AWARD NUMBER: W81XWH-20-1-0269

TITLE: Detection and treatment of Ovarian cancer by targeting tumor extracellular hydroxyapatite: a new paradigm

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CONTRACTING ORGANIZATION: Vanderbilt University Medical Center

REPORT DATE: MAY 2021

TYPE OF REPORT: ANNUAL

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE	*Form Approved* *OMB No. 0704-0188*

1. REPORT DATE: MAY 2021	**2. REPORT TYPE: ANNUAL**	**3. DATES COVERED: 05/01/2020-04/30/2021**

4. TITLE AND SUBTITLE Detection and treatment of Ovarian cancer by targeting tumor extracellular hydroxyapatite: a new paradigm	**5a. CONTRACT NUMBER:** **5b. GRANT NUMBER: W81XWH-20-1-0269** **5c. PROGRAM ELEMENT NUMBER**
6. AUTHOR(S): Mohammed N Tantawy and J. Oliver McIntyre	**5d. PROJECT NUMBER** **5e. TASK NUMBER** **5f. WORK UNIT NUMBER**
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Vanderbilt University Medical Center 1161 21st Ave South Nashville TN 37232	**8. PERFORMING ORGANIZATION REPORT**
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012	**10. SPONSOR/MONITOR'S ACRONYM(S)** **11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Introduction: In this report, we are evaluating tumor extracellular hydroxyapatite (HAP), $Ca_{10}(PO_4)_6OH_2$, as an imaging biomarker of ovarian can a target for therapy. We have shown that HAP-binding radiotracers such as FDA-approved ^{18}F-NaF can be used with PET imaging and ^{99m}Tc-MDP with SPECT imaging to detect breast tumors; in this context, detection of tumor-associated HAP exhibited high specificity and a high signal-to-background ratio (SBR) as HAP is absent in normal soft tissue. In ovarian cancer, conventional imaging modalities lack clear metrics for assessing tumor burden before and after surgical debulking and for assessing tumor response to therapy. This is mainly due to lack of specificity and/or low SBR ratio from standard imaging. Additionally, we had developed a nanoparticulate sulfonated polystyrene solution (NSPS) to break-up HAP *in vivo* inducing a localized alkalosis status in the tumor microenvironment inhibiting tumor growth.

Methods: Female C57Bl/6 mice received intraperitoneal injection of ID8 mouse ovarian tumor cells that constitutively express a luciferase reporter (ID8-luc). After four weeks, bioluminescence imaging (BLI) was used to assess tumor burden and the mice were injected with ~11 MBq/0.1 mL of ^{18}F-NaF and imaged 60 min later for 20 min in a microPET/CT. Within 24 hours, the mice were injected with ~37 MBq/0.1 mL of ^{99m}Tc-MDP and imaging 60 min later in a NanoSPECT/CT for about 20 min. The imaging protocols were repeated weakly. When tumor burden was detected across the different modalities, the mice were treated with weekly 2 mg/kg cisplatin, 6.7 mg/kg NSPS (one time), or vehicle, PBS, weekly (controls). After several weeks of cisplatin treatment, some of the mice were taken off cisplatin and treated with PBS as a model for tumor recurrence. Regions of interest (ROIs) were drawn around the abdominal cavity and the radiotracer concentration within the ROI was deduced. Furthermore, tumor extracellular HAP expression was assessed via IHC in clinically annotated tissue microarray (TMA) of patient samples from the Vanderbilt University Medical Center (VUMC) Tissue Repository for Ovarian Cancer (TROC).

Results: Due to the absence of HAP in normal soft tissue, peritoneal ovarian tumors were detected with high specificity and SBR using ^{18}F-NaF PET which corresponded with BLI and pathology. We demonstrate that imaging tumor-extracellular HAP provided a suitable marker for tracking tumor response to therapy. ^{18}F-NaF uptake was reduced in the cisplatin and NSPS treated mice indicating tumor regression but increased in the PBS treated mice indicating tumor progression. Increase ^{18}F-NaF uptake was observed later in the cisplatin then PBS treated mice indicating that tumor extracellular HAP can also be an indicator of tumor recurrence. Due to entrapment of ^{99m}Tc-MDP in the liver, we could not reliably identify tumor burden using ^{99m}Tc-MDP SPECT imaging. Among 117 cases from the VUMC TROC, HAP staining was more common among serous than non-serous and among late stage than early stage patients.

Conclusion: We show that imaging the deposition of tumor extracellular HAP may be an effective alternative tool for detecting ovarian tumors, particularly in late-stage serous disease. HAP-targeting ligands may yield a metric of disease status distinct from that obtained by conventional CT-based imaging of calcium deposits. Improvements in the clinical monitoring of tumor burden before and after surgical debulking and patient response to therapy has the potential to benefit women diagnosed with this devastating disease.

15. SUBJECT TERMS

NONE LISTED

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	15	

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1. Introduction

Hydroxyapatite (HAP), $Ca_{10}(PO_4)_6OH_2$, once thought to be solely an ubiquitous component of bone, has also been shown to be produced by some types of malignancies via a classical mechanism; alkaline phosphates (ALP) on the surface of cells hydrolyses beta-glycerophosphate (□G) to glycerol and inorganic phosphate (Pi). Pi then combines with calcium in the cell to produce HAP crystals, and then this HAP is deposited in the extracellular space contributing to the structural matrix that influences the tumor microenvironment (1-9). Capitalizing on the presence of extracellular HAP and our expertise in imaging, we have shown that HAP-binding targeting radiotracers - such as FDA-approved ^{18}F-labeled sodium fluoride (^{18}F-NaF) and ^{99m}Tc-labeled methyl diphosphate (^{99m}Tc-MDP) can be used with positron emission tomography (PET) and single photon emission computer tomography (SPECT), respectively, to detect breast tumors; detection of tumor-associated HAP exhibited high specificity and a high signal-to-background ratio (SBR) as HAP is absent in normal soft tissue (10, 11). Furthermore, we had developed a nanoparticulate sulfonated polystyrene solution (NSPS) designed to chelate calcium and subsequently break up tumor extracellular HAP into its basic components, $Ca^{++} + PO_4^- + OH^-$. Dissolution of HAP is predicted to release phosphate and hydroxyl ions in the tumor extracellular milieu inducing a localized microenvironment alkalosis (high pH). Therefore, in this proposal we aimed at testing the hypothesis that tumor extracellular hydroxyapatite is viable target for detecting and treating ovarian cancer. We proposed a battery of *in vitro* and *in* vivo studies to test this hypotheses.

2. Keywords

Hydroxyapatite, ^{18}F-NaF, ^{99m}Tc-MDP, PET, SPECT, ID8-luc, HGSOC, ovarian cancer.

3. Accomplishments

A. Major goals and accomplishments

Research-Specific Tasks:

Specific Aim 1: Investigate the efficacy of NSPS in ovarian cancer	Months	Investigator	% accomplished
•Submission of institution approved animal protocols and related material for DoD's ACURO approval. •Received ACURO approval before initiating animal experiments.		Dr. Tantawy	100% completed
Major Task 1: Treatment with NSPS			
Subtask 1: Cell culture Cell lines. **ID8 mouse ovarian cancer cells** were obtained directly from their academic source (Dr. Kathy Roby, Dr. Iain McNeish and Dr. Sandra Orsulic, respectively). We have used this cell line and verified the presence of tumor extracellular hydroxyapatite. 3D culture as tumor spheroids will be established and cell viability following treatmetn with NSPS versus vehicle (PBS) will be measured using a commercially available Cultrex Spheroid Colorimetric Assay	1-6	Dr. Tantawy Dr. Wilson Dr. McIntyre RA	10% completed: Cell culture for injection in mice. 3D culture not started due to COVID19 restrictions preventing more than one person per room or in person meetings
Subtask 2: Intraperitoneal (i.p.) injection of ID8-luc cells into C57Bl/6	1-7	Dr. Wilson Dr. Yull	50% completed
Subtask 3: Treat mice with a one-time i.p. bolus injection of NSPS. Euthanize and harvest tissue at 0.25, 0.5, 1, 2, 4, 7 and 24 hours (n = 3 per time point) for pharmacokinetic (PK) analysis. Ntot = 21 mice	2-12	Dr. Tantawy RA	10% completed. This task requires both personnel be

			present in the same room. Due to covid19 lock downs, this was not possible until recently.
Subtask 4: Repeat subtasks 2 and 3 and treat with one time NSPS i.p injection but euthanize and harvest tissue at 7, 14, 21, and 28 days post NSPS (n = 3 per time point) for assessment of tumor growth. Analyze the results statistically. Ntot = 12 mice	13-21	Dr. Tantawy Dr. Wilson RA	Started May 2021
Subtask 5: LS/MS/MS and pharmacokinetic analysis. Statistical analysis	6-21	Dr. Tantawy Dr. Rook Dr. Beeghly-Fadiel Dr. McIntyre	0%, see comment in subtask3 above.
Milestone(s) Achieved: NSPS efficacy verified both in vitro as demonstrated by lower cell colonies compared to controls and in vivo via pathology and histology Establish pharmacokinetic parameters. Publish results in peer review journal.			
Specific Aim 2: HAP imaging in mouse models of ovarian cancer			
Major Task 2: Mouse models and imaging			
Subtask 1: Inject ID8-luc cells into C57Bl/6 mice, i.p. (n = 30)	1-3	Dr. Wilson Dr. Yull	60% Progress here has been slow due to COVID19 lockdown
Subtask 2: One week later, the Center for Small Animal Imaging (CSAI) at Vanderbilt Medical Center will image the mice weekly for 3 weeks with ^{18}F-NaF PET, ^{99m}Tc-MDP SPECT, and bioluminescence.	2-4	CSAI	60%
Subtask 2: At 4 weeks post ID8-luc injection, administer 5 mg/kg cisplatin (intravenous) weekly for 4 weeks (n = 10) or NSPS (i.p.) once (n = 10) or vehicle (PBS) once, i.p. (n = 10). Maintain weekly imaging for 4 more weeks	3-8	Dr. Tantawy Dr. Wilson CSAI	40%
Subtask 3: Draw regions-of-interest around tumors, establish time-activity curves and then carry out statistical analyses comparing each time point to the previous time point and comparing cisplatin treatment results to NSPS treatment results.	3-18	Dr. Tantawy Dr. Beeghly-Fadiel	40%
Milestone(s) Achieved: Tumor extracellular HAP imaging can be used to detect tumor burden, tumor distribution, tumor progression, and tumor regression following cisplatin and NSPS treatments. Publish results in peer review journal.			
Specific Aim 3: Elucidate correlations between extracellular HAP in tumors and clinically relevant indicators in ovarian cancer patients			
•Submission of institution’s IRB approval and related material for DoD’s HRPO approval. •Received HRPO approval or exempt finding, before initiating human subjects/HAS related studies.		Dr. Tantawy	100%

Major Task 3: Send ovarian tissue microarrays (TMAs) to Vanderbilt's Translational Pathology Shared Resources (TPSR) for sectioning and staining.			
Subtask 1: Stain with alizarin red S and von Kossa	1-6	TPSR	100%
Subtask 2: Unstained sections loaded onto calcium fluoride slides will be imaged via Raman spectroscopy by CSAI.	2-12	CSAI	10% due to COVID19 lockdown
Subtask 3: Digital images (40x) of the stained slides will be carried out by the Digital Histology Shared Resources (DHSR) at Vanderbilt Medical Center.	1-6	DHSR	100%
Subtask 3: Determine which sections scored positive for alizarin red S and con Kossa and if a 960 cm^{-1} Raman shift was detected. Correlate results with clinically relevant indicators.	1-21	Dr. Tantawy Dr. Watkins Dr. Crispens Dr. Beeghly-Fadiel	40% Still ongoing
Milestone(s) Achieved: Tumor extracellular HAP is correlated with patient survival rate, chemotherapy sensitivity, and other clinically relevant metrics that reinforce the need to image patients with HAP-targeting radioligands to better inform physicians and improve patient management. Publish results in peer review journal.			

B. Significant results and key outcomes.

We stained nearly 200 formalin-fixed paraffin-embedded human ovarian carcinomas in tissue microarrays (TMAs) with von Kossa (for phosphates) and alizarin red S (for calcium), please see figure 1. Preliminary analysis of the stained TMAs indicates tumor extracellular HAP to be more common among serous than non-serous and among late stage than early-stage patients.

To demonstrate that tumor extracellular HAP is a viable candidate imaging biomarker for detecting ovarian tumor burden we imaged groups of mice bearing luciferase-tagged ID8 ovarian cells (ID8-Luc), a well-established murine model of HGSOC (12-14). Since the ID8 cells were luciferase-tagged, we injected the mice with luciferin and imaged them in a bioluminescence optical scanner. The bioluminescence images (BLI's) were used to detect tumors and quantified to assess tumor burden. Tumor burden in the ^{18}F-NaF PET studies corresponded with BLI (see figure 2). As HAP is also present in bone and due to the large surface area of bone, bone uptake of ^{18}F-NaF and ^{99m}Tc-MDP was ~9 times higher than tumor uptake. Therefore, the bone signal appears saturated in figures 2b&c. However, due to the entrapment of ^{99m}Tc-MDP in the liver, it was difficult to accurately detect tumor burden in the mice using ^{99m}Tc-MDP SPECT (see figure 2c).

To investigate whether ^{18}F-NaF PET can track treatment progress of ovarian cancer via detecting changes in tumor-extracellular HAP, we imaged mice bearing peritoneal ID8-luc tumors weekly starting at 2 weeks post tumor cell injection. Then the mice were treated weekly with either PBS (controls) or cisplatin chemotherapy or treated once with NSPS. In the control, PBS treated mice (Group 1; n =3), we measured an increase in ^{18}F-NaF uptake in the tumors during tumor progression indicating an increase in tumor extracellular HAP (see figure 3). Following cisplatin (Group 2; n = 3) or NSPS treatment (n = 2; see figures 3 and compare figure 4B and 4C), we measured a reduction pattern in ^{18}F-NaF uptake in the tumors of the treated mice indicating a reduction in tumor extracellular HAP.

In addition, we measured a decrease in ^{18}F-NaF uptake in the mice that were treated weekly with cisplatin for 2 weeks then an increase in radiotracer uptake within 2 weeks of switching to PBS (Group 3; n = 2) indicating tumor recurrence (see figure 3). Therefore, we were able to track tumor extracellular HAP and tumor progression in the controls. Furthermore, we imaged the mice that were treated with NSPS with ^{18}F-FDG (for metabolism) PET before and after treatment (24 hours apart). The ^{18}F-FDGPET imaged were co-registered to the ^{18}F-NaF PET images to determine tumor location. We measured a significant decrease in ^{18}F-

FDG in the tumor following NSPS treatment indicating loss of tumor metabolic activity (compare figures 4D and 4E).

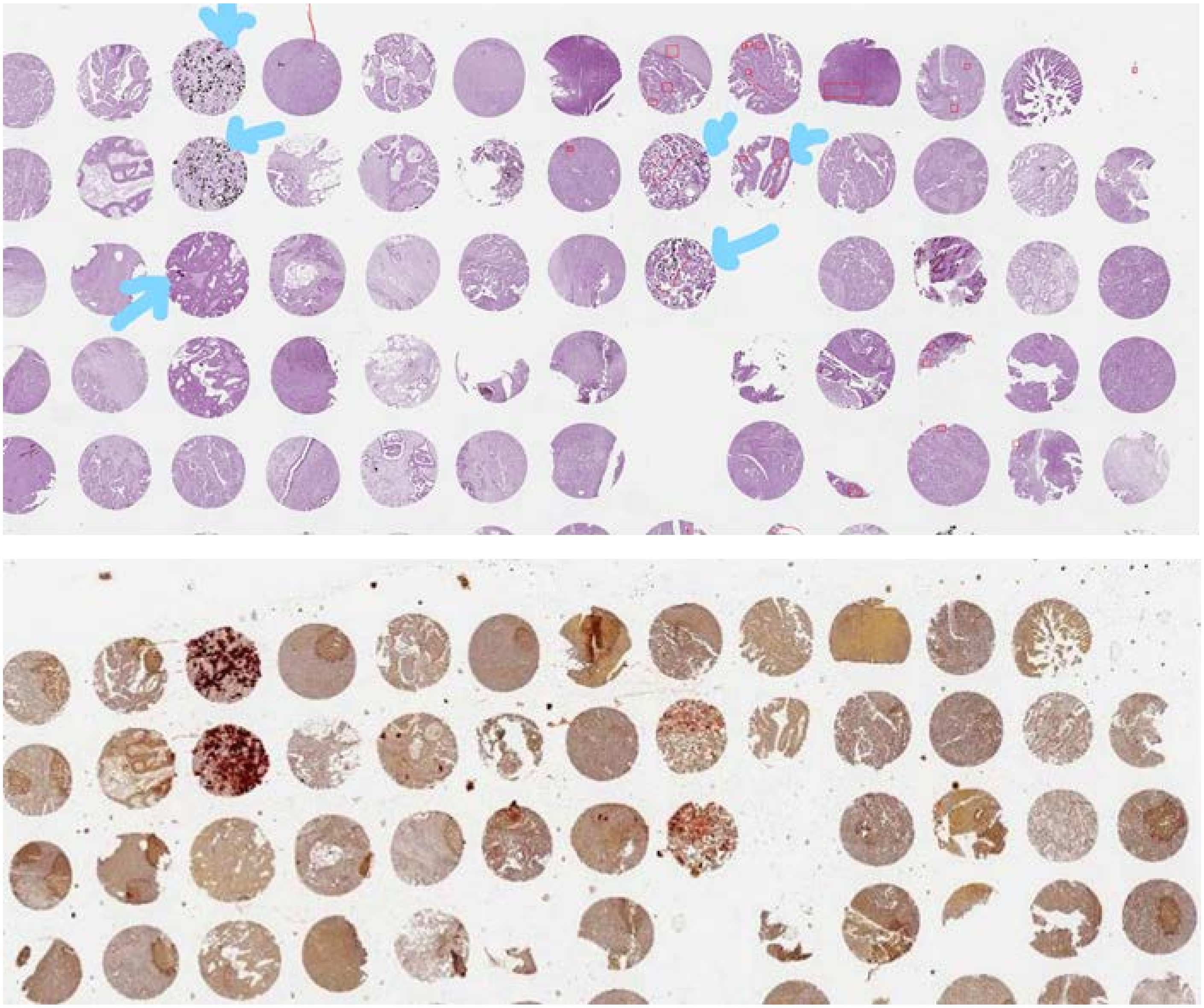

Figure 1. Stained tissue microarrays containing ovarian carcinomas. The TMAs were stained with von Kossa (Top) for phosphates and alizarin red S (bottom) for calcium. The dark spots are positive stains and the arrows point to some examples. If a section is positive for von Kossa and alizarin red S then that is a strong indication of the presence of tumor extracellular hydroxyapatite (HAP). If von Kossa is positive but alizarin red S is negative then further analysis is required (e.g. more samples of the same patient, Raman spectroscopy, stain for alkaline phosphates on the cell surface which helps in the production of HAP…. etc.). If alizarin red S is positive but von Kossa is negative, then that particular sample is most likely to be lacking HAP.

As HAP is absent in normal soft tissue, no adverse effects of NSPS were observed. And while HAP is present on the surface of the bone, lack of vascularization combined with large skeletal surface area, crystalized (compact) formation of the bone HAP matrices, and continuous bone remodeling (15) make it difficult to dissolve. By contrast, tumor extracellular HAP are loosely scattered and not yet crystalized making it easier to dissolve. We did not measure changes in ^{18}F-NaF uptake in skeletal bone after NSPS treatment compared to that prior to treatment. Our next step is to extend the imaging protocol to several weeks following NSPS for efficacy and toxicity evaluation of NSPS.

C. Opportunities for training and professional development.

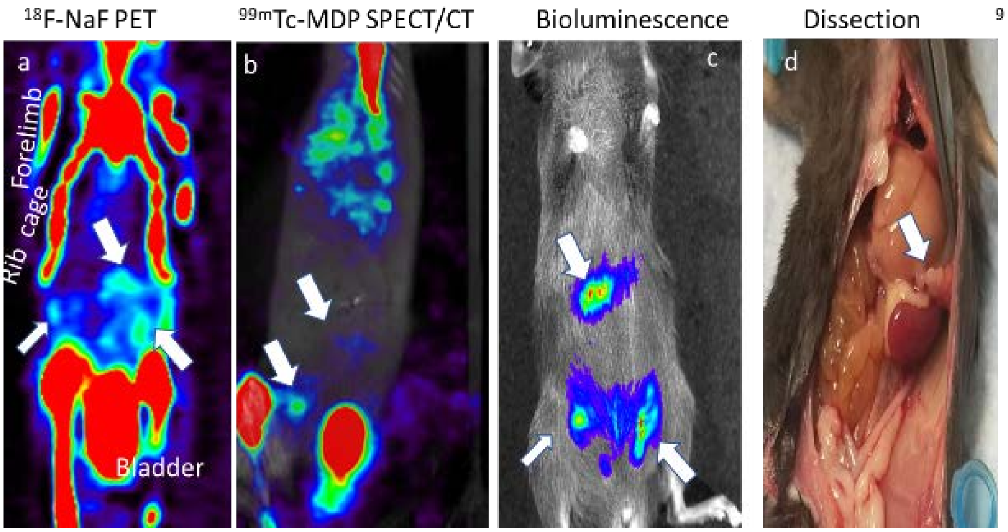

Figure 2: Tumor extracellular HAP as an imaging biomarker. (a) Mouse bearing peritoneal ID8-lucerase-taged tumors imaged with ^{18}F-NaF PET. White arrow points to tumor. Bone signal appears saturated as bone HAP uptake is ~9 times greater than that of tumor-extracellular HAP. (b) Same mouse imaged with ^{99m}Tc-MDP. Not only is there high uptake of the radiotracer in the bone, but the ^{99m}Tc-MDP is also trapped in the liver due to the presence of minerals. (c) Bioluminescence image of the same mouse using luciferin. (d) Pathology image of the same mouse.

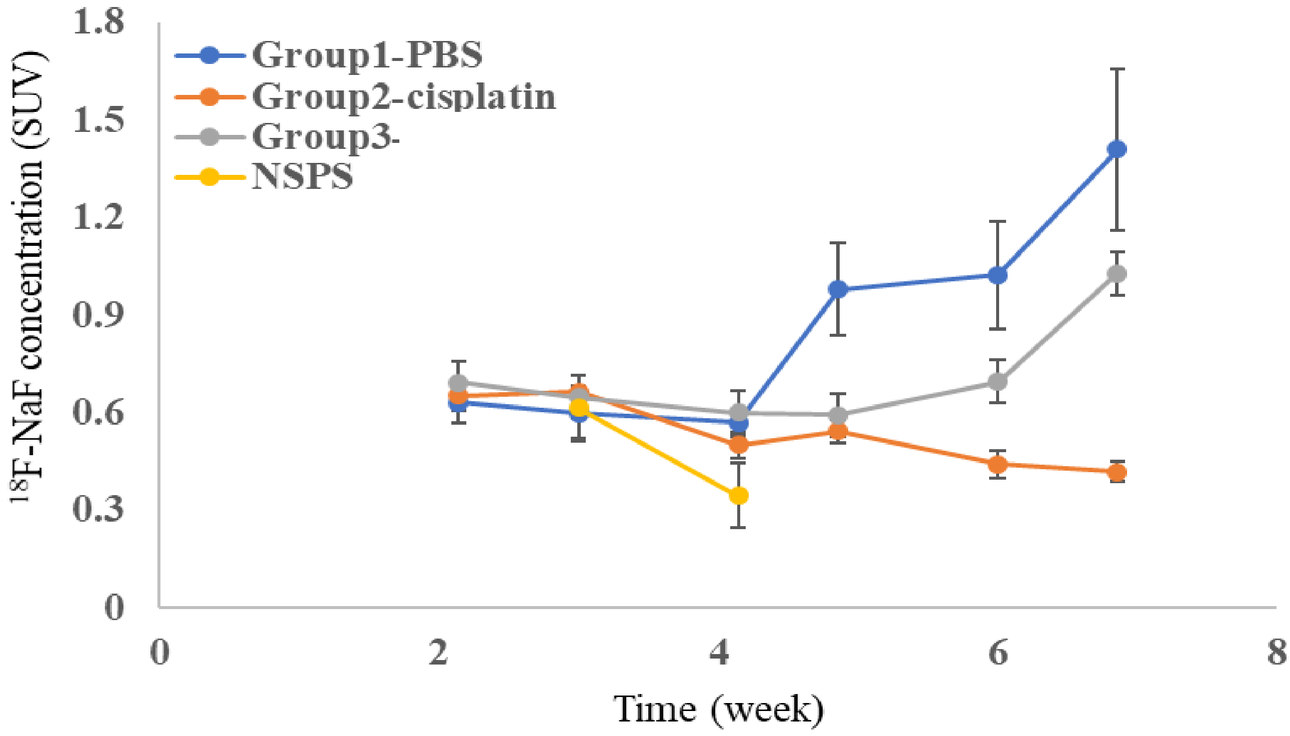

Figure 3. Tumor treatment tracked by imaging tumor extracellular HAP. Groups of mice (n = 3 per group) were injected with ID8-luc tumors, ip and, after two weeks, were imaged weekly with 18F-NaF PET. Two weeks later the mice received weekly PBS (group 1, control) or cisplatin (group 2 & group 3) or received our novel HAP-targeting therapeutic, NSPS. Tumor burden was assessed from retention of ^{18}F within the abdomen (peritoneal cavity). In Group 3, the mice were first treated with cisplatin for 2 weeks then switched to PBS as a model of tumor recurrence. The last group of mice received a one-time treatment of NSPS. The mice were imaged before treatment and one week after treatment then euthanized.

The studies carried out so far afforded important educational opportunities for PI including how to culture cells, how to maintain a colony of mice bearing peritoneal tumors, and grantsmanship. PI also attended and participated in March 2021, for the first time, in the Society of Gynecology annual meeting. Though the meeting was virtual, the PI gained more knowledge about ovarian cancer and the strategy available and being developed for treating this disease.

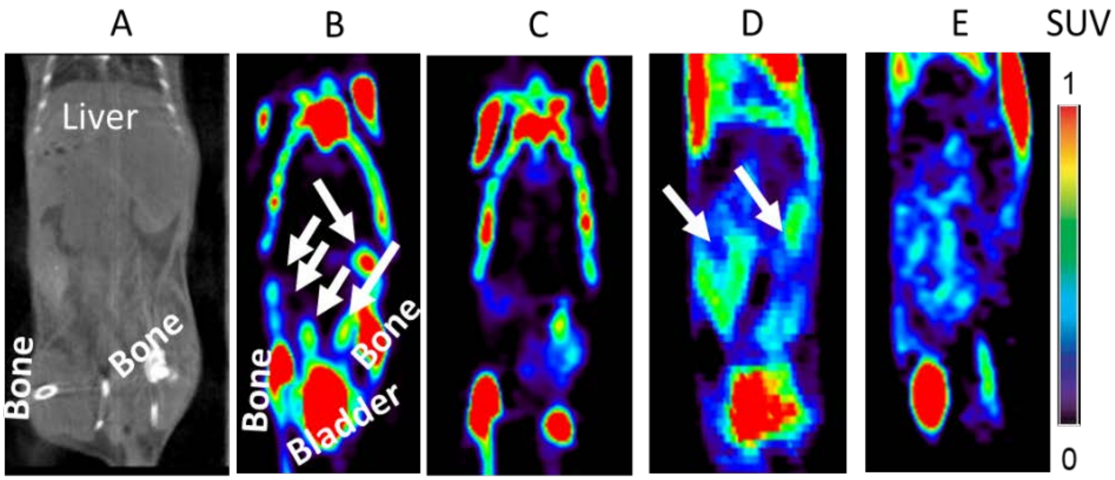

Figure 4. Treatment of mouse model of ovarian cancer with NSPS. (A) CT of a mouse bearing peritoneal ID8 tumors. (B) ^{18}F-NaF PET image of the same mouse at baseline. White arrows point to tumors. Radiotracer concentration in the peritoneal cavity and bone were 0.21 and 3.46 SUV, respectively. This is why the bone signal appears saturated. In the image. (C) ^{18}F-NaF PET image of the same mouse within 72 hours post NSPS treatment. Radiotracer concentration in the peritoneal cavity and bone were 0.11 and 3.65 SUV, respectively. (D) ^{18}F-FDG PET of the same mouse at baseline (before treatment). FDG concentration in the peritoneal cavity was 0.35 SUV (E) ^{18}F-FDG PET of the same mouse within 24 hours post NSPS treatment. FDG concentration in the peritoneal cavity was 0.05 SUV.

D. Dissemination of results to communities of interest.

1. Preliminary results have been reported to our local ovarian cancer interest group (VOCAL) at Vanderbilt University Medical Center.
2. Poster presentation at the 2021-March annual meeting of the Society of Gynecologic Oncology

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

1. We will continue to follow the plans outlined in our SOW.
2. As the pandemic restriction are relaxing, VUMC will allow for multiple personnel to occupy a room which will allow us to move more rapidly in research compared to the past year
3. A new research assistant will start with us before the end of June. He will have some %effort on this grant to assist us with cell culture, animal monitoring, and imaging
4. We plan on starting the 3D cell *in vitro* studies in the summer of 2021

4. **Impact**

A. Impact on the development of the principal discipline

The treatment paradigm studied here can potentially be applied to other types of cancers that form solid tumors. The treatment paradigm also has the potential to treat other types of calcification-involved illnesses include arterial calcifications, liver and kidney calcification, neurodegenerative diseases that are due to the presence of excess neuro-extracellular calcium.

B. Impact on other disciplines

The treatment paradigm studied here can potentially be applied to other types of cancers that form solid tumors. The treatment paradigm also has the potential to treat other types of calcification-involved illnesses include arterial calcifications, liver and kidney calcification, neurodegenerative diseases that are due to the presence of excess neuro-extracellular calcium.

C. Impact on technology transfer

The proposed studies address two of the Ovarian Cancer Research Program (OCRP) objectives: (i) developing reliable diagnostic approaches and treatment; and (ii) providing the tools to study initiation, progression, treatment tracking, and recurrence of ovarian cancer.

There has been considerable progress in the management of patients with ovarian cancer, particularly those with high-grade serous ovarian carcinoma (HGSOC). While treatment now builds on the well-established backbone of surgery and paclitaxel/platinum-based chemotherapy (16) with surgery being carried out upon diagnosis or after neo-adjuvant chemotherapy (NACT), in a significant number of patients, the accurate assessment of initial tumor burden is challenging. Unfortunately, not infrequently patients are first selected for surgery when NACT (before surgery) would have been more appropriate. Further, in HGSOC patients, the assessment of tumor recurrence following surgery and/therapy is less than optimal using current imaging modalities (CT, MRI and/or FDG-PET). The validation of more accurate assessment of tumor burden in HGSOC patients using 18F-NaF PET imaging as we propose would significantly enhance the clinical management of these patients.

Detection and early prediction of treatment response: imaging tumor extracellular HAP using 18F-NaF PET (already FDA approved) has not only shown to be able to detect peritoneal tumors with high specificity and signal-to-background ratio (SBR), but was also effective in assessing response to therapy by sensitively detecting changes in tumor extracellular HAP. This would allow patients to be spared multiple courses of expensive and toxic treatments and quickly move on to potentially more effective therapies.

There has been significant improvement in HGSOC patient survival using combination paclitaxel/platinum-based chemotherapy, particularly with the advent of second-line targeted therapies including bevacizumab and PARP inhibitors that have demonstrated progression-free survival benefits in certain clinically and molecularly defined subgroups. Despite this progress, about 70% of patients are likely to develop recurrent disease and require further treatment (16) with limited therapeutic options due to development of resistance to standard therapies. Likewise, current results with checkpoint inhibitors have been disappointing though strategies to enhance efficacy are being explored in current clinical trials. To address the limitations of current therapies, we successfully tested our novel NSPS therapeutic, designed to target HAP in the microenvironment of tumors, for treating HGSOC. Preliminary data demonstrates that application of NSPS lead to rapid tumor cell death. Our unique treatment strategy has the potential to significantly enhance the treatment options for HGSOC patients, particularly as second-line therapy.

D. Impact on society beyond science and technology

Nothing to report.

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5. Changes/problems

A. Changes in approach and reasons for change

We received the award at the height of the COVID-19 pandemic. Vanderbilt University and Vanderbilt University Medical Center went under strict restrictions including not allowing more than 1 person in a room or lab at any time. Our meetings were switched to virtual meetings and it took us a few months to receive and adjust to new lab rules and practices under the COVID-19 restrictions. Our research assistant also quit during the pandemic and it was difficult to find a replacement right away especially as covid19 cases were rising on the National and State level. We will have a new RA starting in June 2021. Given these unprecedented circumstances, we made significant progress in our research but were not able to accomplish all of our year 1 goals.

B. Changes that had a significant impact on expenditures

The PI was promoted to Research Associate Professor on Jan 1st, 2021. This promotion came with a significant increase in pay. We may have to reduce PI and other personnel %effort by end of summer. We do not anticipate that such changes would impact our work flow for this proposal.

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

Nothing to report.

6. **Products**

   1. New technique developed for detecting ovarian cancer and treatment tracking using ^{18}F-NaF PET. This technique has potential for rapid translation to the clinic as ^{18}F-NaF is already FDA-approved.
   2. Poster presentation at the 2021-March annual meeting of the Society of Gynecologic Oncology
   3. We used the results displayed above to send a letter of intent to apply for a DoD OCRP Investigator initiated award and received an invitation for a full application.

7. **Participants & other collaborating organizations**

Name	Mohammed N Tantawy
Role	PI
Nearest person months worked	2 months
Contribution to project	Dr. Tantawy is the PI on the project and overlooked all experimental details of the project from animal monitoring to imaging and image analysis

8. **Change in PI funding support**

   Dr. Tantawy, the PI, received an R21 award on 04/23/21. The PI's effort in this award is 16%. Below is the following effort distribution for the PI at least through 08/31/21:

Center for Small Animal Imaging Core	Was 33% now reduced to 17%
Department of Radiology	2% effort
Cancer Center Grant	30%
Peek (Digestive system SPORE)	4%
Harris (Kidney)	5%
Tantawy (DoD pilot)	23%
Tantawy (R21)	16%, start date 04/23/2021

9. **Special reporting requirement.**
   Nothing to report
10. **Appendices**

Society of gynecological oncology 2021 annual meeting accepted abstract for poster presentation:

Title: Tumor extracellular hydroxyapatite - A potential biomarker for imaging ovarian cancer
Authors: Mohammed N. Tantawy, Andrew J. Wilson, Alicia Beeghly-Fadiel, Fiona E. Yull, John C. Gore, Ronald D. Alvarez, Marta A. Crispens and J. Oliver McIntyre.

Abstract:

Objectives: Hydroxyapatite (HAP), $Ca_{10}(PO_4)_6OH_2$, is produced by some types of malignancies, including breast and ovarian cancer, and deposited in the tumor extracellular microenvironment. HAP has a chemical composition and pattern of deposition distinct from solid calcium deposits that are detectable by conventional CT imaging. We have shown that HAP-binding radiotracers such as FDA-approved ^{18}F-NaF can be used with PET imaging to detect breast tumors; in this context, detection of tumor-associated HAP exhibited high specificity and a high signal-to-background ratio (SBR) as HAP is absent in normal soft tissue. In ovarian cancer, conventional imaging modalities lack clear metrics for assessing tumor burden before and after surgical debulking and for assessing tumor response to therapy. This is mainly due to lack of specificity and/or low SBR ratio from standard imaging. To address this limitation, and to determine whether tumor extracellular HAP could be an effective imaging biomarker for assessing peritoneal tumor burden using ^{18}F-NaF PET, we performed evaluative studies using a well-established synergic mouse model of ovarian cancer progression.

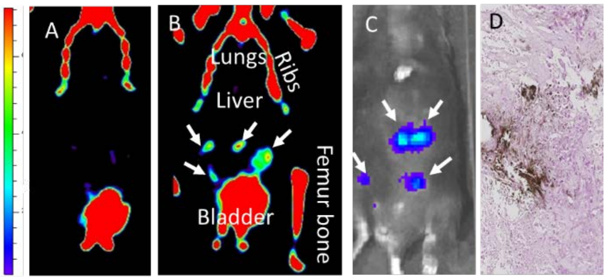


Figure A1. ^{18}F-NaF PET imaging of mouse model of ovarian cancer. (A) ^{18}F-NaF PET images of a normal healthy C57Bl/6 mouse. Total abdominal radiotracer concentration (Tot ARC) = 0.36 ± 0.08 microCi (n = 8, mean ± STD). **(B)** Mouse injected with ID8-luc ovarian tumors and imaged at 4 weeks with ^{18}F-NaF PET. Since normal soft tissue lacks HAP, the HAP-containing tumors are detected with high specificity. Tot ARC = 1.22 ± 0.18 μCi (n = 9). Total bone uptake of $^{18}F^-$ was 11.4 ± 3.0 μCi. **(C)** Luciferase bioluminescence imaging of the same mouse in B. Tumor distribution appears similar to the ^{18}F-NaF PET images. **(D)** A section of the one of the ID8 peritoneal tumors stained with von Kossa. The red/black spots are positive stain for calcium.

Methods: Female C57Bl/6 mice received intraperitoneal injection of ID8 mouse ovarian tumor cells that constitutively express a luciferase reporter (ID8-luc). After four weeks, bioluminescence imaging (BLI) was used to assess tumor burden and the mice were injected with ~11 MBq/0.1 mL of ^{18}F-NaF and imaged 60 min later for 20 min in a microPET/CT. HAP expression in harvested mouse tumors was determined by von Kossa and alizarin red S histological staining and confirmed by Ramen spectroscopy. HAP expression was similarly assessed using a clinically annotated tissue microarray (TMA) of patient samples from the Vanderbilt University Medical Center (VUMC) Tissue Repository for Ovarian Cancer (TROC).

Results: Among 117 cases from the VUMC TROC, HAP staining was more common among serous than non-serous and among late stage than early stage patients. We then confirmed HAP as a viable imaging target in ovarian tumors in the ID8-luc syngeneic model. Due to the absence of HAP in normal soft tissue (**Fig A1.A)**, peritoneal ovarian tumors were detected with high specificity and SBR (**Fig A1.B**) using ^{18}F-NaF PET which correlated well with BLI (**Fig A1.C**) and pathology. HAP presence was confirmed via histology including von Kossa (**Fig A1.D)** and alizarin red S staining and via Raman spectroscopy (not shown). As expected, bone uptake of ^{18}F-NaF was ~ 9 time greater than tumor uptake due to high HAP density and large surface area of bone. Therefore, the bone signal appears saturated in the images.

Conclusion: We show that imaging the deposition of tumor extracellular HAP may be an effective alternative tool for detecting ovarian tumors, particularly in late-stage serous disease. HAP-targeting ligands may yield a metric of disease status distinct from that obtained by conventional CT-based imaging of calcium deposits. Improvements in the clinical monitoring of tumor burden before and after surgical debulking and patient response to therapy has the potential to benefit women diagnosed with this devastating disease.

Poster:

SGO 2021 VIRTUAL ANNUAL MEETING ON WOMEN'S CANCER®

Tumor extracellular hydroxyapatite - A potential biomarker for imaging ovarian cancer

Mohammed N. Tantawy[1,2], Andrew J. Wilson3, Alicia Beeghly-Fadiel[4], Fiona E. Yull[5], John C. Gore[1,2], Ronald D. Alvarez[3], Marta A. Crispens[3,6] and J. Oliver McIntyre[1,2]

[1]Department of Radiology and Radiological Sciences, [2]Vanderbilt University Institute of Imaging Science, [3]Department of Obstetrics and Gynecology, [4]Division of Epidemiology, [5]Pharmacology, [6]Division of Gynecologic Oncology; Vanderbilt Medical Center and Vanderbilt University; Nashville TN

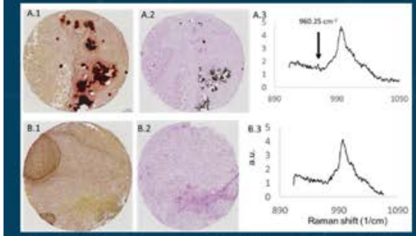
Society of Gynecologic Oncology

Introduction

- Carbonated calcium hydroxyapatite (HAP), $Ca_{10}(PO_4)_6OH_2$ type II microcalcifications [1]:
  - Major component of bone
  - Produced by and deposited in the extracellular matrix of some malignancies [1] including ovarian cancer [2]:
    - ALP on surface of cells hydrolyses βG → glycerol + Pi
    - Pi + Ca in the cell → HAP - HAP is deposited in the extracellular matrix contributing to the structure of the tumor microenvironment in a number of solid tumors.

Goals, Methods, & Results

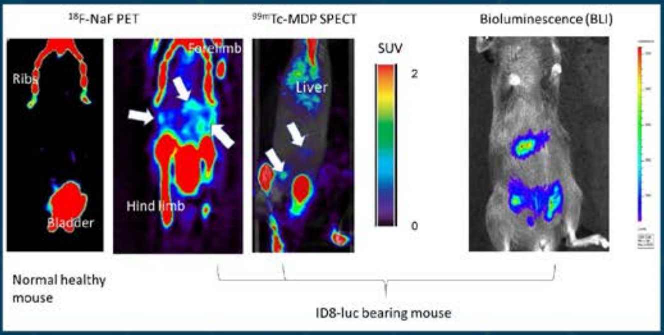
1-Investigate correlations (if any) between tumor extracellular HAP and ovarian cancer in clinical samples:
117 cases from the Vanderbilt University Medical Center (VUMC) Tissue Repository for Ovarian Cancer (TROC) were stained with von Kossa and alizarin red S and also analyzed using Raman microspectroscopy.



(A.1) A sample from a patient with HGSOC stained with alizarin red S (for calcium) and (A.2) von Kossa (for phosphates). Dark spots indicate positive stains. (A.3) A Raman shift at ~960 cm^{-1}, characteristic of hydroxyapatite, was clearly detected despite silica interference from a glass slide (a large peak between 990 and 1040 cm^{-1} (13)). Taken together, this indicates the presence of extracellular HAP. (B.1) A sample from a patient with HGSOC but scored negative for alizarin red S and (B.2) von Kossa. (B.3) No Raman shift was detected at ~960 cm^{-1} indicating absence of extracellular HAP.

HAP staining was more common among serous than non-serous ovarian cancer (consistent with [2]) and among carcinomas from late stage than early stage patients.

2-Investigate tumor extracellular HAP as an *in vivo* imaging metric for tumor burden, distribution, and monitoring treatment response to therapy in the ID8 mouse model of HGSOC [3] using ^{18}F-NaF PET or ^{99m}Tc-MDP SPECT:

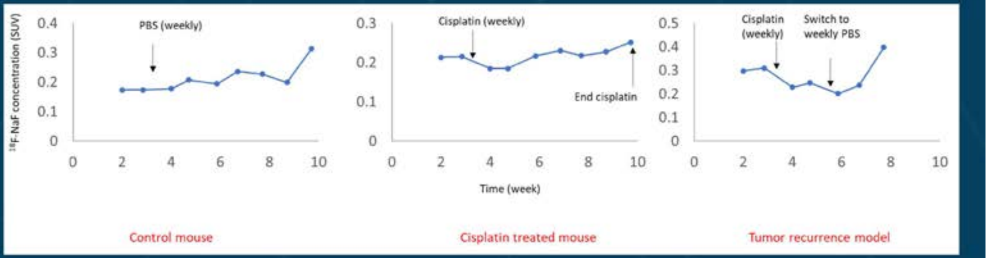


In vivo imaging & treatment schedule

^{18}F-NaF (FDA approved): 18 MBq (retro-orbital) → 1 hour uptake → 20 min PET (Inveon microPET, Siemens)

^{99m}Tc-MDP (FDA approved): 37 MBq (retro-orbital)→ 1 hour uptake → 20 min SPECT/CT (NanoSPECT/CT, Mediso)

Luciferin: 0.1 mL → BLI (IVIS, Perkin Elmer)



Typical uptake of ^{18}F-NaF in peritoneal cavity (lower abdomen) in ID8-luc tumor-bearing mice measured weekly after tumor establishment; mice were treated with cisplatin (or PBS control) as indicated.

Conclusion

- **^{18}F-NaF PET targeting tumor extracellular HAP→ detect peritoneal tumor burden with high specificity and contrast-to-background ratio as HAP is generally absent in normal soft tissue.**
- **^{18}F-NaF PET imaging appears to correlate with BLI but not ^{99m}Tc-MDP SPECT imaging that was confounded by liver uptake.**
- **Preliminary results suggest that ^{18}F-NaF PET imaging has potential for tracking: tumor progression, regression, recurrence, and early identification of tumor resistance to cisplatin chemotherapy**

References

1. Cox RF *et al.* Br J Cancer. 2012;106(3):525-37.
2. Wen J *et al.* Journal of Cancer. 2018;9(6):1006-16
3. Wilson AJ *et al.* Molecular cancer. 2015;14:192

Acknowledgement

This work was supported by DoD grant # W81XWH-20-1-0269. The Inveon microPET was funded by NIH 1S10 OD016245 and the IVIS was funded by 1S10 OD021804-01A1