Application Information Form

Completed  Oct 29 2021

Applicant Information

The information below is pulled directly from your ASCO profile. If you need to make any changes to your information, visit profile.asco.org. Changes made to your profile do not save in this form in real-time but will be reflected before submission of your full application.

Please make sure that your profile has the most up-to-date information before you submit your full application.

Upon completing this form, click Mark as Complete at the bottom of the page.

First Name

Middle Name

Last Name
Degree

MD

Primary Organization Name

Address 1

Address 2

City

State/Province

Zip/Postal Code
Country

Primary Email Address

ORCID ID

ASCO Member ID

Do you have a medical degree (MD, DO) or the international equivalent?

Enter your medical degree completion date.

Enter the date you completed or will complete your final medical subspecialty training program.
Do you have a full-time faculty appointment (this includes the Instructor position)?

Enter your initial faculty appointment date (This is your first faculty appointment after completing your training).

**Academic Rank**

**Subspecialty Training**

Hematology Oncology

**Field of Clinical Training**

Select all that apply.

**Responses Selected:**

- Hematology and Oncology
- Internal Medicine
Field of Research Training

Select all that apply.

Responses Selected:

- Computational Biology
- Evolutionary Biology/Systematics
- Genomics
- Molecular Genetics

CDA Project Information Form

Completed  Oct 19 2021

Project Information

Enter general information about the research project being proposed in this section.

At the bottom of the page:

Click **Save and Continue Editing** to save the information you have entered.

Click **Mark as Complete** once you have completed all fields. If you need to edit any information you have previously entered, click (...) at the top right corner of the form and hit Edit.

Research Project Title

Provide a short descriptive title of the research project, not to exceed 250 characters.

Brief Research Project Description/Abstract

Provide a brief abstract of the research project, not to exceed 3000 characters.

Although the incidence of appendiceal tumors has been steadily rising, they remain a rare and heterogeneous mix of tumors. The rarity of appendiceal neoplasms has made it difficult to conduct...
prospective or randomized clinical trials to guide therapy for these tumors, and virtually no preclinical models exist to facilitate drug discovery efforts. As a result, appendiceal tumors are most commonly treated with chemotherapy similarly to colorectal cancer (CRC) despite clear evidence that appendiceal tumors are distinctly different from CRC in terms of both clinical and molecular features. The use of CRC chemotherapy to treat Appendiceal Adenocarcinoma (AA) results in many patients being treated with ineffective chemotherapy, however this practice is likely to continue unless AA specific treatments can be developed. Here we propose to advance the clinical treatment of AA through an integrated combination of clinical studies and experimentation in preclinical models that will address the most critical aspects of the management of appendix cancer, and provide a platform for future drug discovery and development.

In addition to being a rare, AA is also a very heterogeneous disease with marked differences between high- and low-grade appendiceal carcinomas in terms of natural history, response to chemotherapy, and somatic mutations. Low-grade tumors generally follow an indolent clinical course (median OS 137.8 months, 95%CI: 52.7 -223), respond poorly to chemotherapy, and are often managed with serial debulking surgeries; whereas high-grade tumors follow a more a more aggressive clinical course (median OS 41.9 months, 95%CI: 27.3 -56.6), and are generally managed with chemotherapy designed for CRC. Both high- and low-grade AA are unique from other gastrointestinal tumors in that they typically spread to the peritoneum and rarely distant sites such as the liver, lungs, or abdominal lymph nodes. Our overarching hypothesis is that the tumor specific vulnerabilities of AA will be different than CRC, and more specifically the vulnerabilities of high-grade and low-grade AA will likely be unique from each other.

Major dilemmas facing clinicians caring for patients with AA include uncertainty in determining high versus low-grade disease, non-invasive assessment of tumor burden in the peritoneum to measure response to chemotherapy and select candidates for complete cytoreductive surgery (CRS), as well as a lack of effective systemic therapies. There is also virtually no prospective clinical trial data to guide which patients should be treated and with what drug(s). We will directly address these clinical needs by performing a prospective clinical trial to evaluate the effectiveness of intraperitoneal paclitaxel for the treatment of high-grade AA (Aim 1), evaluating the ability of ctDNA to measure disease burden (Aim 2), and evaluating the predictive value of orthotopic patient derived xenograft (PDX) and patient derived organoid (PDO) models of AA (Aim 3).
Lay Abstract

Provide a layperson summary of the project, not to exceed 2500 characters. Describe your work in a way that it will be understood by people who do not have scientific or medical backgrounds. Be clear and avoid technical and scientific terms when possible. It should not include confidential information. If selected to receive an award, Conquer Cancer may use the content of this layperson summary on its website and/or other public facing materials.

Appendix cancer is a rare tumor, and because it is so rare very few clinical trials have ever been performed in appendix cancer. Without having any trials to help oncologists know what drugs are effective, decisions regarding what patients should get chemotherapy, and the specific type of chemotherapy they should get are generally based on each doctor’s personal experience. Traditionally, appendix cancer has been treated with chemotherapy as if it were the same as colon cancer, and in 2021 this is still accepted practice. However, new data in the last five years generated by [redacted] and others has shown convincingly that appendix tumors are very different than colon tumors. Given that appendix tumors are so different than colon tumors, we believe that practice of giving colon cancer chemotherapy to appendix cancer patients is outdated, and that developing chemotherapy specifically for appendix tumors will be a better approach for these patients.

Part of the reason that no appendix cancer chemotherapy has been developed previously is that there are very few models of appendix cancer to allow scientists to test potential treatments in the lab. Our lab is actively working to develop and test these model systems. In addition to testing potential new drugs in model systems, we will also use next-generation sequencing technology to make detailed measurements of appendix tumors. Having these detailed molecular measurements will allow us to understand the differences in each patients’ tumor, and knowing what differences are important will allow us to pick the best chemotherapy plan to fit each patient.

In addition to using our models to test potential new drug treatments, we will perform a clinical trial of the drug [redacted] given directly into the peritoneal space, which is where appendix cancer spreads to. This trial is based on similar trials in related GI cancers such as gastric (stomach) cancer and small bowel cancer. All patients on the trial will be treated with the experimental therapy (paclitaxel), and compared against similar patients previously treated with standard colon cancer chemotherapy.

Taken a whole, we are confident that the proposed research will lead to the development of one or more drugs that are effective for appendix cancer. Although this idea would need further testing in the future, we believe that this will lead to better results for our appendix cancer patients.
SPECIFIC AIMS

List succinctly the specific objectives of the proposed research project. The aims should state concisely and realistically what the research intends to accomplish and/or what hypothesis is to be tested, and should list measurable objectives for the proposed project. At least one specific aim is required.

How many aims do you have?

3

Specific Aim 1

[Redacted text]

[Redacted text]

[Redacted text]

[Redacted text]

A similar Phase I trial of IP [Redacted text] with weekly dosing is currently ongoing for gastric cancer at [Redacted text], which demonstrates the feasibility of this design. Primary end point of the Phase II portion will be objective response rate at 3 months as measured by modified peritoneal [Redacted text] with 15 patients treated at RP2D (combined over Phase I/II) power will be 87% to detect a 30% response rate with one-sided type I error of 0.05; we will reject the null if at least 3 of 15 patients respond.
Appendiceal cancer is notoriously difficult to measure with standard CT scans, which underestimate tumor burden. Current standard-of-care practice is to perform a diagnostic laparoscopy to assess disease burden determine if complete cytoreductive surgery (CRS) is feasible; however surgical staging is not always practical. ctDNA has shown promise as a non-invasive method to assess and longitudinally track tumor burden in many disease types, although our preliminary data suggests that a commonly used first-generation 70-gene ctDNA assay has sensitivity of only 7.1% in AA. Here we will test if a high sensitivity tumor-informed/personalized ctDNA assay can reliably detect ctDNA in pre-operative AA patients. We will further evaluate correlation of ctDNA with clinical outcomes including survival and tumor response seen radiographically and pathologically.

Preclinical models are critical for the drug development process, and the near complete lack of models in AA is one of the major reasons no specific therapy exists for this tumor. To rectify this problem we have begun to systematically generate PDX and PDO models of AA. To better model the unique natural history of AA, which involves early and extensive peritoneal spread but we implant tumors into the peritoneal cavity to create orthotopic models. Preliminary evaluation of orthotopic PDX generated thus far indicate that these models recapitulate the histologic appearance and transcriptional state of human AA tumors. Ultimately a preclinical model is useful if it predicts clinical response; here we will directly evaluate the ability of PDX and PDO models to predict drug response by comparing to the outcomes of trial patients (Aim 1).
Subject Area

Select one Subject Area from the drop-down list that best describes your research grant project. If "Other" is selected, provide information in the text field.

Gastrointestinal (Noncolorectal) Cancer

Focus Area(s)

Scroll through the list to find research areas that may apply to your research project. You may check several research areas, but at least one focus area is required. If "Other" is selected, provide information in the text field.

Responses Selected:

- Bioinformatics
- Biomarkers
- Chemotherapy
- Clinical Trials
- Drug Development
- Model Systems (not mouse)
- Mouse Models of Cancer
- Other
- Targeted Therapeutics

Research Classification

Select from the drop down list your research classification.

...
Type of Research Study

Select from the drop down list the type of your research study.

Clinical Trial Phase

If the research project is “clinical,” indicate the phase of the clinical trial (Phase I, Phase I/II, Phase II, Phase II/III, Phase III).

Clinical Trial Number

Indicate the clinical trial number.

(No response)

ASSURANCES

Animal Use

Indicate whether animals will be used in the research.

Yes

Assurance Status

Approved
IACUC Approval Date

IACUC Expiration Date

Assurance Number

Human Subjects
Indicate whether human subjects will be involved in the research.

Assurance Status

USE OF DRUG(s)

Will your research involve the use of drug(s)?
Name of Drug(s)

Drug manufacturer(s)

RESUBMISSION

Indicate if this application is a resubmission from a prior cycle. If yes, you will be required to upload a one-page resubmission document addressing the feedback of the reviewers of your prior application.

How many mentors do you have?

If you select "2", the task "Mentor Invite 2" will appear once you hit "Mark as Complete" below.

Is at least one of your mentors an ASCO member?

CDA Upload Research Strategy

Completed Oct 21 2021

Upload your research strategy, limited to six (6) typewritten, single-spaced pages, with one-inch margins and 11 point Arial font type. All pertinent tables, pictures, and graphs MUST be included within the 6-page limit. Please refer to the Request for Proposals for details on what must be included in the Research Strategy.

If the document you uploaded exceeds the page limit, Conquer Cancer will return your application.
Use this file naming convention: [year program abbreviation] Research Strategy [Last name]
For example: 20xxCDA Research Strategy Smith

CDA Upload Biostatistical Plan

Completed  Oct 21 2021

Upload a detailed biostatistical plan limited to one (1) typewritten, single-spaced page with one-inch margins and 11-point Arial font type. Please refer to the Request for Proposals for details on what must be included in the Biostatistical Plan.

If the document you uploaded exceeds the page limit, Conquer Cancer will return your application.

Use this file naming convention: [year program abbreviation] Biostatistical Plan [Last name]
For example: 20xxCDA Biostatistical Plan Smith

In addition, upload a letter of support from a biostatistician in the Additional Supporting Documentation section.

CDA Upload Cited References

Completed  Oct 21 2021

Upload a bibliography of any references cited in the Research Plan. The Cited References has no page limit, must be typewritten with single-space, one-inch margins and using an 11-point Arial font type.

Use this file naming convention: [year program abbreviation] Cited References [Last name]
For example: 20xxCDA Cited References Smith

CDA Patient Advocate Form

Completed  Oct 12 2021

Patient Advocate Form

Applications will be reviewed by a patient advocate. The applicant is required to work and communicate with a patient advocate early during the development of the project and the application.
Please answer the questions below (maximum of 200 words). In addition, upload a letter of support from a patient advocate in the Additional Supporting Documentation section.

**Describe the clinical problem being addressed, its scope, and the impact your research could potentially have on this patient population.**

There is a critical need for effective non-surgical treatments for appendiceal adenocarcinoma; for low-grade tumors no effective therapy exists, for high-grade tumors only a subset of patients respond to chemotherapy that has been designed for colorectal cancer. This proposal outlines a systematic approach that has a high likelihood of (eventually) leading to the first ever appendix cancer specific treatment.

**If the study is successful what will be the next steps in moving your research into clinical practice. Describe the potential barriers to accrual and/or retention.**

If the phase I/II trial of [redacted] is successful the next step will be a larger trial to confirm the findings. Given the different natural histories of high- and low-grade appendix cancer the subsequent confirmatory trials would likely need to be performed separately. These studies would likely need to be multi-center trials and thus would need to be a part of a consortium. I have been in discussion with the leadership of the [redacted] regarding building a such a consortium for a [redacted]. To our knowledge, a prospective [redacted] has never been conducted in appendix cancer.

I have also discussed with the [redacted] forming a committee to write for the first-time [redacted] for the treatment of appendix cancer.

Potential barriers include the ability to place IP catheters for drug delivery, however, given established track record of IP chemo delivery in ovarian and gastric cancers this is felt to be feasible. This the dosing schedule has been changed to facilitate the [redacted] population, many of whom drive in several hours each visit. The inclusion / exclusion criteria have also been designed to capture as many patients as possible.
How do you plan to engage patient advocates and relevant stakeholders in the design/implementation of your study and dissemination of the results?

We have already had been in discussion with a patient advocate from the [redacted] and have incorporated several suggestions into our clinical protocol (see letter of support). Part of the mission of the [redacted] is to support patient and physician educational programs with the goal of increasing the knowledge, understanding, and awareness of appendix cancer and pseudomyxoma peritonei. There for we plan to continue to engage with the [redacted] to disseminate the results of our research. Plans are already underway for [redacted] to speak at the [redacted].

How will the results of this study improve a patient's quality of life?

One of the most common ways that patients die of appendiceal cancer is malignant bowel obstruction, if successful, the chemotherapy we are proposing will reduce tumor burden and potentially avoid this terrible complication. Additionally, it is hoped that delivering the taxane chemotherapy IP instead of IV will decrease side effects, in particular hair loss that is common with IV administration of [redacted]. Additionally, moving from weekly to every other week infusions will improve quality of life by reducing travel time.

What burdens will the trial impose on patients? What have you done in designing the study to minimize the burden to patients?

All chemotherapy has side effects and this is the largest burden for the patient. IP delivery of chemotherapy will require placement of an IP catheter, however this has been commonly done before in other tumor types, most notably ovarian cancer. Travel to study site to get chemotherapy is frequently noted as the biggest burden on patients, [redacted] where the majority of our patients travel from out of town. To minimize this burden we are testing biweekly as opposed to weekly dosing.

CDA Budget Form

Completed  Oct 20 2021

Budget

Enter the amount requested in the appropriate categories in each column. **DO NOT USE A COMMA** when
Budget justifications for each category requested must be entered in the "Description of Costs" column. You may upload additional justification in the CDA Upload Additional Supporting Documentation, if needed.

NOTE: Enter N/A in the "Description of Costs" for categories not being requested.

The following budget limitations apply:

- **Total Award**: The grant amount is $200,000 for one year. The budgeted amount must not exceed $200,000. All funds will be paid directly to the Sponsoring Institution. During the award period, at least 80% of the yearly budget must be expended by the end of each reporting year as a condition of approval of the next grant payment installment.

- **Research Support**: At least $59,966 per year should support costs directly related to the research project such as personnel salary, supplies, equipment, and other expenses. Patient care costs that are reimbursable by a third-party payor, professional membership dues, tuition fees, and other fees for academic courses are unallowable costs. Salary limits will be equivalent to the NIH applicable limit.

- **Travel**: Up to $2,500 may be allotted specifically for travel to the ASCO Annual Meeting and for any other travel essential to conducting the study.

- **Indirect Costs**: Up to $4,200 per year (or 6.3% of the yearly total award amount) may be applied to overhead or facilities and administrative costs of the recipient's institution in administering the recipient's research project.

### Costs - Years 1-3

<table>
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<tr>
<th></th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Description of Costs (Required)</th>
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<td>Consortium/Contractual Costs</td>
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<td>Equipment</td>
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<td>Other Expenses</td>
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<td>Patient Care Costs (Inpatient)</td>
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<td>Patient Care Costs (Out-patient)</td>
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<td></td>
<td>cover all costs of the trial in aim 1 that can't be billed as SOC, including the PK measurements, tumor molecular test</td>
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<td>Personnel Costs</td>
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<td>will have 2% effort per MDA requirement, 30% to cover the salary of one research scientist, 2% effort for (biostats)</td>
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<td>Subcontracts</td>
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<td></td>
<td>mice, organoid reagents, and drugs being tested in Aim 3</td>
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<td>Travel</td>
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**Total 3 Year Budget**

$200000.0

**CDA Upload Project Timeline**
Use this project timeline template and upload once completed. Please ensure the document is set to proper print area and that all columns are visible on each page.

Use this file naming convention: [year program abbreviation] Project Timeline [Last name]

For example: 20xxCDA Project Timeline Smith

CDA Upload Resubmission Documentation

Completed Oct 28 2021  Hidden from applicant

Upload an introduction to address the reviewers' feedback and critiques on your previous application. The introduction must be a one (1) page typewritten, single-spaced page with one-inch margins and 11-point Arial font type. Please refer to the Request for Proposals for details on what must be included in the Resubmission Document.

If the document you uploaded exceeds the page limit, Conquer Cancer will return your application.

Use this file naming convention: [year program abbreviation] Resubmission Doc [Last name]

For example: 20xxCDA Resubmission Doc Smith

CDA Personal Statement Form

Completed Oct 19 2021

Personal Statement

Answer the questions below limiting your responses to no more than 2000 characters.

At the bottom of the page:

Click Save and Continue Editing to save the information you have entered.

Click Mark as Complete once you have completed all fields. If you need to edit any information you have previously entered, click (...) at the top right corner of the form and hit Edit.
Applicant's career plan

Provide a brief description of your career plan.

I have spent the last ten years studying cancer genomics, a time period that has coincided with a tremendous influx in the availability of tumor genome sequences and now multi-omics profiled tumor samples. If we as a community of cancer researchers have learned anything sequencing these thousands of tumors, it is that cancer is a remarkably heterogeneous disease at the molecular level. Not surprisingly, when these very heterogeneous cohorts of patients are treated with the same chemotherapy only a fraction will respond favorably. While the promise of ‘precision oncology’ remains out of reach for most solid tumor patients, I feel that the necessary tools now exist to make meaningful, if incremental, progress towards this end. For this reason I am broadly creating a research program that will link cancer molecular profiling (genome, transcriptome, methylome, proteome, ect) to the phenotype of chemotherapy response for colorectal and appendiceal tumors.

After more than a decade of [redacted] I have finally achieved my first career goal which was establishing my own lab as an independently funded investigator. With the [redacted] now established we seek to ensure our long-term success of our lab by continuing to pursue clinically impactful science at the interface of cancer genomics, pre-clinical modeling of cancer, and clinical oncology. The ultimate goal of the lab is to translate our research discoveries into new cancer therapies. Recognizing that as a new lab we need to focus our efforts we have chosen to two specific priorities: (1) developing effective therapies appendiceal cancer as we now propose to do in this CDA award, and (2) performing functional genomic screening to systematically identify genetic interactions (and opportunities for synergistic drug combinations) in colorectal cancer.
Impact of award on applicant’s career

Provide a brief explanation on how receiving this award would affect your career.

I was fortunate enough to be awarded a [REDACTED] from [REDACTED] during my final year of clinical fellowship, and that award was critical as it allowed me dedicated research time as a postdoctoral fellow. Additionally, the [REDACTED] brought me into the network of young physician-scientists at [REDACTED] which has been a tremendous resource as I have moved forward in my career. Now as junior faculty, this Career Development Award will provide the resources needed to take recent discoveries in our lab into clinical testing, which is our ultimate goal. Successfully completing even a small, proof-of-principle, clinical trial as we propose here will lend further validation to the notion that appendiceal tumors must be treated differently from colorectal tumors. This point is important as it will likely encourage other physicians and scientists to pursue research in appendiceal cancer. Specifically to myself and our lab, the validation of our experimental methods (in this case pre-clinical models) and computation methods will give our lab growing confidence to continue to expand our research to address the many unmet needs in colorectal and appendiceal cancer.

Additionally, as was the case with the [REDACTED] being selected for a CDA award will bring with it opportunities to network with other up and coming research oncologists and the ASCO leadership. It is my sincere belief that this CDA award will allow me to launch into the next phase of my career, transitioning from merely being a laboratory head to a being a thought leader in the admittedly small but hopefully growing field of appendiceal cancer research.

Percentage time of research activities

Provide the percentage time you will spend on total research activities.

80
Applicant's role

Describe briefly your role versus your mentor's role in the proposed research study.

I have designed the research plan with assistance of [Name]. I designed the [Name] appendiceal cancer PDX experiment that serves as the pre-clinical basis to support the proposed human appendiceal cancer clinical trial. For the ctDNA aim I wrote the statement-of-work that is the basis for the research collaboration [Name], and I have been leading that collaboration. For the pre-clinic model (PDX and PDO) aim I have set up the collaboration with [Name] to get direct access to fresh tumor from the operating room. However, Dr [Name] is PI of the IRB approved protocol we use for prospective tissue collection. [Name] has also been involved in setting up the collaboration with [Name] to generate PDO, although [Name] have taken the lead.

[Name] has reviewed the aims and research plan, as well as the clinical protocol and provided significant advice regarding the design of the trial and execution of the aims. [Name] will continue to provide advice in real-time regarding the execution of the research plan (all aims). I will be PI of the clinical trial in Aim 1. For other Aims I will lead a team that consists of both computational and experimental scientists, and be responsible for their supervision, training, and ultimately all data collection, analysis and publication.

Sources of salary support

List the sources of your salary support.

[Name]
[Name]
[Name]
Collection and support of data

Briefly describe who will collect and analyze the data.

Data from the clinical trial in Aim 1 will be collected with assistance of a trial coordinator, this same person will assist faculty with the paperwork needed for trial enrollment. I will be primary supervisor of this trial coordinator as study PI. Data analysis will be performed primarily by a clinical postdoctoral fellow in my lab (拿出来), likely with assistance of a surgical fellow.拿出来 will assist with biostatistics (see letter of support).

Data from ctDNA studies and molecular profiling of appendiceal tumors will be transferred from genomic core labs to a computational postdoctoral fellow in my lab, and analysis of said data will be performed by myself in concert with my computational team.

Data from PDO and PDX experiments will be collected by a research technician who is experienced in mouse and organoid experimentation. Analysis will be performed by this technician in collaboration with myself and postdoctoral fellows.
Clinical potential of research project

Briefly describe the clinical potential of this research project.

YIA Project Accomplishments

If the applicant was previously awarded a Conquer Cancer Young Investigator Award (YIA) and the proposed CDA project is a continuation, briefly describe the key accomplishments of the YIA project. If the applicant is not a previous Conquer Cancer YIA recipient, please indicate N/A.

Other funding sources

List other funding agencies/organization where this research proposal was or will be submitted. If none, please indicate N/A.

CDA Upload Biosketch
Upload your biosketch using the most recent NIH Biosketch template. Please refer to these instructions. If the document you uploaded exceeds the page limit, Conquer Cancer will return your application.

Use this file naming convention: [year program abbreviation] Biosketch [Last name]

For example: 20xxCDA Biosketch Smith

CDA Mentor Invite 1

Invite your Mentor to submit a recommendation for your application. If your Mentor has an ASCO user account, use your Mentor's email address associated with his/her ASCO account to ensure access to the recommendation task in the Application Portal. If you used an incorrect email address, you may withdraw your request and create a new request using the correct email address.

To resend or withdraw your request, click the ellipsis (...) and select from the options.

Once your Mentor submits the recommendation, you will receive a notification. Click Mark as Complete.

Recommenders
Recommender:

Content:
Form

CDA Mentor Invite 1

First Name

Middle name
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<th>Institution Name</th>
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<tr>
<th>Email Address</th>
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**ASCO ID**

Enter your ASCO Member ID. If you are not an ASCO Member, enter N/A.

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Biosketch (Maximum of 5 pages)

Upload your biosketch using the most recent NIH Biosketch template. Please refer to these instructions.

Use this file naming convention: [year program abbreviation] Biosketch [your last name]

For example: 20xx CDA Biosketch Smith

Letter of Support (Must be signed and on official institutional letterhead)

Upload your letter of support. For more details on what to include in the letter of support, please refer to the Request for Proposal (RFP) posted on asco.org/cda.

Use this file naming convention: [year program abbreviation] LOS [your last name]

For example: 20xx CDA LOS Smith

CDA Mentor Invite 2

Incomplete  Hidden from applicant

Invite your Mentor to submit a recommendation for your application. If your Mentor has an ASCO user account, use your Mentor’s email address associated with his/her ASCO account to ensure access to the recommendation task in the Application Portal. If you used an incorrect email address, you may withdraw your request and create a new request using the correct email address.

To resend or withdraw your request, click the ellipsis (...) and select from the options.

Once your Mentor submits the recommendation, you will receive a notification. Click Mark as Complete.

Recommenders

CDA Sponsor Invite

Incomplete  Hidden from applicant

Invite your Sponsor to submit a recommendation for your application. Use your Sponsor’s email address associated with his/her ASCO account to ensure access to the recommendation task in the Application Portal.
Portal. If you used an incorrect email address, you may withdraw your request and create a new request using the correct email address.

To resend or withdraw your request, click the ellipsis (...) and select from the options.

Once your Sponsor submits the recommendation, you will receive a notification. Click **Mark as Complete**.

**Recommenders**

**CDA Upload Mentorship Plan**

**Completed**  Oct 19 2021

Upload a Mentorship Plan limited to two (2) typewritten, single-spaced page with one-inch margins and 11-point Arial font type, **signed by the applicant and mentor(s)**. Please refer to the Request for Proposals for details on what must be included in the Mentorship Plan.

*If the document you uploaded exceeds the page limit, Conquer Cancer will return your application.*

Use this file naming convention: [year program abbreviation] Mentorship Plan [Last name]

For example: 20xxCDA Mentorship Plan Smith

**CDA Upload Institutional Letter of Support**

**Completed**  Oct 18 2021

Upload a signed letter on official institution letterhead written by the Department Chair or Dean at your sponsoring institution that includes a statement confirming institutional support that will enable you to perform the proposed research. If your mentor is the Department Chair, the Institutional Letter of Support must come from the Dean. Please refer to the Request for Proposals for details on what must be included in the Institutional Letter of Support.

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**CDA Upload Clinical Protocol**

**Completed**  Oct 21 2021

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CDA Publication Form

Completed Oct 18 2021

Publication(s)

Up to two prior publications that highlight the applicant's experience and qualifications may be included. The applicant must be at least a co-author on these publications. Please enter the publication information in this section including the title, the year published, the type of publication, publication status, and funding (whether the project was funded by Conquer Cancer or not).

Scroll to the bottom of the page to upload the actual publication.

How many publications are you including in your application?

Publication 1

Publication Title

Publication ID

Enter your PubMed ID number.
Publication Year

If the status of your publication is "In Press" or "In Preparation", please enter "0000" in the Publication Year field.

Publication Type

Research Article

Publication Name

Publication Status

Select the status of your publication.

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URL

Enter the URL in PubMed format (http://www.ncbi.nlm.nih.gov/pubmed/PMID) where PMID is your publication's PubMed ID number.

For example:  https://www.ncbi.nlm.nih.gov/pubmed/18276894
Funding Status

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Upload Publications

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For example: 20xxCDA Publication1 Smith

Publication 2

Publication Title

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For example: 20xxCDA Publication2 Smith

CDA Upload Additional Supporting Documentation

Completed Oct 20 2021

Upload any additional documents relevant to your application (See Request for Proposals for examples). Letters of Support from collaborating biostatistician(s) and patient advocate are required.

Use this file naming convention: [year program abbreviation] Supporting Doc[number] [Last name]

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CDA Institution Approval

Completed  Oct 20 2021

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To resend or withdraw your request, click the ellipsis (...) and select from the options.

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**Recommenders**

**Recommender:**

**Content:**

Form

**Institution Approval**

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This is the organization providing administrative, research, and financial oversight of the grant.
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This is the name of the institution that must be used in the award agreement if the applicant is selected to receive the award.

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Institution Common Name

This is the name of the institution that will be used in public facing materials (e.g. recipient listings, press release, etc.) if the applicant is selected to receive the award. Select the institution name from the drop-down list. If the institution name is not listed or needs to be listed differently, select "Other" and indicate the institution name.

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SPONSORING INSTITUTION CERTIFICATION AND ACCEPTANCE

I certify that the information herein and all the components of the grant application are true, complete, and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, and administrative penalties. I have also read, understood, and agree to the above Application Information Use and Sharing.

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DATE

[Signature]

[Date]
I. Significance & Background

New treatments are needed for appendiceal cancer. Appendiceal tumors encompass a rare and diverse group of neoplasms; appendiceal adenocarcinoma (AA) is the most common histologic subtype. Epidemiologic studies based on Surveillance, Epidemiology, and End Results (SEER) data have shown a steady increase in incidence from approximately 0.2 cases per 100,000 in the 1970s, to current estimates of just over 1 per 100,000.\(^1,2\) In comparison, this is 40-fold less common than colorectal cancer (CRC), which in the US has an incidence of approximately 40 per 100,000.\(^3\) Cases of early-onset AA, defined as diagnosis before age 50, have increased by 24% between 2011 to 2016, and in 2016 represented 40% of all appendiceal cancer.\(^4\) In contrast, the increase in early-onset colorectal cancer (CRC) was only 2.2% for that same time period.\(^4\) Historically, appendiceal tumors have been grouped together with CRCs due to anatomic vicinity, similar embryological origin, and common expression of the transcription factor CDX2;\(^5,6\) as of 2021 the National Comprehensive Cancer Network (NCCN) guidelines still suggest that AA be treated with chemotherapy similarly to CRC. The rarity of AA has made it difficult to conduct prospective or randomized clinical trials to guide therapy for these tumors, and virtually no pre-clinical models exist to facilitate drug discovery efforts. As a result, appendiceal tumors are most commonly treated with CRC chemotherapy despite a growing consensus that AA is a clinically and molecularly distinct entity.\(^7-11\) The use of CRC chemotherapy to treat AA results in many patients being treated with ineffective chemotherapy, however this practice is likely to continue unless AA cancer specific treatments can be developed.

Molecular & clinical differences between AA subtypes and CRC. Unlike CRC, which has a predictable spread into lymph nodes and then to the liver (70% of cases),\(^12\) AA rarely involves lymph nodes and even high-grade tumors almost never spread hematogenously.\(^9\) Rather both high- and low-grade AA spread directly into the peritoneal space where they cause peritoneal carcinomatosis, which is frequently mucinous in nature, giving rise to the clinical syndrome of Pseudomyxoma Peritonei (PMP)\(^13,14\). AA has a more indolent natural history relative to CRC, with median OS of 76 months (95%CI: 58.1 - 93.5) for metastatic patients but this median disguises marked differences between high- and low-grade tumors.\(^15\) Low-grade tumors generally follow an indolent clinical course (median OS 138 months, 95%CI: 53-223), respond poorly to chemotherapy, are more likely to be mucinous, and are most often managed with serial debulking surgeries combined with Heated Intraperitoneal Chemotherapy (HIPEC). High-grade tumors follow a more aggressive clinical course (median OS 42 months, 95%CI: 28-57), are less likely to be mucinous, and are generally managed with CRC chemotherapy.\(^13,16,17\) Cohort sequencing studies by us and others\(^7-9\) have revealed APC mutation, which is a hallmark feature of CRC, is uncommon in all subtypes of AA. Frequency of GNAS mutation is much higher in AA, with a particularly strong enrichment in mucinous tumors (\(\chi^2\) p < 0.0001)\(^7\) (Fig. 1A). Transcriptional analysis shows that AA clearly separates from CRC does not resemble any of the known consensus molecular subtypes (CMS) of CRC, consistent with AA being a separate disease entity from CRC\(^7,15\) (Fig. 1B).

When AA patients are not candidates for surgical resection they are referred to medical oncologists where they present a particularly vexing problem given AA tumors generally respond poorly to systemic cytotoxic chemotherapy and patients often succumb to bowel obstruction or cancer cachexia from diffuse peritoneal carcinomatosis. There is also virtually no prospective clinical trial data.
to guide which patients should be treated and with what drug(s). We will directly address these clinical needs by performing a prospective clinical trial to evaluate the effectiveness of intraperitoneal paclitaxel for the treatment of high-grade AA (Aim 1), evaluating the ability of circulating tumor DNA (ctDNA) to measure disease burden (Aim 2), and evaluating the predictive value of orthotopic patient-derived xenograft (PDX) and patient-derived organoid (PDO) models of AA (Aim 3).

*Given the strength of the preliminary data supporting our hypotheses, we feel there is a high likelihood that the proposed work will lead to the development of a chemotherapy treatment specifically for AA, something that has never been done previously.*

II. Innovation

**The molecular heterogeneity of cancer.** It is now known after sequencing over 10,000 tumors in The Cancer Genome Atlas (TCGA) that cancer is tremendously diverse at the molecular level. Tumors that are similar in terms of both anatomic origin and histologic appearance may have little overlap in somatic mutations; similarly they can harbor dramatically different transcriptional states. It is clear from many anecdotal examples, such as the success of BRAF inhibition in BRAF V600E melanoma, but its subsequent failure in BRAF V600E colon cancer, that chemo-genetic relationships are not absolute, but rather dependent on factors including cell lineage and the presence of other genomic aberrations. Given that we and others have established that there are real differences in somatic mutation profiles as well as transcriptional state between AA and CRC (Fig. 1) it cannot be assumed that the drug-response relationships seen in CRC will hold in AA. Thus the continued development of AA specific pre-clinical models is needed to identify AA tumor specific vulnerabilities. Using the Cellsigner method to perform unsupervised clustering of transcriptomic profiles from all TCGA tumors and cancer cell lines from the lab we find that AA tumors clustered closely together and the AA PDX clustered with human tumors. The AA cluster was separated from CRC, from any other tumor type, and from any cell-line model, indicating that no representative cell line models of AA exist, in contrast to findings for CRC which clusters with many known CRC cell lines (Fig. 2).

**Prospective clinical trials in AA.** Leveraging our clinical volume and status as a tertiary referral center, with ~200 unique AA patients seen each year, the GI Medical Oncology group has begun conducting prospective clinical trials in AA. A total of 20 patients with AA were prospectively treated on a single-arm trial of cobimetinib plus (Fig. 3). Unfortunately, no objective responses were reached, approximately half of the patients achieved stable disease. Pre- and post-treatment biopsies were available for four patients, RNAseq profiling suggested that MEK was not effectively inhibited in any of the four (Fig. 3). These data suggest mucin produced by AA may prevent drugs from reaching the tumor cells or cobimetinib may have been otherwise under-dosed to reach the peritoneal space.
Semi-Automated aggregation of ‘real world’ data. Recognizing both the difficulty in conducting prospective trials in a rare disease such as AA as well as the value of ‘real world’ data from an institution as large as ours, we have started an active collaboration with the [redacted] and his team have adapted Foundry specifically for the application of mining clinical and molecular data for CRC and AA patients. As of our last query we have some clinical annotation on over 6,000 unique appendiceal cancer patients.

Patient-derived micro-organosphere (PDMS) technology for the systematic generation of organoid models in AA. Appendiceal tumors are known to grow poorly in standard 2-dimensional (2D) culture conditions, a major reason why no commercially available AA cell lines exist. To overcome these limitations, we will employ PDMS technology, recently developed by our collaborator (see letter of support, Fig. 4). This technology uses droplet-based microfluidics to partition and isolate individual cancer cells into miniaturized micro-reactors to generate microfluidic micro-organospheres (MOS). The droplets are then patterned into high-density well plates and dosed with drug compounds, or pooled for CRISPR screening, in vivo implantation, or other profiling experiments. Previously shown is that immortalized 2D cell lines are not always predictive of patient outcomes. Patient-derived organoids (PDOs), which preserve cell-to-cell contacts important for epithelial tumors like AA, are uniquely positioned to fill this gap as, compared to cell lines, they more accurately depict patient tumors while compared to PDXs, they improve initiation times, cost, and efficiency scales. PDOs have shown high fidelity as tumor avatars, allowing for high-throughput screening of many drugs, and have been proposed as a functional precision-medicine technology capable of guiding treatment decisions in the clinic. Recent co-clinical trials in metastatic CRC and other gastrointestinal (GI) cancers found that PDOs successfully predicted drug response in their host patient 80-90% of the time, and non-response 100% of the time. The main barrier to greater use of PDOs has been difficulty in generation. To circumvent these barriers, we leverage advances in emulsion microfluidics and droplet generators to develop PDMS, a miniaturized version of organoids that can be established rapidly and efficiently in terms of both time and cost.

III. Approach
Specific Aim 1.

We hypothesize that [redacted] will be active in high-grade AA, and that IP delivery will increase therapeutic window.

Taxane chemotherapy has been ineffective for CRC, however, the mechanism of this resistance has been mechanistically linked to APC loss-of-function. Unlike CRC, where APC is mutated in 73.5% of tumors, APC mutation is uncommon in AA (9.0%), as are mutations in other known Wnt pathway genes. Based on strong pre-clinical data linking taxane resistance to APC mutation and subsequent chromosomal instability (CIN), a Phase II trial of [redacted] was conducted in both CpG Island methylator phenotype (CIMP)-high CRC and Small Bowel Adenocarcinoma (SBA). Reasonable activity was seen in SBA, with two partial responses and three patients with stable disease out of 10 treated, interestingly no responses were seen in CIMP-high CRC. Based on these data as well as a small retrospective case series taxane chemotherapy has been included in the NCCN guidelines for the treatment of SBA. Given that APC mutation is even less frequent in AA than SBA (9.0% vs. 26.8%) it is reasonable to surmise that taxanes will have activity in AA. Given these data in SBA four AA patients who had progressed on three or four prior lines of CRC therapy were treated with [redacted]; two of four achieved tumor marker response and one had stabilization of tumor markers (data-not-yet-published). A recent pre-clinical study with PDX models of both high- and low-grade AA showed near complete response with IP
Similarly, we have shown that given IP is active in an orthotopic PDX model of high-grade AA (Figs. 5,6). IP administration is attractive given that AA almost never extends beyond the peritoneum, and most complications from AA, such as bowel obstruction, are a result of peritoneal disease. Taxanes have been given safely in ovarian cancer for many years\textsuperscript{42,43} and recently have been shown to be effective in peritoneal carcinomatosis from gastric cancer\textsuperscript{44}. It has been suggested that the hydrophobicity of taxanes leads to uptake preferentially in the lymphatics leading to prolonged retention in the peritoneal cavity relative to hydrophilic drugs\textsuperscript{45}.

On the basis of these preliminary data we propose a Phase I/II trial of IP cabazitaxel in high-grade AA to establish the optimal dose when given q14 days, then to evaluate efficacy at the Recommended

Figure 5. Schema for mouse IP PTX experiments. All the tumors obtained from the implanted, allowed to grow for 4 weeks, then treated with either saline or PTX. The tumors were then measured at weekly intervals for 8 weeks.

Figure 6. Results of mouse IP PTX experiments. Left: representative MRI images of AA tumor growing in mouse peritoneal space. Middle: Spider plot serial tumor growth, note stabilizes disease. Right: All four AA tumors treated with saline show progression, vs. one treated tumor.

A similar Phase I trial of cabazitaxel with weekly dosing is currently ongoing for gastric cancer at [see letter of support], which demonstrates the feasibility of this design. Thus far no dose limiting toxicity has been seen on and the dose has progressed from level 0 (40 mg/m2) to the maximum 100 mg/m2. The main eligibility criteria will be pathologically confirmed high-grade AA with peritoneal carcinomatosis, PCI > 20 or otherwise not a candidate for CRS/HIPEC, and no tumor outside of the abdominal cavity. In the experience patients with high-grade AA and PCI < 20 can do well with CRS/HIPEC\textsuperscript{46}. These patients with limited disease, who represent 25% or less of the high-grade AA patients seen at [see letter of support], would go for CRS/HIPEC rather than on trial. Similarly, low-grade AA patients, who can generally do well with serial debulking surgeries would also be excluded. Given that the majority of AA patients are treated with either FOLFOX or FOLFIRI prior to referral to it is expected that most patients will have been treated with at least one prior chemotherapy, but given the lack of prospective data showing the efficacy of CRC chemotherapy in AA prior systemic chemotherapy will not be required. As was done [see letter of support], patients will first have a staging diagnostic laparoscopy during which the IP port will be placed and research biopsies obtained if the patient remains a trial candidate (see Clinical Protocol, Fig. 7). Treatment will occur biweekly for 12 weeks (6 doses) with serial sampling of blood and peritoneal fluid after which patients will undergo repeat diagnostic laparoscopy to evaluate for potential CRS/HIPEC and potential port removal if there is evidence of progressive disease (defined as any increase in the peritoneal
carcinomatosis index). If there is a partial response patients will be allowed to remain on treatment (IP catheter not removed). Given that surgical staging is considered standard-of-care for AA both laparoscopies will be billed to insurance, as will be the _____ . So far on _____ there have been no insurance denials, so we expect similar with the proposed AA trial.

Given much of the _____ population travels to _____ from a great distance, after discussion with our patient advocate it was felt that biweekly dosing would increase patient enrollment and significantly decrease patient burden. There is also data to suggest _____ may be able to stay in the IP space for 14 days or longer 47; the weekly dosing schedule of _____ was selected to match historical trials in gastric cancer 48. Given the change to q14 day dosing a Phase I design is required to establish the _____ . The starting dose of 150 mg/m2 q14 days will deliver same total dose over 28 days as 100 mg/m2 given days 1,8,15, which appears safe in _____ . dose will increase in 50 mg/m2 increments to max of 300 mg/m2. Of note, pharmacokinetic data from _____ is expected by the end of _____ these data may alter dosing plan. To minimize the total n, all patients treated at RP2D will be included on the Phase II portion of the trial; primary end point will be objective response rate at 3 months as measured by modified peritoneal (_____ ). With 15 patients treated at RP2D power will be 87% to detect a 30% response rate with one-sided type I error of 0.05; we will reject the null if at least 3 of 15 patients respond (see details in Biostatistical Plan). Secondary end points include PFS, OS, pharmacokinetics of pacitaxel in blood and peritoneal fluid, pathologic response and change in PCI, rate of initially unresectable patients converted to resectable, correlation of ctDNA and peritoneal fluid tumor DNA (ptDNA) with outcome, and patient Quality of Life surveys.

Specific Aim 2: ___

___ hypothesize that a high-sensitivity assay will be able to detect ctDNA in AA.

Appendiceal cancer is notoriously difficult to measure with standard CT scans. Sensitivity in detecting peritoneal recurrence is as low as 42%, as even diffuse disease will often form a thin plaque not readily detected on cross sectional imaging 49. Therefore, patients with a low-burden of disease oftentimes cannot be identified without a diagnostic laparoscopy; requiring general anesthesia, an outpatient surgical procedure and all of the cost and risk associated with each. Similarly, for patients with widespread peritoneal disease who are not surgical candidates, it is difficult to track response to systemic therapy using any form of cross-sectional imaging such as CT or MRI. Since appendiceal tumors normally form thin sheets rather than solid tumors, often times patients with a high burden of disease will not have measurable lesions by _____ , which often leaves them ineligible for clinical trials. Although the serum tumor markers CA19-9, CA-125 and CEA are frequently elevated, these do not always reliably track with disease burden 51.

tDNA has shown great promise as a non-invasive method to longitudinally tract tumor burden in some cancer types 52-56; however efforts to use first-generation ctDNA assays such as _____ in AA by us and others have shown limited sensitivity 57. While identification of ctDNA in peritoneal metastases of CRC has been shown to correlate with overall and recurrence free survival 58 there has only been a negative study in AA 59. The largest study of ctDNA in AA used the Guardant360 assay on samples without matched tumor NGS, finding GNAS mutation in only 2.6% of cases vs. an expected 34.1% from tumor (ratio 13.1), but found TP53 mutation in 23.4% of cases vs. and expected 35.1% (ratio 1.5) 60,61. Given the far greater sensitivity for TP53 (more commonly mutated in high-grade) relative to GNAS (mostly mutated in low-grade) these data suggest high-grade AA is more likely to shed ctDNA. We identified 56 MDA patients tested with the same ctDNA assay before receiving any
treatment, 9 of 26 (34.6%) high-grade vs. 6 of 30 (20%) low-grade patients had any detectable ctDNA. There was a trend toward worse OS for ctDNA+ high-grade patients (HR: 5.8, 95% CI: .95-35.5) but not enough deaths to evaluate low-grade. Matched tumor NGS was available for 23 patients, of 42 mutations seen in tumor, only 3 were found in ctDNA, a sensitivity of only 7.1%.

Given the limited sensitivity of first generation ctDNA assays (especially in low-grade AA) we have partnered with Natera to pilot a high-sensitivity assay to detect ctDNA in AA (see letter of support). Designed specifically to detect minimal residual disease, the Natera assay first performs exome sequence on each patients' tumor tissue and identifies a bespoke panel of 16 variants then highly amplifies those variants with PCR. It detected baseline ctDNA in 98% of samples in a cohort of mixed solid tumors. We have already collected pre and post-operative blood samples for testing on 30 patients under an approved IRB protocol (is PI, is co-I), and will collect serial blood and peritoneal fluid samples from the IP.

Given that it is unknown if AA, a disease that almost never spreads via hematogenous routes, even sheds DNA into the blood, we will consider a definitive answer to that question to be a success. Therefore we will first run the assay on pre-operative blood samples. If our hypothesis that high-grade tumors are more likely to shed ctDNA than low-grade proves correct, it may be possible to use quantitative ctDNA measurement as a biomarker predictive of survival, which would also aid in the selection of patients for CRS/HIPEC. If ctDNA can be reliably detected in pre-treatment samples, we will test the serial blood samples from IP trial to see if drop in ctDNA correlates with treatment response, and if clearance of ctDNA predicts DFS and OS. Finally, if ctDNA is not reliably detectable in AA as an alternate strategy we will collect peritoneal fluid and determine if peritoneal fluid tumor DNA (pftDNA) can be substituted for ctDNA as a biomarker.

Specific Aim 3.

We hypothesize that both PDX and PDO models will be predictive of drug response in actual AA patients.

Preclinical models are critical for the drug development process, and the near complete lack of models in AA is one of the major reasons no specific therapy exists for AA. We have begun to systematically generate PDX and PDO models of AA. We have shown that our orthotopic PDX recapitulate the histology of the AA from which they were derived (Fig. 8) and have similar transcriptomic profiles (Fig. 2). Now we will objectively test how well PDX and PDO models predict the drug response of actual human AA tumors by generating both PDX and PDO for each of the patients treated on the IP trial (aim 1). That trial will only include high-grade patients where we have seen a take rate of 83% (5 of 6). We anticipate that drug response to paclitaxel in both PDX and PDO will correlate with response in the trial patients, as has been the case for PDX models of other tumor types. If this is the case it will allow for expanded testing in these model systems to identify potential other drugs that might synergize with paclitaxel. First choices to test include taxanes and other drugs given the success of those combination in other GI cancers. Combining multiple drugs to overcome tumor resistance mechanisms has long been a corner stone of chemotherapy, but is not practical unless these combinations can be tested in preclinical models. Traditionally a Phase III study would randomize patients to individual components to demonstrate proof-of-activity for each agent or doublet, however given the rarity of AA this is unlikely to ever be possible. The FDA has recently provided guidance that PDX models from on-study biopsies can be an acceptable means of establishing proof-of-component for each drug in a combination. Such a trial design is currently open in our department.

Figure 8. PDX models of AA. Histology of AA PDX showing a mucinous AA (top) and a colonic type (non-mucinous) AA (bottom) over serial passage.
Statistical Analysis:

**Phase I:** This is an open label, Phase I trial to determine the maximum tolerated dose (MTD) of IP given q14 days, there are four dose levels.

The **BOIN** design identifies the MTD through minimizing the incorrect decisions of dose escalation and de-escalation (i.e., erroneously escalating/deescalating the dose when the current dose is actually higher/lower than the MTD), thereby optimizing the dose assignment for each enrolled patient. The BOIN design is simple to implement and has been shown to have superior performance through simulations. The BOIN design is algorithm-based, which is similar to the traditional "3+3"; however, its overall performance is substantially better. Its average performance is comparable to model-based design, such as the CRM (continual reassessment method) in terms of selecting the correct MTD, yet with a substantially lower risk of assigning patients to sub-therapeutic or overly toxic doses.

The target toxicity rate for the MTD is $\phi = 0.3$ and the maximum sample size is 24. We will enroll and treat patients in cohorts of size 3. To guide dose-escalation decisions, if the observed DLT rate at the current dose is $\leq 0.236$, the next cohort of patients will be treated at the next higher dose level; if it is $\geq 0.359$, the next cohort of patients will be treated at the next lower dose level. These boundaries were created when minimizing decision errors such that $\phi_1 = 0.18$ is the highest toxicity probability that is considered sub-therapeutic (underdosing) and $\phi_2 = 0.42$ is the lowest toxicity probability that is deemed overly toxic (overdosing). For the purpose of overdose control, doses $j$ and higher levels will be eliminated from further examination if $\Pr(p_j > 0.3 | data) > 0.95$, where $p_j$ is the true DLT rate of dose level $j, j = 1, \cdots, 5$. When the lowest dose is eliminated, stop the trial for safety.

**Phase II:** The primary objective in the Phase II portion of this trial is to determine the proportion of patients with an objective response after 3 months. Peritoneal disease is difficult to measure by cross-sectional imaging as it frequently exists and a contiguous erratically shaped area in the peritoneal cavity. As current RECIST criteria do not consider mucinous/cystic disease as measurable, standard RECIST criteria do not apply well to peritoneal disease. Previously a modified novel quantitative measuring system designed for mucinous peritoneal disease was developed, termed mucinous peritoneal RECIST (mpRECIST). This metric, which measure up to 5 areas of tumor in the abdominal cavity, has been used successfully in prior trials at [reference]. All patients who are treated at the MTD in Phase I will be counted toward the sample size in the Phase II portion of this trial. There will be a total of 15 patients treated at the MTD over phase I and II combined. Based on historical data, we estimate that the proportion of patients who will demonstrate an objective response without going on trial is 0.05, i.e. the null hypothesis is a 0.05 ORR. Treatment is expected to raise this proportion to 30%. Using an exact binomial test with one-sided type I error of 0.05, **we will reject the null if at least 3 of 15 patients achieve response.** This test has power of 87%. We will also estimate the ORR along with an exact 90% confidence interval. Assuming 3 of the 15 patients would achieve response, the observed response rate would be 20% and the corresponding 90% exact confidence interval would be (5.7%, 44%).

Safety data will be summarized using frequency tables by grade and attribution. Preliminary anti-tumor activity (i.e., response rate) will be summarized by dose level. Survival outcomes will be assessed through Kaplan-Meier plots. PK and PD data will be described through summary statistics such as mean, median, and quantiles. Correlations between toxicity events and baseline demographic variables may be explored and will be subject to stringent multiple testing correction if presented.
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<td><strong>Milestone/Activity</strong></td>
<td><strong>Description</strong></td>
<td><strong>Expected Completion Date</strong></td>
<td><strong>Is Deliverable</strong></td>
<td><strong>Status</strong></td>
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<td><strong>INSTRUCTIONS:</strong> Please enter the major milestone(s)/activity(ies) of your research project and include a description, the expected completion date, whether it is a deliverable or not, and the status. You may hover over each column heading for additional instructions. A sample is included in line 3. <strong>NOTE:</strong> If you are selected to receive an award, you will be asked to update this template and upload during each reporting period. You may insert row(s) to add milestone(s) or activity(ies). Do not delete previous entries from your application. Use reporting only.</td>
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<td>Milestone/Activity</td>
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<td>1-May-2022</td>
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<td></td>
<td>1 Aim: Enroll 1st patient on trial</td>
<td>1-Jul-2022</td>
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<td>1 Aim: Complete enrollment of Phase II portion of trial</td>
<td>1-Jun-2023</td>
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<td>1 Aim: Phase II results</td>
<td>1-Oct-2023</td>
<td>no</td>
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<td>1 Aim: start blood and peritoneal fluid collection from prospective trial</td>
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<td>3 Aim: Finish creation of PDX and PDO models from prospective trial patients</td>
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<td>3 Aim: Analysis correlating response in PDX and PDO models with response in patients</td>
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<td>3 Aim: identification of novel drug targets in appendiceal cancer</td>
<td>2-Jul-2025</td>
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I am a physician-scientist with a scientific background in chemical biology, functional genomics, and systems biology, and clinical training in internal medicine, hematology, and oncology. I am currently an Assistant Professor in the Department of Gastrointestinal Medical Oncology at the [redacted] Cancer Center where I lead a lab of both computational and experimental scientists studying the genomics and transcriptomics of colorectal- and appendiceal cancers. My clinical practice is similarly focused on these cancers. In the [redacted], it is our long-term research goal to better understand the molecular features needed to support the cancer phenotype and to leverage that understanding to better the delivery of chemotherapy. Broadly, our immediate research goals include [redacted].

Specifically related to this proposal, we will leverage our considerable experience with the genomics of appendiceal cancer, functional genomics, and the analysis of bulk and single cell transcriptomic data to study appendiceal adenocarcinoma with the goal of identifying new treatment options for our appendiceal cancer patients. It has been known for years that appendiceal cancer follows a different clinical course relative to colorectal cancer, and we and others have recently shown clear molecular differences between appendiceal cancer and colorectal cancer. We now leverage the clinical and pre-clinical resources of [redacted] to identify effective chemotherapies for appendiceal cancer. In the GI Medical Oncology department, I and others have built the infrastructure needed to allow for the prospective clinical testing of novel therapeutics specifically in appendiceal cancer. We will also leverage our ability to analyze retrospectively the over 2,600 appendiceal cancer patients treated at [redacted] in the last 15 years, and technological advances in the creation of preclinical models and tumor monitoring with ctDNA. Given these resources there is a high likelihood that the proposed research will lead to the discovery of an effective therapeutic strategy for appendiceal cancer. Given that currently no FDA approved therapeutics for appendiceal cancer and no validated biomarkers to guide therapeutic decisions exist, this proposal seeks to address a critical unmet need for the over 4,000 Americans diagnosed with appendiceal adenocarcinoma every year.

Throughout my career, I have had the fortune of training with pioneering scientists in multiple different disciplines, which has prepared me for the translational, multi-disciplinary science that I now propose. Under the direction of [redacted], I learned how small molecule probes...
could be used to study biological systems as well as how to design, optimize and ultimately analyze the data from high-throughput and high-content chemo-genetic screens. Recognizing that the technical ability to perform high-throughput 'omic' analysis of tumors would profoundly change cancer research during the research portion of my fellowship, I joined the laboratory of [masked], an internationally recognized leader in the field of network and systems biology, and under his mentorship, I became adept at performing and analyzing genetic interaction screens and utilizing knowledge of network relationships to draw biologically relevant conclusions from large datasets. Working closely with [masked], one of the original creators of CRISPR technology, we demonstrated that CRISPR could be modified to allow for high-throughput genetic interaction screening in human cells. Now as I build my lab at [masked], I work closely with mentor [masked], internationally recognized as a leader in the biomarker-driven treatment of colorectal cancer and modeling cancer in PDX.

Ongoing and recently completed projects that I would like to highlight include:

[masked]

This proposal seeks to extend functional genomic screening to primary patient derived cell line models. These data will be aggregated with functional genomic data from multiple other sources to build hierarchical models of the cancer. To transition from descriptive to predictive modeling, machine learning algorithms will be used to predict tumor cell vulnerabilities given tumor genotype and/or transcriptome.

[masked]

This proposal focuses on the identification and validation of synthetic lethal interactions to identify additional therapeutic targets in GI malignancies.
B. Positions and Honors

C. Contributions to Science
1. **Molecular Profiles of Appendiceal Adenocarcinoma:** Appendiceal adenocarcinoma is a rare tumor, making prospective and/or randomized trials difficult. In the absence of such data, appendiceal tumors have traditionally been treated with chemotherapy as if there were the same as the more common colon cancer. Working with colleagues at [ obscured ], we have shown definitively that appendix cancer is quite distinct from colon cancer at the molecular level. We further demonstrated important differences between high- and low grade appendiceal adenocarcinomas, as well as differences between adenocarcinomas and tumors with goblet-cell histology. Importantly, molecular features, such as GNAS and TP53 mutation, have been linked to overall survival. Additionally, I have been at the forefront of testing circulating tumor DNA measurement in appendix cancer.

2. **Computational Methods to Develop Prognostic & Predictive Biomarkers for Solid Tumors:** Current treatment guidelines for cancer are largely based on prospective clinical trials, which are effective in identifying which drugs in aggregate will be effective for a population. However, cancer, in particular solid tumors, are remarkably heterogeneous at the molecular level, so it is not surprising that the same chemotherapy regimen will not work for every patient. Currently, oncologists have very limited tools to identify what will be the most effective therapy for an individual patient. However, there is an ever-growing amount of data linking tumor molecular profiles to clinical outcomes. Using network- and systems-biology techniques on large ‘omics’ datasets, as well as traditional wet lab methods to validate and investigate the mechanism underlying these, I have identified several putative prognostic and predictive biomarkers in multiple different tumor types. I also helped develop a method known as Network-based Stratification (NBS), which uses prior knowledge of genetic networks to allow for a single cancer type to be broken down into more homogenous subtypes.

3. **Synthetic Lethal Strategy for Cancer Therapy:** As evidenced by the efficacy of the now FDA-approved PARP inhibitors olaparib and rucaparib in tumors with BRCA1/2 loss-of-function; it is possible to selectively kill cancer cells by identifying specific weaknesses that result from molecular aberrations in cancer. However, the ability to extend the synthetic lethal strategy to other genetic targets is currently restricted by the limited number of synthetic lethal relationships that have been identified, as well as a poor understanding of how the other
molecular changes in a cancer cell influence the relationship between two genes. Thus far, I have attempted to address these issues by using ultra-high-throughput screens in model organisms to identify new synthetic lethal interactions and using cross-species conservation analysis to prioritize the interactions that are most likely to be conserved in cancer cells. Recently I have been using a novel CRISPR-Cas9 method capable of combinatorial gene knockout (developed in collaboration with [redacted] to screen for synthetic lethal interactions directly in human cancer cells.

4. Chemical Biology: Following my first year of medical school, I worked in the laboratory of [redacted]. I optimized conditions for the then new experimental technique of probing small molecule micro-arrays (SMM) with whole cell lysates. SMM are microscope slides that have been imprinted with tens of thousands of small, drug-like molecules. They can be used for the rapid detection of binding interactions between a protein of interest and a complete library of small molecules, particularly useful for identifying lead compounds for so-called 'undruggable' targets. We demonstrated that it was possible to screen SMM with whole-cell lysate without any purification steps, providing the advantage of better maintaining proteins in their native in vivo conformation. I also developed a high-throughput assay to identify fetal hemoglobin expression, which contributed to the discovery that inhibition of HDAC1 or HDAC2 can increase expression of fetal hemoglobin, identifying these proteins as possible therapeutic targets in sickle cell anemia. Based on this work, the HDAC inhibitor vorinostat is now being tested in clinical trials in sickle cell patients who have not responded to hydroxyurea, and the drug company [redacted] is actively developing a HDAC1/2 selective inhibitor.
Career Goals & Objectives:

Having established my own lab as an independent investigator, I now seek to ensure the long-term success of our lab by continuing to pursue clinically impactful science at the interface of cancer genomics, chemical biology, and clinical oncology. As our lab grows in number and capacity, we will be better positioned for our ultimate goal, which is the translation of scientific discovery into clinical practice; thereby improving outcomes for our appendiceal (and colorectal) cancer patients. Rapid technological advances in sequencing, functional genomics, and cancer model systems have opened many new research avenues for translational cancer research; however, they also require the modern cancer investigator be adept at multiple investigative techniques. My professional training thus far at MIT, the Massachusetts Institute of Technology, has provided me with an excellent foundation of knowledge. However, to meet my long-term career goal of translational science, I have identified the following areas for additional training, mentoring, and experience:

1. Design and execution of investigator initiated clinical trials
2. Experimenting in mouse and organoid models of cancer
3. Laboratory management and mentorship of trainees

Mentorship plan:

[Blank space]

Dr. [Name], is internationally recognized leader in colon cancer research and importantly has a long-standing mentoring relationship with the applicant. A director of research in the GI Medical Oncology department, Dr. [Name] and I have been engaged in a productive scientific collaboration ever since. This collaboration has already produced six published papers; additionally, [Name] and I are co-course masters for the graduate school class [Name].

In addition to serving as mentor to [Name], serves in multiple leadership roles at [Institution]. He has extensive expertise in conducting phase I, II, and III clinical trials, experimenting in PDX models, and has a long track record of successfully securing federal grant funding. In addition to having a skill set that very much aligns with [Name], also has extensive experience as mentor having already mentored two faculty who won the Career Development Award and eight fellows who have won the Young Investigator Award. [Name]'s sustained commitment to mentorship is exemplified by his roles as co-PI for the institutional K12 grant, program co-director of the Cancer Biology Program, and several institutional awards for mentoring clinical fellows, junior faculty, and post-doctoral fellows.

In terms of structure, [Name] and [Name] have a dedicated face-to-face meeting every other week specifically to discuss [Name]. During these meetings, [Name] and [Name] will review the research progress, provide feedback and advice, and help with navigating challenges. To foster professional development, [Name] and [Name] will provide guidance on institutional resources and facilitate further collaborations. He will make introductions to increase visibility in the institution and in the field. He will provide opportunities for presenting, teaching and networking.
to build a reputation in the scientific community. He will share his expertise to troubleshoot any issues, ensure coordination of different aspects of the project and review progress towards milestones. He will provide guidance for the safe and timely completion of the clinical trial and supervise the translational research on patient-derived samples and mouse tissues. He is readily available to help with any challenges in biospecimen submission or analysis at

Finally, [REDACTED] will also help with development of a confirmatory trial if anticipated responses are seen in the proposed trial.

Recognizing that it is important for [REDACTED] to build a research enterprise that is separate from [REDACTED] with regards to the appendiceal cancer project described in this proposal Dr [REDACTED] will be a mentor but not a direct collaborator. Of note [REDACTED] has published three papers independently of [REDACTED] since taking his faculty appointment (two related to appendiceal cancer).

**Training Goal 1: Design and execution of investigator initiated clinical trials.** The ultimate goal of research in the [REDACTED] lab is to bring our discoveries into the clinic. Although this can often be achieved via collaboration with clinical investigators, I now recognize that sometimes this translation is best achieved with a trial designed and executed ourselves. In preparing the current protocol for appendix cancer protocol have already gained a wealth of knowledge related to nuts & bolts of designing and executing a clinical trial. As this trial continues to move forward to departmental and IRB review, and ultimately execution I will continue to be coached by not only [REDACTED] but also biostatistician Dr [REDACTED] and our surgical colleagues, as well as [REDACTED] the other appendiceal specialist medical oncologists at [REDACTED]. From this translational 'Dream Team' I will learn the nuances of leading and completing a clinical trial in general as well as specific nuances related to appendiceal cancer.

**Training Goal 2: Become an expert experimenting in mouse and primary cell culture models of cancer.** Recognizing the inherent limitations of traditional immortalized cancer cell lines as a model of human cancer, the [REDACTED] has been building a focus on Patient Derived Xenografts (PDX), and Patient Derived Organoid (PDO) models. To supplement the experience I have gained in the past three years I will continue to work with experts [REDACTED] [REDACTED] (see letter of support). I will also continue to attend relevant national meetings, such as the [REDACTED] and the [REDACTED].

**Training Goal 3: Laboratory management and mentorship of trainees.** Our lab has been so far successful in the recruitment of skilled, motivated scientists. However, I recognize that I will need specific training regarding mentorship and laboratory management generally. [REDACTED] who holds a joint appointment, has recently started a course which I will attend. Additionally, [REDACTED] organizes a lecture series that includes practical topics including recruitment, mentoring, and grant writing.

**Additional Curriculum:** I will continue to attend the weekly Peritoneal Surfaces (Tues AM), Colon Cancer (Mon PM) and Rectal Cancer (Thurs PM) tumor boards, as well the GI Medical Oncology weekly departmental meeting (primarily discussion of clinical trial protocols). I will continue to lead the weekly lab group meeting, co-lead the biweekly Colorectal Cancer Integrromics meeting (focuses on tumor molecular profiling), attend the weekly [REDACTED] student oral presentation seminar, and attend the weekly [REDACTED] group meeting. In terms of conferences I will attend the annual meetings for the [REDACTED] as well as the ASCO GI Cancers Symposium and other relevant topical meetings (such as AACR Special Conference on Colorectal Cancer).
Mem
bers of the Conquer Cancer Selection Committee,

As Chair of the Department of Gastrointestinal Medical Oncology [REDACTED] wholeheartedly support the application of [REDACTED] for this [REDACTED] [REDACTED]. This institutional commitment represents our confidence in the outstanding scientific and clinical qualities displayed by [REDACTED] to execute his proposed research plan. Combining his talent and experience in the treatment of appendix cancer with the resources of [REDACTED] presents a research plan which has the potential to revolutionize the treatment of appendiceal cancer.

[REDACTED] has received excellent basic science and clinical training. He received his undergraduate S.B. degree in Chemistry, with a minor in Political Science, from the [REDACTED] while conducting research in the laboratories of [REDACTED]. His laboratory effort there led to multiple important publications including (1) A robust small-molecule microarray platform for screening cell lysates, published in *Chem Biol*, and (2) Chemical genetic strategy identifies HDAC1 and HDAC2 as therapeutic targets in sickle cell disease, published in the *Journal of Internal Medicine* in two years as part of the Physician-Scientist Training Program (ABIM research pathway) at the University of [REDACTED] in Hematology & Oncology. There he distinguished himself being selected by the faculty as Chief Fellow for the 2012-2013 academic year. Following the completion of his clinical training [REDACTED] working in the laboratory of [REDACTED]. Recognized internationally as a leader in the field of network and systems biology, the Ideker lab specializes in utilizing knowledge of network relationships to draw biological conclusions from large datasets as well as performing high-throughput genetic interaction experiments in human and model organism systems. His work in the [REDACTED] led to multiple high impact papers including (1) ...
His funding has included numerous key or principle investigator efforts including grants from the 

A national search was performed looking for a candidate with unique expertise, leading to his current appointment as [redacted], term tenure track, which began in [redacted]. As one of only four physician-scientists in our department [redacted] continues to be provided 80% protected time for research, and he was awarded a total of $3,000,000 in start up funds from [redacted]. His salary is also fully supported by the department for the first three years of his appointment. Despite setbacks from the COVID19 pandemic which caused an extended shutdown of labs on our campus, [redacted] is well on his way to establishing an independent research program and has successfully recruited a team of thirteen research scientists, postdoctoral fellows, and graduate and undergraduate students.

Having previously demonstrated that appendiceal cancer is quite different from colorectal cancer at the molecular level [redacted] now seeks to for the first time develop chemotherapeutic treatments specifically for appendiceal cancer. Leveraging the clinical volume of [redacted], an international destination for cancer treatment, he has built a clinical practice focused on appendix cancer. Now in the process of generating detailed molecular profiles of these tumors, [redacted] and his team of computational biologists will use the network-based analysis and high-throughput functional genomic methods he helped develop in the Ideker lab to identify tumor specific vulnerabilities in appendiceal cancer. In addition to these discovery efforts, based on exciting preliminary data on the efficacy of [redacted] in appendiceal cancer he proposes a prospective trial of intraperitoneal paclitaxel.

In addition to the protected time and financial resources listed above, [redacted] will also have mentorship from Deputy Department [redacted] (see separate mentor letter) and will also benefit from continued close collaboration with our group of world class peritoneal surgeons, led by [redacted].

In summary, [redacted], a remarkably talented physician and scientist, and can combine these unique skill sets to perform the cutting-edge, translational research that our patients with gastrointestinal tumors so urgently need. [redacted] is an ideal candidate for this award, and I strongly urge you to give his candidature a favorable consideration.
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ABBREVIATIONS

AA  Appendiceal Adenocarcinoma
BUN  Blood Urea Nitrogen
CBC  Complete Blood Count
CT  Computed Tomography
CI  Confidence Interval
ECOG  Eastern Cooperative Oncology Group
EUS  Endoscopic Ultrasound
Gy  Gray
H & P  History and Physical
HIPEC  Hyperthermic Intraperitoneal Chemotherapy
IP  Intraperitoneal
IP PTX  Intraperitoneal Paclitaxel
IRB  Institutional Review Board
MRI  Magnetic Resonance Imaging
OS  Overall Survival
PDX  Patient Derived Xenograft
PDO  Patient Derived Organoid
PET  Positron Emission Tomography
PCI  Peritoneal Carcinomatosis Index
RBC  Red Blood Cells
SOP  Standard Operating Procedure
TNM  Tumor Regional Nodes and Metastases
WBC  White Blood Count
PK  Pharmacokinetic
AUC  Area Under the Curve
ctDNA  Circulating Tumor DNA
pftDNA  Peritoneal Fluid Tumor DNA
mpRECIST  Modified Peritoneal RECIST
Secondary:
1) To assess the safety and tolerability of escalating doses of intraperitoneal paclitaxel in subjects with metastatic high-grade appendiceal adenocarcinoma. 
2) To assess the progression free and overall survival of metastatic high-grade appendiceal adenocarcinoma treated with intraperitoneal paclitaxel via intraperitoneal route every 14 days.
3) To assess the pharmacokinetics of paclitaxel in patients with metastatic high-grade appendiceal adenocarcinoma. 
4) To assess the pathologic response and change in PCI following intraperitoneal treatment in patients with metastatic high-grade appendiceal adenocarcinoma. 
5) To assess the rate of initially unresectable (PCI > 20) patients with metastatic high-grade appendiceal adenocarcinoma able to undergo CRS / HIPEC after intraperitoneal treatment.
6) To assess the rate of conversion from positive to negative cytology in peritoneal fluid following intraperitoneal treatment in patients with metastatic high-grade appendiceal adenocarcinoma.
7) To assess the prognostic value of circulating tumor DNA (ctDNA) and peritoneal tumor DNA in patients with metastatic high-grade appendiceal adenocarcinoma and the correlation of quantitative ctDNA measurement with radiographic and pathologic response.
8) To generate PDX and PDO models of high-grade appendiceal adenocarcinoma.

2.0 Rationale

Standard-of-Care Treatments for AA. Appendiceal tumors encompass a rare and diverse group of neoplasms; appendiceal adenocarcinoma (AA) is the most common histologic subtype. Epidemiologic studies based on Surveillance, Epidemiology, and End Results (SEER) data have shown a steady increase in incidence from approximately 0.2 cases per 100,000 in the 1970s, to current estimates of just over 1 per 100,0001,2. In comparison, this is 40-fold less common than colon cancer, which in the US has an incidence of approximately 40 per 100,0003. Cases of early-onset AA, defined as diagnosis before age 50, have increased by 24% between 2011 to 2016, and in 2016 represented 40% of all appendiceal cancer4. In contrast, the increase in early-onset colorectal cancer (CRC) was only 2.2% for that same time period4. Historically, appendiceal tumors have been grouped together with CRCs, and as of 2021 the National Comprehensive Cancer Network (NCCN) guidelines still suggested that appendiceal tumors be treated with chemotherapy similarly to colon tumors. The rarity of AA has made it difficult to conduct clinical trials, and in the absence of trial data, the NCCN guidelines assume biological similarity due to anatomic vicinity, common embryological origin, and common expression of the transcription factor CDX25,6. However, there is a growing consensus that AA is a clinically and molecularly distinct entity from CRC, and that AA specific therapies (none exist currently) need to be developed7-9.
AA usually presents in one of two ways: found incidentally during pathological review of appendectomy specimens or only after the tumor has spread to the peritoneal space. Symptoms of advanced AA are nonspecific and include abdominal pain and distention, altered bowel motility, and early satiety. Further, a screening colonoscopy—highly effective in the early detection of CRC—has limited benefit for patients with AA due to difficulty visualizing the appendix during colonoscopy and the fact that AA likely does not follow the traditional pattern of well-characterized CRC progression from adenoma to carcinoma\textsuperscript{10,11}. For these reasons, most patients with AA present with diffuse metastasis throughout the peritoneum. Interestingly even high-grade tumors rarely have lymphatic or hematogenous spread\textsuperscript{12}, in stark contrast to CRC which has a predictable spread into lymph nodes and then to the liver (70\% of cases)\textsuperscript{13}. In general AA has a more indolent natural history relative to CRC, with median overall survival (OS) of 75.8 months (95\%CI: 58.1-93.5) for metastatic tumors, which disguises dramatic differences between different subtypes of AA. Three histological subtypes—mucinous, non-mucinous (sometimes called colonic type), and signet-ring cell—have been used to describe AA. Signet-ring cell tumors consistently have the worst prognosis, but reports comparing the prognosis of mucinous relative to non-mucinous adenocarcinoma do not concur\textsuperscript{14-16}. Despite these nuances and compared to all other clinic-molecular parameters, histological grade provides the best prognostic evaluation, with marked survival differences between high- and low-grade tumors (Figure 1)\textsuperscript{17}. Unfortunately, accurately assessing the AA grade can be difficult given issues with sampling errors that may miss focal high-grade lesions and a tendency towards overinterpretation of low grade or even non-invasive neoplasms by community pathologists\textsuperscript{18}.

This diagnostic uncertainty presents a major clinical dilemma as high- vs low-grade AA are treated differently. Low-grade tumors generally follow an initially indolent clinical course and a most often managed with serial debulking surgeries combined with Heated Intraperitoneal Chemotherapy (HIPEC)\textsuperscript{19}. However, upon progression they generally respond poorly to systemic cytotoxic chemotherapy and patients often succumb to bowel obstruction or cancer cachexia from diffuse peritoneal carcinomatosis. Conversely, high grade tumors, which includes signet-ring cell carcinoma (SRCC), follow a more aggressive clinical course (median OS 41.9 months; 95\%CI: 27.3-56.6), and although no standard-of-care systemic treatment exists for advanced, unresectable disease, these patients are generally treated with chemotherapy designed for CRC. The limited data on the response

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Kaplan-Meier overall survival (OS) curves for AA patients by grade (a) and GNAS status (b)}
\end{figure}
of AA to CRC-style chemotherapy suggests limited efficacy, highlighting the need for the development of AA specific therapy.

**Systemic chemotherapy for AA.** A large percentage of patients either fail primary cytoreductive surgery (CRS) or are unable to undergo surgery for a variety of reasons. Additionally, disease recurrence requires repeated and progressively more difficult surgery due to adhesions and fibrosis and therefore after repeated CRS, eventually patients become non-surgical candidates. Management of these patients is challenging. The role of systemic chemotherapy has been evaluated by a limited number of small retrospective studies and one phase II trial. A retrospective analysis of patients with disseminated appendiceal neoplasm who were not considered optimal candidates for cytoreductive surgery (n = 54) and received 2 or more cycles of systemic chemotherapy demonstrated a 55.6% disease control rate with median progression-free survival and overall survival of 7.6 months and 56 months, respectively. Another retrospective analysis of metastatic poorly differentiated/signet ring cell appendiceal adenocarcinomas (n = 78) showed that chemotherapy led to a radiographic response in 44% patients, a median progression-free survival (PFS) of 6.9 months and a median overall survival (OS) of 1.7 years. A phase II study evaluating the combination of systemic mitomycin C and capecitabine in patients with advanced unresectable PMP (n = 40) showed that 38% (95% confidence interval: 25 to 54%) benefited from chemotherapy in the form of either reductions in mucinous deposition or stabilization of progressive pretreatment disease. However, this study only used semiquantitative assessments and did not provide any information regarding the duration over which progressive disease was defined prior to the start of this study. In addition, this study enrolled primarily DPAM (now classified as low-grade under current classification system) patients as this histological subtype represented 68% of the study population. Another observational study of treatment with 5-fluorouracil and oxaliplatin (FOLFOX-4 regimen) in patients with unresectable or recurrent PMP from AA (mix of high and low grade, n = 20) showed that 20% of the patients had partial response, 45% stable disease and 35% had progressive disease; median progression free survival and overall survival were 8 months and 26 months respectively.

Taken as a whole, data regarding the benefit of systemic therapy for AA remains inconclusive given the small number of studies, fact that most were retrospective, and the confounding issues of inconsistent pathological terminology prior to 2016, as well as mixing of high- and low- grade patients within the same study.

**CRS / HIPEC for AA.** Multiple prior investigators have studied hyperthermic intraperitoneal chemotherapy (HIPEC), a surgical technique for combining hyperthermia and chemotherapeutic agents to the peritoneal surface via a heated perfusion circuit for the treatment of AA. Normally for high-grade patients HIPEC would only be performed if a complete cytoreductive surgery (CRS) could first be performed, with the thought that the chemotherapy agent (usually mitomycin C or oxaliplatin) is primarily effective against microscopic minimal residual disease. Of note, there are several recently reported trials in patients with CRC that question the efficacy of HIPEC in more aggressive tumor types. In the recently reported PROPHYLOCHIP study, prophylactic HIPEC after 6 months of adjuvant chemotherapy in colorectal cancer patients at high risk of peritoneal disease failed to improve survival. In the French PRODIGE 7 trial, patients with colorectal peritoneal carcinomatosis were randomized to cytoreductive surgery alone versus cytoreductive surgery with HIPEC demonstrating no difference in survival. Given the MD Anderson experience that patients with high-grade AA and PCI > 20 do poorly with CRS/HIPEC, these patients are not offered CRS/HIPEC and instead referred for systemic chemotherapy.
Molecular differences between AA and CRC and rational for taxane chemotherapy. Cohort sequencing studies have revealed several key molecular differences between AA and CRC. Most notably, the APC mutation—a hallmark feature of CRC seen in 80% of tumors—is uncommon in all subtypes of AA. Similarly, AA has less frequent TP53 mutation. After KRAS, which remains undruggable except for the rare G12C variant, GNAS is the most frequently mutated oncogene in AA. A strong enrichment of GNAS mutation is found in low grade tumors ($\chi^2 p < 0.0001$), which also tend to show mucinous histology. Unfortunately the incidence of currently ‘actionable’ mutations in genes such as HER2 or EGFR is quite rare in AA, as is microsatellite instability-high (MSI-H, less than 3% of AA cases). Taxane chemotherapy has been ineffective for CRC, however, the mechanism of this resistance has been mechanistically linked to APC loss-of-function. Unlike CRC, APC mutation is uncommon in AA (9.0%), as are mutations in other known Wnt pathway genes. Based on strong pre-clinical data linking taxane resistance to APC mutation and subsequent chromosomal instability (CIN) a Phase II trial of nab-paclitaxel was conducted in both CpG Island methylator phenotype (CIMP)-high CRC and Small Bowel Adenocarcinoma (SBA). Reasonable activity was seen in SBA, with two partial responses and three patients with stable disease out of 10 treated, interestingly no responses were seen in CIMP-high CRC. Based on these data as well as a small retrospective case series taxane chemotherapy has been included in the NCCN guidelines for the treatment of SBA. Given that APC mutation is even less frequent in AA than SBA (9.0% vs. 26.8%), it is reasonable to surmise that taxanes will have activity in AA. A recent pre-clinical study with a PDX model of both high and low grade AA showed near complete response with IP cabazitaxel.

Finally, the [footnote] as recently conducted a pilot experiment using IP paclitaxel in an orthotopic PDX model of high-grade AA. Tumor samples were cut into ~ 25-30 mm³ fragments and implanted into the peritoneal cavity of NSG mice (6 pieces/mouse). Four weeks after implantation, tumors in the peritoneum were confirmed by magnetic resonance imaging (MRI) and mice were randomly assigned to treatment groups of four mice and intraperitoneally (IP) administered with 25 mg/kg of **[footnote]**. The treatment schedule were 8 weeks (once a week IP injection, 3 weeks treatment and 1 week resting, performed twice). Tumor growth were imaged by MRI 4th, 8th and 12th weeks after implantation. Three of four mice treated with IP **[footnote]** weekly, Figure 2) showed stable disease vs. zero of four treated with saline control (Figure 3, data-not-yet-published).

Rational for intraperitoneal delivery of **[footnote]**. The limited effect of systemic treatment on peritoneal metastases is thought to be partially due to the peritoneum-plasma barrier, which prevents effective drug delivery from the systemic circulation into the peritoneal cavity. IP administration is attractive given that AA almost never extends beyond the peritoneum, and most

**Figure 2. Schema for mouse IP experiments.** AA was implanted, allowed to grow for 4 weeks, then treated with **[footnote]**. **[footnote]** was given intraperitoneally (IP) on days 0, 4, and 8. MRI was performed at baseline (0w), 4 weeks (4w), and 8 weeks (8w) post-treatment.
complications from AA, such as bowel obstruction, are a result of peritoneal disease. It has been suggested that the hydrophobicity of taxanes leads to uptake preferentially in the lymphatics leading to prolonged retention in the peritoneal cavity relative to hydrophilic drugs. Thus it is expected that intraperitoneal delivery of taxanes can attain a higher drug exposure in the peritoneal cavity but with reduced systemic toxicity. Taxanes have been given safely in ovarian cancer for many years, and have been shown to be effective in peritoneal carcinomatosis from gastric cancer. A

Due to the limited efficacy of systemic chemotherapy in non-operable, metastatic, high-grade AA, we seek to identify if an additional agent, with a novel route of administration (intraperitoneal), has activity against high-grade AA. We now incorporate many of the lessons we have learned from previous and ongoing clinical trials. The purpose of this clinical trial is to first determine the safety and tolerability of escalating doses of intraperitoneal taxanes in patients with appendiceal cancer, and secondly to evaluate the efficacy of intraperitoneal taxanes as measured by mpRECIST.

3.0 Eligibility of Subjects

Inclusion:

1) Age 18 years and above. There will be no upper age restriction.

2) ECOG performance status ≤ 2.

3) Pathologically confirmed diagnosis of high-grade appendiceal adenocarcinoma (moderately differentiated or poorly differentiated using three grade system)

4) Adequate renal, and bone marrow function:

Figure 3. Results of mouse IP PTX experiments. Left: representative MRI images of AA tumor growing in mouse peritoneal space. Middle: Spider plot serial tumor growth, note PTX stabilizes disease. Right: All four AA tumors treated with saline show progression, vs. one PTX treated tumor.
a. Leukocytes >= 3,000/uL
b. Absolute neutrophil count >= 1,500/uL
c. Platelets >= 60,000/UL
d. Serum creatinine <= 1.5 mg/dL

5) Metastatic disease in the peritoneal cavity with PCI > 20

**Exclusion:**

1) Infections such as pneumonia or wound infections that would preclude protocol therapy.

2) Women with a positive urine or serum pregnancy test are excluded from this study; women of childbearing potential (defined as those who are not postmenopausal defined as no menses in greater than or equal to 12 months, have not had a hysterectomy or bilateral salpingoophorectomy, do not have ovarian failure, or have not had a surgical sterilization procedure) must agree to refrain from breast-feeding and practice adequate contraception as specified in the informed consent. Adequate contraception consists of oral contraceptive, implantable contraceptives, injectable contraceptives, a double barrier method, or abstinence. Men with reproductive potential must agree to an appropriate method of birth control, including abstinence or double barrier method (diaphragm plus condom).

3) Subjects with unstable angina or New York Heart Association Grade II or greater congestive heart failure.

4) Subjects deemed unable to comply with study and/or follow-up procedures.

5) Subjects with a known hypersensitivity to protocol systemic chemotherapy that was life-threatening, required hospitalization or prolongation of existing hospitalization, or resulted in persistent or significant disability or incapacity.

6) Previous surgery that would preclude safe diagnostic laparoscopy with port placement.

### 4.0 Research Plan and Methods

#### 4.1 Treatment Plan and Regimen

**Intraperitoneal Chemotherapy**

Before each treatment, patients will receive the following pre-medications to prevent paclitaxel-associated hypersensitivity reactions:

1. Dexamethasone 10 mg intravenously (I.V.) prior to administration of [Redacted]
2. Diphenhydramine 50 mg intravenously (I.V.) prior to administration of [Redacted]
3. Famotidine 20 mg I.V. prior to administration of [Redacted]

(Note: Should listed medication not be in the MDACC formulary, physicians order for
institutional medication may be substituted. Patients may also be provided equivalent oral medications to be taken the day prior and the day of Intraperitoneal administration will be performed by first instilling 500 mL of normal saline into the peritoneum over 30 minutes (+/- 10 minutes), then by 500 mL injection of the IP PTX diluted in normal saline over 30 minutes (+/- 10 minutes) using the intraperitoneal port. At the conclusion of the infusion the port is flushed with 10 mL of sterile saline and heparin lock is performed.

Intraperitoneal port care will be performed according to standard of care.

Studies of IP taxol administration in Japan have used a weekly treatment schedule for 3 consecutive weeks, followed by 1 week without treatment. In a recent review, the investigators recommend dosing of IP PTX of 80 mg/m². Based on these data a Phase I trial of IP PTX in gastric cancer is currently being conducted although these data have not yet been published to date nine patients have treated at dose of 100 mg/m² on days 1, 8, 15 of 28 day cycle without any dose limiting toxicity observed.

We are incorporating whole blood as well as peritoneal fluid collection for PK analysis. Approximately 10 mL of blood will be collected at each time point: completion of infusion, 1 hour post infusion, 24 hrs post infusion, and 14 days post infusion (next visit to infusion center, prior to next subsequent dose). At the same times as collection of blood approximately 10-20 mL of peritoneal fluid will also be obtained via the IP catheter. These samples will only be obtained for the first IP PTX treatment for each patient.

The starting dose in this study will be 150 mg/m² (Dose Level 0) using a biweekly treatment schedule (days 1, 15 of 28 day cycle) for a total of 12 weeks (3 cycles, 6 doses). The dose-escalation scheme will be by increments of 50 mg/m². No intra-patient dose escalation will be done. A minimum of three patients will be required to be treated at a dose level before new patients are treated at the next higher dose.

Only those DLTs that occur within 30 days from administration will be used for dose escalation decisions and MTD finding. See Table 5.1 for dose escalation procedures.

Subjects with metastatic high-grade appendiceal adenocarcinoma that is not amenable to CRS/HIPEC will be offered participation in the study. Discretion of whether CRS is possible will be determined by attending surgical oncologist, but generally all patients with PCI > 20 will be considered unresectable and thus eligible for this study. Subjects should have a contrast CT scan, MRI, or PET/CT scan within 4 weeks (28 days) of enrollment. It is anticipated that most patients will have received systemic therapy prior to enrollment but this is not required. Diagnostic laparoscopy and intraperitoneal port placement, according to standard of care, will be required prior to treatment. Patients will undergo treatment as displayed in Figure 4. At the end of treatment patients will undergo repeat imaging to assess for response to IP PTX treatment. At the discretion of the treating physician if follow up imaging shows clear response to therapy IP paclitaxel can be continued for as long as it is tolerated. In these cases the post treatment diagnostic laparoscopy can be omitted or delayed at the discretion of treating physician. If follow up imaging shows equivocal tumor response or progression the patient will proceed to diagnostic laparoscopy for surgical assessment of tumor response and IP port removal.
For Phase I portion of the trial the dose of IP PTX will be titrated to determine the maximum tolerated dose (MTD). A maximum of 24 patients will be enrolled into this study. The starting dose for IP PTX will be 150 mg/m² via intraperitoneal administration, with dose escalation according to the following table. See section 5 for statistics determining number of patients at each dose.

### Table 2. Nadir Counts

<table>
<thead>
<tr>
<th>Neutrophils (x 10⁹/L)</th>
<th>[\text{Reduction} ]</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 0.5 OR &lt; 0.5 for &lt; 7 days</td>
<td>No change</td>
</tr>
<tr>
<td>Febrile Neutropenia¹ OR infection with neutropenia² OR Neutrophils &lt; 0.5 for &gt;= 7 days</td>
<td>Reduce 1 level or add growth factor (e.g., G-CSF or Peg-G-CSF)</td>
</tr>
</tbody>
</table>

**Dose Reductions - Hematological Toxicity**

The following guidelines outline dose reductions for hematological toxicities within 4 weeks post IP treatment, defined as related to the study drug - paclitaxel (Table 2).
Febrile neutropenia (fever of unknown origin without clinically or microbiologically documented infection) ANC < 1.0 \times 10^9/L and fever >38.50C

Infection (documented clinically or microbiologically) with ANC < 1.0 \times 10^9/L

**Dose Reductions - Non-hematological Toxicity**

The following guidelines outline dose reductions of or non-hematological toxicities (Table 3). Severity should be recorded and graded according to the National Cancer Institute Common Toxicity Criteria (NCI CTC) Version 5.0. IP PTX may be held until appropriate for further treatment, according to standard of care.

<table>
<thead>
<tr>
<th>Toxicity Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grades 0-2</td>
<td>No change</td>
</tr>
<tr>
<td>Grade 3-4</td>
<td>Reduce by 1 dose level</td>
</tr>
</tbody>
</table>


1 Not applicable to nausea/vomiting in the absence of appropriate anti-emetic treatment or infusion-related reactions. Not applicable to uncomplicated skin rash or fatigue. Not applicable to consequent metabolic side effects (for example low potassium as a consequence of diarrhea). Not applicable to toxicity not attributable to therapy.

The IP treatment will consist of (dose escalation, see Table 1) in 500 mL of saline infused over one hour, performed no sooner than 3 weeks after completion of any previous therapy (systemic chemotherapy). Treatment will occur biweekly for 12 weeks (6 doses). At the conclusion of study-related treatment, patients will undergo evaluation for next line therapy, surgery, or continuation of IP until progression of disease based on multidisciplinary review. At the end of the planned 12 week treatment period and 30 day dose limiting toxicity evaluation, patients typically undergo repeat diagnostic laparoscopy to evaluate for potential CRS/HIPEC and to perform port removal. If there is evidence of progressive disease (defined as any increase in the peritoneal carcinomatosis index), the port will be removed and the patient will undergo further treatment according to multidisciplinary recommendations. If there is no evidence of progressive disease, the port may be left in place and the patient can continue treatment until imaging or clinical evidence of disease progression. Continued treatment is at the same dose and schedule. Patients are monitored for SAEs, but continued treatment is not part of the dose level determination of this trial. Alternately, in scenarios where the patient is a candidate for surgery, the port may be removed and the patient will undergo further consideration for CRS/HIPEC. After completion of study-related treatment, subjects will be followed until recurrence and/or death for up to five years. (see Table 4).
<table>
<thead>
<tr>
<th>H&amp;P &amp; Concurrent Meds</th>
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<th>X</th>
<th>X</th>
<th>X</th>
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</thead>
<tbody>
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<td></td>
</tr>
<tr>
<td>Vital Signs&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>ECOG Performance Status</td>
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<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Serum Chemistries&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>CBC&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>X</td>
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<td></td>
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<tr>
<td>Liver Function Tests&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>X</td>
<td>X</td>
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</tr>
<tr>
<td>CEA, Ca19-9, CA-125</td>
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<td>X</td>
<td>X</td>
<td></td>
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<tr>
<td>ctDNA &amp; pftDNA</td>
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<td>X</td>
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<tr>
<td>Pregnancy Test (urine or serum)</td>
<td>X</td>
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<tr>
<td>Imaging&lt;sup&gt;e&lt;/sup&gt;</td>
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<td></td>
<td>X</td>
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<tr>
<td>Adverse Events&lt;sup&gt;f&lt;/sup&gt;</td>
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<td>X</td>
<td>X</td>
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<tr>
<td>P PTX&lt;sup&gt;g&lt;/sup&gt;</td>
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<td></td>
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<tr>
<td>PK Specimens&lt;sup&gt;j&lt;/sup&gt;</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Quality of Life Assessment</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>
4.2 Pretreatment Evaluation

Within 30 days Prior to Study Enrollment, the following procedures will be performed:

a) Complete blood count: hemoglobin, hematocrit, red blood cells [RBC], white blood cells [WBC], platelets, and differential blood cell counts such as: neutrophils, lymphocytes, monocytes, eosinophils, basophils.

b) Serum chemistries (BUN, chloride, CO2, creatinine, glucose, potassium, sodium).

c) Liver function tests: alanine transaminase, aspartate transaminase, alkaline phosphatase, direct bilirubin, indirect bilirubin, total bilirubin, albumin.

d) Imaging can include CT Chest/Abdomen/Pelvis, Abdominal/Pelvis MRI, or PET/CT scan and may be within 8 weeks of enrollment or pre-treatment evaluation.

4.3 On-treatment Evaluation

Within 3 days prior to the 2nd, 3rd, 4th, 5th, and 6th IP paclitaxel treatment, patients will undergo laboratory assessment with a CBC, chemistries, tumor marker, and liver function tests. Patients will also be screened for adverse events by phone or computer contact, or clinic.
4.4 Post-treatment Evaluation

- Post-treatment Outpatient Evaluation

Within 30 days of treatment completion, the subject will have:
1. Standard of care laboratory analysis, which may be performed at an outside laboratory or hospital.
2. Patients will also be contacted after 30 days for evaluation of adverse events that occurred within 30 days of treatment completion. This may be performed via telephone or email assessment.
3. Multidisciplinary conference evaluation to determine next treatment which may include a treatment break, continuation of IP PTX, CRC/HIPEC, or next line systemic therapy.

- Safety

1. Patients will be contacted after 30 days from treatment completion to obtain a final assessment of adverse events. This may be performed in person or via telephone/email.
2. Safety and tolerability of IP PTX is a secondary end point of this study.

- Criteria for Removal from Study:

1. Inability of subject to comply with study requirements.
2. Determination by the investigator that it is no longer safe or in the patient’s best interest for the patient to continue therapy.

- Follow-up

Patients that continue IP PTX will be monitored for SAEs attributable to therapy, and progressive disease or toxicity requiring treatment discontinuation. Patients will be followed for survival as shown in the study calendar (see Table 4). After completion of the treatment, subjects will be followed with imaging approximately every 6 months for five years. This may include outside imaging.

4.5 Emergency Procedures

- Procedures in Case of Medical Emergency:

The Principal Investigator(s) is responsible for ensuring that procedures and expertise are available to handle medical emergencies during the study.

- Procedures in Case of Overdose:

There is currently no known antidote for the systemic chemotherapy in this study. The treatment of AEs associated with overdose should be supportive for the underlying adverse symptoms.

Doses of study treatment in excess of that specified in the clinical study protocol are considered to be an overdose. Overdose, with or without associated symptoms should be handled in...
the same way as a deviation and sent to IRB. Signs or symptoms of an overdose that meet the criteria of serious should be reported as a SAE in the appropriate timeframes and be documented as clinical sequelae to an overdose.

### 4.6 Data Collection

Data collection will include:

- Patient name
- Medical record number
- Patient age and gender
- Initial diagnosis date and stage IV diagnosis date
- Tumor grade and associated histological features (signet ring, mucinous, ect), tumor location, PCI
- Imaging findings at diagnosis
- Type and duration of systemic chemotherapy
- Laparoscopic findings
- Type and dose of chemoradiation therapy, if given
- Treatment associated morbidity and mortality
- Pathologic details
- Dates and sites of disease progression
- Date of last follow-up and vital status
- Whole blood and peritoneal sample collection for PK analysis after IP paclitaxel. Approximately 5-20 ml of sample will be collected at each time point.

### 5.0 Statistics and Justification of Sample Size

**Phase I**

This is an open label, Phase I trial to determine the maximum tolerated dose (MTD) via intraperitoneal route in subjects with metastatic, high-grade appendiceal adenocarcinoma. A maximum of 24 patients will be enrolled into this study. The starting dose for the table.

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<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>-1</td>
<td>100</td>
</tr>
<tr>
<td>0 (Starting Dose)</td>
<td>150</td>
</tr>
<tr>
<td>1</td>
<td>200</td>
</tr>
<tr>
<td>2</td>
<td>250</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
</tr>
</tbody>
</table>
Dose limiting toxicity (DLT) will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAEv5) by organ system. DLT will be defined as any grade III/IV non-hematologic or neutropenia-associated (infection or fever treated in the hospital) toxicity attributable to this therapy that occurred within 30 days after treatment. The Bayesian Optimal Interval (BOIN) design\(^54\) will be used to determine the MTD of the BOIN design identifies the MTD through minimizing the incorrect decisions of dose escalation and de-escalation (i.e., erroneously escalating/deescalating the dose when the current dose is actually higher/lower than the MTD), thereby optimizing the dose assignment for each enrolled patient. The BOIN design is simple to implement and has been shown to have superior performance through simulations. The BOIN design is algorithm-based, which is similar to the traditional “3+3”; however its overall performance is substantially better. Its average performance is comparable to model-based design, such as the CRM (continual reassessment method) in terms of selecting the correct MTD, yet with a substantially lower risk of assigning patients to sub-therapeutic or overly toxic doses (Figure 5).

The target toxicity rate for the MTD is \(\phi = 0.3\) and the maximum sample size is 24. We will enroll and treat patients in cohorts of size 3. To guide dose-escalation decisions, if the observed DLT rate at the current dose is \(\leq 0.236\), the next cohort of patients will be treated at the next higher dose level; if it is \(\geq 0.359\), the next cohort of patients will be treated at the next lower dose level. These boundaries were created when minimizing decision errors such that \(\phi_1 = 0.18\) is the highest toxicity probability that is considered sub-therapeutic (underdosing) and \(\phi_2 = 0.42\) is the lowest toxicity probability that is deemed overly toxic (overdosing). For the purpose of overdose control, doses \(j\) and higher levels will be eliminated from further examination if \(\Pr(p_j > 0.3 \mid data) > 0.95\), where \(p_j\) is the true DLT rate of dose level \(j, j = 1, \ldots, 5\). When the lowest dose is eliminated, stop the trial for safety.

The steps to implement the BOIN design are as follows:

1. Patients in the first cohort are treated at dose level 2.
2. To assign a dose to the next cohort of patients, conduct dose escalation/de-escalation according to the rule displayed in Table 1. When using Table 1, please note the following:
   a. “Eliminate” means eliminate the current and higher doses from the trial to prevent treating any future patients at these doses because they are overly toxic.
   b. When we eliminate a dose, automatically de-escalate the dose to the next lower level. When the lowest dose is eliminated, stop the trial for safety. In this case, no dose should be selected as the MTD.
   c. If none of the actions (i.e., escalation, de-escalation or elimination) is triggered, treat the new patients at the current dose.
   d. If the current dose is the lowest dose and the rule indicates dose de-escalation, treat the new patients at the lowest dose unless the number of DLTs reaches the elimination boundary, at which point terminate the trial for safety.
e. If the current dose is the highest dose and the rule indicates dose escalation, treat the new patients at the highest dose.

3. Repeat step 2 until the maximum sample size of 24 is reached, or stop the trial if the number of evaluable patients treated at the current dose reaches 12 and the decision according to Table 1 is to stay at the current dose.

**Figure 5. Flowchart for trial conduct using the BOIN design**

- Start at dose level 2
- Treat a cohort of 3 patients
- Is the stopping rule met?
  - Yes: Stop the trial and select the MTD
  - No: Compute the DLT rate
    - $\leq 0.236$: Escalate the dose
    - $> 0.359$: De-escalate the dose
    - Within $(0.236, 0.359]$: Retain the current dose

\* DLT rate = \( \frac{\text{Total number of patients who experienced DLT at the current dose}}{\text{Total number of evaluable patients treated at the current dose}} \)
Table 5. Dose escalation/deescalation rule for the BOIN design

<table>
<thead>
<tr>
<th>Number of patients treated at the current dose</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Deescalate if # of DLT =&gt;</td>
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<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<td>3</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of patients treated at the current dose</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
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</thead>
<tbody>
<tr>
<td>Escalate if # of DLT &lt;=</td>
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<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Deescalate if # of DLT =&gt;</td>
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<td>4</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>6</td>
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</tr>
<tr>
<td>Eliminate if # of DLT =&gt;</td>
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<td>5</td>
<td>6</td>
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</tbody>
</table>

Note. “# of DLT” is the number of patients with at least 1 DLT. When none of the actions (i.e., escalate, de-escalate or eliminate) is triggered, stay at the current dose for treating the next cohort of patients. “NA” means that a dose cannot be eliminated before treating 3 evaluable patients.

After the trial is completed, select the MTD based on isotonic regression as specified in this computation is implemented by the shiny app “BOIN” available at http://www.trialdesign.org. Specifically, select as the MTD the dose for which the isotonic estimate of the toxicity rate is closest to the target toxicity rate. If there are ties, select the higher dose level when the isotonic estimate is lower than the target toxicity rate and select the lower dose level when the isotonic estimate is greater than or equal to the target toxicity rate.

Once we determine the MTD, an additional up to 6 patients will be enrolled for additional experience with safety and efficacy. We will use the elimination boundaries in Table 1 for toxicity monitoring.

Operation Characteristics
Table 2 shows the operating characteristics of the trial design based on 1000 simulations of the trial using shiny app “BOIN” (BOIN V2.6.5.0) available at http://www.trialdesign.org. The operating characteristics show that the design selects the true MTD, if any, with high probability and allocates more patients to the dose levels with the DLT rate closest to the target of 0.3.
Table 6. Operating characteristics of the BOIN design

<table>
<thead>
<tr>
<th>Scenario</th>
<th>True DLT</th>
<th>Selection %</th>
<th>% Pts Treated</th>
<th>Number of Patients</th>
<th>% Early Stopping</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3</td>
<td>61.9</td>
<td>46.6</td>
<td>1</td>
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</tr>
<tr>
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<td>1.6</td>
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<tr>
<td>4</td>
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<td>0.3</td>
<td>0.12</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: “% Early Stopping” refers to early stopping due to excessive DLT.

Statistical Analysis:

Phase I: Safety data will be summarized using frequency tables by grade and attribution. Preliminary anti-tumor activity (i.e., response rate) will be summarized by dose level. Survival outcomes will be assessed through Kaplan-Meier plots. PK and PD data will be described through summary statistics such as mean, median, and quantiles.

Phase II: The primary objective in the Phase II portion of this trial is to determine the proportion of patients with an objective response after 3 months. Peritoneal disease is difficult to measure by cross-sectional imaging as it frequently exists and a contiguous erratically shaped area in the peritoneal cavity. As current RECIST criteria do not consider mucinous/cystic disease as measurable, standard RECIST criteria do not apply well to peritoneal disease. Previously a modified novel quantitative measuring system designed for mucinous peritoneal disease was developed, termed mucinous peritoneal RECIST (mpRECIST). This metric, which measure up to 5 areas of tumor in the abdominal cavity, has been used successfully in prior trials at [reference].

All patients who are treated at the MTD in Phase I will be counted toward the sample size in the Phase II portion of this trial. Additionally, we will use the elimination boundaries in
the BOIN table to perform toxicity monitoring. Correlations between toxicity events and baseline demographic variables may be explored and will be subject to stringent multiple testing correction if presented.

There will be a total of 15 patients. Based on historical data, we estimate that the proportion of patients who will demonstrate an objective response without going on trial is 0.05, i.e. the null hypothesis is a 0.05 ORR. Treatment is expected to raise this proportion to 30%. Using an exact binomial test with one-sided type I error of 0.05, we will reject the null if at least 3 patients achieve response. This test has power of 87%. We will also estimate the ORR along with an exact 90% confidence interval. Assuming 3 of the 15 patients would achieve response, the observed response rate would be 20% and the corresponding 90% exact confidence interval would be (5.7%, 44%). Additional secondary endpoints and safety data will be summarized as previously described.

Pharmacokinetic analysis (PK): PK studies will be conducted to determine systemic exposure of within patients when it is administered intraperitoneally, and to assess how long remains in the IP space after administration. Whole blood and peritoneal samples will be collected at various time points after IP infusion as described to measure the plasma and peritoneal concentration profile. Plasma concentrations will be measured using a validated reverse phase high-performance liquid chromatography, and detection using tandem mass spectrometry. PK parameters such as peak concentration (Cmax), corresponding time to peak (Tmax), area under the concentration-versus time curve (AUC), and half-life (t1/2) will be estimated via non-compartmental analysis using the WinNonlin PK software program.

There is likelihood that there will be patient-to-patient variability in IP exposure of and systemic absorption, which may results in variable outcomes and toxicity. We hypothesize that patients who have significantly higher exposure will have better response and similarly patients with greater systemic absorption will have more toxicities. To investigate whether variability in PK has an impact on outcomes, correlative analysis will be performed with PK parameters (AUC, Cmax, t1/2) with treatment response (decrease in peritoneal carcinomatosis index and adverse events using Wilcoxon rank-sum tests.

6.0 Procedure to Obtain Informed Consent
The investigator must obtain documentation of consent from each potential subject prior to participating in a clinical trial.

The protocol and informed consent documentation for this study must conform to institutional regulations and local and national laws and regulations. As soon as a potential subject is considered for this study and prior to any other study procedures, each prospective subject will be given a full explanation of the purpose of the study, the procedures to be carried out and the potential hazards. Once this essential information is provided to the subject and once the Investigator believes that the subject understands the implications of participating in the study, the subjects will be asked to provide informed consent and authorization to access medical records needed for study documentation. Subjects will be assured that they may withdraw from
the study at any time without jeopardizing their medical care. They will be given a copy of their
Informed Consent Form.

7.0 Data Confidentiality

Data will be available to the PI and people directly involved with the collection and analysis of
data related to this project. IRB approval will be obtained for any exchange of data within and
outside of [redacted].

Collection of Identifiers:
Identifiers (patient names, medical record numbers, and dates) will be collected. Names and MRN
will be replaced by study numbers in the analytic file. The key linking these numbers will be
retained in a locked file by the investigator designated personnel. Dates will be retained as a
limited data set. Data will not be shared with any party outside of [redacted] and will not be
retained or disseminated for other research without prior IRB approval.

Training of Personnel:
All [redacted] personnel will be fully trained to maintain the patient health information
confidentially. Training will be documented as required by institutional policy.

Data Storage:
The PI and research staff will attempt to minimize risk through only storing information containing
subject identifiers in locked file storage, on password-protected computers, on encrypted servers
behind an institutional firewall and according to current institutional and federal data security
requirements. In addition, access to patient identifiers will be limited to the minimum number of
necessary research personnel, and only to those research personnel directly involved with
obtaining patient information and assigning random study identifiers. Keys containing information
linking study subjects to personal identifiers will be maintained in locked storage for paper records
or behind institutionally approved firewall and electronic security measures for electronic keys,
and available ONLY to the PI and research personnel directly involved in creating random study
identifiers. Information containing subject personal identifiers will not be removed from
concerning this research study.

Data Sharing:
Study data will not be shared with any individuals or entities that are not involved in the study
without IRB approval. No identifying information will be shared with outside collaborating sites or
outside collaborating research staff without prior IRB approval and a data use or material transfer
agreement has been implemented. If approval is obtained, sharing of data would be done after
approval of the PI and only by secure mechanisms, as approved [redacted] Security.

Final Disposition of Study Records:
These data will be used for this research study. Data that is in hard-copy form will be retained on
site until the study is terminated, and may be stored indefinitely, per institutional standards, in
long-term off-site storage with an [redacted] approved, secured contract site. Electronic data
will be retained indefinitely on [redacted] servers behind the institutional firewall. Data will not
be shared with any party outside of [redacted] without IRB approval and will not be retained
or disseminated for other research without prior IRB approval. Study data and paper records will not be destroyed but will be retained permanently.

8.0 References
On behalf of the Research Foundation, I wish to extend our thanks for the innovative research that you are doing in appendix cancer. We are happy to support it.

As you know, appendix cancer is a rare but life-threatening disease for which there are currently limited treatment options. Unfortunately, due to the subtleties of this disease, appendix cancer is often not discovered until has spread outside of the appendix (i.e., classified as Stage 4). The primary treatment available for these patients is an extensive, highly invasive, and costly procedure referred to as cytoreductive surgery plus heated intraperitoneal chemotherapy (CRS+/HIPEC). This procedure is most effective in only a subset of patients, primarily those with a low-grade pathology.

For those patients who have a high-grade pathology and/or for those either ineligible for CRS+HIPEC due to disease burden or for whom CRS+HIPEC is not successful, there are limited treatment options. Sadly, the only therapeutic regimen available to the large majority of those appendix cancer patients is the systemic chemotherapy regimen used to treat colorectal cancer. Yet, appendix cancer is an entirely separate disease from, and behaves differently than, colorectal cancer.

To put it simply, there is an urgent need for new drug development for appendiceal cancer patients.

On behalf of the Foundation, I have reviewed your clinical protocol to test the safety and efficacy of given via intraperitoneal route. Your plan to space out the treatment to every two weeks will help to mitigate the travel burden on patients. We also have discussed the inclusion and exclusion criteria, which we are pleased have been designed in a way to maximize patient enrollment.

Once again, thank you for all that you and your colleagues are doing to innovate and explore new therapies for the treatment of appendix cancer.

Sincerely,
We at [Company Name] are happy to support your research in appendiceal cancer. As you know, [Company Name] has formed a partnership with [Partner Name] to explore how personalized and tumor informed circulating tumor DNA (ctDNA) assays such as our [Assay Name] can be best utilized to advance the care of cancer patients. Your proposal to evaluate the utility of the [Assay Name] specifically in the management of appendiceal cancer was selected by an independent review committee for inclusion in the initial round of pilot studies between [Dates].

We are excited to analyze preliminary test results in appendiceal cancer, which is already being processed, and believe that personalized and tumor-informed testing will provide clinical utility in appendiceal cancer patients. Given the difficulties of measuring disease burden in peritoneal space, we share your optimism that serial ctDNA measurement may be the best way to track tumor response to therapy in appendiceal cancer.

We look forward to our continued collaboration, and wish you success in your grant application.
Dear [Name]

We at [Institution] are excited to support your research in colorectal and appendiceal cancer. The [Collaboration Name] collaboration, officially announced in April of this year, has the potential to revolutionize clinical translational research by taking the volumes of data generated in the standard of care treatment of patients and organizing into a usable format that is both interpretable by scientists and clinicians and also machine readable. Over the past year we have continually worked together to adapt the [Software Name] specifically to address the needs of researching gastrointestinal cancers, and the software is now being actively used by the full colorectal faculty. We look forward to our continued collaboration, including a joint publication highlighting the capabilities of the [Collaboration Name]
It is with great pleasure that I provide this letter of collaboration in support of application for the 2022 Conquer Cancer Career Development Award. I am delighted to provide my expertise in trial design and statistical analysis for his Conquer Cancer Career Development Award proposal entitled. I appreciated the opportunity to work closely with over the last several months to develop the design of this study and to help define clear, testable hypotheses for investigation. Together, we have ensured that the project design, sample size, and endpoints are statistically sound and feasible to accomplish.

As you know, I am an In my statistical methodological work, I am focused on integrating different types of high-dimensional data, e.g. environmental, clinical, or other omics data, to better understand the genetic and genomic etiology of disease. I also maintain a robust clinical trial collaboration portfolio and have published many Commentary in high-profile medical journals regarding the proper analysis and interpretation of trial results. Thus, I will be well-suited to identify novel technical challenges or discuss possible extensions that arise from the results of this work.

I have appreciated the opportunity to both participate in the design of this study and to help define clear, testable hypotheses for investigation. I will also assist in implementing principled and robust analysis plans throughout all the proposed aims. For example, I will assume primary responsibility for ensuring that robust and adequate strategies for multiple testing correction are used in your exploratory analyses. I will additionally participate in the interpretation of analysis results and provide input into manuscript preparation.

I eagerly anticipate the success of this proposal as a foundation for our future collaborative work. Please

Sincerely,
Dear [Name],

As a surgeon specializing in the treatment of peritoneal malignancies I am excited to support your innovative proposal focused on the development of new treatments for appendiceal cancer. Although we have made and will continue to make advancements in the surgical resection of appendiceal cancer, we are both well aware that so many of our patients present with such a high burden of disease they will never be helped with surgery. These patients are in desperate need of an effective therapy; whether it be traditional chemotherapy, targeted therapy, or immune therapy. The proposal you have outlined has a high likelihood of identifying such a therapy.

As you have recognized, the development of treatment for appendiceal cancer has been limited by a lack of cell line and mouse models. To address this issue, I have been supplying you with fresh tumor samples directly from the OR to implant into mice. This collaboration is already off to a good start with nine new PDX models already implanted in less than a year. This is in addition to the banking of specimens for later molecular profiling, and the banking of blood for measurement of circulating tumor DNA.

With regard to immediate clinical translation I am excited to support your efforts to start a trial of intraperitoneal (IP) treatment for high-grade appendix cancer. Unfortunately the majority of the high-grade appendiceal patients have extensive peritoneal spread at the time of diagnosis and are not candidates for complete surgical resection. Given the limited efficacy of systemic chemotherapy for this disease these patients have few treatment options, so your trial is filling an important clinical need. [Hospital] has already opened a Phase I trial to study the [drug] to treat gastric cancer here at [Hospital]. Although as you know there are important differences between gastric and appendix cancer, this speaks to the feasibility of starting similar trial in appendiceal cancer as we are planning. Specifically Dr. [Name] trial has established the feasibility of IP catheter placement, the safety of weekly dosed
paclitaxel, and logistically that both the cost of drug and diagnostic laproscopy can be billed to insurance as a standard-of-care charge. Given our treatment volume at [redacted] I conservatively estimate that we would see eight patients a month meeting the eligibility criteria for your trial.

[redacted] has been an innovator in appendix cancer for many years, including the first ever prospective trial specifically in appendix cancer led by [redacted], and the nearly completed randomized any chemotherapy vs. observation trial led by myself and [redacted]. Your proposal continues in this tradition, and here at [redacted] we have the supporting resources to make it successful.

Wishing you best of success with your ASCO Career Development Award proposal!
I am delighted to support your research in appendiceal cancer.

As a physician-scientist with training in both experimental and computational biology, and clinical expertise in colorectal and appendix cancer you are uniquely situated to perform the exciting, translational research you have proposed. We are happy to support your efforts with our expertise in the generation of patient derived droplet organoids (PDO) from clinical samples. As you know we have an IRB approved protocol and material transfer agreement already in place, and have in fact already successfully created PDOs from peritoneal metastases from a colon cancer as well as an appendiceal cancer send by your team from [redacted]. As described in your proposal, based on this initial success we are expanding PDO creation efforts from appendiceal tumors.

Our labs have a growing history of successful collaboration, including the joint submission of a U54 proposal, your contribution to my [redacted], and your contribution to the [redacted] we are submitted around the [redacted]. We have one co-authored paper so far and of course worked closely together planning the first annual Translational Colorectal Cancer: from Genomics to Therapy conference.

In summary, I enthusiastically look forward to our continued collaboration, and wish you success in your grant application.
Dear [Name],

I am excited to support your proposed clinical trial testing the safety and efficacy of the intraperitoneal delivery of [agent] for appendiceal cancer patients. As we have discussed your interest in intraperitoneal (IP) chemotherapy treatment for appendix cancer is closely aligned with my interest in using the same agents to treat metastatic gastric cancer. I share your optimism that IP drug delivery could increase the therapeutic window of chemotherapy.

My Phase I trial testing the safety of escalating doses of [agent] in gastric cancer patients has been going quite well with no dose limiting toxicity seen thus far after nine patients. The success of this trial speaks to the feasibility of opening a similar trial in appendiceal cancer here at [Institution] as you propose to do. Of note we have not had any issues with billing the paclitaxel and imaging as part of standard clinical care, greatly reducing the cost of the trial. To aid in the design of your trial we are happy to share the pharmacokinetic data we generate from the gastric phase I trial when it becomes available. Of note the day 1, 8, and 15 dosing used in the gastric phase I trial was selected to match historical data previously generated in Japan, there is reason to believe the biweekly dosing as you will test will be safe and equally efficacious.

I look forward to our continued collaboration and wish you success with your ASCO Career Development Award proposal.
INTRODUCTION

The rarity of appendiceal neoplasms has made it difficult to conduct prospective or randomized clinical trials to guide therapy for these tumors. The small number of appendiceal tumors that are detected, in many cases as an incidental finding in < 1% of appendectomy specimens, comprise multiple histopathologic subtypes, including noninvasive mucinous neoplasms, mucinous and nonmucinous adenocarcinomas, carcinoids, goblet cell carcinoids (GCCs, now also called goblet cell tumors), and signet ring cell carcinomas. Early-stage cancers can be treated definitively with surgery, and selected patients derive long-term benefit from cytoreductive surgery and heated intraperitoneal chemotherapy (HIPEC). However, there is no standard of care for the systemic treatment of advanced, unresectable disease.

In the absence of randomized phase III data, the majority of medical oncologists use colorectal cancer (CRC) chemotherapy regimens for the treatment of unresectable epithelial appendiceal neoplasms, as is currently recommended by the National Comprehensive Cancer Network guidelines. Support for the use of fluoropyrimidine-based combinations with platinum agents or mitomycin-C comes from retrospective single-institution reviews, case reports, and single-arm prospective studies. Although there are reports of similar response and survival outcomes for contemporary regimens, including infusional...
Table 1. Clinical and Demographic Patient Characteristics by Subtype

<table>
<thead>
<tr>
<th>Subtype</th>
<th>No.</th>
<th>Median Age (years)</th>
<th>Sex Ratio M:F (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucinous adenocarcinoma</td>
<td>320</td>
<td>54</td>
<td>43:57</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>208</td>
<td>56</td>
<td>42:58</td>
</tr>
<tr>
<td>Goblet cell carcinoma</td>
<td>84</td>
<td>54</td>
<td>36:64</td>
</tr>
<tr>
<td>Pseudomyxoma peritonei</td>
<td>54</td>
<td>54</td>
<td>46:54</td>
</tr>
<tr>
<td>Signet ring carcinoma</td>
<td>37</td>
<td>56</td>
<td>49:51</td>
</tr>
</tbody>
</table>

fluorouracil, leucovorin, and oxaliplatin; fluorouracil, leucovorin, and irinotecan; and targeted agents in appendiceal adenocarcinomas compared with CRC, it is known that appendiceal neoplasms have a better prognosis after cytoreductive surgery with HIPEC treatment. There is also a growing body of data showing that there are clear molecular differences between appendiceal and colorectal cancers. Here we present a cohort of 703 molecularly profiled appendiceal neoplasms, the largest such cohort to date in this rare disease. Comparing the mutational landscapes across histologic subtypes we find significant differences in KRAS, GNAS, and FALT mutation prevalence and confirm that mutational profiles of appendiceal neoplasms are distinct from CRC and other GI cancers. In addition, we identify that patients can be risk stratified using the combined mutation status of GNAS and TP53 and that outcomes are favorable for patients with KRAS wild-type disease when treated with irinotecan.

RESULTS

Mutation Landscape of Appendiceal Neoplasms

The 703 cases were categorized into four different histopathological subtypes consistent with the recently updated consensus classification from the Peritoneal Surface Oncology Group International; in addition, cases of pseudomyxoma peritonei (PMP) were included, because this syndrome usually arises from the appendix. The majority were either mucinous adenocarcinomas (MAd, 46%) or adenocarcinomas (Ad, 30%), with the rest being GCCs (12%), PMPs (7.7%), or signet ring cell carcinoma (SRC, 5.3%; Appendix, Table 1). The majority of specimens submitted for sequencing came from intraperitoneal metastatic deposits, although there were also primary appendiceal tumors and a small number of lung, liver, and bone metastases (Fig 1A).

Mutation analysis revealed KRAS to be the most frequently mutated gene in MAd (77%), Ad (56%), and PMP (81%) and the second most frequently mutated gene in SRCC (35%). In contrast, KRAS mutations were significantly less frequent in GCCs (13%; χ² P < .001), where TP53 (33%) was the most frequently mutated gene (Fig 1B; Appendix Fig A1A). GNAS mutations were the second most frequent alteration in MAs (52%) and PMP (72%) and third most frequent in Ad (25%). GNAS mutations were significantly less frequent in SRCC (8%) and GCC (6%) compared with the rest of the cohort (χ² P < .001). TP53 mutations were most common in Ad (47%) and SRCCs (43%), slightly less common in MAs (33%) and GCCs (33%), and significantly less common in PMP (7%); χ² P < .001). Mutations in KRAS were almost exclusively at codon 12, and GNAS mutations at codon 201, consistent with gain-of-function, whereas mutations in TP53 were spread across the gene and included many frameshift mutations, consistent with loss of function (Appendix Figs A1B-A1D). FALT mutations were significantly more frequent in GCCs (17%; χ² P < .001). BRAF, BRCA1, CDKN1B, CDKN2A, MYC, PTEN, and TGFB2 mutations were present in < 10% of cases across all subtypes (Table 2). Given its unique mutation profile relative to the other histologies, GCCs were excluded from comutation and mutual exclusivity analysis. GNAS and KRAS were the only gene pair significantly comutated (odds ratio, 6.8; Bonferroni corrected P = 8.6x10⁻⁴⁷); GNAS and TP53 were the only gene pair significantly mutually exclusive (odds ratio, 0.20; Bonferroni corrected P = 6.7x10⁻¹⁰; Fig 1C; Data Supplement).

Pathway-Based Analysis of Mutation Profiles

Genetic aberrations were subsequently grouped by signaling pathway (Appendix Table A1). Components of the RAS/RAF signaling pathway (ie, BRAF, HRAS, KRAS, and NRAS) were the most frequently altered genes in epithelial appendix cancers, occurring in > 80% of MAs and PMPs, 60% of Ads, but only 33% of GCCs (χ² P < .001; Fig 1D). Alterations in homologous recombination deficiency genes were observed in > 50% of all subtypes but were most prevalent in SRCC (80%).
Fig 1. Genomic profiles of appendiceal tumors. (A) Distribution of tissue site submitted for sequencing. (B) Frequency of mutation for selected genes, separated by histologic subtype. (C) Mutation plot from targeted sequencing of 703 appendix cancer tumors. A selection of genes relevant to the disease is represented. (D) Frequency of alteration for specific pathways. Ad, adenocarcinoma; GCC, goblet cell carcinoid; MAd, mucinous adenocarcinoma; PMP, pseudomyxoma peritonei; SRCC, signet ring cell carcinoma.
Table 2. Comparison of Mutation Frequencies of Key Genes in Appendiceal, Pancreatic, and Colorectal Cancer

<table>
<thead>
<tr>
<th>Gene</th>
<th>MAD</th>
<th>Ad</th>
<th>PMP</th>
<th>SRCC</th>
<th>CRC</th>
<th>PDAC</th>
<th>GCC</th>
<th>Neuroendocrine Pancreas</th>
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<tr>
<td>MSI-H</td>
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<td></td>
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<tr>
<td>&gt; 20 mutations/Mb</td>
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<td>3.4</td>
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<td>2.7</td>
<td>6.0</td>
<td>1.0</td>
<td>1.2</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Data presented as % frequency.
Abbreviations: Ad, adenocarcinoma; CRC, colorectal cancer; GCC, goblet cell carcinoid; MAD, mucinous adenocarcinoma; MSI-H, microsatellite instability-high; PDAC, pancreatic ductal adenocarcinoma; PMP, pseudomyxoma peritonei.

Comparison of Genomic Aberrations in Appendix, Colorectal, and Pancreas Cancers

Given the clinical practice of treating metastatic appendiceal cancers with CRC regimens, we compared genomic alteration profiles of the appendiceal subtypes with those of 10,000 CRCs profiled by the same laboratory (Table 2). CRCs and appendiceal Ads had similar frequencies of KRAS (51% vs 56%, respectively) and SMAD4 (16% vs 18%, respectively) mutations. However, all appendiceal subtypes had significantly less-frequent alterations in TP53 and APC relative to CRC ($\chi^2 P < .001$). GNAS mutations were significantly more common in MAD, Ad, and PMP ($\chi^2 P < .001$) but not GCC or SRCC (Table 2). The high frequency of KRAS mutations observed in multiple appendiceal subtypes prompted inquiry into possible parallels with pancreatic ductal adenocarcinoma (PDAC), which harbors KRAS mutations in up to 95% of cases. Sequencing of 2,800 pancreatic tumors revealed KRAS mutations in 87% of PDACs. TP53 mutations were significantly more frequent in PDACs (71%) compared with appendiceal cancers ($\chi^2 P < .001$).

Given their unique alteration landscape relative to other appendiceal subtypes, GCCs, which demonstrate both glandular and neuroendocrine differentiation, were also compared with pancreatic neuroendocrine tumors (PNETs). PNETs and GCCs exhibited similar frequencies of KRAS, GNAS, and APC mutations, but GCCs had significantly more frequent mutations of SMAD4, ARID1A, and TP53 ($\chi^2 P < .001$).

Conversely, GCCs exhibited significantly lower rates of RB1 alteration than PNETs (2% vs 11%; $\chi^2 P < .001$). High tumor mutational burden ($\geq 20$ mutations/Mb) and microsatellite-unstable tumors were both slightly more frequent in CRC compared with appendiceal cancers. There tended to be a higher frequency of microsatellite-unstable or tumor mutational burden–high SRCCs and Ads compared with MAds (Table 2; Appendix Fig A1E).

Histopathologic and Molecular Features Predictive of Survival

To determine the influence of histologic and molecular features on clinical outcomes, a retrospective review of a single-institution case series was performed. Similar to the full 703-patient cohort, the majority of cases were either Ads (n = 17) or MAds (n = 33), with fewer low-grade appendiceal mucinous neoplasms that manifested as PMPs (n = 13) and few SRCCs (n = 9). Median follow-up was 29.9 months, with 42 (55%) patients alive at the time of analysis. Data for chemotherapy treatment were available for 60 patients, showing that the majority were treated with a fluoropyrimidine and either oxaliplatin or irinotecan (Appendix; Table 3).

Overall survival (OS), determined from time of initial diagnosis, was similar for Ad and MAD (log-rank P = .29; Appendix Fig A2A), so these groups were combined in subsequent analyses. There was a trend toward better OS for low-grade appendiceal mucinous neoplasms/PMPs and worse OS
for SRCCs ($P = .11$; Fig 2A). Consistent with prior reports, tumor grade was strongly predictive of survival, with low-grade tumors (well differentiated) having median OS more than double that of high-grade (poorly differentiated) tumors (115.5 vs 53.6 months; log-rank $P = .0012$; Fig 2B).\textsuperscript{17} Associating mutations with survival, tumors with $TP53$ mutation had significantly better OS than $TP53$ wild-type tumors (37.1 vs 115.5 months; log-rank $P = .0020$; Fig 2D).\textsuperscript{18} $KRAS$ mutation status was not significantly associated with survival (log-rank $P = .22$; Fig 2E). However, use of irinotecan in any line of therapy was associated with a survival advantage in $KRAS$ wild-type tumors (log-rank $P = .041$; Fig 2F) but not in $KRAS$ mutant tumors (log-rank $P = .32$; Appendix Fig A2B). Tumor grade was significantly associated with $GNAS$ and $TP53$, but not $KRAS$ mutation status. Low-grade tumors were enriched for $GNAS$ mutations (72% vs 18% for high grade; $\chi^2 P < .001$; Appendix Fig A3A), consistent with our prior report,\textsuperscript{14} whereas high-grade tumors were enriched for $TP53$ mutations (56% vs 6.9% for low grade; $\chi^2 P < .001$; Appendix Fig A2B).

To assess the relative contributions of mutation and grade to the observed OS differences, a Cox proportional hazard analysis was performed including age, sex, $KRAS$, $GNAS$, $TP53$, and grade as covariates. This identified age (hazard ratio [HR], 1.06/yr; $P = .017$), grade (HR, 10.48; $P = .047$), and $TP53$ mutation status (HR, 4.51; $P = .022$) as the only significant predictors of survival (Wald Test $P = .0086$). Consistent with the Cox analysis, the rare high-grade tumors with a $GNAS$ mutation had OS similar to that of other high-grade tumors (median, 54.5 months vs 53.6 months for all high-grade tumors; Figs 2B and 3A). However, the effect of $TP53$ mutation on survival was independent of grade, with the rare low-grade, $TP53$-mutant tumors having OS similar to other $TP53$-mutant tumors (median, 24.6 months; Fig 3B). Combining $TP53$ and $GNAS$ mutation status as a prognostic biomarker allowed for stratification of patients into three groups with distinctly different survival outcomes (Fig 3C). Tumors with only a $GNAS$ mutation had the best prognosis (median OS, 115.5 months), followed by those with mutation in neither gene (75.8 months), whereas tumors with a $TP53$ mutation had the worst prognosis (37.1 months; log-rank $P = .0031$). The survival separation seen between $TP53$- and $GNAS$-mutated tumors was similar to that between high- and low-grade tumors (Fig 3D), suggesting that mutation status and grade have similar prognostic value.
The mutational profile of 703 appendix neoplasms provides insight into the molecular aberrations that differentiate histologic subtypes and identifies putative prognostic and predictive biomarkers that may help guide treatment in this rare malignancy. Most striking are differences in mutational spectrum between GCCs and epithelial appendiceal cancers, especially Ad, MAd, and PMP subtypes. Compared with Ad, MAd, and PMP, KRAS and GNAS mutations were much less frequent in GCCs, whereas mutations in FAT3 and ARID1A were more frequent (Figs 1B and 1C).

These differences were also seen in the pathway analysis, in which GCCs had less-frequent alterations of the RAS/RAF pathway relative to epithelial appendiceal cancers (Fig 1D). The mutational spectrum of GCC was similar to PNETs with respect to GNAS, KRAS, APC, and RB1 mutation frequency, perhaps not surprising given these tumors are known to display neuroendocrine features (Table 2). In addition, although not significant after multiple hypothesis corrections, there was a trend toward comutation for GNAS and TP53 in GCCs (OR, 8.7; Data Supplement), opposite of what was seen in epithelial appendiceal...
cancers, providing additional evidence that GCCs are a distinct disease entity.\textsuperscript{19}

The profiles of epithelial appendiceal cancers were generally similar to each other, showing frequent mutation in \textit{KRAS} and \textit{GNAS} followed by \textit{TP53} and \textit{SMAD4}, consistent with previously reported case series\textsuperscript{13,14,20-26} (Appendix \textit{Table A2}). These data also show that all of the appendiceal subtypes are molecularly distinct from CRC, with more frequent \textit{GNAS} mutation and significantly lower prevalence of \textit{APC} and \textit{TP53} mutations, which are key pathogenic alterations in CRC. This is a clinically important point, because this case series confirms that most patients with appendix cancer are treated with CRC chemotherapy regimens (Table 3). The mutation profiles of Ad and SRCC bore the most resemblance to CRC, with Ad and CRC sharing similar frequencies of mutation in \textit{KRAS}, \textit{SMAD4}, and \textit{ARID1A}; of note, appendiceal Ads have been referred to as colonic-type adenocarcinoma, given clinical behavior more similar to CRC.\textsuperscript{20,27} In this series, SRCC had the worst prognosis of the epithelial appendiceal tumors and also has a clinical course similar to CRC.\textsuperscript{28}

The frequent cotemutation of \textit{KRAS} and \textit{GNAS} in MAd, Ad, and PMP parallels the molecular profile of intraductal papillary mucinous neoplasms.\textsuperscript{14,29} Notably, both intraductal papillary mucinous neoplasms and, in particular, \textit{GNAS} mutant appendiceal cancers are characterized by mucin production and a generally indolent clinical course. The differences in mutation profiles between the epithelial appendiceal tumors can be at least partially explained by grade, with significant association of \textit{GNAS} mutation with low-grade tumors and \textit{TP53} mutation with high-grade tumors (Appendix Figs A3A and A3B). Consistent with this is the higher incidence of \textit{TP53} mutation and lower incidence of \textit{GNAS} mutation in SRCCs, which are by definition all high grade.

Histologic grade was also a strong predictor of survival (Fig 2B), consistent with prior reports.\textsuperscript{17,30} We did not observe a significant difference in OS between Ad and MAd; however, the distribution of tumor grade was similar between these two subtypes in our study. In contrast, in prior case series that reported better survival for MAd, MAds were enriched for
low-grade tumors. Although GNAS mutant tumors were significantly associated with better survival, Cox proportional hazard analysis confirmed that this effect was due to the association of GNAS mutation and low histologic grade. Conversely, TP53 mutation was an independent predictor of poor survival, with low-grade, TP53-mutant tumors having survival similar to that of high-grade tumors. Accurately assessing the grade of appendiceal tumors is difficult. Frequently, not all tumor deposits can be surgically removed, giving rise to possible sampling errors where focal high-grade lesions could be missed. In addition, because appendiceal tumors are so rare, they are difficult to diagnose pathologically and are frequently overinterpreted by community pathologists. Although GNAS mutation is not an independent predictor of survival, because GNAS and TP53 mutations occur mutually exclusive of each other and are associated with low- and high-grade tumors, respectively, the two genes can be substituted for grade to predict survival. The survival stratification achieved with the GNAS-TP53 biomarker is similar to grade, an important observation, given that it is now much easier to obtain a mutation profile than an expert pathology review in the community oncology setting.

The absence of a GNAS mutation in the majority of high-grade tumors and the mutual exclusivity of TP53 and GNAS mutations both strongly suggest that most high-grade appendiceal tumors occur de novo, rather than progressing from low-grade tumors, confirming, on a larger scale, our previous observations. However, there were a minority of tumors with both TP53 and GNAS mutation (n = 41; 6.7%), suggesting that transformation from low grade to high grade can occur. Serial biopsy or serial measurement of circulating tumor DNA would be needed to confirm that these TP53 mutations did in fact occur after the formation of a low-grade, GNAS-mutant tumor. However, given the low propensity for appendiceal tumors to spread beyond the abdominal cavity, there may be limited tumor DNA in circulation, potentially making blood-based tumor detection difficult. Indeed, three of the four University of California, San Diego, patients who underwent circulating tumor DNA sequencing had no reportable alterations.

Regarding predictive biomarkers, KRAS wild-type status was associated with better survival in the subset of patients treated with irinotecan. A retrospective study in metastatic CRC reported better response to irinotecan in patients with wild-type versus mutant plasma KRAS. However, a larger prospective study found that mutant KRAS was associated with poor survival but not with response to irinotecan in CRC. Regarding targeted therapies, there are unfortunately few clinically actionable mutations in appendiceal cancers, although the RAS/RAF signaling pathway is frequently altered in epithelial appendiceal cancers. Data on therapeutic targeting of the RAS/RAF cascade in appendiceal tumors are limited, although a recent case report described clinical benefit in a patient with appendiceal MAd harboring a GNAS R201H mutation who was treated with trametinib. Because only eight patients in this cohort received an anti-EGFR antibody, we were unable to assess interactions between KRAS mutation status and response to these agents.

A major limitation of this study is its retrospective design. With regard to the 703-patient cohort, clinical information such as precise TNM stage was not available, but the fact that > 80% of specimens submitted came from metastases indicates that the majority of patients had stage IV disease. Although specimens were independently reviewed by pathologists to confirm the diagnosis before undergoing sequencing, subtype definitions are potentially subject to variability and overlap, because there was no consensus classification system for appendiceal neoplasms and PMP until recently. For example, PMP is an inherently imprecise term used to describe the clinical syndrome of mucinous peritoneal dissemination from an appendiceal neoplasm. PMP encompasses a spectrum of both high- and low-grade lesions but does not reference the histopathologic characteristics of the appendiceal primary from which it arises. Newer classification schemes separate PMPs with low-grade and high-grade features (also known as disseminated peritoneal adenomucinosis and peritoneal mucinous carcinomatosis, respectively) and PMP with signet ring cells. Because this study did not distinguish between these subtypes, we are unable to report on genomic differences associated with grade in the larger 70-patient cohort. With respect to the 76-patient University of California, San Diego, cohort, this analysis is also limited by its single-institution and retrospective nature,
small sample size, and relatively limited time of follow-up. Chemotherapy treatment data were not available for those patients who were referred to an academic center for surgery but received chemotherapy in a community setting. In addition, analysis of interactions between genotype and specific drugs are confounded by the fact that most patients received multiple lines of therapy.

In conclusion, appendiceal neoplasms have molecular profiles that are distinct from CRC and are characterized by frequent GNAS and KRAS mutations, especially in low-grade tumors.

This study of unprecedented size in this rare disease highlights important molecular differences between different subtypes of appendix cancer and identifies GNAS and TP53 mutation status as a prognostic biomarker. This comprehensive portrait of the molecular landscape of appendix cancer will help with the design of future clinical studies to develop and test therapeutic strategies specific to this disease.
Appendix

Tumor tissue from 703 patients with appendiceal cancer was submitted to a Clinical Laboratory Improvement Amendments–certified laboratory sequencing and variant calling. Pathology reports and a subset of hematoxylin and eosin slides were reviewed by board-certified pathologists to independently confirm the diagnosis and subtype of appendiceal cancer. However, grade was not reported in the majority of cases. A minimum of 50 ng of DNA was extracted and a hybrid-capture method used to capture 3,769 exons from 315 cancer-related genes and 47 introns of 28 genes commonly rearranged in cancer; this material was then sequenced to high (average, 756X) uniform coverage allowing for evaluation of genomic alterations, including base substitutions, indels, amplifications, copy number alterations, and fusions/rearrangements. Actionable genomic alterations were defined as those identifying anticancer drugs on the market or in registered clinical trials. Tumor mutational burden was calculated from a minimum of 1.11 Mb sequenced DNA and reported as mutations per megabase. Microsatellite status was determined by evaluating the insertion/deletion characteristics at 114 homopolymer repeat loci in targeted regions of the genes.

Approval for this study, including a waiver of informed consent and Act waiver of authorization, was obtained from the . Separately, the Institutional Review Board granted approval for a retrospective study patients with appendix cancer. Clinical characteristics and outcomes, including tumor histology, grade, stage, overall survival (OS), and chemotherapy given were determined from review of the electronic medical record. All patients with appendix cancer and somatic mutation profiling were identified. A total of 80 patients with stage IV appendix cancer and somatic mutation profiling were identified. Sequencing failed quality control in two cases, and two patients with only blood-based cell-free DNA sequencing were excluded, leaving 76 patients available for analysis. Because there were only four goblet cell carcinoids, these were removed from survival analysis.

For mutual exclusivity and comutation analysis, goblet cell carcinoid tumors and microsatellite instability-high tumors were removed, and a Fisher’s exact test was performed for all gene combinations, followed by Bonferroni multiple hypothesis correction. Kaplan-Meier plotting, log-rank, and χ² statistical tests were performed using Prism version 7.04 proportional hazard analysis for predictors of overall survival was performed including age, sex, KRAS, GNAS, TP53, and grade as covariates
**Fig A1. Mutation burden and spectrum in appendiceal tumors.** (A) Tumor mutation frequency (mutations per megabase) for each subtype. Incidence and location of mutations in (B) KRAS, (C) GNAS, and (D) TP53. (E) Tumor mutation burden (mutations per megabase) for each histologic subtype. Ad, adenocarcinoma; GCC, goblet cell carcinoid; MA, mucinous adenocarcinoma; PMP, pseudomyxoma peritonei; TM8, tumor mutational burden.
Fig A2. Clinical and molecular features predictive of survival. (A) Overall survival of adenocarcinoma (Ad) versus mucinous adenocarcinoma (MAd). (B) Overall survival of patients with KRAS mutations, stratified by irinotecan use.

Fig A3. Correlation of grade with GNAS, TP53 mutation status. (A) GNAS mutation frequency, by grade. (B) TP53, by grade. (C) Adenocarcinoma versus mucinous adenocarcinoma frequency, by grade.
### Table A1. Gene Lists for Pathway Analysis

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Abbreviations: HRD, homologous recombination deficiency; PI3K, phosphoinositide 3-kinase.
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Abbreviations: Ad, nonmucinous adenocarcinoma; GCC, goblet cell carcinoid; IHC, immunohistochemistry; MAd, mucinous adenocarcinoma; MSS, microsatellite stable; NGS, next-generation sequencing; PCR, polymerase chain reaction; PMP, pseudomyxoma peritonei.

- 16% mucinous adenocarcinomas with PMP.
- With PMP.
- n = 3.
- Appendiceal adenocarcinomas (mucinous/nonmucinous not differentiated).
- Appendiceal adenocarcinomas with/without goblet cell/signet ring features.
- Low-grade MCP only.
- High-grade MCP only.
- All-female population.
Regarding Blood-Based Next-Generation Sequencing Analysis of Appendiceal Cancers

Appendix cancer is a rare disease, and the publication of such a large patient cohort is commendable; however, I am concerned that the conclusion of Shaib et al. [1], namely, that evaluation of circulating tumor DNA (ctDNA) is feasible among patients with appendiceal cancer, is not supported by the presented data.

Specifically, the authors claim that the frequencies of genomic alterations in liquid next-generation sequencing (NGS) are similar to those previously reported for tissue NGS. However, comparing the results reported by Shaib et al. for liquid NGS with the largest cohort of tissue NGS reveals stark differences. Overall, an alteration could only be identified in 184 of 303 patients (60.7%), even counting for the fact that some patients were tested multiple times. Restricting the tissue NGS gene panel to just the 73 genes tested in liquid NGS, at least one alteration was found in 658 of 703 patients (93.6%) in tissue NGS [2].

Second, it is important to note that the mutation spectrum of different subtypes of appendiceal cancer are quite different. Unfortunately, the histology was only known for a fraction of the patients reported in the Shaib et al. study. Working on the assumption that the same distribution seen in the 63 cases with known histology (52% mucinous adenocarcinoma, 22% adenocarcinoma, 22% goblet cell carcinoma) was similar to that of the rest of the 303-patient cohort, we can estimate the number of patients with each histology. Using the reported mutation frequencies in tissue NGS [2] for each subtype, 103 patients would be expected to have GNAS mutation, but only 8 were found by liquid NGS (Table 1). Analysis of other genes (TP53, KRAS, APC, SMAD4, ARID1A, ERBB2) shows that in every case the number of mutations detected in liquid NGS is less than expected based on tissue NGS (Table 2). Interestingly, the disparity is highly variable for different genes, ranging from an expected to observed ratio of 1.5 to more than 30. Given that certain mutations, such as GNAS, are known to be enriched in low-grade tumors, whereas others, such as TP53, are enriched in high-grade, these observed differences suggest that low-grade tumors are less likely to shed DNA into the blood.

To ultimately answer the question of whether liquid NGS is a reasonable surrogate of tissue NGS for all forms of

<table>
<thead>
<tr>
<th>Table 1. Expected mutation frequency for GNAS</th>
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<tbody>
<tr>
<td>% Distribution</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>0.524</td>
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<tr>
<td>0.222</td>
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<tr>
<td>0.222</td>
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<tr>
<td>Expected number of patients with GNAS mutation based on tissue NGS</td>
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<tr>
<td>Reported number of patients with GNAS mutation in liquid NGS</td>
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</table>

*Abbreviation: NGS, next generation sequencing.*

<table>
<thead>
<tr>
<th>Table 2. Expected versus observed mutation frequencies</th>
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<tbody>
<tr>
<td>Gene</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>APC</td>
</tr>
<tr>
<td>ARID1A</td>
</tr>
<tr>
<td>ERBB2</td>
</tr>
<tr>
<td>GNAS</td>
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<tr>
<td>KRAS</td>
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<td>SMAD4</td>
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<td>TP53</td>
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appendiceal cancer, a cohort with paired samples from the same patient will be required. As correctly noted by Shaib et al., there are many advantages to liquid NGS; however, until the above discrepancies are further evaluated, liquid NGS should not replace tissue NGS when tissue NGS is feasible. Of note, newer ctDNA assays with greater sensitivity are being developed [3], and these should be tested in appendiceal cancer.

Disclosures
The author indicated no financial relationships.

REFERENCES