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TITLE: A Proteomic Co-Clinical Trial of BGJ-398 in FGFR-Driven Biliary Cancers

PRINCIPAL INVESTIGATOR: Kevan Shokat

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Regents of the University of California
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14. ABSTRACT

Intrahepatic cholangiocarcinomas (ICC) is a dead malignancy of the liver bile ducts (<10% 5-year survival for patients of all stages) with few therapeutic options for advanced disease. The project involves translational studies relating targeting the Fibroblast Growth Factor Receptor (FGFR) signaling pathway in ICCs harboring alterations in the FGFR pathway. FGFR alterations (most commonly fusions of the FGFR2 gene that result in activation of the FGFR2 kinase) are present in ~20% of ICCs and clinical trials with FGFR kinase inhibitors are showing promise in these patients. However, resistance inevitably arises, limiting therapeutic efficacy. The goals of this project are to understand the basis of FGFR signaling dependency in FGFR-activated ICC, to elucidate mechanisms of clinical acquired resistance, and to improve therapeutic strategies against this subset of patients that improve initial responses and overcome resistance. The scope of work includes conducting sequencing studies of patient samples pre- and post-progression to identify potential resistance mechanisms, generating patient-derived models and utilizing sophisticated proteomics and genetic approaches to decipher FGFR-controlled signaling networks and to validate and understand resistance mechanisms, and to use the signaling information and results from drug screens to identify combinations therapies that prevent or overcome resistance.

15. SUBJECT TERMS- Postdoctoral fellowships research, bio-medical research, training, education

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

Intrahepatic cholangiocarcinomas (ICC) is a dead malignancy of the liver bile ducts (<10% 5-year survival for patients of all stages) with few therapeutic options for advanced disease. The project involves translational studies relating targeting the Fibroblast Growth Factor Receptor (FGFR) signaling pathway in ICCs harboring alterations in the FGFR pathway. FGFR alterations (most commonly fusions of the FGFR2 gene that result in activation of the FGFR2 kinase) are present in ~20% of ICCs and clinical trials with FGFR kinase inhibitors are showing promise in these patients. However, resistance inevitably arises, limiting therapeutic efficacy. The goals of this project are to understand the basis of FGFR signaling dependency in FGFR-activated ICC, to elucidate mechanisms of clinical acquired resistance, and to improve therapeutic strategies against this subset of patients that improve initial responses and overcome resistance. The scope of work includes conducting sequencing studies of patient samples pre- and post-progression to identify potential resistance mechanisms, generating patient-derived models and utilizing sophisticated proteomics and genetic approaches to decipher FGFR-controlled signaling networks and to validate and understand resistance mechanisms, and to use the signaling information and results from drug screens to identify combinations therapies that prevent or overcome resistance.

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

Biliary tract cancer, intrahepatic cholangiocarcinoma, FGFR, kinase, cancer genomics, phosphoproteomics, patient-derived models, signal transduction, acquired resistance, co-clinical trials

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

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

Specific Aim 1: Determine the impact of distinct modes of FGFR activation on signaling output and drug response

Major Task 1: Characterize basal signaling from treatment naïve FGFR-driven biliary cancer cell models. Time line: months 1-24. Completion to date: 66% (4/6 components)

Major Task 2: Analyze differential signaling feedback driven by genetic activation of FGFR2. Time line: months 1-24. Compeletion to date: 33% (1/3 components)

Major Task 3: Characterize novel mechanisms of FGFR-driven liver cancer growth. Time line: months 3-36. Completion to date: 12.5% (0.5/4 components)

Specific Aim 2: Co-clinical trials to determine the cell biologic & molecular impact of FGFRi in vivo, and assess effect of concurrent genetic alterations.

Major Task 4: Murine co-clinical trial of BGJ-398. Time line: 1-28 months. Completion to date: 25% (0.5/2 major component; minor component = regulatory approval also complete)

Major Task 5: Phenotypic and biochemical characterization of BGJ-398 response. Time line: months 12-30. Completion to date: 25% (0.5/2 components). Ahead of schedule

Major Task 6: Model effect of concurrent genetic alterations on BJG-398 response. Time line: months 6-36. Completion to date: 2.5% (0.1/4 components).

Specific Aim 3: Characterize polyclonal drug resistance and identify novel therapeutic vulnerabilities in FGFR2-driven BTC.

Major Task 7: Characterize genetic and signaling changes driven by FGFR inhibition for hepatobiliary cancer patients in clinical trials. Time line: Months 1-36. Completion to date: ~33% (~1.33/4 components)

Major Task 8: Characterize signaling effects of treatment associated mutations. Time Line: Months 13-36. Completion to date: ~10% (ahead of schedule)

Major Task 9: Overcoming resistance with next generation FGFRi. Time line: months 12-30. Completion to date: not started

Major Task 10: Overcoming resistance with combination drug screens. Time line: months 13-36. Completion to date: not started

What was accomplished under these goals?

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

Specific Aim 1: Determine the impact of distinct modes of FGFR activation on signaling output and drug response

Major Task 1: Characterize basal signaling from treatment naïve FGFR-driven biliary cancer cell models

a) Obtain local IRB approval and federal HRPO for use of human tumor tissue, including making PDXs/cell lines and genetic analysis.

We obtained these approvals: Local IRB: DFCI Protocol No.: 13-416.

HRPO: Assigned Number: A-20290.1 (Bardeesy) A-20291.1 (Zhu)

b) Convert extant FGFR-driven PDXs to 2-D cell lines (at least two new lines).

There is a critical need to establish new models of ICC, given the lack of such models in standard repositories. There are no published FGFR-driven ICC models. We have established three new patient-derived lines harboring FGFR2 fusions: ICC13-7 (FGFR2-OPTN) ICC10 (FGFR2-PHGDH), and ICC11 (FGFR2-PHGHD). We also identified an existing ICC cell line that massively overexpresses FGFR1 (CCLP). The creation and characterization of these models is a significant advance in the translational study of this important subset of ICC. Data are summarized in ***Figure 1.***

c) Develop isogenic immortalized biliary lines preferably using MMNK1 or H89 cells (available through academic cell banks) with WT, point mutants or fusions of FGFR2.
This sub-aim and (d), below, seek to define FGFR signaling outputs in the biliary epithelium driven by different ICC-associated FGFR2 fusions or point mutations in order to help define their underlying oncogenic mechanisms We have established and characterized MMNK1 derivative cell lines expressing FGFR2-wild type, FGFR2-S252W, FGFR2-BICC1, FGFR2-OPTN, and FGFR2-PHGDH via lentiviral transduction. This system provides a physiologically relevant context non-malignant context (biliary epithelial cells) to explore FGFR pathway activation using different activated forms of FGFR2 observed in human cancer.

d) Proteomic analysis of global phosphorylation of isogenic cells at baseline and following treatment with BGJ-398 and/or TAS-120.
Since FGFR2 is a kinase, we seek to understand the signaling cascades it generates to drive malignancy. To this end, we have conducted phosphoproteomics on two of the cell lines from (c) at baseline or following TAS-120 treatment. While further data analysis will commence in Y2, we already see very significant signaling difference between the two FGFR2 allelles, supporting the rationale of these studies. ***Please see Figures 2-5.***

Major Task 2: Analyze differential signaling feedback driven by genetic activation of FGFR2

a) Characterize sensitivity and kinetics of response to reversible and covalent FGFR2 inhibitors (BGJ-398, LY2874455, Debio-1347 and TAS-120) by measuring cell viability and changes in FRS phosphorylation.
Using our novel FGFR-activated ICC cell lines, we have sought to define whether FGFR signaling is required to support proliferation in vitro and to characterized the central downstream signaling pathways driven by FGFR in this context. These information are essential for understanding the mechanisms by which FGFR alterations promote ICC development and maintenance, and will be critical in the development of more effective treatment approaches as well as in the prediction of resistance mechanisms. We have tested a panel of FGFR inhibitors for their effects on the growth of CCLP and ICC13-7 cells as well as on the downstream signaling program. While ICC cell lines lacking molecular alterations that activate FGFR signaling are insensitive to FGFR inhibitors (e.g. for BGJ398, the IC50 is >1000 nM for each line), we found that CCLP and ICC13-7 are sensitive at less than 10 nM. Moreover, FGFR inhibition leads to a rapid and durable decrease in the key FGFR2 substrate FRS2, as well as of downstream phosphorylation of SHP2, MEK and ERK, where AKT activity was not effected. Thus, FGFR signaling is essential for maintenance of MEK/ERK signaling in FGFR-driven ICC, while the PI3K-AKT is not controlled by FGFR in these cells. ***Please see Figure 1B-F.***

Major Task 3: Characterize novel mechanisms of FGFR-driven liver cancer growth

a) Continued establishment of biliary & hepatocellular cancer PDX and cell line models & test for FGFRi response (at least 10 models derived from resected patients at Mass. Gen. Hospital)
Given the paucity of ICC models in general and of FGFR-driven models in particular, it is essential for us to continue to expand our collection of such models, in order to fully decipher the underlying signaling program in this subset of ICC and to understand how it compares with other ICC subsets. We have established 7 new PDX lines since the commencement of this grant, including 2 with FGFR alterations. Please see ***Figure 3***.

b) Genomic characterization of new tumors using mutation panel of 400 cancer genes. We have conducted genomic characterization of these models. Please see ***Figure 6.***

Specific Aim 2: Co-clinical trials to determine the cell biologic & molecular impact of FGFRi in vivo, and assess effect of concurrent genetic alterations

Major Task 4: Murine co-clinical trial of FGFR inhibitors

a) Obtain ACURO approval for therapeutics studies in mice.
We have obtained approval: Protocol [ACURO Assigned Number]: CA160216 Title: Mouse Models of Cancer

b) Murine co-clinical trial testing FGFR inhibitors in in vivo models harboring FGFR alterations. The availability of preclinical models of FGFR-activated ICC allows us to overcome the challenges of studying how FGFR inhibitors affect ICCs at the molecular and cell biological level in patient samples (i.e. repeat biopsies are very limited and are usually confined to a single pre-treatment and post-progression biopsy, and thus the acute effects of a medicine cannot be evaluated mechanistically). To address this, we have completed a trial with the FGFR inhibitor, TAS-120, in our FGFR2-KIAA expressing PDX model, MG69. Mice were treated with the drug or vehicle control when tumor reached ~ 400 mm3. Mice were treated for 14 days for efficacy studies, with serial measurements of tumor volume. A subset of mice were euthanized at serial time points to isolate tumors for molecular analyses and histology (3 days and 14 days). We observed a complete block in tumor growth in this model. Please see ***Figure 7A.*** The analysis of specimens from this study will allow us to have unparalleled understanding of FGFR signaling 'addiction' in ICC.

Major Task 5: Phenotypic and biochemical characterization of FGFR inhibitor response

a) Correlative molecular and histological analysis of samples of co-clinical trials (see Major Task 4, part (b) above.
We have examined the tumors from the MG69 model treated with TAS-120. Histological assessment demonstrates evidence of tumor cell differentiation. Accordingly, we have seen a remarkable complete loss of proliferation (Ki-67 staining) as rapidly as 3 days after the start of treatment. By contrast, we do not observe apoptosis (cleaved caspase-3). Analysis of signaling changes, revealed loss of p-MEK, p-ERK, and p-SHP2, but no change in pAKT, mirroring the in vitro data. These data suggest that while FGFR inhibition has dramatic effects in vivo, completing arresting tumor cell proliferation and shutting down signaling to the MEK/ERK pathway, it does not appear to incite tumor cell death acutely. Thus, while there is true 'oncogene addiction', the persistence of growth inhibited cells may ultimately drive recurrence. These data support the search for drug combination strategies that synergize with FGFR inhibition to drive tumor cell death. (please see ***Figure 7B and C***).

Major Task 6: Model effect of concurrent genetic alterations on BJG-398 response.
a) Perform gene editing to generate ICC cell lines harboring inactivating mutations in common ICC tumor suppressor genes (e.g. PTEN, ARID1A)
The goal of these studies is to test whether specific mutations that co-exist with FGFR pathway alterations in subsets of ICC can influence FGFR inhibitor sensitivity. To address this, we are using gene editing strategies in FGFR inhibitor sensitive ICC cell lines and evaluating the resulting impact on drug sensitivity. To date, we have creates sgRNA vectors to target PTEN. Use of these tools will proceed in the upcoming year.

Major Task 7: Characterize genetic and signaling changes driven by FGFR inhibition for hepatobiliary cancer patients in clinical trials

a) Collect blood and tumor specimens from patients on study of BGJ-398, TAS-120, other FGFR inhibitors. Blood and tumor material will be collected pre-treatment. Blood will be collected approximately monthly throughout the course of treatment. A second biopsy will be performed upon disease progression.
Our robust translational pipeline enables us to isolate and study patient specimens from serial time points during therapy. In the past year, we have prepared a set of pre- and post-treatment specimens from 8 patients.

b) Next generation sequencing of ctDNA and tumor biopsy specimens.
Using 4 of the samples above, we have conducted sequencing that has identified multiple candidate resistance mechanisms. These include multiple mutations in the FGFR2 kinase domain. Please see ***Figure 8***.

c) Develop PDX models of treatment naïve and resistant tumor specimens (at least 3 models from patients on FGFR inhibitor clinical trials).
The availability of new models from patients at various stages of treatment enables powerful co-clinical trials that model the underlying biology of response. We have developed 5 PDX models from patients (1 treatment naïve and 4 resistant). These will be employed in molecular and functional studies in the upcoming year. Please see ***Figure 9***.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

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

The project has provide training opportunities for Dr. Lipika Goyal and John Gordan, who were promoted to attending physicians during the prior year. Both work closely with Dr. Bardeesy, Zhu, and Shokat who serve as mentors and have regular in-person meetings as well as joint teleconferences to trouble-shoot and discuss progress and future directions.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

The work was presented in the 2018 Cholangiocarcinoma Foundation Annual Meeting in Salt Lake City, January 30-Feb 1st, 2018. This meeting consists both of a regular scientific conference with leading researchers from around the world presenting cutting-edge discoveries and clinical progress as well as a full day devoted to outreach and education for patients and their families and caregivers have the opportunity to hear talks given with layman's language relating to the disease and emerging research in the area.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

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

Major focus of the upcoming year are to build upon our phosphoproteomics data, genomic information regarding potential resistance mechanisms using our wealth of novel models to fully decipher the circuits downstream of FGFR that support ICC growth, to credential candidate resistance mechanisms and to understand them functionally and to uncover approaches to prevent and overcome resistance based on signaling changes identified in our work and on new drug screens. Finally, since ICC is genetically heterogeneous in general, and among the subset of ICC with FGFR alterations, and since the mechanisms of resistance we are uncovering are also diverse, we will continue to prioritize model development in order to have systems that appropriately mirror the diverse presentation of the disease in the patient population.

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

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

Medicines that inhibit the FGFR signaling pathway (FGFR inhibitors) are showing promise in patients with ICC that harbor FGFR alterations. Unfortunately, patients eventually relapse due to the acquisition of drug resistance. Our work has provide key insights into the main resistance mechanisms assoiciated with different FGFR inhibitors. These findings help to physicians anticipate when treatment failure is occurring and guide treatment with alternative FGFR inhibitors. In addition, the wealth of model systems we have developed together with advanced methods in understanding protein function (phosphoproteomics) provide us with unprecedented opportunities to understand why FGFR inhibitors are initially so effective in these patients and why they ultimately fail. They also enable us to use genetic methods and drug screening approaches to discover the next generation of therapies that will boost the effect of the FGFR inhibitors, preventing resistance from occurring or overcoming it once it arises.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

Our work is focused on liver cancers with FGFR alterations. However, since the FGFR signaling pathway is also deregulated in multiple other cancer types (bladder, breast, stromach, lung, and others), the insights from our data will help understand FGFR inhibitor response and resistance in these other settings as well.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

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

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

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

Nothing to report

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

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

Nothing to report

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

Nothing to report

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

Nothing to report

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

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

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

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

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

Nothing to report.

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

Nothing to report

  Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

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

Nothing to report

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

Nothing to report

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

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*

- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

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

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Nabeel Bardeesy
Project Role: PI
Researcher Identifier;
Nearest person month worked:
Contribution to project: Led the model development and characterization efforts. Assayed FGFR sensitivity in vitro and in vivo. Conducted in vitro and in vivo signaling analyses. Credentialled FGFR kinase domain mutations.
Name: Andrew Zhu
Project Role: PI.
Researcher Identifier;
Nearest person month worked:
Contribution to project: Supervised translational efforts using patient samples.

Name: Kevan Shokat
Project Role: PI
Researcher Identifier
Nearest person month worked:
Contribution to project: Supervised proteomics efforst
Name: Krishna Tummala
Project Role: Postdoc
Researcher Identifier;
Nearest person month worked:
Contribution to project: Conducted signaling studies, established and studied models.
Name: John Gordan
Project Role: Instructor
Researcher Identifier;
Nearest person month worked:
Contribution to project: Developed biliary cell models and conducted and analyzed phosphoproteomics
Name: Lipika Goyal
Project Role: Instructor
Researcher Identifier;
Nearest person month worked:
Contribution to project: Conducted and coordinate clinical studies including sample acquisition and sequencing analysis.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

Dr. Bardeesy was awarded the grant below. It does not impact effort on the present DOD grant.

R01 CA215498-01A1 Bardeesy (PI)
Functions of the LKB1 tumor suppressor in control in metabolism and epigenetics
The goal of this project is to define to circuits downstream of LKB1 mediating tumor suppression, including the roles for altered cell metabolism and its interplay to epigenetic regulation.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

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

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

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

We have submitted reports for the PI (Bardeesy) and partnering PI's (Zhu and Shokat)

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

9. APPENDICES:

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

Figure 1

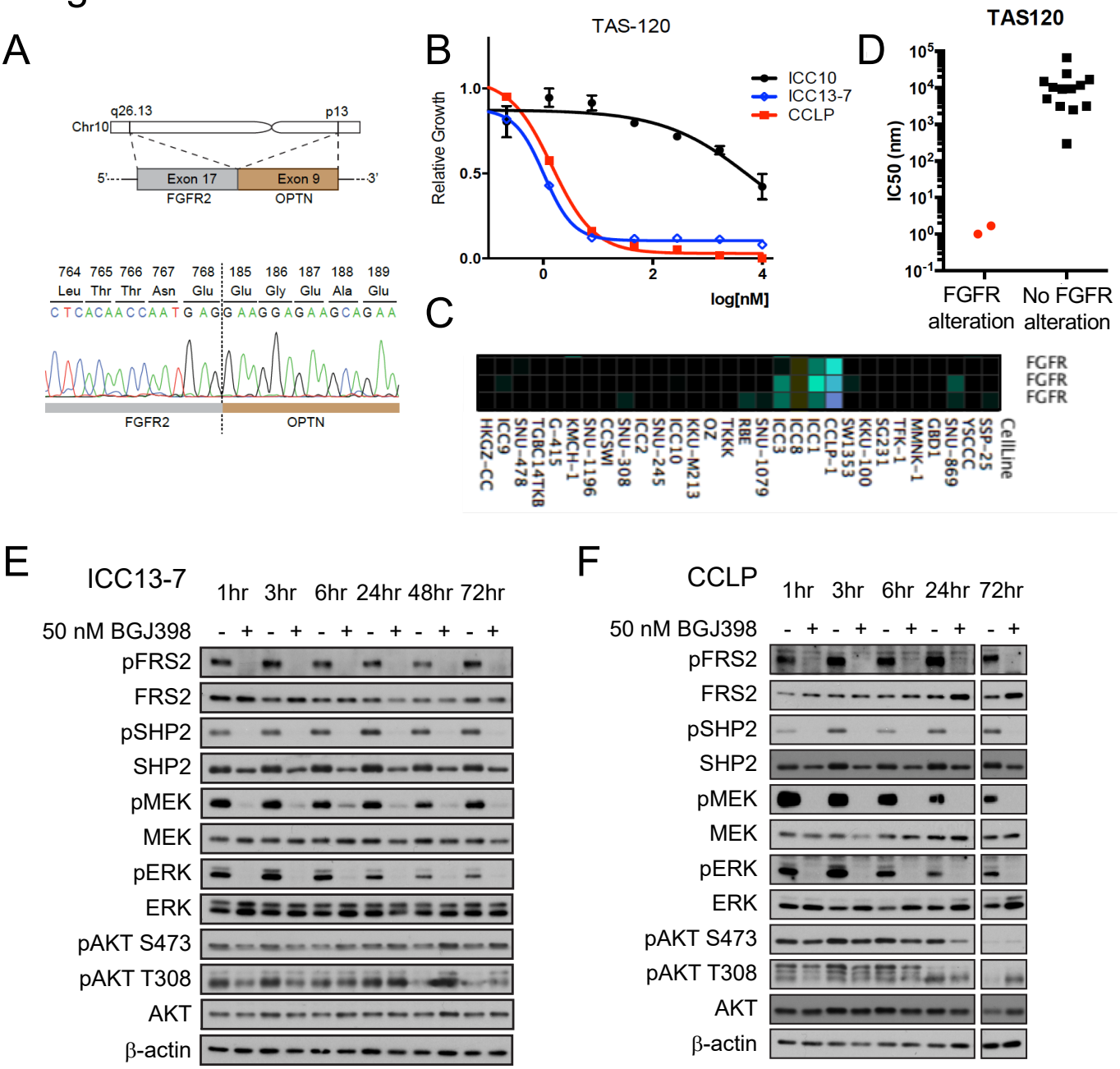

Figure 1. Conversion of existent FGFR-driven ICC to 2-D cell lines.
A. We derived the ICC13-7 cell line from a PDX model. The graphic shows the structure of the FGFR2-OPTN fusion that we detected in this cell line. We also generated a 2-D cell line (ICC10) from a second PDX model and found that it had an FGFR2-PHGDH fusion (not shown).
B. We tested the response of ICC10, ICC13-7, and CCLP cells to TAS-120 (IC50 is graphed).
C. Heatmap of a set of ICC cell lines screened for sensitivity to three FGFR inhibitors (TAS-120, Debio1347 and BGj398). Only CCLP is sensitive in the set shown.
D. Graph of IC50 data ICC cell lines with FGFR alterations (ICC13-7 and CCLP) and those lacking such alterations.
E,F. Immunoblot of signaling effects of BGJ398 treatment of ICC13-7 cells (E) and CCLP cells (F).

Dr. Bardeesy led these studies with assistance of Dr. Zhu and Shokat/Gordan

Figure 2

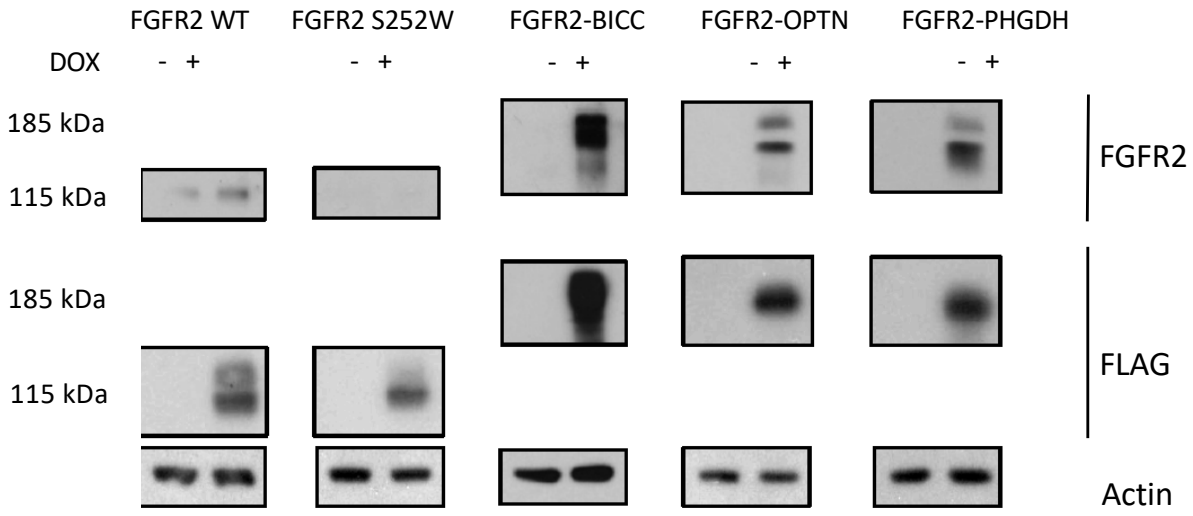

Figure 2. Generation of isogenic FGFR2 WT, FGFR2 S252W, FGFR2-BICC, FGFR2-OPTN, and FGFR2-PHGDH MMNK1 cells.

FGFR2 WT and mutants were cloned into a doxycycline (dox)-inducible puromycin-resistant destination vector with Gateway Cloning, packaged into lentivirus, and transduced into MMNK1 cells. Transduced cells were stably selected with puromycin and plated into 10cm plates with either 1mg/mL dox or no dox. After over 24 hours of dox induction, cells were harvested, lysed, and analyzed by western blot. Membranes were blotted for FGFR2, FLAG, and Actin.

Drs. Shokat/Gordan led these studies with assistance of Dr. Bardeesy

Figure 3

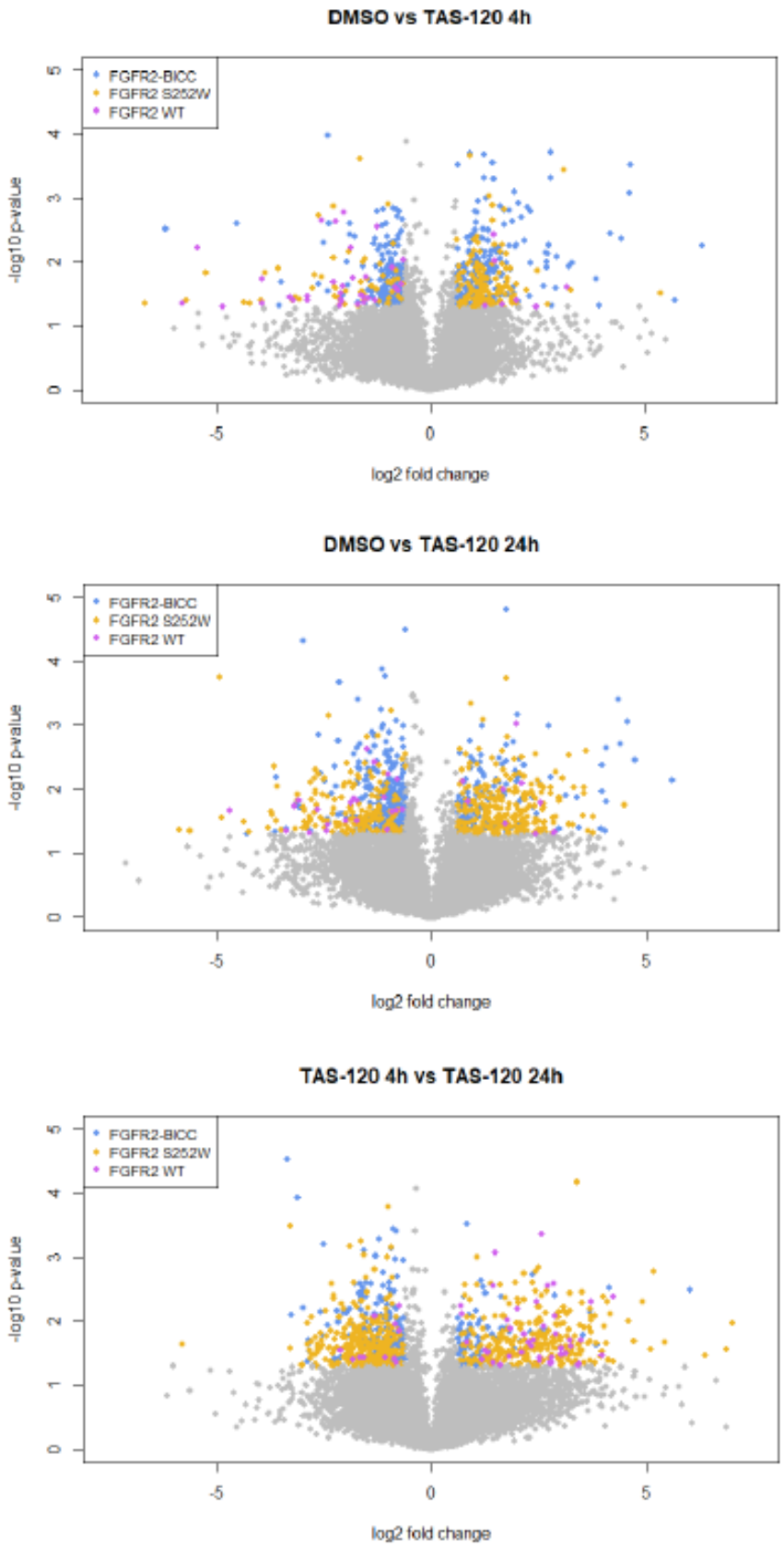

Figure 3. Global phosphorylation analysis in MMNK1 isogenic cells. Changes in phosphorylation sites between conditions in MMNK1 cells containing FGFR2 WT, FGFR2 S252W, and FGFR2-BICC. MMNK1 clones were treated with DMSO or 50nM TAS-120 for 4 hours or 24 hours in triplicate, harvested, and prepped for phosphoproteomics. Volcano plots were generated using the log2 fold changes and p-values of phosphorylation sites between conditions in each MMNK1 clone

Drs. Shokat/Gordan led these studies with assistance of Dr. Bardesesy

Figure 4

Gene/kinase		FGFR2 S252W			FGFR2-BICC		
		DMSO-TAS4h	DMSO-TAS24h	TAS4h-TAS24h	DMSO-TAS4h	DMSO-TAS24h	TAS4h-TAS24h
	MTOR	-0.482	0.191	0.362	0.454	0.257	-0.443
	AKT1	**-0.489**	0.274	**0.423**	-0.314	-0.324	-0.229
	MOS	-0.565	-0.722	-0.619	0.837	**0.972**	0.930
	PIM1	**0.883**	**0.965**	0.442	-0.845	**-0.918**	0.526
CTNNB1	P35222_T551	0.606	0.404	-0.203	-0.288	0.168	0.455
RB1	P06400_T823	**-1.63**	**1.75**	**3.38**	-0.408	-0.508	-0.0997
PAK2	Q13177_S2	0.493	1.18	0.69	1.18	**-1.68**	**-2.86**
PGK1	P00558_S203	**-0.782**	**0.605**	**1.39**	-1.15	**-1.67**	-0.521
BAD	Q92934_S118	-0.60	1.19	1.79	-1.05	**-1.04**	0.007
PKN1	Q16512_S562	-0.158	-2.20	**-2.05**	-0.389	-0.359	0.031
SCRIB	Q14160_S1508	-0.486	1.49	1.98	-0.609	-0.682	-0.072
PXN	P49023_S302	-0.816	0.379	1.20	0.531	0.706	0.175

Figure 4. Log2 fold changes of selected kinases and phosphorylation sites of interest between MMNK1 cells containing FGFR2 S252W and FGFR2-BICC.

Phosphoproteomics datasets for MMNK1+FGFR2 S252W and MMNK1+FGFR2-BICC were analyzed and compared for differences in potential key effectors of FGFR2. Significant log2 fold changes (p-value < 0.05) are in bold.

Drs. Shokat/Gordan led these studies with assistance of Dr. Bardeesy

Figure 5

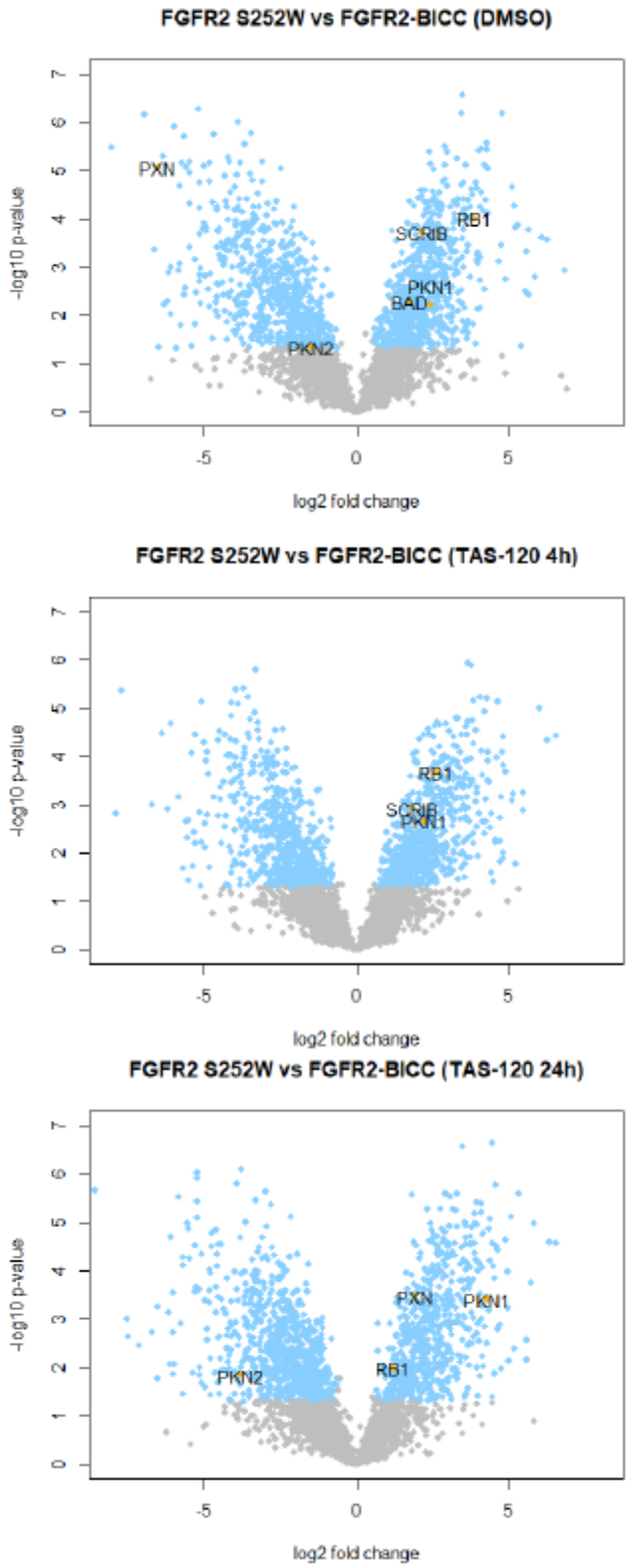

Figure 5. Selected significant phosphorylation changes between MMNK1+FGFR2 S252W and MMNK1+FGFR2-BICC at baseline and following TAS-120 treatment. Phosphoproteomic data for MMNK1 cells with FGFR2 S252W and FGFR2-BICC were re-analyzed with MSstats, comparing the phosphorylation changes between the two mutants for each condition. Proteins of significantly altered phosphorylation sites of interest were annotated.

Dr. Shokat/Gordan led these studies with assistance from Dr. Bardeesy

Figure 6

ID	Genetics
SS106	GNAS; KRAS; CTNNB1
MG10	BRAF
MG94	BRAF
MG59-7	FGFR2-OPTN
CHCA1	TP53 and CDKN2A
CHCA5	BRCA2, EGFR, STK11, CDKN2A, MSH6, SMAD4, TP53
MG69	FGFR2-KIAA1217

Figure 6. Generation of new biliary tract cancer patient-derived xenograft (PDX) models

List of new biliary tract cancer PDXs and associated mutations in major oncogenes/tumor suppressors.

Dr. Bardeesy Lab led these studies with assistance from Dr. Zhu

Figure 7

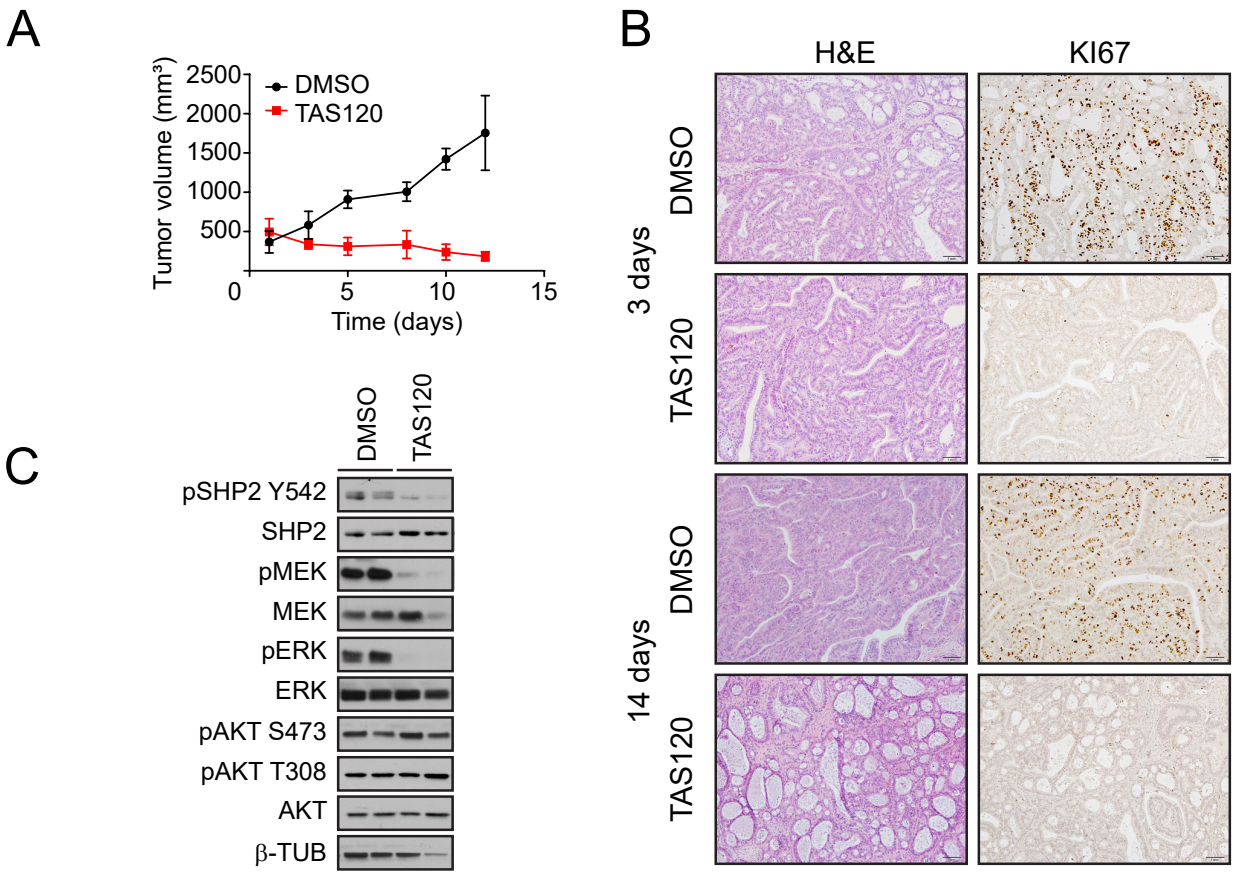

Figure 7. Murine co-clinical trial of FGFR inhibitors

A. Tumors were implanted in NOG-SCID mice and treatment was begun when tumor reached ~300 mm3, using vehicle or TAS-120. Tumor volumes were measured at the indicated days.
B. Histologic images (H&E staining) and measurement of proliferation (Ki67 staining) of tumors isolated after 3 days and 14 days of treatment.
C. Immunoblot data showing signaling inhibition upon TAS-120 treatment (samples are from 3 days treatment).

Dr Bardeesy led these studies with assistance from Dr. Zhu

Figure 8

Table 1a: Clinical Data of Patients with FGFR2 Fusion Positive Cholangiocarcinoma Receiving FGFR Inhibitors

Patient ID	FGFR2 Fusion	1st FGFR Inhibitor	PFS (Months)	ORR	Intervening Therapies Between 1st and 2nd FGFRi	Interval Between 1st and 2nd FGFRi (Months)	2nd FGFR Inhibitor	PFS (Months)	ORR
1	FGFR2-ZMYM4	BGJ398	5.57	-49.9%	None	1.60	TAS120	7.23	+8.30%
2	FGFR2-SORBS1	BGJ398	12.57	-68.2%	None	1.20	TAS120	15.83	-76.7%
3	FGFR2-NRAP	BGJ398	7.13	-40.0%	T8 palliative radiation, Pembrolizumab, Resection of T8 metastasis, FOLFOX	7.43	TAS120	13.03+ (Ongoing)	-47.7%
4	FGFR2-INA	Debio1347	12.63	-46.0%	Gem/Docetaxel, T11 palliative radiation	3.27	TAS120	5.10	-22.1%

Table 1b: FGFR2 mutations detected in cfDNA and tumor biopsies

Patient ID	FGFR2 Fusion	Post-progression BGJ398/Debio1347, Prior to TAS-120		Post-progression TAS-120	
		cfDNA	Tumor Biopsy	cfDNA	Tumor Biopsy
1	FGFR2-ZMYM4	V564F, K659M, E565A, N549H, N549K	V564F, K659M	V564F, K659M, E565A, N549H, N549K, V562L	V562L
2	FGFR2-SORBS1	K659M, K714R	None detected	V564F	V564F
3	FGFR2-NRAP	None detected	No biopsy obtained	Response ongoing	Response ongoing
4	FGFR2-INA	H682L, L617V	Biopsy #1: H682L Biopsy #2: N549H, N549T, M537I	V564L, E565A, N549H, N549T, L617V	No biopsy obtained

Figure 8. Evaluation of resistance mechanisms in FGFRi clinical trials

1a. Clinical characteristics and outcomes of patients with FGFR2 Fusion Positive Cholangiocarcinoma receiving FGFR inhibitors.
1b. Detection of FGFR2 mutations in ctDNA and tumor biopsies

Dr. Zhu led these studies.

Figure 9

ID	predicted FGFRi response
MG98-2	resistant
MG98-3	resistant
MG98-8	resistant
MG98-6	resistant
MG26	sensitive

Figure 9. Development of PDX models from patients enrolled in FGFRi clinical trials

The MG98 series of models were derived from a patient who acquired resistance to the FGFRi, TAS-120. The MG26 model is from a patient who subsequently went on to an FGFRi clinical trial.

Dr. Bardeesy led these studies with assistance of Dr. Zhu.

AD_____

AWARD NUMBER: GRANT12248945, GRANT12248894, GRANT12248883
Log Number: CDMRP Log Number: CA160216

TITLE: A Proteomic Co-clinical Trial of BGJ-398 in FGFR-Driven Biliary Cancers

PRINCIPAL INVESTIGATOR: Kevan Shokat

RECIPIENT: Emilee Senkevitch

REPORT DATE: August 28, 2018

TYPE OF REPORT: Annual Report

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

DISTRIBUTION STATEMENT: A

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STANDARD FORM 298: Sample SF 298 is provided at https://mrmc.amedd.army.mil/rrpindex.asp. The abstract shall be provided in Block 14 and shall state the purpose, scope, and major findings and be an up-to-date report of the progress in terms of results and significance. Abstracts will be submitted to the Defense Technical Information Center (DTIC) and shall not contain proprietary information. Subject terms are keywords that may have been previously assigned to the proposal abstract or are keywords that may be significant to the research. The number of pages shall include all pages that have printed data (including the front cover, SF 298, table of contents, and all appendices). Count pages carefully to ensure legibility and that there are no missing pages as this delays processing of reports. Page numbers shall be typed; do not hand number pages.

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

Intrahepatic cholangiocarcinomas (ICC) is a dead malignancy of the liver bile ducts (<10% 5-year survival for patients of all stages) with few therapeutic options for advanced disease. The project involves translational studies relating targeting the Fibroblast Growth Factor Receptor (FGFR) signaling pathway in ICCs harboring alterations in the FGFR pathway. FGFR alterations (most commonly fusions of the FGFR2 gene that result in activation of the FGFR2 kinase) are present in ~20% of ICCs and clinical trials with FGFR kinase inhibitors are showing promise in these patients. However, resistance inevitably arises, limiting therapeutic efficacy. The goals of this project are to understand the basis of FGFR signaling dependency in FGFR-activated ICC, to elucidate mechanisms of clinical acquired resistance, and to improve therapeutic strategies against this subset of patients that improve initial responses and overcome resistance. The scope of work includes conducting sequencing studies of patient samples pre- and post-progression to identify potential resistance mechanisms, generating patient-derived models and utilizing sophisticated proteomics and genetic approaches to decipher FGFR-controlled signaling networks and to validate and understand resistance mechanisms, and to use the signaling information and results from drug screens to identify combinations therapies that prevent or overcome resistance.

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

Biliary tract cancer, intrahepatic cholangiocarcinoma, FGFR, kinase, cancer genomics, phosphoproteomics, patient-derived models, signal transduction, acquired resistance, co-clinical trials

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

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

Specific Aim 1: Determine the impact of distinct modes of FGFR activation on signaling output and drug response

Major Task 1: Characterize basal signaling from treatment naïve FGFR-driven biliary cancer cell models. Time line: months 1-24. Completion to date: 66% (4/6 components)

Major Task 2: Analyze differential signaling feedback driven by genetic activation of FGFR2. Time line: months 1-24. Compeletion to date: 33% (1/3 components)

Major Task 3: Characterize novel mechanisms of FGFR-driven liver cancer growth. Time line: months 3-36. Completion to date: 12.5% (0.5/4 components)

Specific Aim 2: Co-clinical trials to determine the cell biologic & molecular impact of FGFRi in vivo, and assess effect of concurrent genetic alterations.

Major Task 4: Murine co-clinical trial of BGJ-398. Time line: 1-28 months. Completion to date: 25% (0.5/2 major component; minor component = regulatory approval also complete)

Major Task 5: Phenotypic and biochemical characterization of BGJ-398 response. Time line: months 12-30. Completion to date: 25% (0.5/2 components). Ahead of schedule

Major Task 6: Model effect of concurrent genetic alterations on BJG-398 response. Time line: months 6-36. Completion to date: 2.5% (0.1/4 components).

Specific Aim 3: Characterize polyclonal drug resistance and identify novel therapeutic vulnerabilities in FGFR2-driven BTC.

Major Task 7: Characterize genetic and signaling changes driven by FGFR inhibition for hepatobiliary cancer patients in clinical trials. Time line: Months 1-36. Completion to date: ~33% (~1.33/4 components)

Major Task 8: Characterize signaling effects of treatment associated mutations. Time Line: Months 13-36. Completion to date: ~10% (ahead of schedule)

Major Task 9: Overcoming resistance with next generation FGFRi. Time line: months 12-30. Completion to date: not started

Major Task 10: Overcoming resistance with combination drug screens. Time line: months 13-36. Completion to date: not started

What was accomplished under these goals?

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

Specific Aim 1: Determine the impact of distinct modes of FGFR activation on signaling output and drug response

Major Task 1: Characterize basal signaling from treatment naïve FGFR-driven biliary cancer cell models

a) Obtain local IRB approval and federal HRPO for use of human tumor tissue, including making PDXs/cell lines and genetic analysis.

We obtained these approvals: Local IRB: DFCI Protocol No.: 13-416.

HRPO: Assigned Number: A-20290.1 (Bardeesy) A-20291.1 (Zhu)

b) Convert extant FGFR-driven PDXs to 2-D cell lines (at least two new lines).

There is a critical need to establish new models of ICC, given the lack of such models in standard repositories. There are no published FGFR-driven ICC models. We have established three new patient-derived lines harboring FGFR2 fusions: ICC13-7 (FGFR2-OPTN) ICC10 (FGFR2-PHGDH), and ICC11 (FGFR2-PHGHD). We also identified an existing ICC cell line that massively overexpresses FGFR1 (CCLP). The creation and characterization of these models is a significant advance in the translational study of this important subset of ICC. Data are summarized in ***Figure 1.***

c) Develop isogenic immortalized biliary lines preferably using MMNK1 or H89 cells (available through academic cell banks) with WT, point mutants or fusions of FGFR2.
This sub-aim and (d), below, seek to define FGFR signaling outputs in the biliary epithelium driven by different ICC-associated FGFR2 fusions or point mutations in order to help define their underlying oncogenic mechanisms We have established and characterized MMNK1 derivative cell lines expressing FGFR2-wild type, FGFR2-S252W, FGFR2-BICC1, FGFR2-OPTN, and FGFR2-PHGDH via lentiviral transduction. This system provides a physiologically relevant context non-malignant context (biliary epithelial cells) to explore FGFR pathway activation using different activated forms of FGFR2 observed in human cancer.

d) Proteomic analysis of global phosphorylation of isogenic cells at baseline and following treatment with BGJ-398 and/or TAS-120.
Since FGFR2 is a kinase, we seek to understand the signaling cascades it generates to drive malignancy. To this end, we have conducted phosphoproteomics on two of the cell lines from (c) at baseline or following TAS-120 treatment. While further data analysis will commence in Y2, we already see very significant signaling difference between the two FGFR2 allelles, supporting the rationale of these studies. ***Please see Figures 2-5.***

Major Task 2: Analyze differential signaling feedback driven by genetic activation of FGFR2

a) Characterize sensitivity and kinetics of response to reversible and covalent FGFR2 inhibitors (BGJ-398, LY2874455, Debio-1347 and TAS-120) by measuring cell viability and changes in FRS phosphorylation.
Using our novel FGFR-activated ICC cell lines, we have sought to define whether FGFR signaling is required to support proliferation in vitro and to characterized the central downstream signaling pathways driven by FGFR in this context. These information are essential for understanding the mechanisms by which FGFR alterations promote ICC development and maintenance, and will be critical in the development of more effective treatment approaches as well as in the prediction of resistance mechanisms. We have tested a panel of FGFR inhibitors for their effects on the growth of CCLP and ICC13-7 cells as well as on the downstream signaling program. While ICC cell lines lacking molecular alterations that activate FGFR signaling are insensitive to FGFR inhibitors (e.g. for BGJ398, the IC50 is >1000 nM for each line), we found that CCLP and ICC13-7 are sensitive at less than 10 nM. Moreover, FGFR inhibition leads to a rapid and durable decrease in the key FGFR2 substrate FRS2, as well as of downstream phosphorylation of SHP2, MEK and ERK, where AKT activity was not effected. Thus, FGFR signaling is essential for maintenance of MEK/ERK signaling in FGFR-driven ICC, while the PI3K-AKT is not controlled by FGFR in these cells. ***Please see Figure 1B-F.***

Major Task 3: Characterize novel mechanisms of FGFR-driven liver cancer growth

a) Continued establishment of biliary & hepatocellular cancer PDX and cell line models & test for FGFRi response (at least 10 models derived from resected patients at Mass. Gen. Hospital)
Given the paucity of ICC models in general and of FGFR-driven models in particular, it is essential for us to continue to expand our collection of such models, in order to fully decipher the underlying signaling program in this subset of ICC and to understand how it compares with other ICC subsets. We have established 7 new PDX lines since the commencement of this grant, including 2 with FGFR alterations. Please see ***Figure 3***.

b) Genomic characterization of new tumors using mutation panel of 400 cancer genes. We have conducted genomic characterization of these models. Please see ***Figure 6.***

Specific Aim 2: Co-clinical trials to determine the cell biologic & molecular impact of FGFRi in vivo, and assess effect of concurrent genetic alterations

Major Task 4: Murine co-clinical trial of FGFR inhibitors

a) Obtain ACURO approval for therapeutics studies in mice.
We have obtained approval: Protocol [ACURO Assigned Number]: CA160216 Title: Mouse Models of Cancer

b) Murine co-clinical trial testing FGFR inhibitors in in vivo models harboring FGFR alterations. The availability of preclinical models of FGFR-activated ICC allows us to overcome the challenges of studying how FGFR inhibitors affect ICCs at the molecular and cell biological level in patient samples (i.e. repeat biopsies are very limited and are usually confined to a single pre-treatment and post-progression biopsy, and thus the acute effects of a medicine cannot be evaluated mechanistically). To address this, we have completed a trial with the FGFR inhibitor, TAS-120, in our FGFR2-KIAA expressing PDX model, MG69. Mice were treated with the drug or vehicle control when tumor reached ~ 400 mm3. Mice were treated for 14 days for efficacy studies, with serial measurements of tumor volume. A subset of mice were euthanized at serial time points to isolate tumors for molecular analyses and histology (3 days and 14 days). We observed a complete block in tumor growth in this model. Please see ***Figure 7A.*** The analysis of specimens from this study will allow us to have unparalleled understanding of FGFR signaling 'addiction' in ICC.

Major Task 5: Phenotypic and biochemical characterization of FGFR inhibitor response

a) Correlative molecular and histological analysis of samples of co-clinical trials (see Major Task 4, part (b) above.
We have examined the tumors from the MG69 model treated with TAS-120. Histological assessment demonstrates evidence of tumor cell differentiation. Accordingly, we have seen a remarkable complete loss of proliferation (Ki-67 staining) as rapidly as 3 days after the start of treatment. By contrast, we do not observe apoptosis (cleaved caspase-3). Analysis of signaling changes, revealed loss of p-MEK, p-ERK, and p-SHP2, but no change in pAKT, mirroring the in vitro data. These data suggest that while FGFR inhibition has dramatic effects in vivo, completing arresting tumor cell proliferation and shutting down signaling to the MEK/ERK pathway, it does not appear to incite tumor cell death acutely. Thus, while there is true 'oncogene addiction', the persistence of growth inhibited cells may ultimately drive recurrence. These data support the search for drug combination strategies that synergize with FGFR inhibition to drive tumor cell death. (please see ***Figure 7B and C***).

Major Task 6: Model effect of concurrent genetic alterations on BJG-398 response.
a) Perform gene editing to generate ICC cell lines harboring inactivating mutations in common ICC tumor suppressor genes (e.g. PTEN, ARID1A)
The goal of these studies is to test whether specific mutations that co-exist with FGFR pathway alterations in subsets of ICC can influence FGFR inhibitor sensitivity. To address this, we are using gene editing strategies in FGFR inhibitor sensitive ICC cell lines and evaluating the resulting impact on drug sensitivity. To date, we have creates sgRNA vectors to target PTEN. Use of these tools will proceed in the upcoming year.

Major Task 7: Characterize genetic and signaling changes driven by FGFR inhibition for hepatobiliary cancer patients in clinical trials

a) Collect blood and tumor specimens from patients on study of BGJ-398, TAS-120, other FGFR inhibitors. Blood and tumor material will be collected pre-treatment. Blood will be collected approximately monthly throughout the course of treatment. A second biopsy will be performed upon disease progression.
Our robust translational pipeline enables us to isolate and study patient specimens from serial time points during therapy. In the past year, we have prepared a set of pre- and post-treatment specimens from 8 patients.

b) Next generation sequencing of ctDNA and tumor biopsy specimens.
Using 4 of the samples above, we have conducted sequencing that has identified multiple candidate resistance mechanisms. These include multiple mutations in the FGFR2 kinase domain. Please see ***Figure 8***.

c) Develop PDX models of treatment naïve and resistant tumor specimens (at least 3 models from patients on FGFR inhibitor clinical trials).
The availability of new models from patients at various stages of treatment enables powerful co-clinical trials that model the underlying biology of response. We have developed 5 PDX models from patients (1 treatment naïve and 4 resistant). These will be employed in molecular and functional studies in the upcoming year. Please see ***Figure 9***.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

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

The project has provide training opportunities for Dr. Lipika Goyal and John Gordan, who were promoted to attending physicians during the prior year. Both work closely with Dr. Bardeesy, Zhu, and Shokat who serve as mentors and have regular in-person meetings as well as joint teleconferences to trouble-shoot and discuss progress and future directions.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

The work was presented in the 2018 Cholangiocarcinoma Foundation Annual Meeting in Salt Lake City, January 30-Feb 1st, 2018. This meeting consists both of a regular scientific conference with leading researchers from around the world presenting cutting-edge discoveries and clinical progress as well as a full day devoted to outreach and education for patients and their families and caregivers have the opportunity to hear talks given with layman's language relating to the disease and emerging research in the area.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

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

Major focus of the upcoming year are to build upon our phosphoproteomics data, genomic information regarding potential resistance mechanisms using our wealth of novel models to fully decipher the circuits downstream of FGFR that support ICC growth, to credential candidate resistance mechanisms and to understand them functionally and to uncover approaches to prevent and overcome resistance based on signaling changes identified in our work and on new drug screens. Finally, since ICC is genetically heterogeneous in general, and among the subset of ICC with FGFR alterations, and since the mechanisms of resistance we are uncovering are also diverse, we will continue to prioritize model development in order to have systems that appropriately mirror the diverse presentation of the disease in the patient population.

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

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

Medicines that inhibit the FGFR signaling pathway (FGFR inhibitors) are showing promise in patients with ICC that harbor FGFR alterations. Unfortunately, patients eventually relapse due to the acquisition of drug resistance. Our work has provide key insights into the main resistance mechanisms assoiciated with different FGFR inhibitors. These findings help to physicians anticipate when treatment failure is occurring and guide treatment with alternative FGFR inhibitors. In addition, the wealth of model systems we have developed together with advanced methods in understanding protein function (phosphoproteomics) provide us with unprecedented opportunities to understand why FGFR inhibitors are initially so effective in these patients and why they ultimately fail. They also enable us to use genetic methods and drug screening approaches to discover the next generation of therapies that will boost the effect of the FGFR inhibitors, preventing resistance from occurring or overcoming it once it arises.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

Our work is focused on liver cancers with FGFR alterations. However, since the FGFR signaling pathway is also deregulated in multiple other cancer types (bladder, breast, stromach, lung, and others), the insights from our data will help understand FGFR inhibitor response and resistance in these other settings as well.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

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

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

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

Nothing to report

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

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

Nothing to report

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

Nothing to report

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

Nothing to report

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

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

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

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

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

Nothing to report.

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

Nothing to report

  Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

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

Nothing to report

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

Nothing to report

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

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*

- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

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

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Nabeel Bardeesy
Project Role: PI
Researcher Identifier;
Nearest person month worked:
Contribution to project: Led the model development and characterization efforts. Assayed FGFR sensitivity in vitro and in vivo. Conducted in vitro and in vivo signaling analyses. Credentialled FGFR kinase domain mutations.
Name: Andrew Zhu
Project Role: PI.
Researcher Identifier;
Nearest person month worked:
Contribution to project: Supervised translational efforts using patient samples.

Name: Kevan Shokat
Project Role: PI
Researcher Identifier
Nearest person month worked:
Contribution to project: Supervised proteomics efforst
Name: Krishna Tummala
Project Role: Postdoc
Researcher Identifier;
Nearest person month worked:
Contribution to project: Conducted signaling studies, established and studied models.
Name: John Gordan
Project Role: Instructor
Researcher Identifier;
Nearest person month worked:
Contribution to project: Developed biliary cell models and conducted and analyzed phosphoproteomics
Name: Lipika Goyal
Project Role: Instructor
Researcher Identifier;
Nearest person month worked:
Contribution to project: Conducted and coordinate clinical studies including sample acquisition and sequencing analysis.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

Dr. Bardeesy was awarded the grant below. It does not impact effort on the present DOD grant.

R01 CA215498-01A1 Bardeesy (PI)
Functions of the LKB1 tumor suppressor in control in metabolism and epigenetics
The goal of this project is to define to circuits downstream of LKB1 mediating tumor suppression, including the roles for altered cell metabolism and its interplay to epigenetic regulation.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

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

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

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

We have submitted reports for the PI (Bardeesy) and partnering PI's (Zhu and Shokat)

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

9. APPENDICES:

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

Figure 1

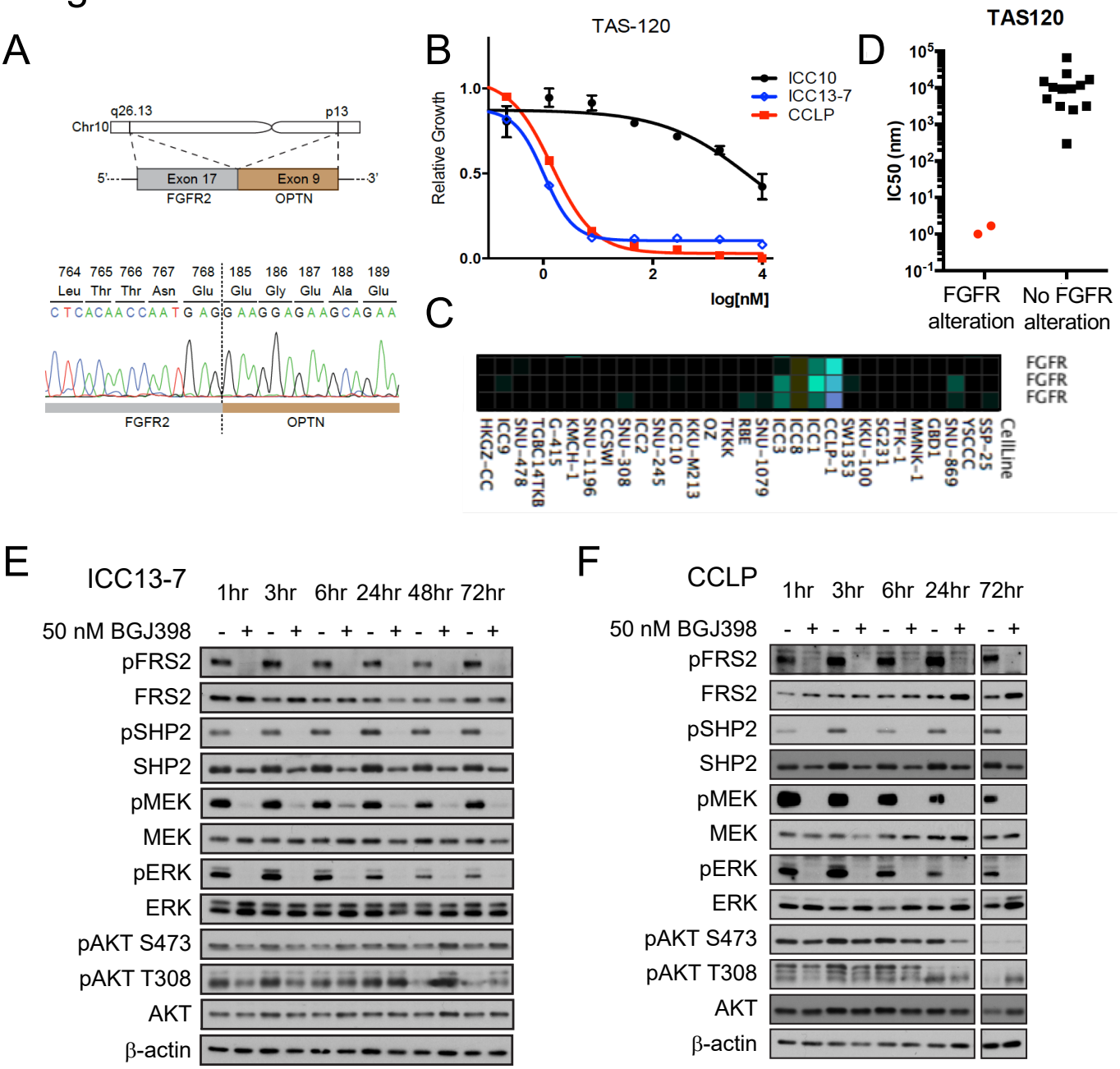

Figure 1. Conversion of existent FGFR-driven ICC to 2-D cell lines.
A. We derived the ICC13-7 cell line from a PDX model. The graphic shows the structure of the FGFR2-OPTN fusion that we detected in this cell line. We also generated a 2-D cell line (ICC10) from a second PDX model and found that it had an FGFR2-PHGDH fusion (not shown).
B. We tested the response of ICC10, ICC13-7, and CCLP cells to TAS-120 (IC50 is graphed).
C. Heatmap of a set of ICC cell lines screened for sensitivity to three FGFR inhibitors (TAS-120, Debio1347 and BGj398). Only CCLP is sensitive in the set shown.
D. Graph of IC50 data ICC cell lines with FGFR alterations (ICC13-7 and CCLP) and those lacking such alterations.
E,F. Immunoblot of signaling effects of BGJ398 treatment of ICC13-7 cells (E) and CCLP cells (F).

Dr. Bardeesy led these studies with assistance of Dr. Zhu and Shokat/Gordan

Figure 2

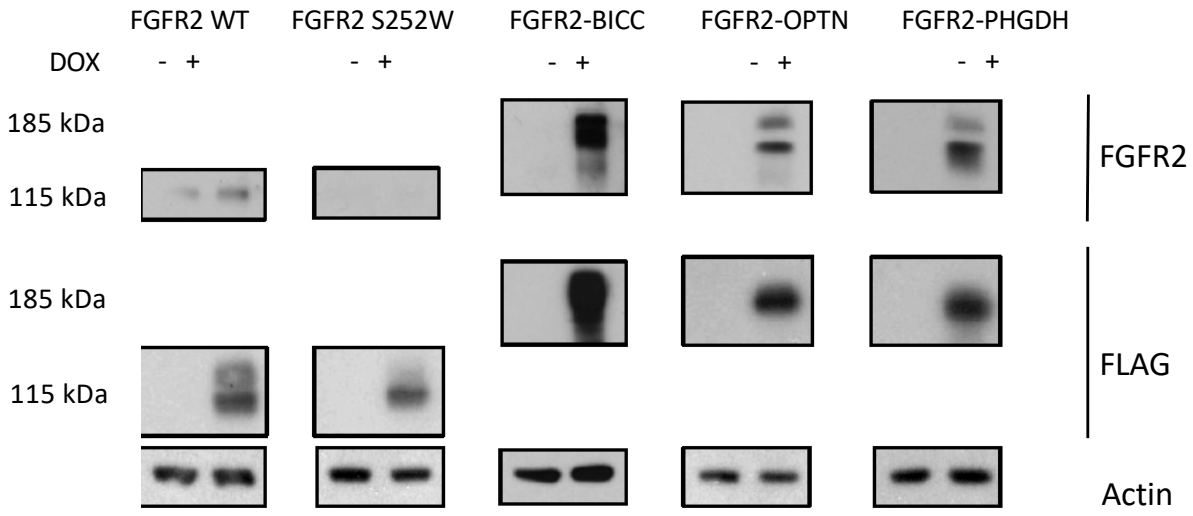

Figure 2. Generation of isogenic FGFR2 WT, FGFR2 S252W, FGFR2-BICC, FGFR2-OPTN, and FGFR2-PHGDH MMNK1 cells.

FGFR2 WT and mutants were cloned into a doxycycline (dox)-inducible puromycin-resistant destination vector with Gateway Cloning, packaged into lentivirus, and transduced into MMNK1 cells. Transduced cells were stably selected with puromycin and plated into 10cm plates with either 1mg/mL dox or no dox. After over 24 hours of dox induction, cells were harvested, lysed, and analyzed by western blot. Membranes were blotted for FGFR2, FLAG, and Actin.

Drs. Shokat/Gordan led these studies with assistance of Dr. Bardeesy

Figure 3

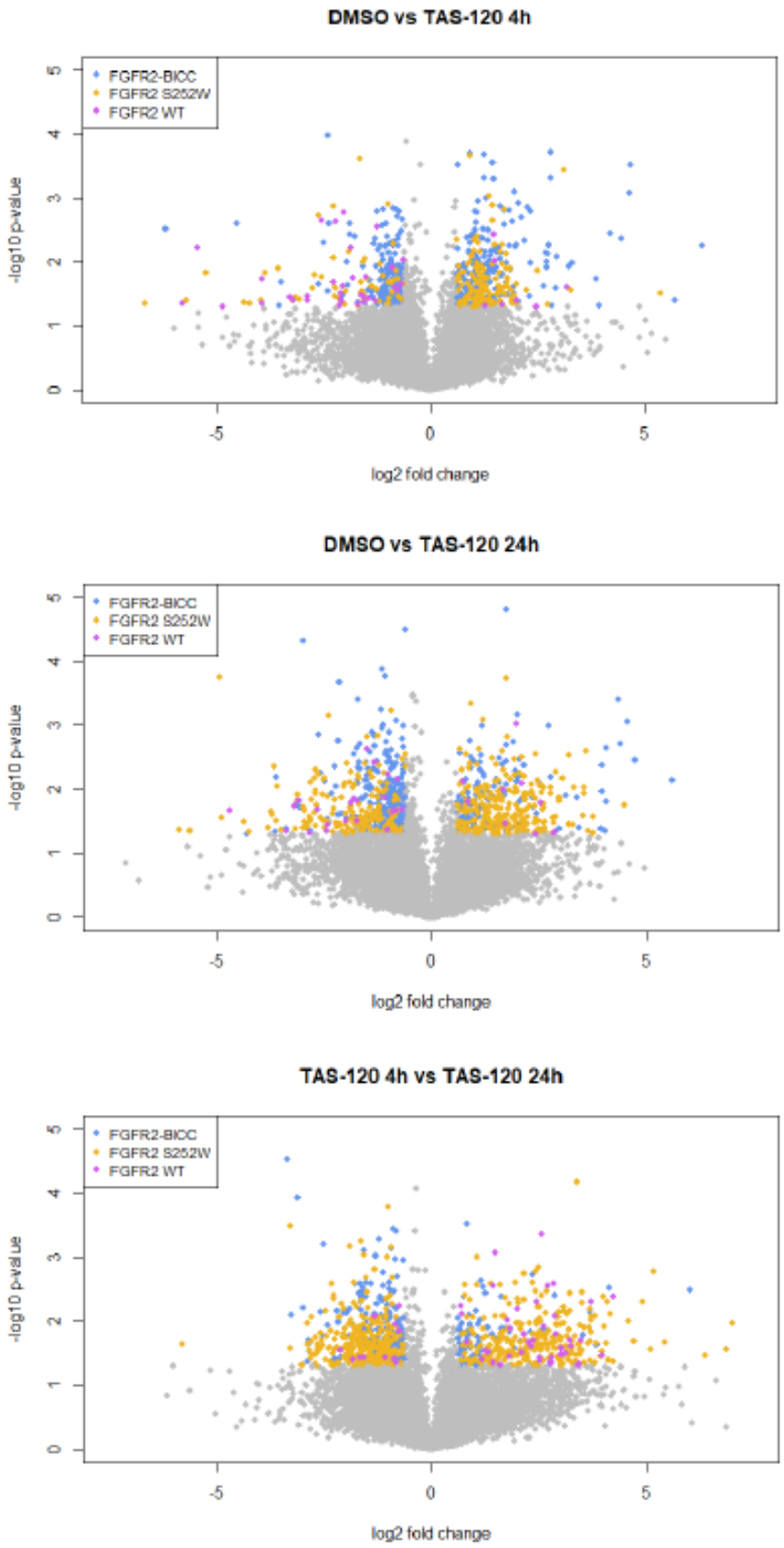

Figure 3. Global phosphorylation analysis in MMNK1 isogenic cells. Changes in phosphorylation sites between conditions in MMNK1 cells containing FGFR2 WT, FGFR2 S252W, and FGFR2-BICC. MMNK1 clones were treated with DMSO or 50nM TAS-120 for 4 hours or 24 hours in triplicate, harvested, and prepped for phosphoproteomics. Volcano plots were generated using the log2 fold changes and p-values of phosphorylation sites between conditions in each MMNK1 clone

Drs. Shokat/Gordan led these studies with assistance of Dr. Bardesesy

Figure 4

Gene/kinase		FGFR2 S252W			FGFR2-BICC		
		DMSO-TAS4h	DMSO-TAS24h	TAS4h-TAS24h	DMSO-TAS4h	DMSO-TAS24h	TAS4h-TAS24h
	MTOR	-0.482	0.191	0.362	0.454	0.257	-0.443
	AKT1	**-0.489**	0.274	**0.423**	-0.314	-0.324	-0.229
	MOS	-0.565	-0.722	-0.619	0.837	**0.972**	0.930
	PIM1	**0.883**	**0.965**	0.442	-0.845	**-0.918**	0.526
CTNNB1	P35222_T551	0.606	0.404	-0.203	-0.288	0.168	0.455
RB1	P06400_T823	**-1.63**	**1.75**	**3.38**	-0.408	-0.508	-0.0997
PAK2	Q13177_S2	0.493	1.18	0.69	1.18	**-1.68**	**-2.86**
PGK1	P00558_S203	**-0.782**	**0.605**	**1.39**	-1.15	**-1.67**	-0.521
BAD	Q92934_S118	-0.60	1.19	1.79	-1.05	**-1.04**	0.007
PKN1	Q16512_S562	-0.158	-2.20	**-2.05**	-0.389	-0.359	0.031
SCRIB	Q14160_S1508	-0.486	1.49	1.98	-0.609	-0.682	-0.072
PXN	P49023_S302	-0.816	0.379	1.20	0.531	0.706	0.175

Figure 4. Log2 fold changes of selected kinases and phosphorylation sites of interest between MMNK1 cells containing FGFR2 S252W and FGFR2-BICC.

Phosphoproteomics datasets for MMNK1+FGFR2 S252W and MMNK1+FGFR2-BICC were analyzed and compared for differences in potential key effectors of FGFR2. Significant log2 fold changes (p-value < 0.05) are in bold.

Drs. Shokat/Gordan led these studies with assistance of Dr. Bardeesy

Figure 5

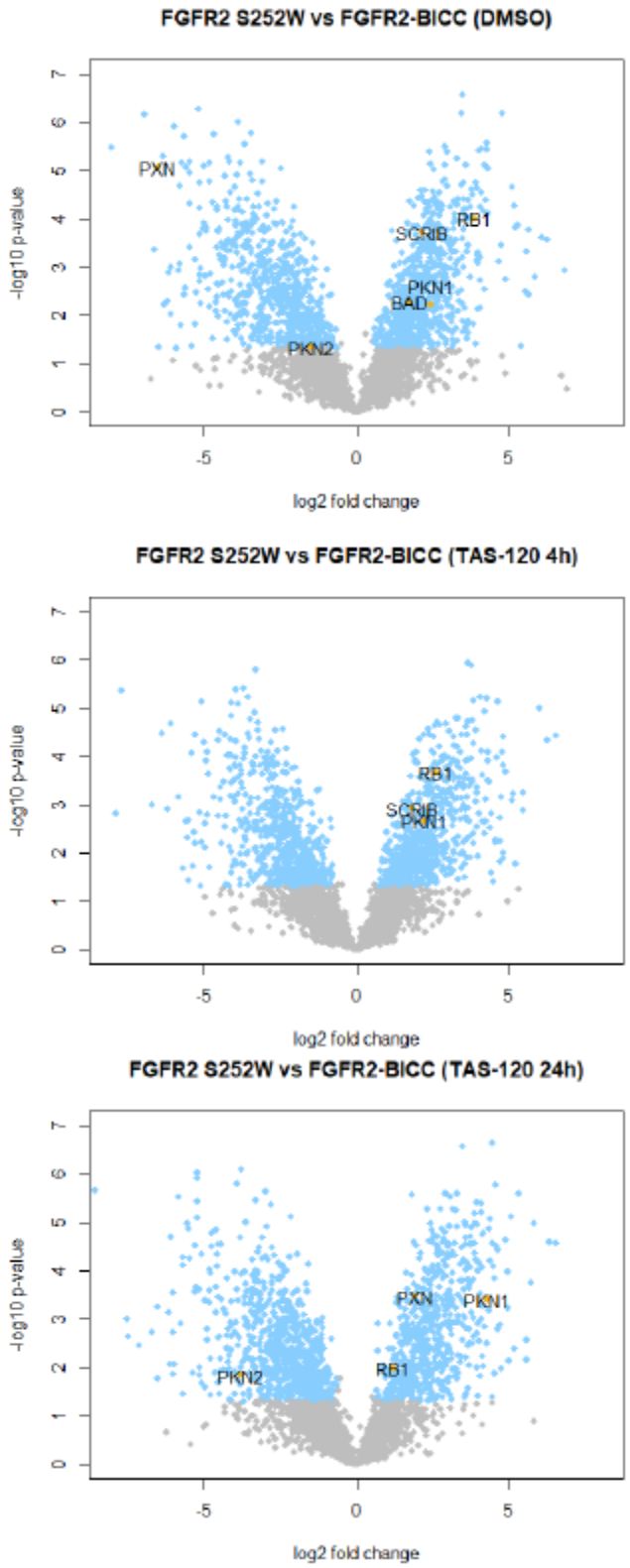

Figure 5. Selected significant phosphorylation changes between MMNK1+FGFR2 S252W and MMNK1+FGFR2-BICC at baseline and following TAS-120 treatment. Phosphoproteomic data for MMNK1 cells with FGFR2 S252W and FGFR2-BICC were re-analyzed with MSstats, comparing the phosphorylation changes between the two mutants for each condition. Proteins of significantly altered phosphorylation sites of interest were annotated.

Dr. Shokat/Gordan led these studies with assistance from Dr. Bardeesy

Figure 6

ID	Genetics
SS106	GNAS; KRAS; CTNNB1
MG10	BRAF
MG94	BRAF
MG59-7	FGFR2-OPTN
CHCA1	TP53 and CDKN2A
CHCA5	BRCA2, EGFR, STK11, CDKN2A, MSH6, SMAD4, TP53
MG69	FGFR2-KIAA1217

Figure 6. Generation of new biliary tract cancer patient-derived xenograft (PDX) models

List of new biliary tract cancer PDXs and associated mutations in major oncogenes/tumor suppressors.

Dr. Bardeesy Lab led these studies with assistance from Dr. Zhu

Figure 7

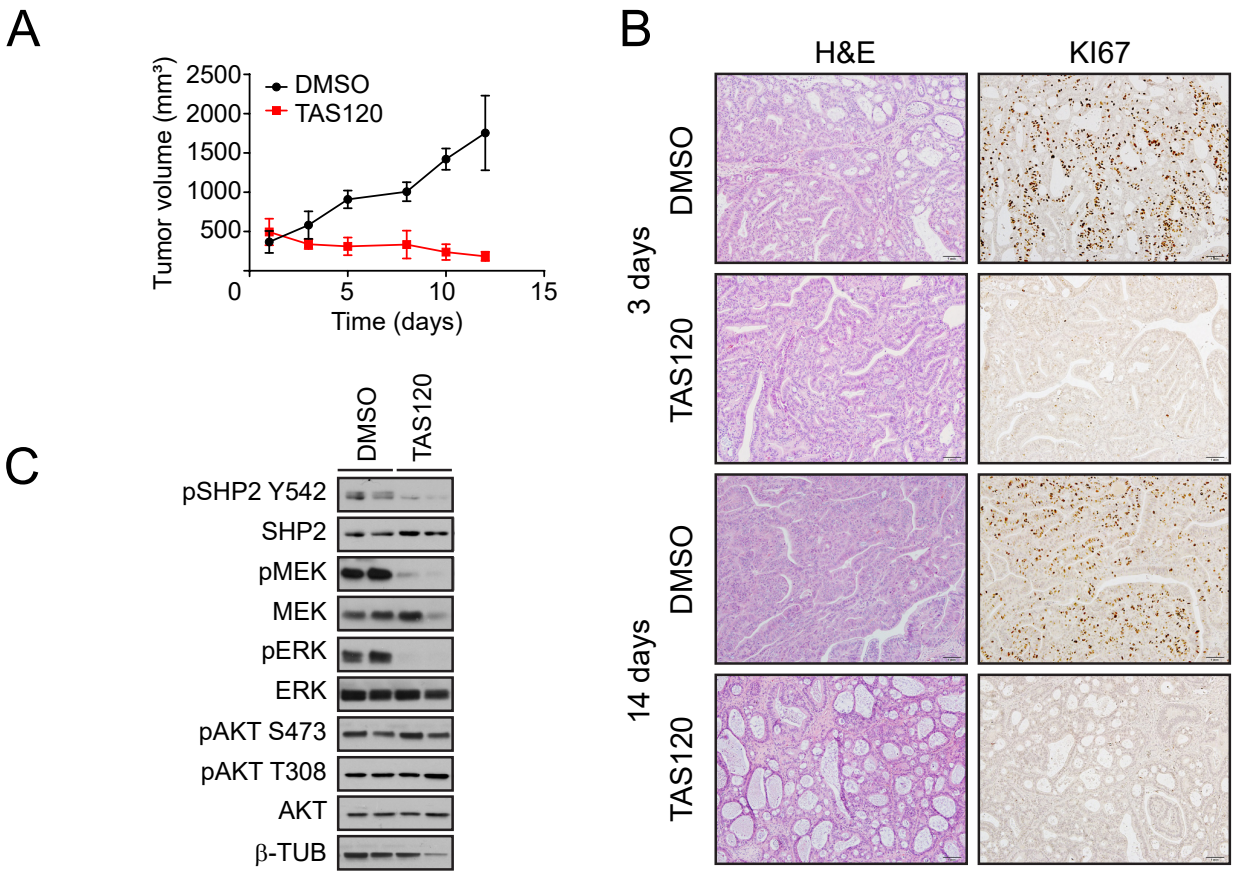

Figure 7. Murine co-clinical trial of FGFR inhibitors

A. Tumors were implanted in NOG-SCID mice and treatment was begun when tumor reached ~300 mm3, using vehicle or TAS-120. Tumor volumes were measured at the indicated days.
B. Histologic images (H&E staining) and measurement of proliferation (Ki67 staining) of tumors isolated after 3 days and 14 days of treatment.
C. Immunoblot data showing signaling inhibition upon TAS-120 treatment (samples are from 3 days treatment).

Dr Bardeesy led these studies with assistance from Dr. Zhu

Figure 8

Table 1a: Clinical Data of Patients with FGFR2 Fusion Positive Cholangiocarcinoma Receiving FGFR Inhibitors

Patient ID	FGFR2 Fusion	1st FGFR Inhibitor	PFS (Months)	ORR	Intervening Therapies Between 1st and 2nd FGFRi	Interval Between 1st and 2nd FGFRi (Months)	2nd FGFR Inhibitor	PFS (Months)	ORR
1	FGFR2-ZMYM4	BGJ398	5.57	-49.9%	None	1.60	TAS120	7.23	+8.30%
2	FGFR2-SORBS1	BGJ398	12.57	-68.2%	None	1.20	TAS120	15.83	-76.7%
3	FGFR2-NRAP	BGJ398	7.13	-40.0%	T8 palliative radiation, Pembrolizumab, Resection of T8 metastasis, FOLFOX	7.43	TAS120	13.03+ (Ongoing)	-47.7%
4	FGFR2-INA	Debio1347	12.63	-46.0%	Gem/Docetaxel, T11 palliative radiation	3.27	TAS120	5.10	-22.1%

Table 1b: FGFR2 mutations detected in cfDNA and tumor biopsies

Patient ID	FGFR2 Fusion	Post-progression BGJ398/Debio1347, Prior to TAS-120		Post-progression TAS-120	
		cfDNA	Tumor Biopsy	cfDNA	Tumor Biopsy
1	FGFR2-ZMYM4	V564F, K659M, E565A, N549H, N549K	V564F, K659M	V564F, K659M, E565A, N549H, N549K, V562L	V562L
2	FGFR2-SORBS1	K659M, K714R	None detected	V564F	V564F
3	FGFR2-NRAP	None detected	No biopsy obtained	Response ongoing	Response ongoing
4	FGFR2-INA	H682L, L617V	Biopsy #1: H682L Biopsy #2: N549H, N549T, M537I	V564L, E565A, N549H, N549T, L617V	No biopsy obtained

Figure 8. Evaluation of resistance mechanisms in FGFRi clinical trials

1a. Clinical characteristics and outcomes of patients with FGFR2 Fusion Positive Cholangiocarcinoma receiving FGFR inhibitors.
1b. Detection of FGFR2 mutations in ctDNA and tumor biopsies

Dr. Zhu led these studies.

Figure 9

ID	predicted FGFRi response
MG98-2	resistant
MG98-3	resistant
MG98-8	resistant
MG98-6	resistant
MG26	sensitive

Figure 9. Development of PDX models from patients enrolled in FGFRi clinical trials

The MG98 series of models were derived from a patient who acquired resistance to the FGFRi, TAS-120. The MG26 model is from a patient who subsequently went on to an FGFRi clinical trial.

Dr. Bardeesy led these studies with assistance of Dr. Zhu.

AD_____

AWARD NUMBER: GRANT12248945, GRANT12248894, GRANT12248883
Log Number: CDMRP Log Number: CA160216

TITLE: A Proteomic Co-clinical Trial of BGJ-398 in FGFR-Driven Biliary Cancers

PRINCIPAL INVESTIGATOR: Andrew Zhu

RECIPIENT: Emilee Senkevitch

REPORT DATE: August 28, 2018

TYPE OF REPORT: Annual Report

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

DISTRIBUTION STATEMENT: A

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STANDARD FORM 298: Sample SF 298 is provided at https://mrmc.amedd.army.mil/rrpindex.asp. The abstract shall be provided in Block 14 and shall state the purpose, scope, and major findings and be an up-to-date report of the progress in terms of results and significance. Abstracts will be submitted to the Defense Technical Information Center (DTIC) and shall not contain proprietary information. Subject terms are keywords that may have been previously assigned to the proposal abstract or are keywords that may be significant to the research. The number of pages shall include all pages that have printed data (including the front cover, SF 298, table of contents, and all appendices). Count pages carefully to ensure legibility and that there are no missing pages as this delays processing of reports. Page numbers shall be typed; do not hand number pages.

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

Intrahepatic cholangiocarcinomas (ICC) is a dead malignancy of the liver bile ducts (<10% 5-year survival for patients of all stages) with few therapeutic options for advanced disease. The project involves translational studies relating targeting the Fibroblast Growth Factor Receptor (FGFR) signaling pathway in ICCs harboring alterations in the FGFR pathway. FGFR alterations (most commonly fusions of the FGFR2 gene that result in activation of the FGFR2 kinase) are present in ~20% of ICCs and clinical trials with FGFR kinase inhibitors are showing promise in these patients. However, resistance inevitably arises, limiting therapeutic efficacy. The goals of this project are to understand the basis of FGFR signaling dependency in FGFR-activated ICC, to elucidate mechanisms of clinical acquired resistance, and to improve therapeutic strategies against this subset of patients that improve initial responses and overcome resistance. The scope of work includes conducting sequencing studies of patient samples pre- and post-progression to identify potential resistance mechanisms, generating patient-derived models and utilizing sophisticated proteomics and genetic approaches to decipher FGFR-controlled signaling networks and to validate and understand resistance mechanisms, and to use the signaling information and results from drug screens to identify combinations therapies that prevent or overcome resistance.

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

Biliary tract cancer, intrahepatic cholangiocarcinoma, FGFR, kinase, cancer genomics, phosphoproteomics, patient-derived models, signal transduction, acquired resistance, co-clinical trials

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

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

Specific Aim 1: Determine the impact of distinct modes of FGFR activation on signaling output and drug response

Major Task 1: Characterize basal signaling from treatment naïve FGFR-driven biliary cancer cell models. Time line: months 1-24. Completion to date: 66% (4/6 components)

Major Task 2: Analyze differential signaling feedback driven by genetic activation of FGFR2. Time line: months 1-24. Compeletion to date: 33% (1/3 components)

Major Task 3: Characterize novel mechanisms of FGFR-driven liver cancer growth. Time line: months 3-36. Completion to date: 12.5% (0.5/4 components)

Specific Aim 2: Co-clinical trials to determine the cell biologic & molecular impact of FGFRi in vivo, and assess effect of concurrent genetic alterations.

Major Task 4: Murine co-clinical trial of BGJ-398. Time line: 1-28 months. Completion to date: 25% (0.5/2 major component; minor component = regulatory approval also complete)

Major Task 5: Phenotypic and biochemical characterization of BGJ-398 response. Time line: months 12-30. Completion to date: 25% (0.5/2 components). Ahead of schedule

Major Task 6: Model effect of concurrent genetic alterations on BJG-398 response. Time line: months 6-36. Completion to date: 2.5% (0.1/4 components).

Specific Aim 3: Characterize polyclonal drug resistance and identify novel therapeutic vulnerabilities in FGFR2-driven BTC.

Major Task 7: Characterize genetic and signaling changes driven by FGFR inhibition for hepatobiliary cancer patients in clinical trials. Time line: Months 1-36. Completion to date: ~33% (~1.33/4 components)

Major Task 8: Characterize signaling effects of treatment associated mutations. Time Line: Months 13-36. Completion to date: ~10% (ahead of schedule)

Major Task 9: Overcoming resistance with next generation FGFRi. Time line: months 12-30. Completion to date: not started

Major Task 10: Overcoming resistance with combination drug screens. Time line: months 13-36. Completion to date: not started

What was accomplished under these goals?

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

Specific Aim 1: Determine the impact of distinct modes of FGFR activation on signaling output and drug response

Major Task 1: Characterize basal signaling from treatment naïve FGFR-driven biliary cancer cell models

a) Obtain local IRB approval and federal HRPO for use of human tumor tissue, including making PDXs/cell lines and genetic analysis.

We obtained these approvals: Local IRB: DFCI Protocol No.: 13-416.

HRPO: Assigned Number: A-20290.1 (Bardeesy) A-20291.1 (Zhu)

b) Convert extant FGFR-driven PDXs to 2-D cell lines (at least two new lines).

There is a critical need to establish new models of ICC, given the lack of such models in standard repositories. There are no published FGFR-driven ICC models. We have established three new patient-derived lines harboring FGFR2 fusions: ICC13-7 (FGFR2-OPTN) ICC10 (FGFR2-PHGDH), and ICC11 (FGFR2-PHGHD). We also identified an existing ICC cell line that massively overexpresses FGFR1 (CCLP). The creation and characterization of these models is a significant advance in the translational study of this important subset of ICC. Data are summarized in ***Figure 1.***

c) Develop isogenic immortalized biliary lines preferably using MMNK1 or H89 cells (available through academic cell banks) with WT, point mutants or fusions of FGFR2.
This sub-aim and (d), below, seek to define FGFR signaling outputs in the biliary epithelium driven by different ICC-associated FGFR2 fusions or point mutations in order to help define their underlying oncogenic mechanisms We have established and characterized MMNK1 derivative cell lines expressing FGFR2-wild type, FGFR2-S252W, FGFR2-BICC1, FGFR2-OPTN, and FGFR2-PHGDH via lentiviral transduction. This system provides a physiologically relevant context non-malignant context (biliary epithelial cells) to explore FGFR pathway activation using different activated forms of FGFR2 observed in human cancer.

d) Proteomic analysis of global phosphorylation of isogenic cells at baseline and following treatment with BGJ-398 and/or TAS-120.
Since FGFR2 is a kinase, we seek to understand the signaling cascades it generates to drive malignancy. To this end, we have conducted phosphoproteomics on two of the cell lines from (c) at baseline or following TAS-120 treatment. While further data analysis will commence in Y2, we already see very significant signaling difference between the two FGFR2 allelles, supporting the rationale of these studies. ***Please see Figures 2-5.***

Major Task 2: Analyze differential signaling feedback driven by genetic activation of FGFR2

a) Characterize sensitivity and kinetics of response to reversible and covalent FGFR2 inhibitors (BGJ-398, LY2874455, Debio-1347 and TAS-120) by measuring cell viability and changes in FRS phosphorylation.
Using our novel FGFR-activated ICC cell lines, we have sought to define whether FGFR signaling is required to support proliferation in vitro and to characterized the central downstream signaling pathways driven by FGFR in this context. These information are essential for understanding the mechanisms by which FGFR alterations promote ICC development and maintenance, and will be critical in the development of more effective treatment approaches as well as in the prediction of resistance mechanisms. We have tested a panel of FGFR inhibitors for their effects on the growth of CCLP and ICC13-7 cells as well as on the downstream signaling program. While ICC cell lines lacking molecular alterations that activate FGFR signaling are insensitive to FGFR inhibitors (e.g. for BGJ398, the IC50 is >1000 nM for each line), we found that CCLP and ICC13-7 are sensitive at less than 10 nM. Moreover, FGFR inhibition leads to a rapid and durable decrease in the key FGFR2 substrate FRS2, as well as of downstream phosphorylation of SHP2, MEK and ERK, where AKT activity was not effected. Thus, FGFR signaling is essential for maintenance of MEK/ERK signaling in FGFR-driven ICC, while the PI3K-AKT is not controlled by FGFR in these cells. ***Please see Figure 1B-F.***

Major Task 3: Characterize novel mechanisms of FGFR-driven liver cancer growth

a) Continued establishment of biliary & hepatocellular cancer PDX and cell line models & test for FGFRi response (at least 10 models derived from resected patients at Mass. Gen. Hospital)
Given the paucity of ICC models in general and of FGFR-driven models in particular, it is essential for us to continue to expand our collection of such models, in order to fully decipher the underlying signaling program in this subset of ICC and to understand how it compares with other ICC subsets. We have established 7 new PDX lines since the commencement of this grant, including 2 with FGFR alterations. Please see ***Figure 3***.

b) Genomic characterization of new tumors using mutation panel of 400 cancer genes. We have conducted genomic characterization of these models. Please see ***Figure 6.***

Specific Aim 2: Co-clinical trials to determine the cell biologic & molecular impact of FGFRi in vivo, and assess effect of concurrent genetic alterations

Major Task 4: Murine co-clinical trial of FGFR inhibitors

a) Obtain ACURO approval for therapeutics studies in mice.
We have obtained approval: Protocol [ACURO Assigned Number]: CA160216 Title: Mouse Models of Cancer

b) Murine co-clinical trial testing FGFR inhibitors in in vivo models harboring FGFR alterations. The availability of preclinical models of FGFR-activated ICC allows us to overcome the challenges of studying how FGFR inhibitors affect ICCs at the molecular and cell biological level in patient samples (i.e. repeat biopsies are very limited and are usually confined to a single pre-treatment and post-progression biopsy, and thus the acute effects of a medicine cannot be evaluated mechanistically). To address this, we have completed a trial with the FGFR inhibitor, TAS-120, in our FGFR2-KIAA expressing PDX model, MG69. Mice were treated with the drug or vehicle control when tumor reached ~ 400 mm3. Mice were treated for 14 days for efficacy studies, with serial measurements of tumor volume. A subset of mice were euthanized at serial time points to isolate tumors for molecular analyses and histology (3 days and 14 days). We observed a complete block in tumor growth in this model. Please see ***Figure 7A.*** The analysis of specimens from this study will allow us to have unparalleled understanding of FGFR signaling 'addiction' in ICC.

Major Task 5: Phenotypic and biochemical characterization of FGFR inhibitor response

a) Correlative molecular and histological analysis of samples of co-clinical trials (see Major Task 4, part (b) above.
We have examined the tumors from the MG69 model treated with TAS-120. Histological assessment demonstrates evidence of tumor cell differentiation. Accordingly, we have seen a remarkable complete loss of proliferation (Ki-67 staining) as rapidly as 3 days after the start of treatment. By contrast, we do not observe apoptosis (cleaved caspase-3). Analysis of signaling changes, revealed loss of p-MEK, p-ERK, and p-SHP2, but no change in pAKT, mirroring the in vitro data. These data suggest that while FGFR inhibition has dramatic effects in vivo, completing arresting tumor cell proliferation and shutting down signaling to the MEK/ERK pathway, it does not appear to incite tumor cell death acutely. Thus, while there is true 'oncogene addiction', the persistence of growth inhibited cells may ultimately drive recurrence. These data support the search for drug combination strategies that synergize with FGFR inhibition to drive tumor cell death. (please see ***Figure 7B and C***).

Major Task 6: Model effect of concurrent genetic alterations on BJG-398 response.
a) Perform gene editing to generate ICC cell lines harboring inactivating mutations in common ICC tumor suppressor genes (e.g. PTEN, ARID1A)
The goal of these studies is to test whether specific mutations that co-exist with FGFR pathway alterations in subsets of ICC can influence FGFR inhibitor sensitivity. To address this, we are using gene editing strategies in FGFR inhibitor sensitive ICC cell lines and evaluating the resulting impact on drug sensitivity. To date, we have creates sgRNA vectors to target PTEN. Use of these tools will proceed in the upcoming year.

Major Task 7: Characterize genetic and signaling changes driven by FGFR inhibition for hepatobiliary cancer patients in clinical trials

a) Collect blood and tumor specimens from patients on study of BGJ-398, TAS-120, other FGFR inhibitors. Blood and tumor material will be collected pre-treatment. Blood will be collected approximately monthly throughout the course of treatment. A second biopsy will be performed upon disease progression.
Our robust translational pipeline enables us to isolate and study patient specimens from serial time points during therapy. In the past year, we have prepared a set of pre- and post-treatment specimens from 8 patients.

b) Next generation sequencing of ctDNA and tumor biopsy specimens.
Using 4 of the samples above, we have conducted sequencing that has identified multiple candidate resistance mechanisms. These include multiple mutations in the FGFR2 kinase domain. Please see ***Figure 8***.

c) Develop PDX models of treatment naïve and resistant tumor specimens (at least 3 models from patients on FGFR inhibitor clinical trials).
The availability of new models from patients at various stages of treatment enables powerful co-clinical trials that model the underlying biology of response. We have developed 5 PDX models from patients (1 treatment naïve and 4 resistant). These will be employed in molecular and functional studies in the upcoming year. Please see ***Figure 9***.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

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

The project has provide training opportunities for Dr. Lipika Goyal and John Gordan, who were promoted to attending physicians during the prior year. Both work closely with Dr. Bardeesy, Zhu, and Shokat who serve as mentors and have regular in-person meetings as well as joint teleconferences to trouble-shoot and discuss progress and future directions.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

The work was presented in the 2018 Cholangiocarcinoma Foundation Annual Meeting in Salt Lake City, January 30-Feb 1st, 2018. This meeting consists both of a regular scientific conference with leading researchers from around the world presenting cutting-edge discoveries and clinical progress as well as a full day devoted to outreach and education for patients and their families and caregivers have the opportunity to hear talks given with layman's language relating to the disease and emerging research in the area.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

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

Major focus of the upcoming year are to build upon our phosphoproteomics data, genomic information regarding potential resistance mechanisms using our wealth of novel models to fully decipher the circuits downstream of FGFR that support ICC growth, to credential candidate resistance mechanisms and to understand them functionally and to uncover approaches to prevent and overcome resistance based on signaling changes identified in our work and on new drug screens. Finally, since ICC is genetically heterogeneous in general, and among the subset of ICC with FGFR alterations, and since the mechanisms of resistance we are uncovering are also diverse, we will continue to prioritize model development in order to have systems that appropriately mirror the diverse presentation of the disease in the patient population.

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

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

Medicines that inhibit the FGFR signaling pathway (FGFR inhibitors) are showing promise in patients with ICC that harbor FGFR alterations. Unfortunately, patients eventually relapse due to the acquisition of drug resistance. Our work has provide key insights into the main resistance mechanisms assoiciated with different FGFR inhibitors. These findings help to physicians anticipate when treatment failure is occurring and guide treatment with alternative FGFR inhibitors. In addition, the wealth of model systems we have developed together with advanced methods in understanding protein function (phosphoproteomics) provide us with unprecedented opportunities to understand why FGFR inhibitors are initially so effective in these patients and why they ultimately fail. They also enable us to use genetic methods and drug screening approaches to discover the next generation of therapies that will boost the effect of the FGFR inhibitors, preventing resistance from occurring or overcoming it once it arises.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

Our work is focused on liver cancers with FGFR alterations. However, since the FGFR signaling pathway is also deregulated in multiple other cancer types (bladder, breast, stromach, lung, and others), the insights from our data will help understand FGFR inhibitor response and resistance in these other settings as well.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

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

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

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

Nothing to report

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

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

Nothing to report

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

Nothing to report

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

Nothing to report

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

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

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

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

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

Nothing to report.

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

Nothing to report

  Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

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

Nothing to report

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

Nothing to report

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

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*

- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

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

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Nabeel Bardeesy
Project Role: PI
Researcher Identifier;
Nearest person month worked:
Contribution to project: Led the model development and characterization efforts. Assayed FGFR sensitivity in vitro and in vivo. Conducted in vitro and in vivo signaling analyses. Credentialled FGFR kinase domain mutations.
Name: Andrew Zhu
Project Role: PI.
Researcher Identifier;
Nearest person month worked:
Contribution to project: Supervised translational efforts using patient samples.

Name: Kevan Shokat
Project Role: PI
Researcher Identifier
Nearest person month worked:
Contribution to project: Supervised proteomics efforst
Name: Krishna Tummala
Project Role: Postdoc
Researcher Identifier;
Nearest person month worked:
Contribution to project: Conducted signaling studies, established and studied models.
Name: John Gordan
Project Role: Instructor
Researcher Identifier;
Nearest person month worked:
Contribution to project: Developed biliary cell models and conducted and analyzed phosphoproteomics
Name: Lipika Goyal
Project Role: Instructor
Researcher Identifier;
Nearest person month worked:
Contribution to project: Conducted and coordinate clinical studies including sample acquisition and sequencing analysis.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

Dr. Bardeesy was awarded the grant below. It does not impact effort on the present DOD grant.

R01 CA215498-01A1 Bardeesy (PI)
Functions of the LKB1 tumor suppressor in control in metabolism and epigenetics
The goal of this project is to define to circuits downstream of LKB1 mediating tumor suppression, including the roles for altered cell metabolism and its interplay to epigenetic regulation.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

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

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

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

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

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

We have submitted reports for the PI (Bardeesy) and partnering PI's (Zhu and Shokat)

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

9. APPENDICES:

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

Figure 1

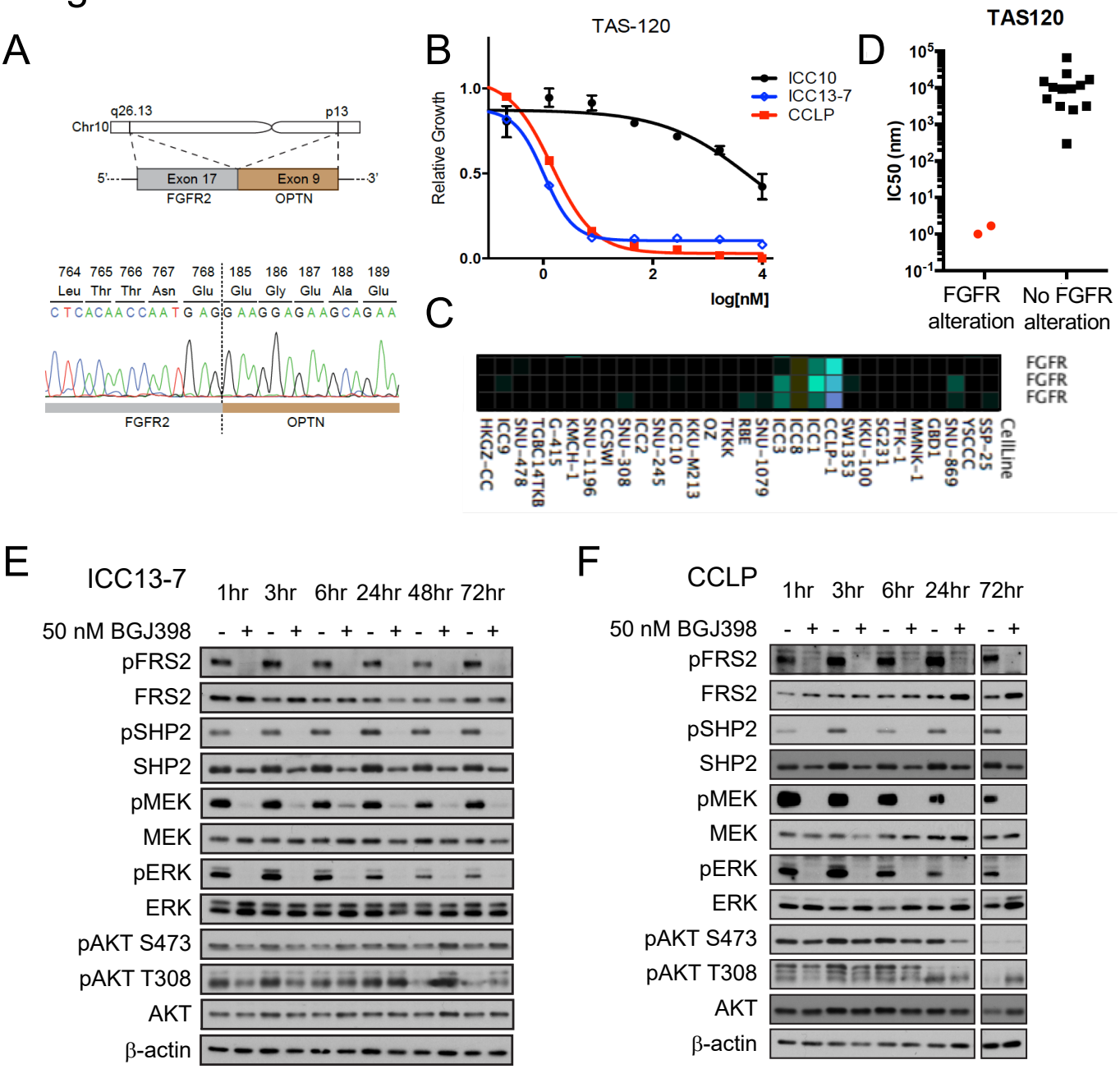

Figure 1. Conversion of existent FGFR-driven ICC to 2-D cell lines.
A. We derived the ICC13-7 cell line from a PDX model. The graphic shows the structure of the FGFR2-OPTN fusion that we detected in this cell line. We also generated a 2-D cell line (ICC10) from a second PDX model and found that it had an FGFR2-PHGDH fusion (not shown).
B. We tested the response of ICC10, ICC13-7, and CCLP cells to TAS-120 (IC50 is graphed).
C. Heatmap of a set of ICC cell lines screened for sensitivity to three FGFR inhibitors (TAS-120, Debio1347 and BGj398). Only CCLP is sensitive in the set shown.
D. Graph of IC50 data ICC cell lines with FGFR alterations (ICC13-7 and CCLP) and those lacking such alterations.
E,F. Immunoblot of signaling effects of BGJ398 treatment of ICC13-7 cells (E) and CCLP cells (F).

Dr. Bardeesy led these studies with assistance of Dr. Zhu and Shokat/Gordan

Figure 2

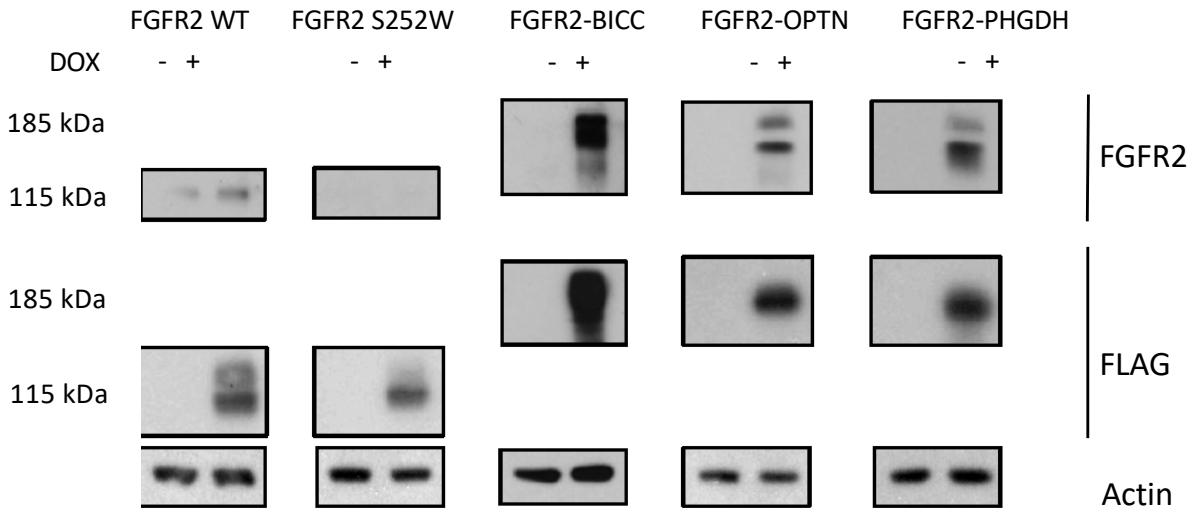

Figure 2. Generation of isogenic FGFR2 WT, FGFR2 S252W, FGFR2-BICC, FGFR2-OPTN, and FGFR2-PHGDH MMNK1 cells.

FGFR2 WT and mutants were cloned into a doxycycline (dox)-inducible puromycin-resistant destination vector with Gateway Cloning, packaged into lentivirus, and transduced into MMNK1 cells. Transduced cells were stably selected with puromycin and plated into 10cm plates with either 1mg/mL dox or no dox. After over 24 hours of dox induction, cells were harvested, lysed, and analyzed by western blot. Membranes were blotted for FGFR2, FLAG, and Actin.

Drs. Shokat/Gordan led these studies with assistance of Dr. Bardeesy

Figure 3

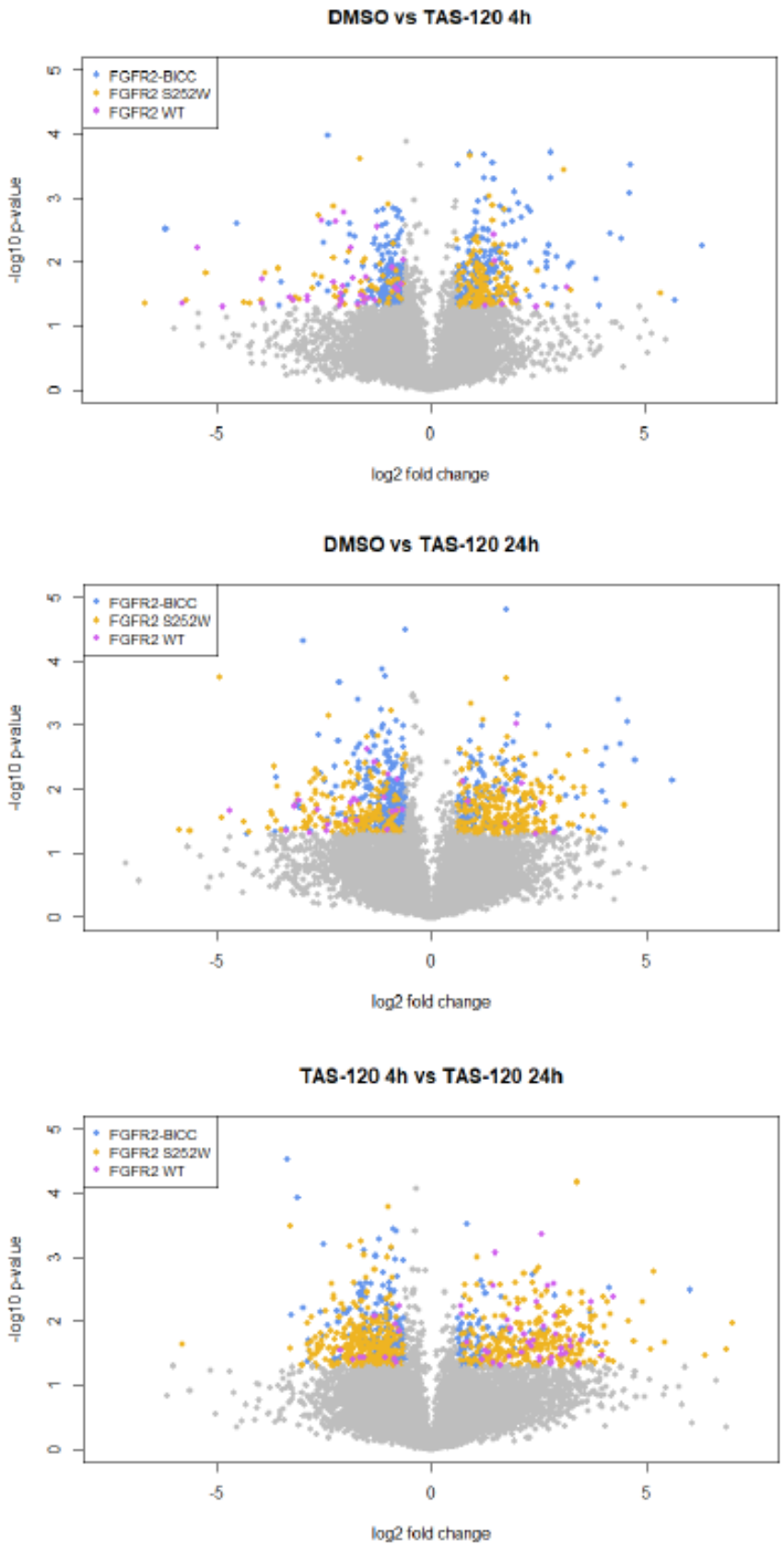

Figure 3. Global phosphorylation analysis in MMNK1 isogenic cells. Changes in phosphorylation sites between conditions in MMNK1 cells containing FGFR2 WT, FGFR2 S252W, and FGFR2-BICC. MMNK1 clones were treated with DMSO or 50nM TAS-120 for 4 hours or 24 hours in triplicate, harvested, and prepped for phosphoproteomics. Volcano plots were generated using the log2 fold changes and p-values of phosphorylation sites between conditions in each MMNK1 clone

Drs. Shokat/Gordan led these studies with assistance of Dr. Bardesesy

Figure 4

Gene/kinase		FGFR2 S252W			FGFR2-BICC		
		DMSO-TAS4h	DMSO-TAS24h	TAS4h-TAS24h	DMSO-TAS4h	DMSO-TAS24h	TAS4h-TAS24h
	MTOR	-0.482	0.191	0.362	0.454	0.257	-0.443
	AKT1	**-0.489**	0.274	**0.423**	-0.314	-0.324	-0.229
	MOS	-0.565	-0.722	-0.619	0.837	**0.972**	0.930
	PIM1	**0.883**	**0.965**	0.442	-0.845	**-0.918**	0.526
CTNNB1	P35222_T551	0.606	0.404	-0.203	-0.288	0.168	0.455
RB1	P06400_T823	**-1.63**	**1.75**	**3.38**	-0.408	-0.508	-0.0997
PAK2	Q13177_S2	0.493	1.18	0.69	1.18	**-1.68**	**-2.86**
PGK1	P00558_S203	**-0.782**	**0.605**	**1.39**	-1.15	**-1.67**	-0.521
BAD	Q92934_S118	-0.60	1.19	1.79	-1.05	**-1.04**	0.007
PKN1	Q16512_S562	-0.158	-2.20	**-2.05**	-0.389	-0.359	0.031
SCRIB	Q14160_S1508	-0.486	1.49	1.98	-0.609	-0.682	-0.072
PXN	P49023_S302	-0.816	0.379	1.20	0.531	0.706	0.175

Figure 4. Log2 fold changes of selected kinases and phosphorylation sites of interest between MMNK1 cells containing FGFR2 S252W and FGFR2-BICC.

Phosphoproteomics datasets for MMNK1+FGFR2 S252W and MMNK1+FGFR2-BICC were analyzed and compared for differences in potential key effectors of FGFR2. Significant log2 fold changes (p-value < 0.05) are in bold.

Drs. Shokat/Gordan led these studies with assistance of Dr. Bardeesy

Figure 5

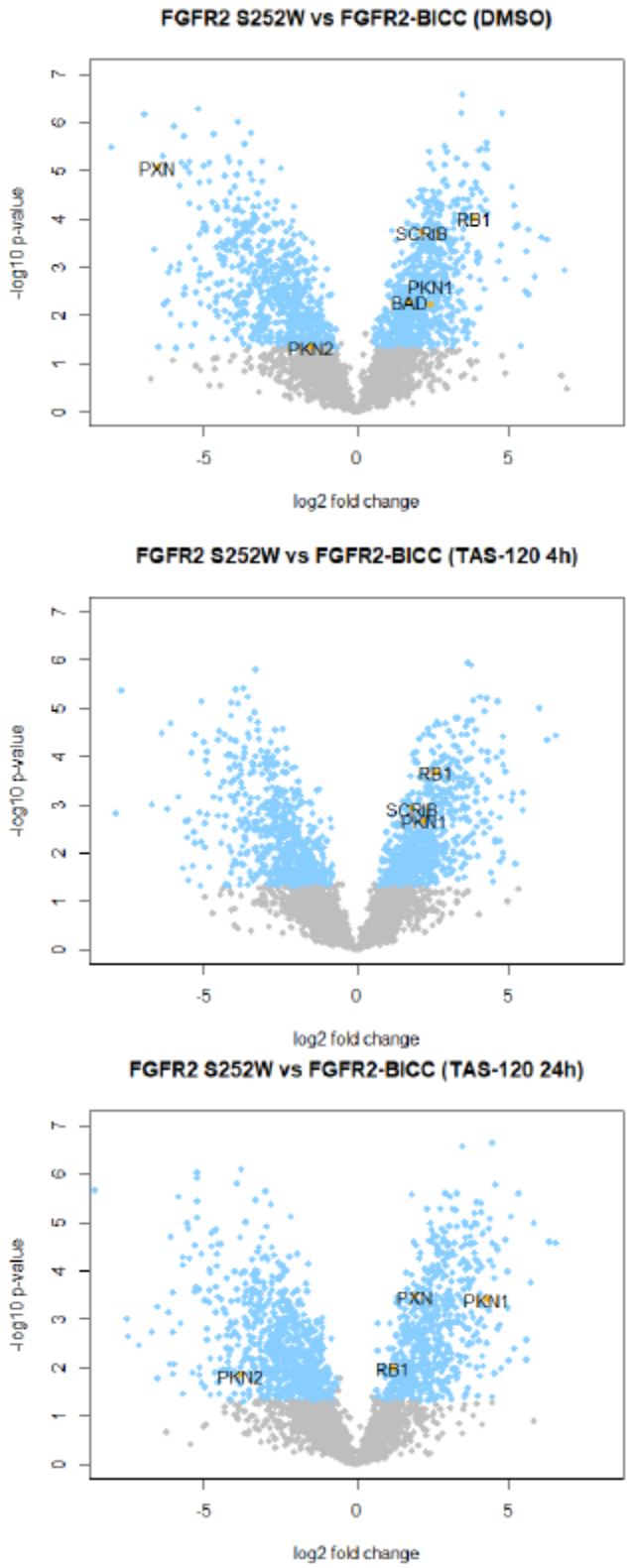

Figure 5. Selected significant phosphorylation changes between MMNK1+FGFR2 S252W and MMNK1+FGFR2-BICC at baseline and following TAS-120 treatment. Phosphoproteomic data for MMNK1 cells with FGFR2 S252W and FGFR2-BICC were re-analyzed with MSstats, comparing the phosphorylation changes between the two mutants for each condition. Proteins of significantly altered phosphorylation sites of interest were annotated.

Dr. Shokat/Gordan led these studies with assistance from Dr. Bardeesy

Figure 6

ID	Genetics
SS106	GNAS; KRAS; CTNNB1
MG10	BRAF
MG94	BRAF
MG59-7	FGFR2-OPTN
CHCA1	TP53 and CDKN2A
CHCA5	BRCA2, EGFR, STK11, CDKN2A, MSH6, SMAD4, TP53
MG69	FGFR2-KIAA1217

Figure 6. Generation of new biliary tract cancer patient-derived xenograft (PDX) models

List of new biliary tract cancer PDXs and associated mutations in major oncogenes/tumor suppressors.

Dr. Bardeesy Lab led these studies with assistance from Dr. Zhu

Figure 7

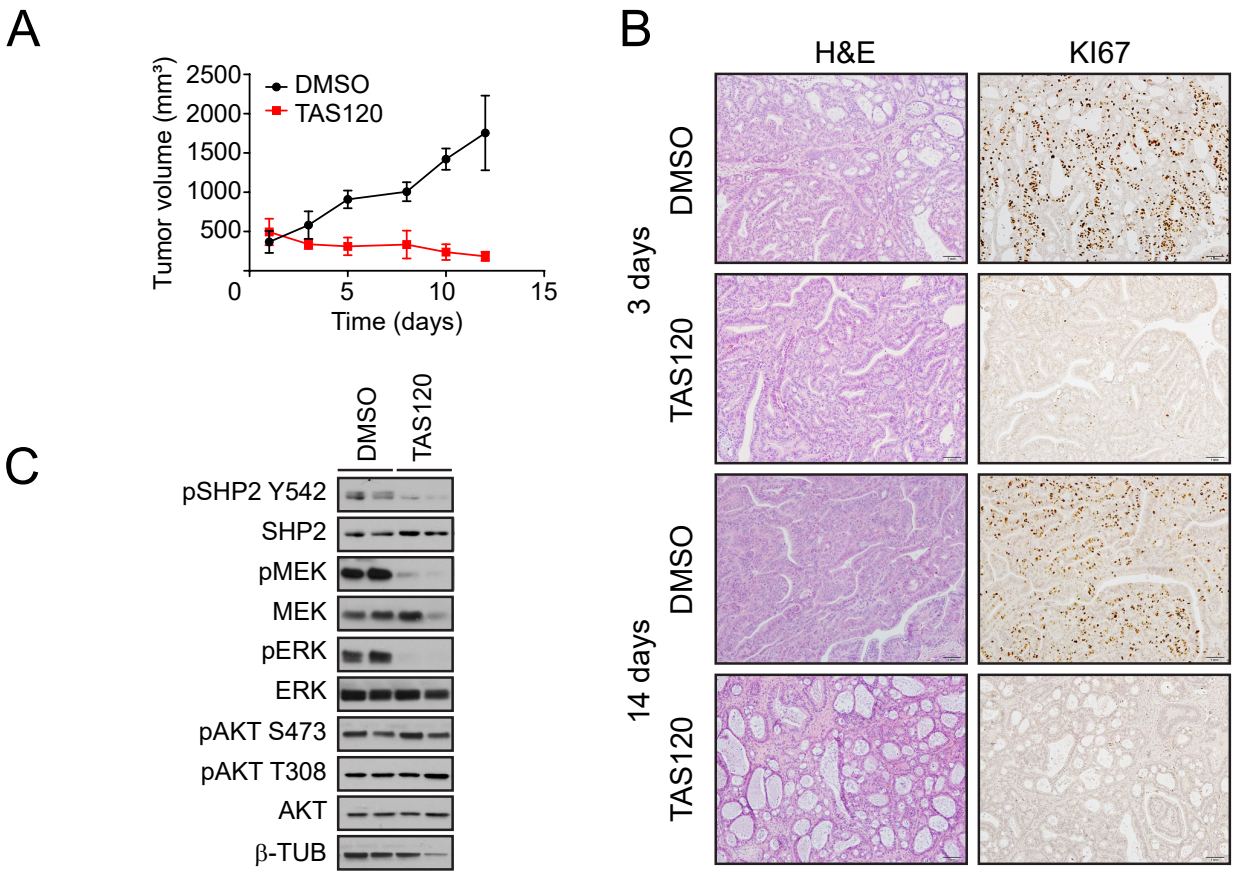

Figure 7. Murine co-clinical trial of FGFR inhibitors

A. Tumors were implanted in NOG-SCID mice and treatment was begun when tumor reached ~300 mm3, using vehicle or TAS-120. Tumor volumes were measured at the indicated days.
B. Histologic images (H&E staining) and measurement of proliferation (Ki67 staining) of tumors isolated after 3 days and 14 days of treatment.
C. Immunoblot data showing signaling inhibition upon TAS-120 treatment (samples are from 3 days treatment).

Dr Bardeesy led these studies with assistance from Dr. Zhu

Figure 8

Table 1a: Clinical Data of Patients with FGFR2 Fusion Positive Cholangiocarcinoma Receiving FGFR Inhibitors

Patient ID	FGFR2 Fusion	1st FGFR Inhibitor	PFS (Months)	ORR	Intervening Therapies Between 1st and 2nd FGFRi	Interval Between 1st and 2nd FGFRi (Months)	2nd FGFR Inhibitor	PFS (Months)	ORR
1	FGFR2-ZMYM4	BGJ398	5.57	-49.9%	None	1.60	TAS120	7.23	+8.30%
2	FGFR2-SORBS1	BGJ398	12.57	-68.2%	None	1.20	TAS120	15.83	-76.7%
3	FGFR2-NRAP	BGJ398	7.13	-40.0%	T8 palliative radiation, Pembrolizumab, Resection of T8 metastasis, FOLFOX	7.43	TAS120	13.03+ (Ongoing)	-47.7%
4	FGFR2-INA	Debio1347	12.63	-46.0%	Gem/Docetaxel, T11 palliative radiation	3.27	TAS120	5.10	-22.1%

Table 1b: FGFR2 mutations detected in cfDNA and tumor biopsies

Patient ID	FGFR2 Fusion	Post-progression BGJ398/Debio1347, Prior to TAS-120		Post-progression TAS-120	
		cfDNA	Tumor Biopsy	cfDNA	Tumor Biopsy
1	FGFR2-ZMYM4	V564F, K659M, E565A, N549H, N549K	V564F, K659M	V564F, K659M, E565A, N549H, N549K, V562L	V562L
2	FGFR2-SORBS1	K659M, K714R	None detected	V564F	V564F
3	FGFR2-NRAP	None detected	No biopsy obtained	Response ongoing	Response ongoing
4	FGFR2-INA	H682L, L617V	Biopsy #1: H682L Biopsy #2: N549H, N549T, M537I	V564L, E565A, N549H, N549T, L617V	No biopsy obtained

Figure 8. Evaluation of resistance mechanisms in FGFRi clinical trials

1a. Clinical characteristics and outcomes of patients with FGFR2 Fusion Positive Cholangiocarcinoma receiving FGFR inhibitors.
1b. Detection of FGFR2 mutations in ctDNA and tumor biopsies

Dr. Zhu led these studies.

Figure 9

ID	predicted FGFRi response
MG98-2	resistant
MG98-3	resistant
MG98-8	resistant
MG98-6	resistant
MG26	sensitive

Figure 9. Development of PDX models from patients enrolled in FGFRi clinical trials

The MG98 series of models were derived from a patient who acquired resistance to the FGFRi, TAS-120. The MG26 model is from a patient who subsequently went on to an FGFRi clinical trial.

Dr. Bardeesy led these studies with assistance of Dr. Zhu.