

A phase I/II study of first line gemcitabine, cisplatin and MEK162 in advanced biliary tract carcinoma.

PROTOCOL FACE PAGE FOR
 MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

Principal Investigator/Department:	Ghassan Abou-Alfa, MD	Medicine
Co-Principal Investigator(s)/Department:	Leonard Saltz, MD	Medicine
Investigator(s)/Department:	Andrea Cercek, MD Andrew Epstein, MD David Ilson, MD, PhD Yelena Janjigian, MD David Kelsen, MD Nancy Kemeny, MD Geoffrey Ku, MD Eileen O'Reilly, MD Diane Reidy-Lagunes, MD Neil Segal, MD Zsofia Stadler, MD Rona Yaeger, MD Kenneth Yu, MD Jedd Wolchok, MD, PhD Michael Berger, MD Jinru Shia, MD James Harding, MD Anna Varghese, MD Elizabeth Won, MD Scott Gerst, MD Murk Heinemann, MD Marinela Capanu, PhD Maria Pacis, NP Armin Shahrokni, MD Ellen Hollywood, NP Kinh Gian Do, MD Erica Kaufmann, NP Patricia Gabriel, NP Robin Brenner, RN Pamela Raasch, RN Nitya Raj, MD Jaclyn Rosenthal, NP Luis A. Diaz, MD Karuna Ganesh, MD, PhD Michal Segal, RN	Medicine Medicine Medicine Medicine Medicine Medicine Medicine Medicine Medicine Medicine Medicine Medicine Medicine Medicine Pathology Pathology Medicine Medicine Medicine Radiology Surgery Epidemiology & Biostatistics Nursing Medicine Nursing Radiology Nursing Nursing Nursing Nursing Nursing Medicine Nursing Medicine Medicine Medicine Nursing
Consenting Professional(s)/Department:	Ghassan Abou-Alfa, MD Andrea Cercek, MD	Medicine Medicine Medicine Medicine

	David Ison, MD, PhD	Medicine
	Andrew Epstein, MD	Medicine
	Yelena Janjigian, MD	Medicine
	David Kelsen, MD	
	Nancy Kemeny, MD	Medicine
	Geoffrey Ku, MD	Medicine
	Eileen O'Reilly, MD	Medicine
	Diane Reidy-Lagunes, MD	Medicine
	Leonard Saltz, MD	Medicine
	Neil Segal, MD	Medicine
	Zsofia Stadler, MD	Medicine
	Rona Yaeger, MD	Medicine
	Kenneth Yu, MD	Medicine
	James Harding, MD	Medicine
	Anna Varghese, MD	Medicine
	Elizabeth Won, MD	Nursing
	Nitya Raj, MD	Nursing
	Ellen Hollywood, NP	Nursing
	Maria Pacis, NP	Nursing
	Erica Kaufmann, NP	Nursing
	Patricia Gabriel, NP	Medicine
	Jaclyn Rosenthal, NP	Medicine
	Luis A. Diaz, MD	
	Karuna Ganesh, MD, PhD	

Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

Memorial Sloan-Kettering Cancer Center
 1275 York Avenue
 New York, New York 10065

Table of Contents

1.0	PROTOCOL SUMMARY AND/OR SCHEMA	4
2.0	OBJECTIVES AND SCIENTIFIC AMS	7
3.0	BACKGROUND AND RATIONALE	8
4.0	OVERVIEW OF STUDY DESIGN/INTERVENTION	11
4.1	Design	11
4.2	Intervention	12
5.0	THERAPEUTIC/DIAGNOSTIC AGENTS	15
6.0	CRITERIA FOR SUBJECT ELIGIBILITY	16
6.1	Subject Inclusion Criteria	16
6.2	Subject Exclusion Criteria	17
7.0	RECRUITMENT PLAN	18
8.0	PRETREATMENT EVALUATION	19
9.0	TREATMENT/INTERVENTION PLAN	20
10.0	EVALUATION DURING TREATMENT/INTERVENTION	26
12.0	CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT	33
13.0	CRITERIA FOR REMOVAL FROM STUDY	35
14.0	BIostatISTICS	35
15.0	RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES	38
15.1	Research Participant Registration	38
15.2	Randomization	38
16.0	DATA MANAGEMENT ISSUES	38
16.2	Data and Safety Monitoring	39
17.0	PROTECTION OF HUMAN SUBJECTS	39
17.1	Privacy	39
17.2	Serious Adverse Event (SAE) Reporting	39
17.2.1	Reporting	41
	Ethics and Good Clinical Practice	42
18.0	INFORMED CONSENT PROCEDURES	42
19.0	REFERENCES	42
20.0	APPENDICES	45

1.0 PROTOCOL SUMMARY AND/OR SCHEMA

Title	A phase VII study of first line gemcitabine, cisplatin and MEK162 in advanced biliary tract carcinoma (BTC)
Primary Objective	To evaluate the safety and activity of MEK162 in combination with gemcitabine and cisplatin in patients with advanced BTCs naïve to systemic therapy
Secondary Objective	To assess tissue biomarkers and perform tumor whole genome sequencing to discern genotypic differences by anatomic location, histology and response to study therapy
Rationale	<p>Median overall survival for patients with advanced biliary carcinomas is 8 months. Gemcitabine and cisplatin is considered the most active combination regimen and current standard of care for treatment of patients with advanced disease, however improvements in systemic therapy are urgently needed. The Ras/Raf/Mek/Erk cascade controls normal processes necessary for cell proliferation and survival and thus has the potential to cause malignant transformation if left unchecked. Mutations in KRAS and BRAF, both of which are upstream of MEK, indicate a role for this signaling pathway in the pathogenesis of BTCs. Direct inhibition of MEK1/2 by is considered a valid strategy to study for therapeutic targeting of the MEK pathway in BTC. Preliminary results of a phase II trial evaluating the MEK inhibitor AZD6244 in a mixed population of pretreated (39%) and treatment-naïve patients with BTC reported an objective response rate of 14% (including one complete response), stable disease in 60%, median progression-free and overall survival times of 5.4 and 8.2 months, respectively. MEK162 is an oral, selective small molecule inhibitor of MEK1/2. In vivo studies performed on human tumor explants have shown that MEK162 activity is potentiated by various chemotherapy agents including gemcitabine and cisplatin, providing a rationale for the combination to be evaluated in this phase VII trial.</p>
Study Design/Intervention	<p>Eligible patients must have evidence of recurrent disease, metastatic disease or locally advanced biliary tract carcinoma not amenable to surgical resection. Measurable disease (RECIST) is required in baseline imaging studies. Histologic or cytologic confirmation of diagnosis is mandatory. Patients will sign written informed consent.</p> <p><u>Phase I portion:</u></p>

	<p>In the phase I component of the study, a classic 3+3 cohort dose escalation scheme will be used to identify the MTD of MEK162 when administered with gemcitabine at dose 800 mg/m² and cisplatin given at dose 20 mg/m² week 2 & 3 of a 3 week cycle. The final cohort will receive gemcitabine 1000mg/m² and cisplatin 20mg/m² week 2 and 3 of a 3 week cycle in combination with MEK162 at the MTD as determined above. MEK162 will be self-administered orally BID from week 1 of each treatment cycle. MEK162 will be held for 2 days prior to chemotherapy administration and resumed the day following chemotherapy. A minimum of 2 patients and a maximum of 18 patients will be enrolled in the phase I part of this study.</p> <p><u>Phase II portion:</u></p> <p>In the phase II part of the study, patients will receive MEK162 at 45mg BID plus gemcitabine (800 mg/m²) and cisplatin (20 mg/m²) as determined by the phase I portion. 29 patients will be enrolled on the phase II part; plus the 6 patients treated at the MTD level on the phase I portion will be evaluable for the primary and secondary endpoints of the phase II part.</p> <p><u>For both phase I/II portions:</u></p> <p>Imaging studies for restaging purpose will be obtained every 3 cycles. Medical and laboratory evaluation will be done on weeks 1, 2 and 3 of the first cycle and at week 2 of every cycle thereafter. Vital signs, nursing assessment, CBC and creatinine will be obtained prior to every treatment. Treatment will continue until disease progression, development of unacceptable toxicity or consent withdrawal.</p> <p><u>Correlative studies:</u></p> <p><u>Tissue</u></p> <p>Targeted cancer gene sequencing of 230 genes using next generation techniques will be conducted in the pathology dept at MSKCC on available pre-treatment tissue samples to explore aberrations in the Ras/Raf/Mek/Erk signaling cascade and identify genotypic differences amongst tumors according to anatomic site, histology and response to study therapy. Tumor samples also will be examined for Ki67 staining,</p>
--	---

	<p>expression of pERK by immunohistochemistry and expression of markers of MEK activity (SPRY4 and DSUP6 mRNA by PCR).</p> <p><u>Blood:</u></p> <p>Pharmacodynamic studies exploring the biological efficacy of the different doses of MEK162 will be performed by measuring changes in peripheral blood pERK expression and cell cycle analysis using flow cytometry. Blood samples will be analysed pre and post treatment for circulating free DNA and results correlated with response to therapy.</p>
Statistical Design	<p><u>Phase I portion:</u></p> <p>The primary objective of the phase I portion is to determine the MTD of MEK162 in combination with gemcitabine/cisplatin chemotherapy. A dose escalation scheme will be used whereby patients will be treated in sequential cohorts of 3. If no patients experience a DLT at dose level 1 in an initial group of 3 patients a new cohort of patients will be enrolled at dose level 2. If 1 of 3 patients experience a DLT, the cohort will be expanded to 6. If no further DLT occurs, a new cohort will be enrolled at the next dose-level. If 2 of 6 patients experience a DLT, MTD has been exceeded. The MTD will be defined as the highest dose for which not more than 1 of 6 patients develop DLT. If three or fewer patients are treated at the dose considered to be the MTD, additional patients (to a total of six) will be treated at that level to confirm the MTD. No inpatient dose escalation will occur.</p> <p><u>Phase II portion:</u></p> <p>The primary objective of the phase II part is to evaluate the efficacy of the combination of gemcitabine and cisplatin plus MEK162 in patients with advanced biliary cancers naïve to systemic chemotherapy. The primary efficacy endpoints are the 6 month progression-free survival (PFS) rate and response rate (RR) at one year. For the combination of gemcitabine and cisplatin, the 6 month PFS rate is 59% and RR is 26%. An exact binomial single stage design will be used to discriminate between true 6-month PFS rates of 59% vs. 82%, and between true response rates of 26% and 50%. Thirty five patients will be enrolled. If at least 26 patients were</p>

	<p>observed to survive progression-free for at least 6 months, or at least 14 responses were observed among the 35 patients, this agent would be considered worthy of further testing in this disease.</p> <p>This design yields 91% power to detect a true 6-month PFS rate of at least 82%, and a true RR of at least 50%. It yields a 0.05 probability of a positive PFS result if the true 6-month PFS rate is no more than 59%, and a 0.05 probability of a positive RR result if the true RR is no more than 26%. Therefore, assuming that PFS and RR are independent, the over-all type 1 error is .10. The type 1 error decreases very slightly if PFS and RR are positively correlated, which is very likely.</p> <p>Progression free survival will be calculated from study entry to documented disease progression or death from any cause, whatever occurs first. Time to death (survival) will be calculated from study entry to death or last follow up. Patients who stop therapy for reasons other than progression of disease or death will be counted as events; however they will be encouraged to continue getting restaging radiologic evaluations until progression or death. In that case, their PFS will be recalculated. Progression free survival, time to progression and survival will be estimated using the Kaplan–Meier methodology. The documented response rate and exact 95% confidence intervals will be calculated</p>
Study Subject Number	<p>Phase I: 2-18 Phase II: 29 (6 patients treated at the MTD on phase I portion will be evaluable for the primary and secondary endpoints of the phase II portion.)</p>
Estimated time to completion	24 months

2.0 OBJECTIVES AND SCIENTIFIC AIMS

Primary objective: Phase I

- To evaluate the safety of MEK162 in combination with gemcitabine and cisplatin in patients with advanced BTCs naïve to systemic therapy

Primary objective: Phase II

- To evaluate the activity of MEK162 in combination with gemcitabine and cisplatin in patients with advanced BTCs naïve to systemic therapy

Secondary objectives: Phase I and II

- Perform genetic analysis on tissue samples to evaluate for mutations in the Ras/Raf/Mek/Erk signaling cascade and to look for genotypic differences between tumors that differ in response to therapy and anatomic/histologic classification
- Evaluate potential predictive tissue biomarkers of response to MEK162 and markers of MEK activity including tumor expression of pERK, Ki67, SPRY4 and DSUP6
- Measure peripheral T-lymphocyte pERK expression by flow cytometry.

3.0 BACKGROUND AND RATIONALE

3.1 Biliary tract carcinoma (BTC)

Carcinomas of the biliary tract include gallbladder carcinoma, intrahepatic and extrahepatic cholangiocarcinomas (perihilar and distal cholangiocarcinomas). In the United States, approximately 7500 cases of BTC are diagnosed annually, of which 5000 are gallbladder carcinomas and 2500 are cholangiocarcinomas.¹ The global epidemiology of BTCs is influenced by gender and ethno-geographic variables. Female sex, parity, advanced age and the metabolic syndrome are known risk factors.²⁻⁴ Gallbladder carcinoma is a leading cause of cancer mortality in northern India and Chile where infection with *Salmonella typhi*, a known risk factor, is endemic.^{5,6} Cholangiocarcinomas are especially frequent in areas like northern Thailand where liver fluke infections are common.³

The vast majority of patients diagnosed with BTCs present with advanced disease given the propensity for regional and distant spread. Overall 5-year survival rates range from 5-12% with a median survival of approximately 6 months for those with metastatic disease⁷⁻⁹ The poor prognosis of BTCs highlights a continued urgent need for effective systemic therapies.

3.2 Systemic therapy for advanced BTC

Cytotoxic chemotherapy for BTC is typically composed of a fluoropyrimidine or gemcitabine backbone. While some pooled analyses suggest that both agents have similar activity as monotherapies, others have reported better response rates with gemcitabine compared to fluoropyrimidines (20-30% vs. 10-20%).^{10,11} The combination of gemcitabine and cisplatin yields response rates of 30-50% based on phase II trials.¹⁰ A recent meta-analysis of randomized and non-randomized trials reported superior objective response and tumor control rates with gemcitabine-platinum over fluoropyrimidine-platinum combinations.¹¹ There was also a trend towards improved time to tumor progression with gemcitabine-platinum, but this did not translate into an overall survival benefit.¹¹ The mechanism underlying the apparent synergism of gemcitabine-platinum combinations is thought to result from the inhibitory effect of gemcitabine on repair of cisplatin-induced DNA damage.^{12,13}

The activity of gemcitabine and cisplatin was confirmed in the ABC-02 trial, a randomized phase III trial comparing the combination with gemcitabine alone in patients with advanced (unresectable, recurrent or metastatic) BTC. There were approximately 200 patients in each arm, and gemcitabine

1000 mg/m² and cisplatin 25 mg/m² were given weekly for two weeks followed by a week of rest for a total of four cycles. Compared to gemcitabine, combination therapy significantly improved overall survival (11.7 vs 8.1 months, p<0.001), progression-free survival (8.0 vs. 5.0 months, p<0.001) and disease control (81.4% vs. 71.8%, p = 0.049). Differential activity was not seen among the various sites along the biliary tree.¹⁴ The ABC-02 study established gemcitabine plus cisplatin as a new reference standard for the treatment of advanced BTC. Although other chemotherapy doublets such as gemcitabine and oxaliplatin,^{15,16} gemcitabine and capecitabine,^{17,18} and oral or IV fluoropyrimidines with cisplatin¹⁹⁻²¹ have shown comparable activity, there have been no head-to-head comparisons with gemcitabine and cisplatin.

3.3 Genetic alterations and the Ras/Raf/Mek/Erk Pathway in BTCs

The rarity of BTCs poses a challenge for study recruitment, and many trials have been inadequately powered to detect differences in outcomes between gallbladder carcinomas, intrahepatic and extrahepatic cholangiocarcinomas. In clinical practice, advanced BTCs are currently regarded as a single disease entity treated with the same agents regardless of anatomic site of origin. There is however a growing awareness that BTCs are heterogeneous and efforts are underway to elucidate the common genetic alterations associated with different anatomic sites.²² For example, mutations in CTNNB1 have been documented in gallbladder carcinomas but not cholangiocarcinomas.²² ERBB2/Her2 overexpression has been found in 15% of gallbladder carcinomas, 5% of extrahepatic cholangiocarcinomas and 0% of intrahepatic cholangiocarcinomas.²³ Vascular endothelial growth factor (VEGF) overexpression has been reported in nearly 60% of cholangiocarcinomas and 91% of gallbladder carcinomas and is a poor prognosticator.^{24,25} KRAS mutations have been reported in all BTCs, although the frequency seems to be highest among intrahepatic cholangiocarcinomas at 48-54%.²⁶⁻²⁸ while BRAF mutations have been reported in 20-30% of BTCs in two European series, although none were identified in tumors taken from a North American and Chilean population.^{26,29,30} It is unclear if this discordance reflects artifact or true geographic differences in mutation frequency.²⁶ There is currently no available data on the expression levels or mutation frequency of MEK in different parts of the biliary duct. The Ras/Raf/Mek/Erk cascade controls normal processes necessary for cell proliferation and survival and thus has the potential to cause malignant transformation if left unchecked. Mutations in KRAS and BRAF, both of which sit upstream of MEK, suggest a potential role of this signaling pathway in the pathogenesis of BTCs.^{22,26}

3.4 Targeting the MAP kinase (MEK) pathway in BTC

Clinical evaluation of targeted therapies in advanced BTCs is a relatively new area of investigation and no such therapies have yet been approved for this setting. The anti-tumor activity of MEK inhibition in BTCs has been assessed in several studies.

Sorafenib is an oral multi-target tyrosine kinase inhibitor of the VEGF and platelet-derived growth factor receptors, c-kit, as well as the Raf/Mek/Erk pathway. The results of two phase II trials evaluating single agent sorafenib 400 mg BID in both treatment-naïve and treatment-refractory advanced BTC have been reported.³¹⁻³³ Despite the emerging data implicating aberrant KRAS, BRAF and VEGF expression in BTC, objective response rates on sorafenib have been disappointing at 2-6%, stable disease is achieved in 30-50%, and median survival ranges from 4-6 months.^{31,33}

Direct inhibition of MEK1/2 by AZD6244 appears to be a more promising strategy for targeting the MEK pathway in BTC. Preliminary results of a phase II trial evaluating AZD6244 in a mixed population of pretreated (39%) and treatment-naïve patients reported an objective response rate of 14% (including one complete response), stable disease in 60%, median progression-free and overall survival times of 5.4 and 8.2 months, respectively. Furthermore, pERK positivity was present in 90% of patients who had disease control compared to 0% of patients who progressed on AZD6244. In contrast, staining for pAKT did not correlate with response.³⁴

3.5 MEK162 (ARRY-438162)

MEK162 is an oral, selective small molecule inhibitor of MEK1/2. MEK162 exerts an anti-proliferative effect, induces apoptosis, and decreases phosphorylation of ERK, a downstream effector of MEK, in cancer cell lines and various solid tumor xenografts.³⁵

MEK162 has been evaluated in six clinical trials thus far. Three of these were phase I studies conducted in healthy subjects.³⁵ Two studies were conducted in patients with rheumatoid arthritis given the pro-inflammatory effects of Ras/Raf/Mek signaling, but primary efficacy endpoints were not met.^{36,37} A single oncologic phase I study is underway beginning with a dose escalation phase in advanced solid tumors followed by an expansion phase in patients with metastatic BTCs or colorectal carcinomas (www.clinicaltrials.gov, NCT00959127). Preliminary pharmacodynamic analyses have shown that MEK162 suppresses pERK and significantly decreases markers of MEK activity and cell proliferation from pre-treatment levels, particularly at doses of 45 mg and 60 mg BID. The most frequently reported grade 1-2 toxicities include asymptomatic elevations in creatine kinase, stomatitis/mucositis and diarrhea. Retinal events including photopsia, darkened vision and altered color perception occurred in 14% of patients and reversed with cessation of MEK162. Efficacy data are currently pending.³⁵

The occurrence of retinal events, including more serious complications such as retinal vein occlusion, has been reported with multiple other MEK inhibitors being evaluated for their anti-neoplastic activity.³⁸⁻⁴⁵ In a phase I study of the oral MEK1/2 inhibitor, PD-0352901, patients who developed retinal vein occlusion had predisposing risk factors including hypertension, diabetes, dyslipidemia and glaucoma.³⁸ While the mechanism underlying ocular toxicity with MEK inhibition is poorly understood, pre-clinical studies on rat models have revealed increased retinal gene expression of markers of inflammation, oxidative stress, endothelial damage and procoagulability.⁴⁶ Furthermore, the severity and type of ocular toxicity may be dosing schedule dependent. As an example, while retinal vein occlusions were observed on a continuous schedule of PD-0352901, none were seen with an intermittent schedule.^{38,41}

3.5 Combining MEK162 with chemotherapy

Although incremental increases in survival have been observed with gemcitabine-based doublets in advanced BTC, the possibility of further improving outcomes with the addition of targeted agents is being actively explored. In vivo studies performed on human tumor explants have shown that MEK162 activity is potentiated by various chemotherapy agents including taxanes, gemcitabine and cisplatin.³⁵ In the Calu-6 mouse model, the combination of MEK162 plus gemcitabine and cisplatin has been shown to be tolerable and with improved delayed growth of tumors relative to single agent

MEK 162 or the combination of gemcitabine and cisplatin. Clinical trials have shown that combining targeted agents with a gemcitabine-platinum doublet is feasible, safe, and effective. A phase II trial of bevacizumab, gemcitabine and oxaliplatin yielded an objective response rate of 40%, median progression-free and overall survival times of 7 and 12.7 months, respectively.⁴⁷ The addition of cetuximab to gemcitabine and oxaliplatin showed a promising improvement in 4-month progression-free survival over chemotherapy alone (61% vs. 44%).⁴⁸ Furthermore, we recently completed a phase II trial at MSKCC of sorafenib, gemcitabine and cisplatin in advanced BTCs (www.clinicaltrials.gov, NCT00919061). It should be noted that in this trial, gemcitabine and cisplatin were initially administered at the same doses as in the ABC-02 study (1000 mg/m² and 25 mg/m², respectively) concurrent with sorafenib at the standard dose of 400 mg BID. However, these doses were poorly tolerated, resulting in a high frequency of hand-foot syndrome, diarrhea and elevated liver enzymes. The study regimen was subsequently modified to gemcitabine 800 mg/m², cisplatin 20 mg/m², and sorafenib 400 mg, providing the rationale for the dose of gemcitabine/cisplatin chosen for use in combination with MEK162. In addition, preclinical studies performed in biliary cancer models have demonstrated schedule dependence to the antitumor effect observed when sequencing the MEK inhibitor AZD6244 with gemcitabine. DNA synthesis was suppressed during treatment with AZD6244, and re-entry into S-phase was delayed by approximately 48hr post-treatment. Strong schedule dependence was seen in all four biliary cancer models tested, suggesting that combined treatment with AZD6244 plus gemcitabine would be more active in biliary cancer patients when gemcitabine is given following a 48hr interruption in AZD6244 dosing, rather than concurrently⁵⁵. Based on this data we plan an interrupted dosing schedule of MEK162 in combination with gemcitabine and cisplatin.

3.6 Summary

MEK1/2 is a therapeutic target in BTC due to the frequent occurrence of activating KRAS and BRAF tumor mutations resulting in oncogenic signaling through the Ras/Raf/Mek/Erk pathway. Clinical activity of single agent MEK1/2 inhibition has been observed in patients with advanced BTC, and pre-clinical evidence indicates enhanced activity of MEK162 when given with chemotherapy. Gemcitabine-cisplatin is the current reference regimen for advanced BTC; the combination of a direct MEK inhibitor with chemotherapy has not previously been clinically evaluated in BTCs. We propose a phase I/II study evaluating the safety and activity of gemcitabine, cisplatin and MEK162 as first-line therapy for advanced BTCs.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This is a non-randomised, open label, single institution phase I/II study of gemcitabine, cisplatin and MEK162 in patients with advanced BTC naïve to systemic therapy.

In the phase I portion, up to 18 patients will be enrolled in classic 3+3 cohort dose escalation design to identify the MTD of MEK162 when administered with gemcitabine and cisplatin

given weeks 2 and 3 of a 3 week cycle . MEK162 will be self-administered orally from week one of each cycle and held 2 days prior to chemotherapy administration. Treatment with MEK162 will resume the day following each chemotherapy administration. Patients will be observed for 6 weeks (2 cycles) prior to determination of the MTD. Patients treated at the MTD in the phase I portion will be evaluable for primary and secondary endpoints of the phase II portion.

In the phase II portion, up to 29 patients will be treated with gemcitabine 800mg/m² , cisplatin 20mg/m² week 2 and 3 of a 3 week cycle and MEK162 at 45mg BID. The dose of gemcitabine used in the phase II portion will be dependent on results of the phase I portion.

Phase I

- Primary endpoint is to determine the MTD of MEK162 in combination with gemcitabine/cisplatin chemotherapy.

Phase II:

- Primary endpoints are six-month progression free survival and objective response rate at one year
- Secondary endpoints are: median PFS, median overall survival, safety/toxicity profile
- Joint primary endpoints of PFS6 and response rate were chosen for the phase II portion as responses to treatment with single agent MEK inhibitor were observed in a previous phase I trial in biliary cancer. The use of a dual primary endpoint therefore ensures that if a significant increase in response rate is observed but PFS6 does not reach significance, the combination will still be considered worthy of further investigation.

Correlative studies:

- Perform genetic analysis on tissue samples to evaluate for mutations in the Ras/Raf/Mek/Erk signaling cascade and to look for genotypic differences between tumors that differ in response to therapy and anatomic/histologic classification⁵⁴
- Evaluate potential predictive tissue biomarkers of response to MEK162 and markers of MEK activity including tumor expression of pERK, Ki67, SPRY4 and DSUP6
- Measure changes in peripheral T-lymphocyte pERK expression and cell cycle by flow cytometry.⁴⁹⁻⁵³
-

4.2 Intervention

Phase I

Patients will receive treatment on the following schedule:

- Gemcitabine over 30 mins IV week 2 & 3 of a 3 week cycle.

- NOTE: Infusions within 10 minutes of the specified time will be allowed at the discretion of the treating nurse (e.g. to allow patients to use the restroom) and will not be considered a protocol violation.
- Cisplatin over 30 mins IV week 2 & 3 of a 3 week cycle
 - NOTE: Infusions within 10 minutes of the specified time will be allowed at the discretion of the treating nurse (e.g. to allow patients to use the restroom) and will not be considered a protocol violation.
- MEK162 orally BID starting week one of each cycle, with treatment held 2 days prior to each chemotherapy administration and resumed one day following chemotherapy.
- Dose escalation schedule for gemcitabine, cisplatin and MEK162 as below:

Table 1: Dose levels for MEK162, gemcitabine and cisplatin

Dose level	MEK162*	Gemcitabine	Cisplatin
1	30 mg PO BID	800 mg/m ²	20 mg/m ²
2	45 mg PO BID	800 mg/m ²	20 mg/m ²
3	MTD	1000mg/m ²	20mg/m ²

A dose escalation scheme will be used whereby patients will be treated in sequential cohorts of 3-6. If no patients experience a DLT at dose level 1 in an initial group of 3 patients a new cohort of patients will be enrolled at dose level 2. If 1 of 3 patients experience a DLT, the cohort will be expanded to 6. If no further DLT occurs, a new cohort will be enrolled at the next dose-level. If 2 of 6 patients experience a DLT, MTD has been exceeded. The MTD will be defined as the highest dose for which not more than 1 of 6 patients develop DLT. If three or fewer patients are treated at the dose considered to be the MTD, additional patients (to a total of six) will be treated at that level to confirm the MTD. No inpatient dose escalation will occur. Dose-limiting toxicities (DLT) are defined as:

1) Ocular disorders defined by:

- Central serous retinopathy (CSR):
 - CTCAE v4.0 grade 2 lasting > 14 days confirmed by ophthalmologic examination
 - CTCAE v4.0 grade ≥ 3 confirmed by ophthalmologic examination
 - CTCAE v4.0 grade ≥ 2 recurrence with same severity following suspension of study therapy followed by resolution and resumption of therapy at the same or lower dose
- CTCAE v4.0 grade ≥ 1 non-CSR retinopathy confirmed by ophthalmologic examination
- CTCAE v4.0 grade ≥ 3 visual disturbances (blurring, floaters, flashing lights) without retinal changes

- CTCAE v4.0 grade ≥ 2 uveitis
- Any other CTCAE v4.0 grade ≥ 3 eye disorders

2) Cardiac dysfunction defined by:

- CTCAE v4.0 grade ≥ 2 cardiac dysfunction; relative decrease in ejection fraction $\geq 20\%$ from baseline value

3) Liver dysfunction defined by:

- Elevation of AST/ALT > 4 times baseline level or CTCAE v4.0 grade 3, whichever is greatest.

4) Skin toxicity defined by:

- Rash/ photosensitivity \geq CTCAE v4.0 grade 3 lasting > 48 hours despite skin toxicity treatment

5) Delay of more than 4 weeks in receiving MEK162 due to toxicity.

6) CTCAE v4.0 grade ≥ 3 non-hematologic toxicity, considered potentially irreversible or life threatening despite best supportive efforts.

7) CTCAE v4.0 grade ≥ 4 hematologic toxicity

Phase II:

Patients will receive treatment on the following schedule:

- Gemcitabine 30 mins IV week 2 & 3 of a 3 week cycle, at dose of $800\text{mg}/\text{m}^2$ as determined by phase I portion.
 - NOTE: Infusions within 10 minutes of the specified time will be allowed at the discretion of the treating nurse (e.g. to allow patients to use the restroom) and will not be considered a protocol violation.
- Cisplatin $20\text{mg}/\text{m}^2$ over 30 mins IV week 2 & 3 of a 3 week cycle
 - NOTE: Infusions within 10 minutes of the specified time will be allowed at the discretion of the treating nurse (e.g. to allow patients to use the restroom) and will not be considered a protocol violation.
- MEK162 orally BID starting week one of each cycle, with treatment held 2 days prior to each chemotherapy and resuming one day post chemotherapy at a dose of 45mg BID as determined by the phase I portion.

For both phase I/II portions:

Imaging studies for restaging purpose will be obtained every 3 cycles. Medical (vital signs and physical exam) and laboratory evaluation (CBC, comprehensive metabolic panel, direct bilirubin, magnesium, amylase, lipase, and ldh) will be done on week 1,2 and 3 of the first cycle and at week 2 of every cycle thereafter. Vital signs, nursing assessment, CBC and creatinine will be obtained prior to every treatment. Cardiac function will be evaluated by transthoracic echocardiogram at baseline and every 3 cycles. Patients will complete a pill diary, which will be collected and reviewed at each clinic visit. All patients will complete the Amsler grid test (test of central visual field) at baseline and week 2 of each cycle thereafter. If a patient has an abnormal Amsler grid test, he/she will undergo ophthalmologic assessment.

Treatment will continue until disease progression, development of unacceptable toxicity or consent withdrawal.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 Gemcitabine

- 5.1.1 Definition: Gemcitabine is a pyrimidine analogue of deoxycytidine in which the deoxyribose moiety contains 2 fluorine atoms at the 2'-position. The drug acts as an inhibitor to ribonucleotide reductase and inhibition of DNA synthesis may result in perturbations of deoxynucleotide pools and interference with DNA chain elongation. The drug is cell-cycle specific and blocks cells in the G1/S interface. Cytotoxicity is schedule-dependent and increases with increasing duration of exposure. The drug is rapidly eliminated from plasma, owing mainly to deamination. Renal clearance of drug is less than 10% of parent drug.
- 5.1.2 Supply: The drug is supplied as either a 200 mg or 1 gram lyophilized powder in a 50-mL sterile single vial for reconstitution.
- 5.1.3 Administration: The drug is administered via a freely running intravenous catheter per institutional guidelines.
- 5.1.4 Toxicity: Nausea, vomiting, alopecia, stomatitis, anorexia, fatigue, elevations of hepatic transaminases, reversible myelosuppression, rash, flu-like symptoms, edema, constipation, paresthesias, hypersensitivity reactions, phlebitis, proteinuria, hematuria, and rarely interstitial pneumonitis, ARDS and hemolytic uremic syndrome..

5.2 Cisplatin

- 5.2.1 Definition: Cisplatin is a planar inorganic metal salt that function as an alkylating agent. In aqueous solution, the drug is equated to a diaquo species as the two chloride groups leave the molecule. The reactive diaquo species binds to N7 residues of guanine bases on DNA resulting in strand scission, and intra-inter strand cross-linking.
- 5.2.2 Supply: Cisplatin is commercially supplied as a lyophilized powder in 10 mg and 50 mg vials, and stored at room temperature. The drug should be reconstituted using 10 ml and 50 ml respectively of sterile water for injection, USP, to yield a concentration of 1 mg/ml. Pre-reconstituted multi-dose vials of 100 mg are also available. Once the multidose vial has been entered, the remaining cisplatin is stable for 28 days when protected from light.
- 5.2.2 Administration: Cisplatin should be given IV drip per institutional guidelines. Needles and IV sets using aluminum should not be used in the administration of cisplatin.
- 5.2.3 Toxicity: Fatigue, asthenia, nausea, vomiting, nephrotoxicity, ototoxicity, myelosuppression, anaphylaxis, peripheral neuropathy, diarrhea, alopecia, hypersensitivity reactions, and rarely myocardial infarction, stroke, seizure, and blindness.

5.3 MEK162

- 5.3.1 Definition: MEK162 (also known as ARRY-438162) is a potent, selective, allosteric small molecule inhibitor of mitogen-activated protein (MAP) kinase kinase (MEK) that is uncompetitive with adenosine triphosphate (ATP).
- 5.3.2 Supply: MEK162 is supplied as film coated tablets. The film coated tablets consist of MEK162, lactose monohydrate, microcrystalline cellulose, colloidal silicon dioxide, croscarmellose sodium, magnesium stearate and a commercial film coating. The capsule-shaped film coated tablets are yellow to dark yellow, and are supplied as a dosage strength of 15mg. MEK162 film coated tablets are packed in a high-density polyethylene bottle with an induction seal and a child resistant closure. MEK162 film coated tablets should be stored below 25°C, protected from light.
- 5.3.3 Administration: MEK162 will be orally administered by the patient themselves, as directed by the physician. Patients must take 90% of MEK162 doses per cycle. If a patient lives out of state and forgets to pick up their MEK162 refill, pharmacy may ship the MEK162 drug to the patient's home address at the discretion of the PI or co-PI.
- 5.3.4 Toxicity: rash-like events (acne, rash, dermatitis acneiform, etc.), elevations of CK, diarrhea, nausea, vomiting, stomatitis/mucositis, edema and visual changes related to retinal events. See investigator brochure (ADDENDUM 1) for full description of human and animal toxicity and pharmacokinetic studies.
- 5.3.5 Management of MEK162 related toxicity:

Rash: treatment with topical or oral antibiotics, topical or oral anti-inflammatories, topical or oral corticosteroids. The use of emollients and sunscreens has not been evaluated with MEK162 but may be used prophylactically or as treatment for rash.

Elevated CK levels: patients may remain on MEK162 per investigator discretion if asymptomatic.

Nausea, vomiting and diarrhea: treat with standard anti-emetic and anti-diarrhoeal medications. Prophylactic treatment for MEK162-related nausea and diarrhea is not required.

Edema: treatment with diuretic medications if clinically indicated.

Stomatitis/mucositis-like events: institutional standard of care treatment

Visual changes or new ocular findings: study drug should be immediately held and an ophthalmologic evaluation initiated. If visual changes or new ocular findings are assessed as not related to study drug after ophthalmologic evaluation, then study drug can be resumed at the previous dose. See section 4.2 for definition of ophthalmologic DLTs

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

6.1 Subject Inclusion Criteria

6.1.1 Histologically / cytologically verified, non-resectable, recurrent, or metastatic biliary tract carcinoma including intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma

and gallbladder carcinoma. Combined cholangiocarcinoma and hepatocellular carcinoma is allowed.

6.1.2 Patients must have measurable disease by RECIST 1.1

6.1.3 KPS \geq 80%

6.1.4 Age \geq 18 years

6.1.5 Adequate bone marrow function defined as: Hb \geq 8 g/dl, ANC \geq 1.5 K/mcL, Platelets \geq 100 K/mcL

6.1.6 Adequate renal function defined as serum creatinine $<$ 1.6 mg/dl and/or measured creatinine clearance from 24-hour urine collection of \geq 60 ml/min

6.1.7 Adequate hepatic function defined as total bilirubin \leq 2 mg/dl, ALT/AST \leq 5 x ULN. Patients with biliary obstruction can join if bilirubin corrects to required limit after a dequate biliary drainage.

6.1.9 Adequate cardiac function defined as ejection fraction \geq 45% as determined by transthoracic echocardiogram or MUGA

6.1.8 Patients who have received prior local therapy, including but not limited to embolization, chemoembolization, radiofrequency ablation, radiation therapy, are eligible provided that measurable disease falls outside the treatment field or within the field but has shown an increase of \geq 20% in the size. Prior local therapy must be completed at least 4 weeks prior to the baseline scan

6.1.9 Women of childbearing potential must have a negative pregnancy test within 7 days prior to study treatment

6.1.10 Men and women of childbearing potential must be willing to consent to using effective contraception while on treatment and for at least 3 months thereafter.

6.1.11 Ability to understand and the willingness to sign a written informed consent document

6.2 Subject Exclusion Criteria

6.2.1 Any previous chemotherapy, biologic therapy, or investigational agent, except for adjuvant therapy or as radio-sensitizing agents limited to 5-fluorouracil/capecitabine and gemcitabine. Patient must have completed adjuvant therapy no less than six months prior to accrual.

6.2.2 Evidence of another active cancer that may influence patient outcome as determined by the Principal Investigator (PI) or co-Principal Investigator (co-PI), except for non-melanoma skin carcinoma, melanoma in-situ, in-situ carcinoma of the cervix curatively treated, treated superficial bladder cancer, and adenocarcinoma of the prostate that has been surgically treated with a post-treatment PSA that is non-detectable.

6.2.3 Known brain metastases or primary central nervous system tumors with seizures that are not well controlled with standard medical therapy.

6.2.5 Uncontrolled intercurrent illness including, but not limited to psychiatric illness/social situations that would limit compliance with study requirements.

6.2.6 Known HIV positive patient

6.2.7 Significant cardiovascular disease including congestive heart failure (New York Heart Association Class II or higher) or active angina pectoris.

6.2.8 History of a myocardial infarction within 6 months.

6.2.9 History of a stroke or transient ischemic attack within 6 months.

6.2.10 Clinically significant peripheral vascular disease.

6.2.11 Major surgical procedure within 4 weeks.

6.2.12 Uncontrolled infection.

6.2.13 Known or suspected allergy to gemcitabine or cisplatin

6.2.14 Pregnant (positive pregnancy test)

6.2.15 Breast-feeding should be discontinued if a nursing mother is to be treated on clinical trial.

6.2.16 Any condition that impairs patient's ability to swallow whole pills

6.2.17 Malabsorption problem that may limit or inhibit the absorption of MEK 162

6.2.18 Patients with a history or current known evidence of central serous retinopathy (CSR), retinal vein occlusion (RVO) or ophthalmopathy at baseline that would be considered a risk factor for CSR or RVO.

6.2.19 History of any organ or bone marrow transplant.

7.0 RECRUITMENT PLAN

This study will be conducted at Memorial Sloan-Kettering Cancer Center. Between 2 and 47 patients will be needed to meet the study endpoints, projected accrual rate is two patients / month.

All patients with biliary tract carcinomas referred for considerations of systemic therapy will be screened regardless of gender or ethnicity. Participation is voluntary. Before any protocol intervention and after discussion of the experimental nature of the therapy, alternatives, potential benefits, side effects, risks and discomforts, patients may sign informed consent. Patients who agree to join, will sign an institution's IRB approved written consent form. Three copies of the informed consent will be signed and dated by the patient or the patients legally authorized representative. One copy will be given to the patient, one copy will be filed in the patient's medical record, and one copy will be stored in the research file. Written consent will be obtained by either the principal investigator or participating investigators. Patients who prefer to be consented using another language than English will be consented in their

preferred language as per the standard operating procedures established by the IRB at MSKCC.

During the initial conversation between the investigator/research staff and the patient, the patient may be asked to provide certain health information that is necessary to the recruitment and enrollment process. The investigator/research staff may also review portions of their medical records at MSKCC in order to further assess eligibility. They will use the information provided by the patient and/or medical record to confirm that the patient is eligible and to contact the patient regarding study enrollment. If the patient turns out to be ineligible for the research study, the research staff will destroy all information collected on the patient during the initial conversation and medical records review, except for any information that must be maintained for screening log purposes.

The protocol will be advertised on the MSKCC website and also will be registered and posted on clinicaltrials.gov. The protocol however does not contemplate the use of any other form of advertisements or payment to participants. In view of the high incidence of certain BTC among woman, and the large referral circle based on previous experience accruing to studies of BTC, there is no envisioned limitation in accruing women and minorities in the research study.

8.0 PRETREATMENT EVALUATION

Test	≤ 28 days prior to registration	≤ 7 days prior to treatment
Complete medical history and physical examination		X
KPS / ECOG		X
Height / Weight / Body surface area		X
Complete blood count with differential		X
Comprehensive biochemistry profile*		X
Amylase/Lipase		X
LDH		X
Magnesium		X
Direct bilirubin		X
CEA / CA 19.9/AFP		X
PT/INR and PTT	X	
Urinalysis	X	
Pregnancy test (females of child-bearing potential)		X
CT Chest, Abdomen, Pelvis**	X	
24-hour urine collection for creatinine clearance****	X	
EKG	X	
Transthoracic echocardiogram / MUGA	X	
Audiogram****	X	
Amsler grid test (eye test)	X	

Confirmation of pathologic diagnosis at MSKCC	X***	
Informed consent	X	
Research blood draw		X

* Including plasma electrolytes, BUN, creatinine, glucose, calcium, total protein, albumin, alkaline phosphatase, AST, AST, total bilirubin.

** MRI of the abdomen and pelvis with non-contrast CT of the chest may be obtained instead of CT chest/abdomen/pelvis with contrast (or with liver tri-phasic) if patients liver disease is not visible on triphasic liver CT, or if the patient is allergic to CT scan contrast despite pre-treatment

*** Prior pathologic confirmation at MSKCC before 4 weeks is acceptable

**** Audiography / 24 hour creatinine clearance only if clinically indicated, i.e. if patient has a baseline complaint of hearing loss / serum creatinine > 1.6 and documentation of 24 hour CCL > 60ml/min is needed to confirm eligibility.

9.0 TREATMENT/INTERVENTION PLAN

9.1 Gemcitabine and cisplatin administration

9.1.1 Patients will receive treatment under the following schedule:

- Gemcitabine: over 30 minutes IV, week 2 & 3 of a 3 week cycle at dose of 800 mg/m²
- Cisplatin: 20 mg /m² over 30 minutes IV, week 2 & 3 of a 3 week cycle

9.1.2 One cycle corresponds to three weeks of treatment (21 days)

9.1.3 Before week 2 of each cycle of therapy a CBC, complete metabolic panel including LDH, magnesium, amylase and lipase will be obtained. The total granulocyte counts must be ≥ 1,500 and the platelet count ≥ 100,000 to proceed with therapy at week 2 of treatment. The serum creatinine must be ≤ 1.6 mg/dl or the CrCl has been measured at ≥ 60 ml/min at screening) to proceed with treatment. Otherwise, hold therapy and re-evaluate weekly.

9.1.4 Before week 3 of each cycle of therapy a CBC and creatinine will be obtained. The total granulocyte counts must be ≥ 1,000 and the platelet count ≥ 75,000 to proceed with therapy. The serum creatinine must be ≤ 1.6 mg/dl to proceed with treatment. Otherwise, hold therapy and re-evaluate weekly. Patients with a baseline serum creatinine of > 1.6 mg/dL but with a creatinine clearance of ≥ 60 ml/min at screening, will be treated as long as creatinine does not increased by more than 30% from their baseline obtained at the start of the study.

9.1.5 A maximum of four weeks delay of treatment is allowed. If gemcitabine and cisplatin treatment is held for toxicity week 2 of any cycle, that cycle and week of treatment is given when toxicity has resolved (i.e missed treatments are made up). If gemcitabine and cisplatin treatment is held for toxicity week 3 of any cycle, that treatment is not made up and treatment continues per previous schedule once toxicity has resolved. If MEK162 treatment is held for toxicity, patients continue on gemcitabine and cisplatin treatment per previous schedule and resume MEK162 when toxicity has resolved.

9.1.6 Patients who require to come off one of the two chemotherapeutic agents, can continue with the other one along with MEK162. At the discretion of the PI and/or Co-PI, patients may be permitted to continue MEK162 alone without gemcitabine or cisplatin after completion of 6 months of combined treatment with MEK162, gemcitabine, and cisplatin. In this situation, MEK162 dosing will continue at the previous dose level.

9.1.7 Ancillary medications:

9.1.7.1 Primary prophylactic G-CSF support is not indicated. Secondary prophylaxis as indicated in section 11.2.

9.1.7.2 Anti-emetic therapy will be given according to institutional guidelines.

9.1.7.3 Erythropoietin may be used according to institutional guidelines or investigator preference.

9.1.8 Treatment will continue until disease progression (clinical or radiological), development of unacceptable toxicity or consent withdrawal.

9.1.9 Dose modifications: see section 11.2

9.2 MEK162 Administration

9.2.1 Starting cycle # 1 day #1 the patient will initiate MEK162 at the appropriate dose level as per table 1 (see section 4.2.). Treatment with MEK162 will be held 2 days prior to chemotherapy administration and resumed the following chemotherapy.

9.2.2 MEK162 in 15 mg tablets will be dispensed through the outpatient pharmacy. Detailed instructions regarding taking the medication will be provided. Adherence will be determined by evaluating patient medication diaries. All non-used tablets will be collected and returned to the pharmacy at the end of each cycle.

9.2.3 MEK162 will be continued, as long as tolerated, until the time of disease progression, or consent withdrawal. Patient whose gemcitabine and cisplatin have been discontinued for toxicity may continue taking MEK162 until recovery.

9.2.4 MEK162 will be supplied by Array BioPharma and provided at no cost to the patient.

9.2.5 See section 11.2 for dose modifications of MEK162 on the phase II portion.

9.3 Correlative studies:

Tissue correlatives: Archival tissue will be obtained on all patients where available; paraffin block or 8-10 unstained slides.

9.3.1 Genotyping:

Archival FFPE tumor tissue and stored buffycoat samples will be used for genotyping studies. Sections will be reviewed by the reference pathologist (Dr. Jinru Shia) DNA will be extracted from reviewed FFPE tumor sections in the genomics core laboratory using Agilent alternative DNA extraction kit. Matched germline DNA will be extracted from stored buffycoat

samples using Gentra Puregene Blood Kit (Qiagen). Tumor samples and matched germline DNA will be sequenced for mutations in over 230 target genes (see Appendix A). These genes include oncogenes and tumor suppressor genes commonly mutated in biliary cancer as well as a wider selection of candidate genes. This targeted gene sequencing platform will be evaluated and interpreted by Dr Michael Berger from the Department of Pathology. Custom oligonucleotides will be used to capture all exons of the target genes, with exon capture performed by hybridization (Agilent SureSelect Target Enrichment System) followed by next generation sequencing (Illumina HSeq). The accuracy and reproducibility of this platform has been demonstrated internally at MSKCC. This method of genotyping will allow the identification of sequence variants, small insertions and deletions and copy number alterations in the target genes. See appendix A for full list of genes to be studied.

9.3.2 Analysis of SPRY4 and DSUP6 gene expression

RNA will be extracted from archival FFPE tumor tissue using High Pure RNA extraction kit in the Geoffrey Beene Translational Core Facility at MSKCC. Reverse transcriptase PCR will be performed using Taqman gene expression assay to assess mRNA expression of markers of MEK activation SPRY4 and DSUP6. Gene expression data will be analysed in conjunction with the bio-informatics core facility.

9.3.2 pERK/Ki67 pre-treatment immunostaining:

Sections (5–6 μm thick) cut from paraffin-embedded pre-treatment tumor biopsies will be analyzed with immunohistochemistry using a rabbit polyclonal antibody for pERK (phospho-p44/42 MAPK [Thr202/Tyr204]; Cell Signaling Technology Inc., MA, USA). Positive and negative controls will ensure that the integrity of all tumor samples and reagents is maintained. Positive controls, selected to encompass a range of pERK staining, include human xenograft MDA-MB-231 (breast) and MiaPaCa (pancreas) cells, and a renal cell carcinoma biopsy. Slides from each biopsy will be stained with a species, isotype (IgG), and concentration-matched negative control antibody. A hematoxylin-eosin slide will also be stained for each sample. Stained slides will be evaluated independently by two pathologists. Localization of pERK staining to cell nuclei or cytoplasm will be evaluated qualitatively. Nuclear pERK staining intensity will be graded semi-quantitatively using a five-point scale that will be based on the percentage of tumor showing positive staining in relation to the total amount of tumor present in the section.: 0, no staining; 1+, weak; 2+, moderate; 3+, strong and 4+, intense. Ki67 will be performed on all samples as per established clinical laboratory protocol. The experiments will be performed in the Pathology Immunohistochemistry Core Laboratory at MSKCC, under the guidance of Dr Jinru Shia. Samples will be preserved in the pathology department at MSKCC.

9.3.3 pERK flow cytometry and cell cycle analysis:

Pharmacodynamic studies exploring the biological efficacy of the different doses of MEK162 will be performed by measuring changes in peripheral blood pERK expression using flow cytometry. The use of pERK for optimal biologic effective dose is meant to be an exploratory investigation to complement but not replace MTD. In addition, PBMCs will be analyzed for

changes in cell cycle by flow cytometry using PI staining These studies will be performed by the Immune Monitoring Core Facility in collaboration with the Wolchok laboratory under the supervision of Dr Margaret Callaghan and Dr Jedd Wolchok.

Blood samples will be analysed for presence of circulating free DNA. The detection of tumor derived DNA circulating within a blood sample provides the opportunity for relatively non-invasive assessment of the genetic alterations harbored in the tumor. Optimally the analysis of cfDNA can be used to determine tumor mutation status for patients in whom a biopsy is not feasible and potentially serial assessment for new genetic alterations that occur during tumor progression and therapeutic resistance. Genotype concordance will be assessed between tumor and cfDNA, the primary endpoint. We will also assess genotype concordance between multiple separate collections of cfDNA as this has not been explored previously. We will further assess clinical correlates that may associate with variance including cfDNA characteristics (fragment size/quantity), tumor burden, and sites of metastatic disease. Supernatant from prepared buffycote samples will be used for this analysis, to be performed in the Jasin laboratory, RRL and the genomics core facility. DNA will be extracted using Qiagen QIAmp CAN columns. Somatic mutations detected in the primary tumor will be assessed in cfDNA through digital PCR

Blood correlatives: Collection and processing of samples:

9.3.5 Buffycoat preparation from blood samples: performed in ETC

Collection of blood:

- Blood should be drawn into a 10-mL Lavendar top EDTA tube
- Mix immediately by gentle inversion 8 to 10 times.
- After collection, store tube upright at room temperature until centrifugation. Blood samples should be centrifuged within one hour of blood collection for best results.

Centrifugation steps:

- For best results, blood samples should be **centrifuged within one hours** of blood collection at room temperature (18-25°C). Store tube upright until centrifugation.
- Remix the blood samples immediately prior to centrifugation by gently inverting the tube 8 to 10 times.
- Centrifuge tube/blood samples at room temperature (18-25°C) in a horizontal rotor (swing-out head) for a **minimum of 20 minutes at 1500 to 1800 RCF** (Relative Centrifugal Force). Some specimens may require 30 minutes. Centrifugation beyond 30 minutes has little additional effect. The tube may be re-centrifuged if the mononuclear “band” or layer is not disturbed. Always check to see that the tube is in the proper centrifuge carrier/adaptor and balanced properly.

Pipetting steps:

- Label a conical centrifuge tube.
- After centrifugation, mononuclear cells and platelets will be in a whitish layer just under the plasma layer
- Using a pipette, **aspirate approximately half of the plasma** from each Lavendar EDTA tube **without disturbing the whitish cell layer** and aliquot into 2ml Eppendorf tubes. The plasma aspirates should be clear so as not to contain any white cells.
- Spin the 2 ml Eppendorf tubes containing plasma at 16,000xg x 10 minutes at 4 degrees.
- Aliquot supernatant into labeled Eppendorf tube and freeze at -80 degrees.
- Using a fresh pipette, **collect the whitish cell layer** from the Lavendar EDTA tube and transfer into a separate properly labeled 15 mL size conical centrifuge tube. This layer will contain the remaining plasma layer plus the PBMC layer (this is the entire remaining layer above the gel). Collection of cells immediately following centrifugation will yield best **results**. The Lavendar EDTA tube will now only contain the unusable layers under the gel, and can be disposed of according to MSKCC SOP on the disposal of biohazardous waste.

You should now have a conical tube containing the whitish cell layers (PBMC layers)

Step 1: Preparation of phosphate buffered saline (PBS) Please allow 15-20 minutes for this step.

- PBS is used for cell washing in step 2. NOTE: This will yield enough washing solution for use on both conical tubes containing PBMCs. The phosphate buffer should be prepared fresh for each subject and kept at room temperature. Any unused buffer should be discarded according to disposal SOPs at your facility.
- Fill the 250 mL graduated disposable beaker (provided in kit) up to the 200 mL mark with deionized/purified lab water. Place one phosphate buffered saline (PBS) tablet into the beaker. Make sure the tablet has dissolved completely and is completely mixed and in solution before proceeding with step 2.

Step 2: Cell Washing Steps

- Using a fresh pipette, add PBS to bring the total volume to 15 mL in each of the graduated conical tubes containing the whitish cell layer (PBMCs). Cap the tubes. Mix cells by inverting the tubes 5 times.
- Centrifuge both conical tubes for 15 minutes at 300 RCF. Aspirate as much supernatant as possible from both tubes without disturbing cell pellets.

- Resuspend cell pellet in both conical tubes by gently vortexing or tapping tube with index finger.
- To each conical tube containing PBMC pellets, add PBS to bring volume to 10 mL in each tube. Cap tubes. Mix cells by inverting tubes 5 times.
- Centrifuge both conical tubes for 10 minutes at 300 RCF. Aspirate as much supernatant from both conical tubes as possible without disturbing cell pellet.
- Cap the conical tubes containing PBMC pellets, make sure tubes are tightly capped to prevent desiccation. Do not freeze tubes in a styrofoam tray because tubes may crack.
- Freeze the dry pellet (do not re-suspend the pellet in additional PBS) in an open tube rack at -80°C. Samples will be stored at MSKCC for planned genetic studies, in -80°C freezer at 3rd floor, room 312 (3rd floor) of the Rockefeller Outpatient Pavilion at 160 E 53rd street. All samples will be labeled with the protocol number (IRB# 13-004), the patient initials, date and time of collection..

9.3.6: Serum sample processing and storage

Serum samples will be obtained for pharmacokinetic studies and for future studies to be specified at that time.

1. Draw 10 ml of blood in red top vacuum tube (no anticoagulant). Allow the tubes to remain at room temperature for 30 to 60 minutes to clot.
2. Centrifuge tubes at 1500 RPM for 15 minutes.
3. Divide the serum samples into 3 equal aliquots.
4. Label cryovials with the protocol number (IRB# 13-004), the patient name, MRN, study number and date of collection.
5. Freeze and store the serum samples at -20° to -80° C as soon as possible once they are aliquoted and labeled.
6. Specimen will be stored in the -80° C freezer located at 160 E 53rd street, 3rd floor, Room 312

9.4.7: Blood samples for pERK studies

1. Draw blood into 4 x CPT tubes
2. Invert all tubes several times immediately after collection
3. Write patient initials, date, and time of collection on each tube
4. Fill in date and time of collection in requisition form
5. Place all collected tubes in biohazard ziplock bag

- Please send all specimens via Stat Messengers to Immune Monitoring Core located at Zuckerman Research Building Room 1513. (There is a blood bin on the table.)

Contact details: Immune Monitoring Core ext 125-2114 (Lab), Attention: Teresa Rasalan ext. 125-2114; Rosemarie Ramsalwak 125-3106 (Pager 2877)

9.4.9: Blood samples for cfDNA studies

- Within one hour of sample collection, centrifuge 10 ml lavender EDTA vacutainer tube at low speed (~3000 RPM) for 10 minutes at 4 degrees.
- Carefully aliquot plasma into 2ml Eppendorf tubes, avoiding buffy coat. Each Eppendorf tube will be labeled with the protocol number (IRB# 13-004), the patient initials, date and time of collection.
- Spin 16,000xg x 10 minutes at 4 degrees.
- Aliquot supernatant into labeled Eppendorf tube and freeze at -80 degrees in the -80°C freezer located in room 312 (3rd floor) of the Rockefeller Outpatient Pavilion at 160 E 53rd street

10.0 EVALUATION DURING TREATMENT/INTERVENTION

Evaluation during treatment to document disease status and treatment toxicity will occur at prespecified intervals according to the following calendar:

Test Type	Week 1 of treatment*	Week 2 of treatment	Week 3 of treatment	Every cycle	Every 3 rd cycle
Clinical					
History & physical	x ^a	x	x ^a	x	
KPS/ECOG	x			x	
Weight	x			x	
Vital signs	x	x	x	x	
Vital signs	x	x	x	x	
Adverse event monitoring	x ^b	x	x ^b	x	
Laboratory					
CBC Comp Direct bilirubin LDH Magnesium Amylase, lipase	x ^a	x	x ^a	x	
CBC, Comp			x ^c	x	
ECHO or MUGA					x ^b

24 hour urine collection for creatinine clearance		As clinically indicated			
CA 19.9/CEA/AFP	x				x
Radiology					
CT ^a Chest/abdomen/pelvis	x				x ^d
Other					
Audiogram		As clinically indicated			
Amsler grid test		x (once per cycle)			
Pre-treatment tissue	x ^e				
Research bloods ^f		See timetable below for research blood collection			

*Week 1 MD visit and labs required for cycle 1 only

^a On week 3 of therapy will only take place during the first cycle

^b On week 3 of therapy will only take place during the first cycle, then as nurse assessment. This is not a formal RN visit, but rather an assessment done by the chemo nurse.

^c Bloods of week 3 for cycle 2 and thereafter

^d MRI of the abdomen and pelvis with non-contrast CT of the chest may be obtained instead of CT chest/abdomen/pelvis with contrast (or with liver tri-phasic) if patients liver disease is not visible on triphasic liver CT, or if the patient is allergic to CT scan contrast despite pre-treatment.

^e Can be obtained anytime after consenting

^f Windows for testing/ treatment are as follows:

Radiology, cardiology investigations: +/- 7 days

Lab and research blood draws, MD visit, clinical evaluation and treatment administration: +/- 3 days

However, MEK162 should always be held for at least 2 days prior to intravenous chemotherapy administration, MEK162 maybe held for an extra 72 hours to facilitate rescheduling of gemcitabine/cisplatin.

^g If treatment is held for toxicity, radiographic imaging and cardiac assessments should continue per original treatment schedule i.e every 9 weeks +/- 1 week from initiation of study treatment.

^hPatients who travel from far may be exempt from Cycle 1 day 3 and Cycle 2 day 3 research blood draws at the discretion of the PI or co-PI.

Investigators will keep track of patient's medical condition for at least three years by calling the patient once a year. This will help checking on patients condition and look at the long-term effects of the study.

Research blood draws:

Time:	Research bloods to be drawn:
Cycle 1 day 1 or ≤ 7 days prior to first study treatment (pre 1 st dose of MEK162)	1,2, 3,4
Cycle 1 day 3 (at least 90 mins post AM dose of	2

MEK162)	
Cycle 1 week 2 (same day as chemotherapy admin)	2
Cycle 2 day 3	2,3 ,4
Cycle 3 week 2	4

1. 1 x 10ml BD vacutainer with EDTA (lavendar top) for buffycoat processing
2. 2. 4 x 8ml CPT tubes with sodium heparin (green & red) for pERK studies
3. 1 x 10ml BD vacutainer serum tube (red-top) for Serum PK samples
4. 1 x 10ml BD vacutainer with EDTA (lavendar top) for cfDNA processing

:11.0 TOXICITIES/SIDE EFFECTS

All toxicities will be rated as per the NCI Common Toxicity Criteria, version 4.

11.1 Anticipated adverse effects are as follows:

11.1.1 Gemcitabine: Nausea, vomiting, alopecia, stomatitis, anorexia, fatigue, elevations of hepatic transaminases, reversible myelosuppression, rash, flu-like symptoms, edema, constipation, paresthesias, hypersensitivity reactions, phlebitis, proteinuria, hematuria, diarrhea or constipation, and rarely interstitial pneumonitis, ARDS and hemolytic uremic syndrome, or sepsis.

11.1.2 Cisplatin: Fatigue, asthenia, nausea, vomiting, loss of appetite, nephrotoxicity, ototoxicity, myelosuppression, anaphylaxis, peripheral neuropathy, diarrhea, alopecia, hypersensitivity reactions, transient elevation of liver enzymes, electrolyte disturbances and rarely myocardial infarction, stroke, seizure, sepsis and blindness.

11.1.3 MEK162: Nausea, vomiting, diarrhoea, abdominal pain, rash, oedema peripheral/generalized/peri-orbital, fatigue, anaemia, dry skin/pruritus, stomatitis, dry mouth, dyspepsia, increased creatine phosphokinase (CK) mucosal inflammation, anorexia and eye events including retinal events, blurred vision, and myodesopsia.

Reproductive risks: Patients should not become pregnant or father a baby while on this study because the drug in this study can affect an unborn baby. Women should not breastfeed a baby while on this study. Patients need to use birth control while on this study. These precautions should be in place for the whole duration of the study therapy and for at least 3 months after the last dose of gemcitabine, cisplatin, or MEK162.

11.2.1 Dose reductions for gemcitabine and cisplatin:

Specific doses for Gemcitabine dose modification: each gemcitabine dose level is decreased by 20% from previous one:

Dose Level	Gemcitabine
Starting dose	800 mg/m ²

Dose level -1	640 mg/m ²
Dose level -2	512 mg/m ² ^a

Dose Level	Gemcitabine
Starting dose	1000 mg/m ²
Dose level -1	800 mg/m ²
Dose level -2	640 mg/m ²

^a Patients who begin at the cohort 3 gemcitabine dose level (1000 mg/m² gemcitabine) and continue on to the phase II portion may be dose reduced to the lowest gemcitabine dose level (512 mg/m²) per discretion of the PI or co-PI.

Specific doses for Cisplatin dose modification: each cisplatin dose level is decreased by 20% from previous one:

Dose Level	Cisplatin
Starting dose	20 mg/m ²
Dose level -1	16 mg/m ²
Dose level -2	13 mg/m ²

Neutropenia:

- Week 2 of therapy of any given cycle, including MEK162 treatment will not be administered unless the ANC is ≥ 1500. Treatment may be held a maximum of 4 weeks.
- Week 3 of therapy of any given cycle, including MEK162 treatment will not be administered unless the ANC is ≥ 1000. Treatment may be held a maximum of 4 weeks.
- If patients experience recurrent grade II hematologic toxicity (platelets or neutrophils) that delays therapy on ≥ 2 occasions, the investigator may choose to decrease the dose of gemcitabine or cisplatin for subsequent cycles of therapy. Gemcitabine should be decreased by 1 dose level first and subsequently cisplatin as needed
- Colony stimulating factors: Patients should not routinely receive prophylactic colony stimulating factors (e.g., G-CSF, GM-CSF) during cycle 1. Subsequent use will be at the discretion of the treating physician.

<u>Dose reductions for neutropenia</u>			
Absolute ANC Nadir	MEK162	Gemcitabine	Cisplatin
Grade 3 (no fever)	No change	No change	No change
Grade 4 (no fever)	No change	Dose level -1	Dose level -1
Grade 3-4 (with fever)	Dose level -1	Dose level -1	Dose level -1
Grade 4 (no fever) 2 nd episode	No change	Dose level -2	Dose level -2
Grade 4 (no fever) 3 rd episode	Dose level -2	Dose level -2	Dose level -2
Grade 3-4 (with fever)	Dose level -2	Dose level -2	Dose level -2

- If the patient requires gemcitabine and cisplatin to be held despite prophylactic use of G-CSF, cisplatin will be reduced one dose level for further cycles.
- If the patient required gemcitabine and cisplatin to be held despite prophylactic use of G-CSF and prior dose reduction of cisplatin for neutropenia, both gemcitabine and cisplatin will be reduced one dose level for further cycles.
- If patients end up receiving either gemcitabine or cisplatin, same guidelines that are pertinent to the drug they remain on apply.

Thrombocytopenia:

- No gemcitabine and cisplatin or MEK162 will be administered unless platelets $\geq 100,000$ week 2 of treatment, and $\geq 75,000$ week 3 of treatment..
- If patients experience recurrent grade II hematologic toxicity (platelets or neutrophils) that delays therapy on ≥ 2 occasions, the investigator may choose to decrease the dose of gemcitabine or cisplatin for subsequent cycles of therapy. Gemcitabine should be decreased by 1 dose level first and subsequently cisplatin as needed.
- If patients end up receiving either gemcitabine or cisplatin, same guidelines that are pertinent to the drug they remain on apply.

<u>Dose reductions for thrombocytopenia</u>			
Nadir Platelet Count	MEK162	Gemcitabine	Cisplatin
Grade 3-4 1 st episode	No change	Dose level -1	Dose level -1
Grade 3-4 2 nd episode	No change	Dose level -2	Dose level -2

Neutropenic Fever:

- Defined as: ANC $< 1000/\text{mm}^3$ with a single temperature of > 38.3 degrees C (101 degrees F) or a sustained temperature of ≥ 38 degrees C (100.4 degrees F) for more than one hour.
- Patients will receive prophylactic pegfilgrastim or filgrastim with each subsequent cycle of gemcitabine and/or cisplatin
- If patients end up receiving either gemcitabine or cisplatin, same guidelines that are pertinent to the drug they remain on apply.

Nephrotoxicity:

- If on week 2 or week 3 of any given cycle of gemcitabine and cisplatin, the creatinine is > 1.6 mg/dl or more than 30% from baseline for patients with baseline creatinine of > 1.6 mg/dL but with CrCl ≥ 60 mL/min, therapy should be held until the serum creatinine ≤ 1.3 mg/dl or $< 30\%$ from baseline for patients with baseline creatinine of > 1.6 mg/dL but with CrCl ≥ 60 mL/min.
- Cisplatin should be dose reduced one dose level for further cycles, with no change in the gemcitabine dose.
- If no recovery occurs within 4 weeks of holding therapy, gemcitabine should be continued as a single-agent and dose reduced one level.
- If patients end up receiving either gemcitabine or cisplatin, same guidelines that are pertinent to the drug they remain on apply.

Neurologic Toxicity:

- If neurologic toxicity \geq grade 2 occurs, at any point of the cycle, then gemcitabine and cisplatin should be held until resolution to grade < 1 , with the cisplatin dose reduced one dose level with no change in the gemcitabine dose.

Cardiovascular Toxicity:

- If during any cycle of therapy a patient develops \geq grade 3 cardiovascular toxicity, then treatment should be permanently discontinued and the PI or co-PI contacted.

Pulmonary Toxicity:

- If during any cycle of gemcitabine and cisplatin therapy a patient develops \geq grade 3 pulmonary toxicity, then study treatment should be permanently discontinued and the PI or co-PI contacted.

Hepatic Dysfunction:

- If bilirubin > 2 (in the absence of Gilbert's disease) or transaminases > 4 X baseline or = CTCAE v4.0 grade 3, whichever is greater, evaluate for biliary obstruction or progressive disease. If drug toxicity suspected, hold until toxicity \leq grade 2 and resume gemcitabine and cisplatin at reduction of one dose level each.

Other Toxicities:

- For any grade 3 or 4 toxicity attributable to gemcitabine and/or cisplatin not mentioned above, despite appropriate and adequate supportive therapy (e.g. antiemetics or anti-diarrheals) the treatment should be withheld until patient's recovery and the possibility of resumption of therapy should be discussed with the Study PI or co-PI, and gemcitabine and/or cisplatin will be reduced one dose level.
- Patients who develop a gastrointestinal perforation, grade 4 hemorrhage, or symptomatic grade 4 venous thromboembolic event will not be eligible for re-treatment.
- Any grade alopecia does not call for any change in therapy.
- Patients who have an abnormal Amsler grid test during treatment will hold MEK162 pending ophthalmologic review, then dose reduction will be as per instructions for ophthalmologic toxicity detailed below (11.2.2). If patients have abnormal Amsler grid tests and are cleared by ophthalmology to resume treatment, they may forego additional ophthalmology evaluations and continue taking MEK162 per the discretion of the PI or co-PI. This will not compromise the assessment of potential MEK162 mediated impact upon vision. Patients will be monitored for changes in vision at clinic visits and referred for ophthalmology assessment of any new ocular symptoms.

Maximum Duration of Treatment Delays/Maximum Dose Reductions:

- Treatment with gemcitabine and cisplatin may be held up to a maximum of 4 weeks from the scheduled cycle initiation date to await resolution of toxicity according to the guidelines

above. If more than 4 weeks are needed for recovery, the study PI or co-PI should be contacted.

11.2.2: Dose reductions for MEK162

For grade 3 and 4 toxicities attributable to treatment with MEK162, treatment should be withheld until patient's recovery to grade ≤ 2 and the possibility of resumption of therapy should be discussed with the Study PI or co-PI.

- Treatment with MEK162 will be held in patients enrolled on the phase II portion with MEK162 related toxicity meeting criteria as below until toxicity resolves to \leq grade 2.
- If the dose of MEK162 used in the phase II portion is 45mg BID, treatment may be re-introduced at a dose reduction to 30mg BID, following resolution of toxicity as above and at the discretion of the study PI or co-PI.
- If the dose of MEK162 used in the phase II portion is 30mg BID, treatment may be re-introduced at a dose reduction to 15mg BID, following resolution of toxicity as above and at the discretion of the study PI or co-PI.
- If toxicity meeting criteria in section 4.2 recurs at MEK162 dose of 15mg BID, study treatment will be discontinued.
- Patients may continue to receive MEK162 if treatment with gemcitabine or cisplatin has been discontinued for nephrotoxicity, neurotoxicity or pulmonary toxicity as per section 11.1.1.
- See section 11.2.1 for specific instructions regarding MEK162 treatment and hematologic toxicity.

1) Ocular disorders defined by:

- Central serous retinopathy (CSR):
 - CTCAE v4.0 grade 2 lasting > 14 days confirmed by ophthalmologic examination
 - CTCAE v4.0 grade ≥ 3 confirmed by ophthalmologic examination
 - CTCAE v4.0 grade ≥ 2 recurrence with same severity following suspension of study therapy followed by resolution and resumption of therapy at the same or lower dose
- CTCAE v4.0 grade ≥ 1 non-CSR retinopathy confirmed by ophthalmologic examination
- CTCAE v4.0 grade ≥ 3 visual disturbances (blurring, floaters, flashing lights) without retinal changes
- CTCAE v4.0 grade ≥ 2 uveitis
- Any other CTCAE v4.0 grade ≥ 3 eye disorders

2) Cardiac dysfunction defined by:

- CTCAE v4.0 grade ≥ 2 cardiac dysfunction; relative decrease in ejection fraction $\geq 20\%$ from baseline value

3) Liver dysfunction defined by:

- Elevation of AST/ALT \geq x 4 times baseline level or CTCAE v4.0 grade 3, whichever is greater.

4) Skin toxicity defined by:

- Rash/ photosensitivity \geq CTCAE v4.0 grade 3 lasting > 48 hours despite skin toxicity treatment

5) Delay of more than 4 weeks in receiving MEK162 due to toxicity.

6) CTCAE v4.0 grade \geq 3 non-hematologic toxicity, considered potentially irreversible or life threatening despite best supportive efforts.

7) CTCAE v4.0 grade \geq 4 hematologic toxicity

Maximum Duration of Treatment Delays/Maximum Dose Reductions:

Treatment with MEK162 may be held up to a maximum of 4 weeks to await resolution of toxicity. If more than 4 weeks are needed for recovery, the study PI or co-PI should be contacted.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

Tumor assessments will be based on Response Evaluation Criteria in Solid Tumors (RECIST 1.1) guidelines.

12.1 Measurability of Tumor Lesions at Baseline

12.1.1 Measurable – Tumor lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of: 10mm by CT scan (irrespective of scanner type) and MRI (no less than double the slice thickness and a minimum of 10mm) or 10mm caliper measurement by clinical exam (when superficial)

12.1.2 Nonmeasurable – leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

12.1.3 Target lesions: All lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected

12.1.4 Non-target lesions: all other lesions (or sites of disease) should be identified as *non-target lesions* and should also be recorded at baseline. It is possible to record multiple nontarget lesions involving the same organ as a single item on the case record form (e.g. “multiple enlarged pelvic lymph nodes” or “multiple liver metastases”). Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

If there is a defined objective that includes the assessment/measurement of therapeutic response to the defined treatment intervention, then detailed description of the measure, methods to be used (e.g., scales and/or definitions to assess response) and the criteria to determine each level of therapeutic response should be included.

If appropriate, indicate what are the limiting factors required to 1) assess response and 2) determine if subject is evaluable and to be included in the study results. This may include total number of treatment interventions required, duration of on-study period, total number of measures utilized to assess therapeutic response, etc.

12.1.5 Guidelines for evaluation of measurable disease: all measurements should be taken and recorded in metric notation. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

12.2 Response criteria

12.2.1 Complete Response (CR) - Target Lesions: Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm (the sum may not be "0" if there are target nodes) Non-target Lesions: disappearance of all non - target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis) . Non-CR: Persistence of 1 or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits

12.2.2 Partial Response (PR) - a 30-99% decrease in the sum of the longest diameter of target lesions, taking as reference the baseline sum longest diameter.

12.2.3 Stable Disease (SD) - neither sufficient shrinkage to qualify for partial response nor sufficient increase to qualify for progressive disease, taking as reference the smallest sum longest diameter since the treatment started, or the persistence of one or more nontarget lesions.

12.2.4 Progressive Disease (PD) – at least a 20% increase in the sum of the longest diameter of target lesions, taking as reference the smallest sum longest diameter recorded since the treatment started and minimum 5mm increase over the nadir, or the appearance of one or more new lesions and/or unequivocal progression of existing nontarget lesions.

12.3 Best Overall Response – the best response recorded from the start of treatment until disease progression/recurrence. The table below should be used to determine best overall response. Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration". Every effort should be made to document the objective progression even after discontinuation of treatment.

Overall Responses for All Combinations of Tumor Responses in Target and Non-target Lesions with or without the Appearance of New Lesions			
Target	Nontarget	New	Overall

Lesions	Lesions	Lesions	Response
CR	CR	No	CR
CR	Non CR/non PD	No	PR
CR	NE	No	PR
PR	Non-PD or NE	No	PR
SD	Non-PD or NE	No	SD
PD	Any	Yes or no	PD
Any	PD	Yes or no	PD
Any	Any	Yes	PD

13.0 CRITERIA FOR REMOVAL FROM STUDY

In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression
- Duration of therapy delay of therapy more than 4 weeks. In the rare instance where the investigator considers the therapy still be beneficial and more than 4 weeks are needed for recovery, the study PI or co-PI should be contacted
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.
- If at any time the patient is found to be ineligible for the protocol as designated in the section on Criteria for Patient/Subject Eligibility (i.e., a change in diagnosis), the patient will be removed from the study.

Patients who are removed from study for any of these reasons will continued to be monitored for survival, unless consent for continued follow-up is withdrawn. Patients will be encouraged to continue to undergo repeat staging radiologic studies scans until progression or death.

14.0 BIostatISTICS

This trial contains a dose escalation phase I portion to determine the MTD of MEK162 in combination with gemcitabine and cisplatin. This will be followed by a non-randomized phase II portion to assess the PFS at six months in patients with advanced biliary cancer treated with gemcitabine, cisplatin and MEK162 at the MTD determined in the phase I trial. Six patients treated at the MTD of MEK162 in the phase I trial will be considered evaluable for the primary and secondary endpoints of the phase II trial. Anticipated accrual is 2 patients per month.

Phase I

In the phase I component of the study, a classic 3+3 cohort dose escalation scheme will be used to identify the MTD of MEK162 when administered with gemcitabine at dose 800 mg/m²

and cisplatin given at dose 20 mg/m² day 1 and 8 of a 21 day cycle. MEK162 will be self-administered orally BID continuously. Three dose levels are planned. A minimum of 2 patients and a maximum of 18 patients will be enrolled in the phase I part of this study.

Table 1: Dose levels for MEK162, gemcitabine and cisplatin

Dose level	MEK162*	Gemcitabine	Cisplatin
1	30 mg PO BID	800 mg/m ²	20 mg/m ²
2	45 mg PO BID	800 mg/m ²	20 mg/m ²
3	MTD (30mg or 45mg PO BID)	1000 mg/m ²	20 mg/m ²

A dose escalation scheme will be used whereby patients will be treated in sequential cohorts of 3. If no patients experience a DLT at dose level 1 in an initial group of 3 patients a new cohort of patients will be enrolled at dose level 2. If 1 of 3 patients experiences a DLT, the cohort will be expanded to 6. If no further DLT occurs, a new cohort will be enrolled at the next dose-level. If 2 of 6 patients experience a DLT, MTD has been exceeded. The MTD will be defined as the highest dose for which not more than 1 of 6 patients develop a DLT. If three or fewer patients are treated at the dose considered to be the MTD, additional patients (to a total of six) will be treated at that level to confirm the MTD. No intrapatient dose escalation will occur. This escalation scheme is summarized in Table 2.

Table 2: Dose escalation scheme using 3 + 3 design.

Number of patients with DLT	Action
0/3	Increase to next dose level
1/3	Accrue 3 more patients to same dose level
≥ 2/3	Stop, recommend previous dose level
1/6	Accrue 3 more patients to next dose level
≥ 2/6	Stop, recommend previous dose level

The dose escalation scheme provides the following probabilities of escalation based on the true chances of DLT at a specific dose level. One can see that the probability of escalation is high if the toxicity risks are low:

Table 3: Probabilities of dose escalation and toxicity:

True probability of toxicity	.10	.20	.30	.40	.50	.60
Probability of escalation	.91	.71	.49	.31	.17	.08

Phase II

A randomized phase II trial conducted in 80 patients with locally advanced or metastatic BTCs reported a 6-month progression-free survival rate (PFS6) of 59% and response rate (RR) of 26% for the combination of gemcitabine and cisplatin.⁵⁵ An exact binomial single stage design will be used to discriminate between true 6-month PFS rates of 59% vs. 82%, and between true response rates of 26% and 50%. Thirty five patients will be enrolled. If at least 26 patients were observed to survive progression-free for at least 6 months, or at least 14 responses were observed among the 35 patients over one year, this agent would be considered worthy of further testing in this disease.

This design yields 91% power to detect a true 6-month PFS rate of at least 82%, and a true RR of at least 50%. It yields a 0.05 probability of a positive PFS result if the true 6-month PFS rate is no more than 59%, and a 0.05 probability of a positive RR result if the true RR is no more than 26%. Therefore, assuming that PFS and RR are independent, the overall type 1 error is .10. The type 1 error decreases very slightly if PFS and RR are positively correlated, which is very likely.

Patients who drop out of the study before 6 months due to toxicity or other reason without documented disease progression will be counted as events (disease progression) for the primary endpoints. However these patients will be encouraged to continue to undergo repeat staging radiologic studies until progression or death.

The PI and co-PI will be monitoring the outcome of the study on frequent basis. If it is found at any time that there are 10 patients that die or progress within the first 6 months, the trial will be stopped as at that point it is mathematically impossible to achieve the desired endpoint of having 26 out of 35 patients alive and progression free at 6 months.

Time to progression will be calculated from study entry to documented disease progression, while progression free survival will be calculated from study entry to documented disease progression or death from any cause, whatever occurs first.

Patients who progress at the 26-week (+/- 2 weeks) planned scan, regardless of the actual calendar date of that scan, will be declared as have progressed at six-months.

Time to death (survival) will be calculated from study entry to death or censored at last follow up.

Time to progression, overall and progression free survival will be estimated using the Kaplan–Meier methodology. The documented response rate and exact 95% confidence intervals will be calculated.

For the correlative study endpoints described in Section 9.3, progression-free-survival, time-to-progression, and overall survival will be correlated with categorical markers using the log-rank test, while Cox regression will be used for continuous markers. Tumor response will be correlated using Fishers exact test for categorical biomarkers and using Wilcoxon rank sum test for continuous markers. The frequency of the CTC in the pre and post treatment biopsies will be summarized descriptively. If CTC are detected, the change in CTC from pre to post treatment biopsies will be associated with response using the Wilcoxon rank sum test. These studies are exploratory. Not all patients may have tissue available so the analyses may involve fewer than 35 patients.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm in the electronic medical record that the patient has received the Notice of Privacy Practice. This must be obtained before the eligibility confirmation and obtaining of the research informed consent. Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (<http://ppr/>). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

15.2 Randomization

No randomization required in this study

16.0 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team. The data collected for this study will be entered into a secure database, the Clinical Research Database (CRDB). Source documentation will be available

to support the computerized patient record.

16.1 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action. Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

16.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials" which can be found at: <http://cancertrials.nci.nih.gov/researchers/dsm/index.html>. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: http://mskweb5.mskcc.org/intranet/_assets/_tables/content/359709/DSMPlans07.pdf

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed and the monitoring procedures will be established at the time of protocol activation

17.0 PROTECTION OF HUMAN SUBJECTS

17.1 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.2 Serious Adverse Event (SAE) Reporting

Any SAE must be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org. The report should contain the following information:

Fields populated from CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
 - A explanation of how the AE was handled
 - A description of the subject's condition
 - Indication if the subject remains on the study
 - If an amendment will need to be made to the protocol and/or consent form.

The PI's signature and the date it was signed are required on the completed report.

For IND/IDE protocols:

The CRDB AE report should be completed as above and the FDA assigned IND/IDE number written at the top of the report. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.

Serious adverse event (SAE) is defined as one of the following:

- Is fatal or life-threatening
- Results in persistent or significant disability/incapacity
- Constitutes a congenital anomaly/birth defect
- Is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above
- Requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:
 - Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition (specify what this includes)

- Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
- Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
- Social reasons and respite care in the absence of any deterioration in the patient's general condition
- Any SAEs that are expected due to the condition being treated, including if the SAE is a primary outcome measure, and whether there has been a clear agreement with regulators not to consider these as SAEs, provided the information is collected elsewhere

17.2.1 Reporting

Reporting requirements to Array:

All events must be reported, by FAX (303-386-1516), to Array Drug Safety within 24 hours of learning of its occurrence. This includes serious, related, labeled (expected) and serious, related, unlabeled (unexpected) adverse experiences. All deaths during treatment or within 30 days following completion of active protocol therapy must be reported within 24 hours.

Any serious adverse event occurring after the patient has provided informed consent and until 4 weeks after the patient has stopped study participation must be reported. This includes the period in which the study protocol interferes with the standard medical treatment given to a patient (e.g. treatment withdrawal during washout period, change in treatment to a fixed dose of concomitant medication).

Serious adverse events occurring more than 4 weeks after study discontinuation need only be reported if a relationship to the Array study drug (or therapy) is suspected.

Follow-up information is sent to the same person to whom the original SAE Report Form was sent, using a new SAE Report Form stating that this is a follow-up to the previously reported SAE and giving the date of the original report. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the blind was broken or not, and whether the patient continued or withdrew from study participation.

If the SAE is not previously documented in the Investigator's Brochure or Package Insert (new occurrence) and is thought to be related to MEK162, an Array Drug Safety representative may urgently require further information from the investigator for Health Authority reporting. Array may need to issue an Investigator Notification (IN), to inform all investigators involved in any study with the same drug that this SAE has been reported. Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in accordance with Directive 2001/20/EC or as per national regulatory requirements in participating countries.

For Comparator Drugs/Secondary Suspects (Concomitant Medications), all serious adverse experiences will be forwarded to the product manufacturer by the investigator.

To ensure patient safety, each pregnancy in a patient on study drug must be reported to Array within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

Pregnancy should be recorded on a Clinical Study Pregnancy Form and reported by the investigator to Array Drug Safety.

Ethics and Good Clinical Practice

This study must be carried out in compliance with the protocol and the principles of Good Clinical Practice, as described in:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996. Directive 91/507/EEC, The Rules Governing Medicinal Products in the European Community.
2. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
3. Declaration of Helsinki and amendments, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects).

The investigator agrees to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

19.0 REFERENCES

1. de Groen PC, Gores GJ, LaRusso NF et al. Biliary tract cancers. *N Engl J Med* 1999, 341:1368-1378.
2. Zhu AX, Hong TS, Hezel AF et al. Current management of gallbladder carcinoma. *The Oncologist* 2010;15:168-181.
3. Parkin DM, Bray F, Ferlay J et al. Global cancer statistics, 2002. *CA Cancer J Clin* 2005 Mar-Apr;55(2):74-108.
4. Charbel H, Al-Kawas FH. Cholangiocarcinoma: epidemiology, risk factors, pathogenesis and diagnosis. *Current Gastroenterology Rep* 2011 Apr;13(2):182-7.
5. Lazcano-Ponce EC, Miquel JF, Munoz N et al. Epidemiology and molecular pathology of gallbladder cancer. *CA: Cancer J Clin* 2001;51:349-364.
6. Leonard GD, O'Reilly EM. Biliary tract cancers: current concepts and controversies. *Expert Opin Pharmacother* 2005;6:211-223.
7. Carriaga MT, Henson DE. Liver, gallbladder, extrahepatic bile ducts, and pancreas. *Cancer* 1995, 75(1 Suppl):171-90.
8. Shaib Y, El-Serag HB. The epidemiology of cholangiocarcinoma. *Semin Liver Dis* 2004, 24(2):115-25.
9. Duffy A, Capanu M, Abou-Alfa GK et al. Gallbladder cancer (GBC): 10-year experience at Memorial Sloan-Kettering Cancer Centre (MSKCC). *J Surg Oncol* 2008;98:485-489.
10. Khan SA, Davidson BR, Goldin R et al. Guidelines for the diagnosis and treatment of cholangiocarcinoma: consensus document. *Gut* 51(Suppl 6):VI1-VI9.
11. Eckel F, Schmid RM. Chemotherapy in advanced biliary tract carcinoma: a pooled analysis of clinical trials. *Br J Cancer* 2007, 96, 896-902.
12. Moufarrij MA, Phillips DR, Cullinane C. Gemcitabine potentiates cisplatin cytotoxicity and inhibits repair of cisplatin-DNA damage in ovarian cancer cell lines. *Mol Pharmacol* 63:862-869, 2003.
13. van Moorsel CJ, Pinedo HM, Veerman G et al. Mechanisms of synergism between cisplatin and gemcitabine in ovarian and non-small-cell lung cancer cell lines. *Br J Cancer* 80:982-990.
14. Valle J, Wasan H, Palmer DH et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N Engl J Med* 2010; 362:1273-1281
15. Andre T, Toumigand C, Rosmorduc O et al. Gemcitabine combined with oxaliplatin (GEMOX) in advanced biliary tract adenocarcinoma : a GERCOR study. *Ann Oncol* 2004 Sep;15(9):1339-43.
16. Andre T, Reyes-Vidal JM, Fartoux L et al. Gemcitabine and oxaliplatin in advanced biliary tree carcinoma : a phase II study. *Br J Cancer*. 2008 Sep 16;99(6):862-7.
17. Knox JJ, Hedley D, Oza A et al. Combining gemcitabine and capecitabine in patients with advanced biliary cancer : a phase II trial. *J Clin Oncol*. 2005;23(10):2332-8.
18. Cho JY, Nam JS, Park MS et al. A phase II study of gemcitabine combined with capecitabine in patients with advanced gallbladder carcinoma. *Yonsei Med J*. 2005;46(4):526-31.
19. Hong YS, Lee J, Lee SC et al. Phase II study of capecitabine and cisplatin in previously untreated advanced biliary tract cancer. *Cancer Chemother Pharmacol* 2007;60:321-328.
20. Kim TW, Chang HM, Kang HJ et al. Phase II study of capecitabine plus cisplatin as first-line therapy in advanced biliary cancer. *Ann Oncol* 2003;14:1115-1120.
21. Ducreux M, Rougier P, Fandi A et al. Effective treatment of advanced biliary cancer using 5-fluorouracil continuous infusion with cisplatin *Ann Oncol* 1998;9:653-656.
22. Hezel AF, Deshpande V, Zhu AX. Genetics of biliary tract cancers and emerging targeted therapies. *J Clin Oncol* 2010 Jul 20;28(21):3531-40

23. Nakazawa K, Dobashi Y, Suzuki S et al. Amplification and overexpression of c-erbB-2, epidermal growth factor receptor, and c-met in biliary tract cancers. *J Pathol* 2005, 206:356–365
24. Yoshikawa D, Ojima H, Wasaki M, et al. Clinicopathological and prognostic significance of EGFR, VEGF, and HER2 expression in cholangiocarcinoma. *Br J Cancer* 2008, 98:418–425.
25. Yamamoto S, Fujii K, Kitadai Y, et al.: Expression of vascular endothelial growth factor in human gallbladder lesions. *Oncol Rep* 1998;5:1065–1069
26. Tannapfel A, Sommerer F, Benicke M et al. Mutations of the BRAF gene in cholangiocarcinoma but not in hepatocellular carcinoma. *Gut* 2003, 52:706–712.
27. Tannapfel A, Benicke M, Katalinic A et al. Frequency of p16(INK4A) alterations and K-ras mutations in intrahepatic cholangiocarcinoma of the liver. *Gut* 2000, 47:721–727.
28. Ohashi K, Nakajima Y, Kanehiro H et al. Ki-ras mutations and p53 protein expressions in intrahepatic cholangiocarcinomas: Relation to gross tumor morphology. *Gastroenterology* 1995, 109:1612–1617.
29. Saetta AA, Papanastasiou P, Michalopoulos NV et al. Mutational analysis of BRAF in gallbladder carcinomas in association with K-ras and p53 mutations and microsatellite instability. *Virchows Arch* 2004 Aug;445(2):179-82
30. Goldenberg D, Rosenbaum E, Argani P et al. The V599E BRAF mutation is uncommon in biliary tract cancers. *Mod Pathol* 2004 Nov; 17(11):1386-91.
31. El-Khoueiry AB, Rankin C, Lenz HJ et al. SWOG 0514: a phase II study of sorafenib (BAY 43-9006) as single agent in patients (pts) with unresectable or metastatic gallbladder cancer or cholangiocarcinomas. *J Clin Oncol* 2007; 25: 18s: (ASCO 2007; abstract 4639).
32. Dealis C, Bertolini F, Malavasi N, et al. A phase II trial of sorafenib (SOR) in patients (pts) with advanced cholangiocarcinoma (CC). *J Clin Oncol* 2008; 26: (ASCO 2008; abstract 4590).
33. Bengala C, Bertolini F, Malavasi N, et al. Sorafenib in patients with advanced biliary tract carcinoma: a phase II trial. *Br J Cancer* 2009; 102: 68-72.
34. Bekaii-Saab TMP, Xiaobai L, Saji M, et al. 100th Annual Meeting of the American Association for Cancer Research (April 18-22, 2009, Denver, CO), A multi-institutional study of AZD6244 (ARRY-142886) in patients with advanced biliary cancers. abstr LB-129.
35. Chang F, Steelman LS, Lee JT et al. Signal transduction mediated by the Ras/Raf/MEK/ERK pathway from cytokine receptors to transcription factors: potential targeting for therapeutic intervention. *Leukemia* 2003; 17:1263-93.
36. McCubrey JA, May WS, Duronio V et al. Serine/threonine phosphorylation in cytokine signal transduction. *Leukemia* 2000; 14:9-21.
37. LoRusso PM, Krishnamurthi SS, Rinehart JJ et al. Phase I pharmacokinetic and pharmacodynamic study of the oral MAPK/ERK kinase inhibitor PD-0325901 in patients with advanced cancers. *Clin Cancer Res* 2010 Mar 15; 16(6):1924-37.
38. Haura EB, Ricart AD, Larson TG et al. A phase II study of PD-0325901, an oral MEK inhibitor, in previously treated patients with advanced non-small cell lung cancer. *Clin Cancer Res* 2010 Apr 15; 16(8):2450-7.
39. Rosen LS, Galatin P, Fehling JM et al. A phase 1 dose-escalation study of XL518, a potent MEK inhibitor administered orally daily to subjects with solid tumors. *J Clin Oncol* 2008; 26(15S):Abstract 14585.
40. Merson C, Chapman MS, Clendeninn Net al. The effect of MEK inhibitor RDEA119 on biomarkers in advanced cancer patients in a phase I clinical trial. Paper presented at: ASCO-NCI-EORTC Annual Meeting on Molecular Markers in Cancer; October 30-November 1, 2008; Hollywood, FL. Abstract 53.

41. Infante JR, Fecher LA, Nallapareddy S et al. Safety and efficacy results from the first-in-human study of the oral MEK 1/2 inhibitor GSK1120212. *J Clin Oncol* 2010;28(suppl). Abstract 2503.
42. Delord, J, Houede N, Awada, A et al. First-in-human phase I safety, pharmacokinetic (PK), and pharmacodynamic (PD) analysis of the oral MEK-inhibitor AS703026 (two regimens [R]) in patients (pts) with advanced solid tumors. *J Clin Oncol* 2010;28(suppl). Abstract 2504.
43. Banerji U, Camidge DR, Verheul HM et al. The first-in-human study of the hydrogen sulfate (Hyd-sulfate) capsule of the MEK1/2 inhibitor AZD6244 (ARRY-142886): a phase I open-label multicenter trial in patients with advanced cancer. *Clin Cancer Res* 2010 Mar 1;16(5):1613-23.
44. Dummer R, Robert C, Chapman PB et al. AZD6244 (ARRY-142886) vs temozolomide (TMZ) in patients (pts) with advanced melanoma: An open-label, randomized, multicenter, phase II study. *J Clin Oncol* 26: 2008 (May 20 suppl; abstr 9033)
45. Huang W, Yang AH, Matsumoto D et al. PD0325901, a mitogen-activated protein kinase kinase inhibitor, produces ocular toxicity in a rabbit animal model of retinal vein occlusion. *J Ocul Pharmacol Ther* 2009 Dec;25(6):519-30.
46. Zhu AX, Meyerhardt JA, Blaszczewski LS et al. Efficacy and safety of gemcitabine, oxaliplatin, and bevacizumab in advanced biliary-tract cancers and correlation of changes in 18-fluorodeoxyglucose PET with clinical outcome: a phase 2 study. *Lancet Oncol* 2010;11:48-54
47. Malka D, Trarbach T, Fartoux L et al. A multicenter, randomized phase II trial of gemcitabine and oxaliplatin (GEMOX) alone or in combination with biweekly cetuximab in the first-line treatment of advanced biliary cancer: Interim analysis of the BINGO trial. *J Clin Oncol* 27:15s, 2009 (suppl; abstr 4520)
48. Chow S, Patel H, Hedley DW. Measurement of MAP Kinase Activation by Flow Cytometry Using Phospho-Specific Antibodies to MEK and ERK: Potential for Pharmacodynamic Monitoring of Signal Transduction Inhibitors. *Cytometry*. 2001 Apr 15;46(2):72-8
49. Hilger RA, Kredke S, Hedley D, Moeller JG, Bauer RJ, Stellberg W, Seeber S, Scheulen ME, Strumberg D. ERK1/2 phosphorylation: A biomarker analysis within a phase I study with the new Raf kinase inhibitor BAY43-9006. *Int J Clin Pharmacol Ther* 2002; 40: 567-568.
50. Chow S, Hedley D, Grom P et al. Whole blood fixation and permeabilization protocol with red blood cell lysis for flow cytometry of intracellular phosphorylated epitopes in leukocyte subpopulations. *Cytometry A* 2005; 67(1):4-17.
51. Krutzik PO, Nolan GP. Intracellular phospho-protein staining techniques for flow cytometry: monitoring single cell signaling events. *Cytometry A* 2003; 55(2): 61-70.
52. Yeh TC, Brown S, Poch G et al. Validation and use of a biomarker for development of the MEK1/2 inhibitor ARRY-142886 (AZD6244).
<http://www.arraybiopharma.com/documents/Publication/PubAttachment98.pdf>
53. Ross JS, Cronin M. Whole cancer genome sequencing by next generation methods. *Am J Clin Pathol* 2011; 136:527-539.
54. Valle J, Wasan H, Palmer DH et al. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N Engl J Med* 2010; 362:1273-1281
55. Xu J, Knox J et al. [Sequence dependence of MEK inhibitor AZD6244 combined with gemcitabine for treatment of biliary cancer. Clin Cancer Res.](#) 2012 Oct 22. (EPUB ahead of print)

20.0 APPENDICES

APPENDIX A: Gene List for Analysis

APPENDIX B: RECIST 1.1:

APPENDIX C: SOP for pERK Phosphoflow studies

APPENDIX D: Amsler grid test

APPENDIX E: ECOG-KPS Conversion Table