy to decrease fucosylated glycans and therefore reduce uptake of fluorofucose PET tracer.

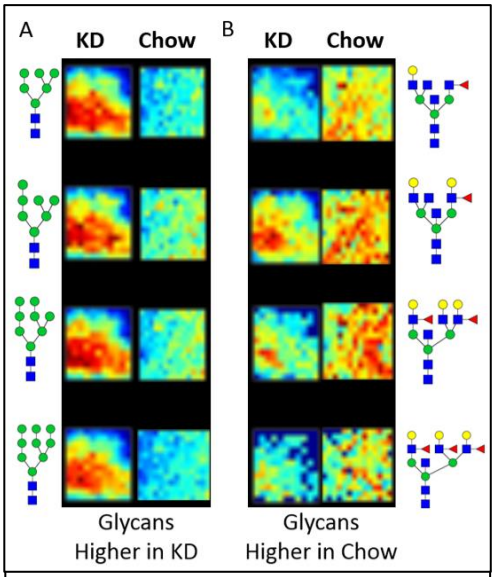

Figure 11. Dietary carbohydrate deprivation remodels the small cell prostate cancer glycome. Tumors from PNEC xenograft mice fed the KD for 4 weeks were imaged with MALDI. A square region of a tumor section on a slide was imaged (square regions) and images for selected glycans are displayed. **A**. Examples of glycans higher in tumors fed the KD and **B**. Examples of glycans higher in tumors fed chow. Intensity scale: blue (lowest) to red (highest).

Thus, using techniques from Drs Drake and Wu, we will be able to perform trials of the ketogenic diet in immunocompetent mice and identify the potential synergy of diet and immunotherapy on prostate cancer viability, with an emphasis on liver metastases.

Aim 3: To assess the effect of pharmacologic inhibition of tumor fucosylation on tumor growth and tumor immunity.

3a. To determine the effect of inhibition of tumor fucosylation on tumor growth.
3b. To determine the effect of co-inhibition of tumor fucosylation in combination with immunotherapy.

To date, we have completed two independent *in vivo* studies to address our tasks in Aim 3a and 3b. In the first experiment, we subcutaneously implanted the sMIC expressing TRAMP-C2 (sMIC-TC2) tumors into the syngeneic MICB transgenic mice. When tumors reached 250-500 mm3 in size, animals were randomized to 4 therapeutic groups: 1) control IgG, 2) 2-FF; 3) anti-sMIC antibody B10 (also B10G5) as previously described in Dr. Wu's publication; 4) a combination of 2-FF and B10 antibody. B10 was given at 6 mg/kg i.p twice weekly. To

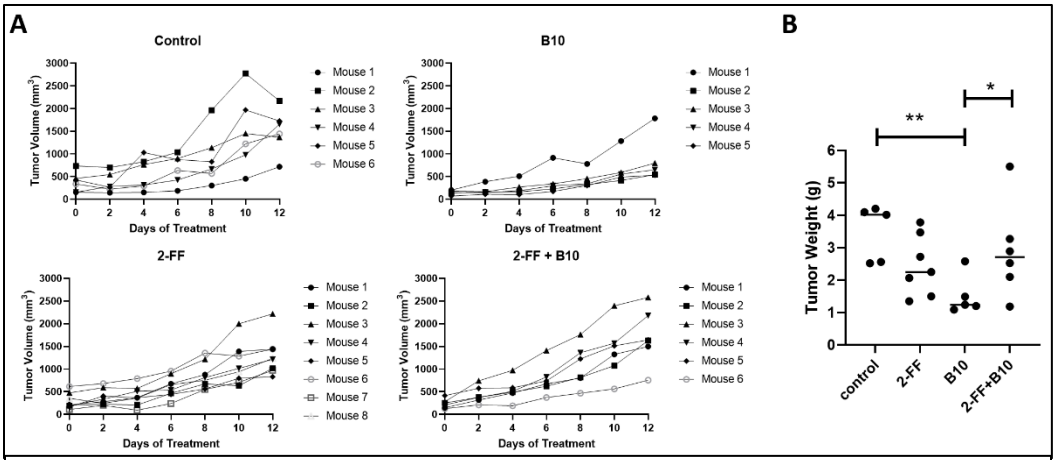

Figure 12. 2-fluorofucose (2-FF) impairs B10 immunotherapy in mice with prostate cancer. A. Tumor volume measurements over 12 days of therapy demonstrate that although B10 antibody therapy has beneficial effects, the addition of 2-FF diminishes those positive effects. B. Tumor weights at the end of the study confirm that 2-FF reduces the effects of B10 immunotherapy. *p<0.05, ** p<0.01.

ensure that 2-FF can act on tumor cells and tumor microenvironment, we injected 2-FF intratumorally at 12.5 uM twice weekly, according to published literature. Tumor volume was monitored twice weekly. As shown in **Figure 12**, single agent therapy with 2-FF presented some, but not significant, inhibition of tumor growth. B10 antibody significantly inhibited tumor growth. However, combination of 2-FF with B10 did not present a significant inhibition of tumor growth. ***Unexpectedly, these observations suggested that 2-FF inhibits the efficacy of immunotherapy.***

To confirm that 2-FF inhibition of fucosylation inhibits anti-tumor immune responses, we performed similar therapeutic experiments with anti-PD1 antibody in the syngeneic TRAMP-C2 tumor model. As shown in **Figure 13**, only nominal inhibition of TRAMP-C2 tumor growth was achieved by 2-FF. Consistently, the tumor growth inhibition effect with anti-PD1 single agent was abolished by addition of 2-FF. ***Together, these data confirmed the inhibitory effect of 2-FF on immunotherapy***. We are still in the process of analyzing the immune cell function in the tumors in these therapeutic settings, to better understand the impact of 2-FF on tumor immune microenvironment. **This is an especially significant finding, as all the published studies with 2-FF treatment of tumors were conducted in immune-deficient mice, lending even further power and significance to our studies that use immunocompetent mice.**

Together, our data suggest that the high fucosylation of lethal prostate cancer variants, especially in visceral/liver metastases may have a direct impact on the function of the immune system. Fucosylation plays many roles in normal immune cell function [28, 29]. Our data suggest that tumor fucosylation may play a potentially significant role in tumor-immune cell interactions, whether it be from direct inhibition of immune cells or a way to hide from the immune system itself. During this final year, we will further characterize this interaction in our immunocompetent models and try different clinically relevant metabolic inhibition strategies to selectively deplete tumor fucosylation and enhance immunotherapy.

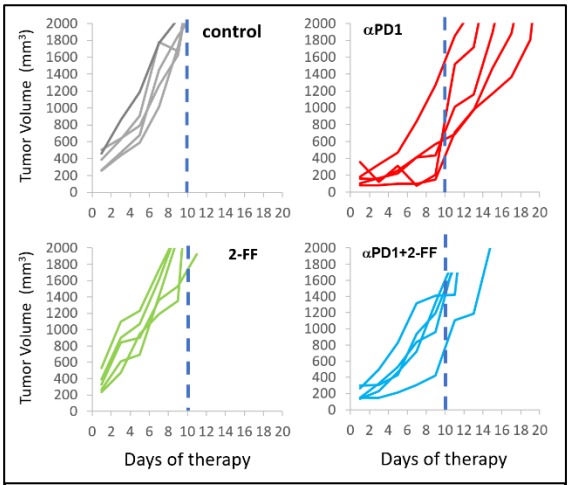

Figure 13. 2-fluorofucose (2-FF) impairs anti-PD1 (αPD1) immunotherapy in mice with prostate cancer. Tumor volume measurements over therapy demonstrate that 2-FF treatment is similar to control and that 2-FF nearly eliminates the advantages of αPD1 immunotherapy. Vertical line indicates the performance of controls as a reference for the treatment groups.

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Opportunities for Training and Professional Development: Our lab is training two undergraduate students, Ms. Hannah Johnston and Ms. Shreya Ashok. Here, they will learn about tumor glycosylation, PET imaging as well as mouse studies.

How were results disseminated to communities of interest?: COVID has limited us in this respect. However, we are in the process of submitting a manuscript based on Aim 1 and will disseminate this on social media and as a pre- print on Biorxiv. As restrictions start to ease, we will present this at the next Prostate Cancer Foundation retreat. We will also circulate this through Dr. Wu and the Northwestern prostate SPORE.

What do you plan to do during the next reporting period to accomplish the goals? This month: submit the manuscript for Aim 1 that will validate fucose as a novel biomarker for neuroendocrine prostate cancer. With Dr. Wu's assistance, we will finish the experiments for Aim 3 and submit a manuscript by the end of the grant period. Dr. Ippolito's lab will complete Aim 2 by the end of the grant period, with an emphasis on using the ketogenic diet to selectively modulate tumor fucosylaion and improve immunotherapy for lethal neuroendocrine prostate cancer.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?
This project not only develops a new PET imaging agent to detect lethal prostate cancer noninvasively, but it characterizes the target molecule, i.e. fucosylated cell surface glycans, on the histopathologic level. Specifically, our group is correlating expression of fucosylated glycans with important clinical metrics such as grade, stage, and development of castrate-resistant disease. We are discovering that there are two specific fucose modifications to these cell surface glycans that are associated with the emergence of lethal neuroendocrine prostate cancer: (i) fucosylation of the core of the cell surface glycan by the fucosyltransferase 8 (FUT8) enzyme and the synthesis of the fucosylated lewis-y antigen on the branches of the glycan.. It advances the idea of a "fucose scoring system", similar to the Gleason scoring system where the more fucoses a tumor has, the more aggressive it is. This has clear implications for the pathology field where both MALDI imaging mass spectrometry and immunohistochemistry could be used to assess these fucose scores on tumors from prostatectomies at initial diagnosis.

There is also an intriguing phenomenon that we are starting to identify: inhibition of the synthesis of fucosylated glycans through diet. Glycans are composed of various types of sugars, many of which are supplied by glucose. We are discovering that the ketogenic diet has the ability to effectively "prune" these fucosylated glycans and convert them to high mannose glycans that are implicated in inflammation and immune activation. Thus, it is possible that we might be able to use diet as a clinically actionable means to enhance tumor killing through immune recognition.

Finally, we are making significant impact in the field of immunotherapy. The majority of mouse models rely on immunocompromised hosts, thus ignoring the importance of the immune system not only for tumorigenic mechanisms, but also for therapeutic strategies. We have discovered that metabolic inhibition through drugs such as 2-fluorofucose (2-FF) may look promising in immunocompromised models through tumor inhibition, but have the opposite effect in models with a competent immune system. Thus, the metabolic mechanisms needed for tumorigenesis may also be shared with immune cells. This opens up the field with new opportunities to develop tumor selective-metabolic inhibitors to kill tumors and enhance immune function.

What was the impact on other disciplines?
Our clinical data demonstrate that there are two key biomarkers for lethal prostate cancers: (i) the presence of a fucosylated lewis-y antigen and (ii) a fucose attached to the core of the glycan by fucosyltransferase 8 (FUT8). These two biomarkers are readily measured with either MALDI imaging mass spectrometry and conventional immunohistochemistry. Therefore, this has clear impact on pathology and how specimens could be assessed on the clinical level. This will also have an impact in oncology, especially as new trials advancing immunotherapy become more widespread. We believe that we may be able to use diet as a means to selectively modulate tumor fucosylation, which can readily be deployed in the clinical setting.

What was the impact on technology transfer?
Our clinical data with MALDI and Lewis-y antigen staining are further advancing the potential efficacy of Lewis-y antigen and core glycan fucosylation by FUT8 as biomarkers for lethal, castrate-resistant prostate cancer. As these biomarkers are measurable with MALDI imaging mass spectrometry, this could advance the role of MALDI in histopathologic assessment.

What was the impact on society beyond science and technology? Nothing to report.

CHANGES/PROBLEMS

Changes in approach and reasons for change:
Initially, we had major limitations in year 1 and part of year 2 due to COVID. Despite that, we have ramped back up fast and have made significant advancements in all facets of the project. Although the 2-FF inhibition had the opposite effect on immunotherapy in Aim 3, this is still a significant advancement for the project for several reasons highlighted above and lends further rationale for the impetus to test different metabolic strategies to inhibit tumor fucosylation, such as with the ketogenic diet as will be expanded as part of Aim 2.

Actual or anticipated problems or delays and actions or plans to resolve them:
In year 1, we had several delays with COVID and lab moves. We moved beyond those delays and made significant progress this year. We had some additional limitations with problems in the imaging facility and were unable to image mice for a while. However, we have achieved the goals and will proceed to complete the rest of the project.

Changes that had a significant impact on expenditures:
In year 1, COVID forced lab shutdown and expenses on the project. My technician was kept out of furlough, doing remote data analyses that moved the clinical aspects of the project forward. In year 2, we lost some time ramping back up as well as had to deal with problems with non-functioning scanners in the imaging facility. Those problems are nearly resolved.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:
None

Significant changes in use or care of human subjects:
N/A

Significant changes in use or care of vertebrate animals:
None

Significant changes in use of biohazards and/or select agents:
None

PRODUCTS

Nothing to report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name	Joseph Ippolito (Washington University)
Project Role	PI
Researcher Identifier	eRA commons: ippolitoj
Nearest Person-month worked	1.2
Contribution to project	Overseeing entire project
Funding support	NA

Name	Richard Drake (MUSC subcontract)
Project Role	Co-PI
Researcher Identifier	eRA commons: RICHARD_R_DRAKE
Nearest Person-month worked	0.6
Contribution to project	Overseeing all MALDI analyses
Funding support	NA

Name	Jennifer Wu (Northwestern subcontract)
Project Role	Co-PI
Researcher Identifier	eRA commons: wumedd
Nearest Person-month worked	0.8
Contribution to project	Overseeing development of TRAMP/MIC model
Funding support	NA

Name	Dong Zhou (Washington University)
Project Role	Co-I
Researcher Identifier	eRA commons: D_ZZZZ
Nearest Person-month worked	1.2
Contribution to project	Synthesis, QI and QA of fluorofucose
Funding support	NA

Name	Elena Nunez (Washington University)
Project Role	technician
Researcher Identifier	NA
Nearest Person-month worked	12
Contribution to project	Cell culture, mouse handling, data analysis
Funding support	NA

Name	Grace Grimsley (MUSC subcontract)
Project Role	Technician
Researcher Identifier	NA
Nearest Person-month worked	1
Contribution to project	MALDI imaging and data analysis, tissue staining
Funding support	NA

Name	Ju Wu (Northwestern subcontract)
Project Role	Staff scientist
Researcher Identifier	NA
Nearest Person-month worked	2.4
Contribution to project	Cell culture, mouse handling, development of TRAMP/MIC
Funding support	NA

SPECIAL REPORTING REQUIREMENTS

N/A

APPENDICES

N/A