Genetics of Biliary Tract Cancers and Emerging Targeted Therapies

Aram F. Hezel, Vikram Deshpande, and Andrew X. Zhu

From the James P. Wilmot Cancer Center, University of Rochester School of Medicine, Rochester NY; and Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA.

Submitted December 6, 2009; accepted April 28, 2010; published online ahead of print at www.jco.org on June 14, 2010.

Supported by a Howard Hughes Medical Institute Early Career Development Award (A.F.H.) and NIH K08 Career Development Award (A.F.H.).

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Corresponding author: Aram F. Hezel, MD, James P. Wilmot Cancer Center, University of Rochester School of Medicine, Rochester, NY, 14642; e-mail: Aram_Hezel@URMC.Rochester.edu.

© 2010 by American Society of Clinical Oncology

0732-183X/10/2821-3531/\$20.00

DOI: 10.1200/JCO.2009.27.4787

ABSTRACT

Biliary tract cancers (BTC), which encompass intra- and extrahepatic cholangiocarcinomas and gallbladder carcinomas, are a genetically diverse collection of cancers. Evidence suggests distinct models of molecular and pathologic progression, and a growing body of genetics data points to a heterogeneous collection of underlying mutations in key oncogenes and tumor suppressor genes. Although tumor genetics have been used to tailor individual treatment regimens and guide clinical decision making in other cancers, these principles have not been applied in BTC. Recent clinical trials with targeted therapies seem promising, although the relationships between subsets of patients with positive responses to therapy and tumor genetics remain unexplored. Here, we summarize the molecular pathogenesis and genetics of BTCs and animal modeling and relate these to recent and ongoing clinical trials with targeted agents.

J Clin Oncol 28:3531-3540. © 2010 by American Society of Clinical Oncology

INTRODUCTION

Biliary tract cancers (BTCs) include a spectrum of invasive adenocarcinomas encompassing both cholangiocarcinoma (CC), which refers to cancers arising in the intrahepatic, perihilar, or distal biliary tree, and gallbladder carcinoma (GBC). BTCs are characterized by early lymph node and distant metastases. Only 10% of patients present with early-stage disease and are considered candidates for surgical resection, which offers the only chance for cure. The prognosis is poor for the majority of patients with locally advanced or metastatic BTC, with a median survival of less than 1 year. BTC afflicts approximately 12,000 people in the United States annually, with a recently appreciated increasing incidence of intrahepatic CC (IHCC).¹ The few clear risk factors share a common theme of chronic tissue damage and inflammation such as gallstones, chronic hepatitis, primary sclerosing cholangitis, and liver fluke infection.^{2,3} Although this collective of diseases (IHCC, extrahepatic CC [EHCC], and GBCs) shares a similar anatomic origin in the biliary system, there are important differences in disease behavior, molecular profiles, and sensitivity to therapy. In general, GBC carries a worse prognosis than CC; however, GBC tends to exhibit a greater response rate (RR) to chemotherapy.⁴ After surgery, GBC is more likely recur distantly than EHCC, for which local recurrence is an appreciable risk.⁵ These differences in clinical behavior are echoed in the molecular features of the disease, which, although overlapping,

are clearly distinct. As the landscape of solid tumor genetics, animal modeling, and clinical research evolves, we see great opportunity to make headway in our basic understanding of BTC biology and in treatment paradigms. Here, we review key molecular features of BTC. We then focus on emerging therapies and discuss the opportunities and challenges presented by the growing ranks of targeted therapies. Other reviews have recently summarized clinical aspects of BTCs including epidemiology, staging, surgical management, and chemotherapeutic treatment of advanced disease to which we refer the reader.^{1,6-12}

HISTOLOGIC PATHOGENESIS

Models of stepwise transformation from a normal epithelial compartment into carcinoma with corresponding accumulation of molecular changes is delineated in colon, brain, breast, and pancreatic cancers.^{13,14} Similarly, such models are in development in BTC, where precancerous changes in the gallbladder and biliary tract are beginning to be linked with molecular changes in established oncogenes and tumor suppressor genes. There are important differences in the pathogenesis of GBC and CC; thus, each entity is discussed separately.

Precursors of CC

There are two appreciated precursors to CC—biliary intraepithelial carcinoma neoplasia (BilIN)

Fig 1. Precursors to biliary tract carcinoma. (A) Biliary dysplasia of the common bile duct (biliary intraepithelial neoplasia-2 [BillN-2]; hematoxylin and eosin [HE]). (B) Biliary dysplasia of the common bile duct (biliary intraepithelial neoplasia-3 [BillN-3]). Compared with BillN-2, BillN-3 shows loss of cell polarity and marked nuclear atypia (HE). (C) Intraductal papillary neoplasm of the bile duct. This papillary neoplasm diffusely involved the intrahepatic biliary tree. The lining epithelium was of the biliary type (HE). The adjacent liver (*) was unremarkable. (D) Gallbladder adenoma lined predominantly by foveolar-type epithelium. There was no evidence of an associated invasive carcinoma (HE). (E) Severe dysplasia/carcinoma in situ of the gallbladder with involvement of Rokitansky-Aschoff sinuses (arrow). There was no evidence of invasive carcinoma (HE). (F) Invasive adenocarcinoma of the gallbladder. There is an extensive intramucosal component (smaller arrow) with focal invasion into the muscularis propria (larger arrow) (HE).

and intraductal papillary neoplasm of the bile duct; the incidence of the former is significantly greater than the latter (Figs 1A to 1C). BillN, which only recently acquired an internationally agreed on classification system, is graded based on the extent of cellular atypia ranging from grades 1 to 3.¹⁵ This mirrors the spectrum of precursor lesions of pancreatic carcinoma, with increasing cellular atypia and architectural aberrancy as the stage advances. Biliary intraductal papillary neoplasm is a macroscopic lesion similar to its pancreatic counterpart intraductal papillary-mucinous neoplasm (Fig 1C).¹⁶⁻¹⁹ Molecularly, these precursors of carcinoma remain poorly characterized, although immunohistochemical analysis suggests that these harbor mutations in *p53* and loss of *SMAD4* (SMAD family member 4).^{20,21} The striking similarity of the precursor lesions between the pancreatic ductal system and the biliary tract raises questions about a shared molecular pathogenesis. For instance, are *KRAS* mutations, which are detected in advanced cancers (discussed later) and are also an early genetic event in the formation of GBC and pancreatic cancer, detectable in the early stages of BillN? A detailed molecular profile of is not yet fully established.

Precursors of GBC

Gallbladder adenomas and dysplasia are two distinct pathologic lesions of the epithelial mucosa (Figs 1D and 1E). Both lesions are found in association with carcinomas, although the frequency of dysplastic changes in association with carcinoma seems to be far greater. In pathologic studies most invasive carcinomas are associated with dysplasia and carcinoma in situ, whereas adenomas are rarely found in

the context of such dysplastic changes.²²⁻²⁵ Thus, based on present evidence, a metaplasia-dysplasia → carcinoma in situ → carcinoma sequence seems to be the more prevalent route over an adenoma → carcinoma sequence.²⁶ Genetic evidence, although limited, also suggests that adenomas, which tend to harbor mutations in *CTNNB1* (catenin $\beta 1$; more commonly known as β -catenin), and epithelial dysplasias, which have been found to harbor *KRAS* mutations, may represent two distinct processes (Table 1). *KRAS* mutations have been identified in dysplastic and hyperplastic lesions associated with anomalous junction of the pancreaticobiliary duct (AJPBD). This anatomic variant of the biliary tract is strongly associated with the risk for the development of GBC, and these tumors consistently demonstrate *KRAS* mutations.^{27,34} Conversely, *CTNNB1* mutations are found in a high number of adenomas but are rare in GBCs and never found in dysplastic lesions.²⁸⁻³⁰ The reciprocal relationship between *CTNNB1* and *KRAS* mutations among adenomas and dysplasia/carcinomas, the frequent association of dysplasia with carcinoma, and the less common finding of adenoma surrounding cancer all suggest that gallbladder adenomas may represent a distinct biologic process. Furthermore, clinically, it is observed that incidentally identified adenomas rarely harbor cancerous changes and seem to represent an entity with negligible clinical risk. This might suggest a paradigm where *CTNNB1*-mutated adenomas represent a relatively low malignant potential, whereas *KRAS*-mutated dysplastic lesions associated with inflammation (such as in cases of AJPBD) may have a greater malignant potential.

Table 1. Mutations in Gallbladder Adenoma, Dysplasia, and Carcinomas

Gene and Reference	Adenoma (%)	Dysplasia (%)	Carcinoma (%)
<i>β-Catenin</i>			
Yanagisawa et al ²⁸	63		5
Rashid et al ²⁹	57		9
Chang et al ³⁰	58	0	0
<i>KRAS</i>			
Hanada et al ²⁷	17	15	38
Kim et al ³¹	0	0	20
Watanabe et al ³²	0		19
Wistuba et al ³³	25		
<i>TP53</i>			
Kim et al ³¹	0	0	36
Wistuba et al ³³	0		
<i>P16INK4A</i>			
Kim et al ³¹	0	0	31

GENETIC AND MOLECULAR FEATURES

BTCs have a spectrum of mutations in established oncogenes and tumor suppressor genes, although the actual frequency of these events has been hard to pinpoint. This is because of heterogeneous sample sets, differing methodologies used to detect mutations, and the high

degree of stromal cells, or desmoplasia, which can dilute out cancer-specific nucleic acids and limit analysis. Despite these confounding factors, established mutations and amplifications of known oncogenes have been clearly demonstrated (Table 2). Beyond those genes and associated pathways that harbor mutations in BTC, a number of other molecules that have demonstrated importance in the disease and represent potential therapeutic targets are also discussed.

KRAS/MAPK Pathway

KRAS is a member of the RAS/RAC family that functions to propagate growth signals via downstream effectors such as RAF and PI3K.⁴⁶ Activation of KRAS is found in subsets of both GBC and CCs with reported rates that range from 3% to 100% among patients with GBC in the setting of AJPBD.^{27,35,36,38,47} The frequency of KRAS mutation is consistently between 40% and 50% among IHCC (Table 2). BRAF (v-ras murine sarcoma viral oncogene homolog B1), which sits at the upstream of the MEK/MAPK signaling pathways and is a key effector of the oncogenic activity of KRAS, was found to be mutated in approximately 20% of patients in two European BTC collections including both GBCs and IHCCs.^{35,40} Importantly, these mutations were mutually exclusive of KRAS mutations, which occurred in 25% and 45% of GBCs and IHCCs, respectively, suggesting that activation of the RAF/MAPK signaling pathway is a key event within the majority of cancers.³⁵ GBCs and CCs from North America and Chile demonstrated differing results. Despite microdissection of specimens and the use of three methods to detect mutations, no BRAF mutations were

Table 2. Mutational Spectrum of Oncogenes

Gene and Reference	Gallbladder Carcinoma (%)	Cholangiocarcinoma (%)		Detection Method
		EHCC	IHCC	
<i>CTNNB1/β-catenin</i>				
Yanagisawa et al ²⁸	5			SEQ
Rashid et al ²⁹	9	0		SEQ
Chang et al ³⁰	0			SEQ
<i>KRAS</i>				
Hanada et al ²⁷	38			PCR-SSCP
Kim et al ³¹	20			PCR-RFLP
Watanabe et al ³²	19			PCR-RFLP
Rashid et al ³⁶	3	15		SEQ
Suto et al ³⁷		10		PCR-SSCP
Tannapfel et al ³⁸			54	SEQ
Tannapfel et al ³⁵			45	SEQ
Ohashi et al ³⁹			48	SEQ
<i>BRAF</i>				
Saetta et al ⁴⁰	33			SEQ
Tannapfel et al ³⁵			22	SEQ
Goldenberg et al ⁴¹	0	0	0	SEQ and GLCR
<i>EGFR</i>				
Leone et al ⁴²	9	18	20	SEQ
Gwak et al ⁴³		6		SEQ
Nakazawa et al ⁴⁴	12	5	10	IHC and FISH
<i>PIK3CA</i>				
Riener et al ⁴⁵	4	0	9	SEQ
<i>ERBB2/HER2</i>				
Nakazawa et al ⁴⁴	16	5	0	IHC and FISH

Abbreviations: EHCC, extrahepatic cholangiocarcinoma; IHCC, intrahepatic cholangiocarcinoma; SEQ, sequencing; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism; RFLP, restriction fragment length polymorphism; GLCR, gap ligase chain reaction; IHC, immunohistochemistry; FISH, fluorescent in situ hybridization.

found.⁴¹ Whether these discrepant results represent a true regional difference in the genetics of BTC in Europe versus the Americas or sampling bias is not clear. Mutational analysis of *BRAF* among additional larger cohorts or in a prospective manner with newer, more sensitive technologies could resolve this question.

EGFR and HER2/NEU

Both the *EGFR* (epidermal growth factor receptor) and *HER2/NEU* (v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma-derived oncogene homolog [avian]) receptor tyrosine kinases have been implicated in BTC pathogenesis. *EGFR* mutations are found in a subset (13.6% to 15%) of BTCs; however, the results of the mutation analyses from the two studies were quite different.^{42,43} The first study identified previously described mutations in the tyrosine kinase domains that have been observed to confer sensitivity to small-molecule inhibitors of *EGFR* in lung cancer.⁴³ The subsequent report identifies mutations not previously described; thus presently, we do not know the biologic significance of these mutations or to what extent they may confer sensitivity to currently available small-molecule inhibitors.⁴² *EGFR* amplifications are also seen in approximately 6% of all BTCs, further prompting consideration of inhibitors of this pathway.⁴⁴ *ERBB2/HER2/NEU* overexpression and gene amplification were also evaluated in this study,⁴⁴ and excellent concordance (79%) between expression measured by immunohistochemistry and gene amplification was observed. GBC had the highest incidence of overexpression at approximately 15% compared with EHCC at approximately 5% and IHCC at 0%. The relevance of this gene in GBC pathogenesis is further demonstrated by transgenic mouse models (described in detail later) created through expression of *ErbB2* in the gallbladder epithelium.⁴⁸

IGF/PIK3CA

PIK3CA (phosphoinositide 3-kinase, catalytic, α -polypeptide) hotspot mutations, which are commonly found in colon, breast, gastric, and brain cancers, are rarely found in BTC.⁴⁵ Despite a low prevalence of activating mutations, immunohistochemical evaluation of downstream *PIK3CA* targets eIF4-E and 4E-BP1, looking

for overexpression and evidence of phosphorylation, respectively, suggests that additional mechanisms may be at play in positively regulating this pathway. Mutations in *PTEN* (phosphatase and tensin homolog) have not been reported. Expression profiling of BTC compared with normal biliary epithelium has identified AKT/mTOR signaling components as being upregulated, including the potential drug target insulin-like growth factor 1 receptor (IGF1-R).⁴⁹ Expression of IGF1-R and its ligands is seen in the majority of GBCs and metastases.^{50,51} Treatment of BTC cell lines with a small-molecule inhibitor of the IGF1-R suggested the efficacy of targeting this pathway.⁵²

Tumor Suppressor Genes

The spectrum of tumor suppressor gene mutations seen in BTC largely mirrors that of other GI malignancies (Table 3) and includes loss of function of *CDKN2A* (cyclin-dependent kinase inhibitor 2A; commonly known as *P16INK4A*),⁵⁸ *TP53* (tumor protein p53),⁵⁹ and *SMAD4*.⁶⁰ *CDKN2A*, which is located at the 9p21 locus, encodes two proteins through splicing of an alternative first exon and is frequently silenced in BTC through hypermethylation, homozygous deletion, and inactivating point mutation.^{38,53,61} Evidence of *TP53* inactivation through either overexpression or mutation is identified in a similarly high frequency of BTCs, irrespective of site of origin.^{36,37,54} *SMAD4* mutations, which function to abrogate the tumor suppressor activities of transforming growth factor β (TGF- β), have been identified in a proportion of BTCs, including both GBC and CC.^{55,56} Concurrent mutations of *KRAS* and *SMAD4* were identified in an adenocarcinoma arising from a choledochal cyst, suggesting cooperation of two events in cancer progression,³⁴ and engineered mutants of both *p53* and *Smad4* have been used in concert to model IHCC in mice.^{62,63} These studies are discussed in detail later in this article. Additional tumor suppressor genes have been implicated in BTC pathogenesis, including *STK11* (serine/threonine kinase 11; also known as *LKB1*), in which case one homozygous deletion was identified among 16 tumors of the common bile duct.⁵⁷ *STK11*, a key regulator of metabolism and cellular polarity, is frequently mutated in non-small-cell lung cancer (NSCLC) and less frequently mutated in pancreatic cancers.^{64,65}

Table 3. Mutational Spectrum of Tumor Suppressor Genes

Gene and Reference	Gallbladder Carcinoma (%)	Cholangiocarcinoma (%)		Detection Method
		EHCC	IHCC	
<i>P16INK4A</i>				
Kim et al ³¹	31			SSCP
Ueki et al ⁵³	62	55		Numerous
Tannapfel et al ³⁸			88	Numerous
<i>TP53</i>				
Kim et al ³¹	36			SSCP
Suto et al ³⁷		33		PCR-SSCP
Tannapfel et al ⁵⁴			37	SEQ
<i>SMAD4</i>				
Hahn et al ⁵⁵		16		PCR-SSCP
Argani et al ⁵⁶		55	13	IHC
<i>STK11/LKB1</i>				
Su et al ⁵⁷		6		SEQ

Abbreviations: EHCC, extrahepatic cholangiocarcinoma; IHCC, intrahepatic cholangiocarcinoma; SSCP, single-strand confirmation polymorphism; PCR, polymerase chain reaction; SEQ, sequencing; IHC, immunohistochemistry.

Additional Genetic Features

Beyond mutations in established oncogenes and tumor suppressor genes, genomic studies using array comparative genomic hybridization demonstrate widespread genomic instability of BTC and many additional alterations. These include recurrent loss of the 9p21 locus encoding the *P16INK4A/P19ARF* tumor suppressor gene and a number of recurrent amplifications including 5p, 7p, and 19q, all loci amplified in subsets of other cancers⁶⁶⁻⁶⁸; 7p and 19q harbor the oncogenes *EGFR* and *AKT*, respectively. *MYCN* (v-myc myelocytomatosis viral related oncogene) is rarely amplified.⁶⁹ Recently, mutations in *KEAP1* (kelch-like ECH-associated protein 1) have been identified. *KEAP1* suppresses NFE2L2 (nuclear factor [erythroid-derived 2]-like 2; also known as NRF2) activity, which transcriptionally controls expression of many genes involved in attenuating oxidative damage.⁷⁰ The expression of additional proteins implicated in the pathogenesis of cancer is similarly characterized in BTC and is reviewed elsewhere.^{9,71}

Microenvironment and Stroma

Beyond the vasculature supporting cancer growth, the microenvironment, which includes activated fibroblasts, macrophages, and T regulatory cells, has been shown to both influence tumor growth and impact the delivery of therapeutics. Hepatic stellate cells (resident myofibroblasts within the hepatic parenchyma) are associated with a poor prognosis in IHCC and enhance growth of BTC cell lines in vitro.^{72,73} T regulatory cells and mast cells, which modulate immune response to cancer growth and may be of prognostic significance in colon cancer, are found in IHCC.^{74,75} The TGF- β pathway, which may regulate both immune response to cancer and fibrosis, has been shown to be upregulated in IHCC. This pathway may serve to both drive stromal proliferation and dampen immune response.⁷⁶ TGF- β may also modulate angiogenesis through the transcriptional activation of vascular endothelial growth factor (VEGF).⁷⁷ The role of tumor stroma and the additional factors involved are reviewed in further depth elsewhere.⁷⁸ Several studies have examined the expression of VEGFs and other angiogenic molecules and their correlation with clinical outcomes. VEGF is expressed by most tumors,^{79,80} and high VEGF expression seems to correlate with hematogenous metastasis in IHCC,⁷⁹ nodal metastases, and poor survival.^{81,82}

MODELS OF BTC

Animal models of cancer have proved invaluable in furthering our understanding of cancer genetics and biologic behavior and are increasingly being used in drug development. There are a number of spontaneous models of BTC in mammalian systems, including mice, rats, and guinea pigs (reviewed in Kiguchi et al⁴⁸), that rely on chemical mutagens. Among these, a furan-induced rat CC model has yielded insight into the potential roles of *c-met* and *ErbB2* oncogenes.⁸³⁻⁸⁷ Immortalized rat cholangiocyte lines have proved to be valuable to understanding *ErbB2* signaling in CC. Furthermore, these cells may be transplanted into syngeneic animals, offering a genetically malleable in vivo model of advanced disease.^{88,89}

Engineered mouse models have proved valuable in the study of cancer genetics, pathogenesis, and biology. Recently, such models have become fundamental to drug development efforts.⁹⁰⁻⁹² Three models of BTC—one of GBC and two of CC—are based on key

genetic events observed in human tumors. As discussed earlier, amplification of *ERBB2* is seen in approximately 15% of GBCs. A transgenic mutant with constitutive expression of *ErbB2* in the gallbladder and biliary tract epithelium develops GBC and CC with a 100% penetrance.⁴⁸ Tumors from this model exhibit elevated MAPK signaling and COX2 expression and have been used to preclinically evaluate the mTOR inhibitor rapamycin.⁹³ Mutations in key tumor suppressor genes have also enabled modeling of BTC. Liver fibrosis and inflammation have been associated with IHCC in humans,^{94,95} and carbon tetrachloride is used as a hepatotoxin in several animal models of both cirrhosis and hepatocellular carcinoma.⁹⁶ With regular carbon tetrachloride exposure causing liver injury and fibrosis, *p53*-mutant mice develop IHCC.⁶³ Mirroring human tumors, expression of *c-met*, *cox2*, and *ErbB2* and loss of E-cadherin are elevated. Because chronic liver injury is significant in the pathogenesis of these tumors, this model may offer some advantage over approaches relying solely on targeted mutations. Homozygous somatic mutation of both *Smad4* and *Pten* in the liver leads to IHCC.⁶² Although deletion of *Smad4* alone does not lead to any hepatic phenotype, mutation of *Pten* does cause biliary hyperplasia and IHCC, albeit with a significantly longer latency. Although *Pten* itself is not mutated in human BTC, deregulation of the pathway may occur through alternative mechanisms such as *AKT* amplification, *PI3K* mutations, and so on, as discussed earlier.

These models provide a critical foundation for further efforts focused on understanding other relevant genetic events involved in BTC pathogenesis. The major oncogenic mutation observed in humans, *KRAS*, has not yet been integrated into modeling efforts, nor have other less common mutations, such as *PIK3CA*, *BRAF*, or *EGFR*. The availability of a spectrum of models mirroring the genetics of the human disease would facilitate both a basic understanding of disease biology and the clinical development of drugs with specific activity given the genetics of each tumor type.

TARGETED THERAPIES

Chemotherapy in locally advanced and metastatic BTC has historically been loosely defined based on reports of activity of single agents and combinations of agents such as gemcitabine, cisplatin, oxaliplatin, and fluorouracil in phase II trials.¹² Only recently has an optimal strategy for the management of advanced disease been defined; a randomized phase III trial demonstrated improved overall survival (OS) with gemcitabine and cisplatin compared with gemcitabine alone.⁹⁷ The trial, which included both CC and GBC, demonstrated that the addition of cisplatin to gemcitabine, compared with gemcitabine alone, afforded a significant progression-free survival (PFS; median, 8.4 v 6.5 months, respectively) and OS benefit (median, 11.7 v 8.3 months, respectively). This study has set the new benchmark against which future studies may be compared.

A number of novel inhibitors of key oncogenic pathways are in development and have been evaluated in BTCs as single agents, as combined targeted agents, and in combination with classic chemotherapy (Tables 4 and 5). All of these early-stage trials include patients with locally advanced or metastatic disease, both GBC and CC. Amplifications and rare activating mutations in *EGFR* are observed in BTC and seem to predict responsiveness to drugs targeting *EGFR* in other cancers.^{105,106} Both small-molecule inhibitors of the kinase domain and blocking antibodies have been used in BTC.^{44,107,108} A

Table 4. Molecularly Targeted Trials

Treatment	Target	No. of Patients	RR (%)	PFS	Reference
Multiple agents (first line)					
GEMOX		50	NA	44% (4 months)	
GEMOX-cetuximab	<i>EGFR</i>	51	NA	61% (4 months)	Malka et al ⁹⁸
GEMOX-bevacizumab	<i>VEGF</i>	35	40	7 months (median)	Zhu et al ¹⁰⁴
Single agents (first and second line)					
AZD6244	<i>MEK1/2</i>	22	14	5.4 months (median)	Bekaii-Saab et al ¹⁰¹
Erlotinib	<i>EGFR</i>	43	7	2.6 months (median)	Philip et al ⁹⁹
Lapatinib	<i>EGFR/HER2</i>	17	0	1.8 months (median)	Ramanathan et al ¹⁰⁰
Sorafenib	<i>BRAF/VEGFR</i>	36	6	2 months (median)	El-Khoueiry et al ¹⁰³
Sorafenib	<i>BRAF/VEGFR</i>	46	2	2.3 months (median)	Bengala et al ¹⁰²

Abbreviations: RR, response rate; PFS, progression-free survival; GEMOX, gemcitabine and oxaliplatin; NA, not available.

randomized phase II study evaluating the impact of cetuximab when added to gemcitabine and oxaliplatin (GEMOX) among patients who did not receive prior chemotherapy suggests a benefit in terms of PFS at 4 months.⁹⁸ One hundred one randomly assigned patients were stratified according to tumor stage, location, and prior treatments. Thirty-six patients were available for an interim analysis. The 4-month PFS rates were 44% (95% CI, 20% to 70%) and 61% (95% CI, 36% to 83%) among patients receiving GEMOX and GEMOX-cetuximab, respectively. Toxicity was balanced between arms with the exception of rash, which was more common among patients on cetuximab. The difference in PFS rate at 4 months seems promising. The study did not report the status of *KRAS* mutation for patients receiving cetuximab-based treatment. In a trial of 42 patients with BTC treated with single-agent erlotinib, three patients achieved responses to therapy, indicating efficacy of the drug in at least some patients.⁹⁹ Many of these patients had already received first-line therapy. Because *EGFR* mutation was not tested in these patients, we do not know whether responses correlated with *EGFR* mutation status. Lapatinib, a dual

EGFR1 and *ERBB2* (*HER2*) inhibitor, has been tested in 17 patients with BTCs.¹⁰⁰ No responses were observed, although five patients had stable disease.

AZD6244, a potent inhibitor of *MEK1/2*, downstream of RAS and RAF, has been tested in 29 patients including 22 who were evaluable for response at the time of reporting.¹⁰¹ Thirty-nine percent of patients had received prior therapy. Three patients achieved responses (one complete response and two partial responses) for an RR of 14%. Median PFS was 5.4 months, and median OS was 8.2 months. Correlation between clinical responses and p-ERK staining was observed. Genetic analysis of *BRAF* and *KRAS* mutation status is ongoing. Among single agents tested in a mixed population of pretreated and treatment-naïve patients, this relatively high RR and PFS are encouraging. Sorafenib, which targets *BRAF* and *VEGF* receptors, has been evaluated in two different phase II studies. A single-agent trial of sorafenib in 46 patients included patients with an Eastern Cooperative Oncology Group performance status of 2, of whom 35% needed to discontinue treatment as a result of toxicity. One patient had a partial

Table 5. Molecularly Targeted Therapies in Development

Treatment	Target	Phase	Sponsoring Institution(s)	ClinicalTrials.gov Identification No.
Oxaliplatin, capecitabine, and sorafenib	<i>BRAF/VEGFR</i>	I/II	University of Wisconsin, Madison, WI	NCT00634751
Gemcitabine, oxaliplatin, and sorafenib	<i>BRAF/VEGFR</i>	I/II	University of Miami Sylvester Comprehensive Cancer Center, Miami, FL	NCT00955721
Erlotinib and docetaxel	<i>EGFR</i>	II	Hoosier Oncology Group, Indianapolis, IN	NCT00532441
BIBW 2992 in cancers with <i>EGFR</i> and/or <i>HER2</i> gene amplification	<i>EGFR/HER2</i>	II	Massachusetts General Hospital Cancer Center, Boston, MA	NCT00748709
FOLFOX6 and bevacizumab	<i>VEGF</i>	II	Georgetown University, Washington, DC	NCT00881504
Gemcitabine, cisplatin, and sorafenib	<i>BRAF/VEGFR</i>	II	Memorial Sloan-Kettering Cancer Center, New York, NY	NCT00919061
Gemcitabine, capecitabine, and vandetanib	<i>VEGFR/EGFR</i>	I/II	University of Colorado at Denver and Health Sciences Center, Denver, CO	NCT00551096
ARRY-438162	<i>MEK</i>	I/II	Sarah Cannon Research Institute, Nashville, TN	NCT00959127

Abbreviation: FOLFOX, infusional fluorouracil, leucovorin, and oxaliplatin.

response (2%), and the median PFS was 2.3 months.¹⁰² Another phase II study of sorafenib as a single agent in 36 treatment-naïve patients reported an RR of 6%, median PFS of 2 months, and median OS of 6 months.¹⁰³

In a single-arm phase II study, the efficacy and safety of bevacizumab, a humanized monoclonal antibody against VEGF, in combination with GEMOX in patients with advanced BTC was established.¹⁰⁴ Of the 35 patients enrolled, 14 (40%) had a partial response, and an additional 10 (29%) had stable disease. The median OS was 12.7 months, and the median PFS was 7.0 months. Treatment was generally well tolerated, and grade 3 and 4 toxicities included neutropenia, elevation of transaminases, peripheral neuropathy, hypertension, anorexia, and thrombocytopenia. Despite the encouraging results, because of the single-arm study design, patient selection bias, and well-known efficacy of GEMOX alone, the relative contribution of bevacizumab remains to be defined. Combining targeted agents that inhibit different pathways critical to cancer growth and survival represents an attractive strategy. An interim report of a phase II study of bevacizumab in combination with erlotinib in 34 patients with CC and GBC observed a promising RR of 20% and a time to progression of more than 7 months.¹⁰⁹

LOOKING FORWARD: IMPROVED MODELS, TUMOR GENOTYPING, AND TARGETED CLINICAL TRIALS

The data on human BTC provide a picture of a collection of distinct diseases that could be classified both by the anatomic location of the tumor's origin (ie, gallbladder, extrahepatic or intrahepatic ducts) and underlying genetics. This genetic heterogeneity is mirrored by differing clinical behavior and responses to therapy.⁴ *KRAS* mutation seems to have the highest incidence, at approximately 40% to 50% in IHCC and lower in GBC and EHCC (Table 2). *ERBB2/HER2* amplifications are found in subsets of GBC. Given the evidence of efficacy of trastuzumab in gastric cancer with such amplifications, the relevance of testing *ERBB2/HER2* should be similarly evaluated in BTC patients. Regarding *BRAF*, the real mutation rate seems impossible to speculate on. However, common activation of the downstream MEK/MAPK pathway and promising single-agent activity of a potent MEK inhibitor should help to prioritize further testing and relate activity to underlying tumor genetics. Rare *EGFR* mutations raise questions concerning responses to small-molecule inhibitors of *EGFR*, as are observed among NSCLC patients with these mutations.¹⁰⁵ Additional mutations in the PI3K pathway point to that pathway's importance.

Improved animal models not only may help to define the biology and behavior of cancers with specific genetics, but may also provide insights into the optimum strategy for using targeted agents and thus streamline clinical efforts. As discussed earlier, engineered mouse models of lung and pancreatic cancers, among others, have proved useful in testing emerging drugs. Creating models of BTC harboring the relevant genetic mutations (eg, *KRAS*, *PIK3CA*) would be a first step. Therefore, the key therapeutic questions (eg, how to best select patients based on tumor genetics and combine new targeted therapies) are similar to those that exist in many other diseases, such as lung, colon, and breast cancer. As these questions are answered in these more common malignancies, we should see progress regarding these issues in BTC.

A newly defined standard of care of gemcitabine in combination with cisplatin in BTC provides a foundation on which to add targeted

agents.⁹⁷ Despite the lack of randomized controlled trials comparing gemcitabine plus cisplatin versus GEMOX versus gemcitabine plus capecitabine, GEMOX and gemcitabine plus capecitabine may represent reasonable alternatives based on phase II studies.¹¹⁰⁻¹¹⁶ One immediate question prompted by early promising data, as well as success in colon cancer, is to what extent *EGFR*-targeting antibodies will offer benefit. The early report of an improved PFS with GEMOX-cetuximab compared with GEMOX alone gives this question further weight.⁹⁸ It will be crucial to retrospectively evaluate *KRAS* status among patients enrolled onto this randomized effort to see whether any benefit is restricted to a *KRAS* wild-type subgroup of patients. Additionally, ongoing studies in genetically selected patients will offer insight into any added benefit of *EGFR*-targeted agents in *KRAS* wild-type populations. Because methodologies for screening such patients are presently clinically adapted and the numbers of patients with BTC with wild-type *KRAS* is approximately 50%, a prospective trial evaluating cetuximab or panitumumab in screened patients is certainly feasible. Even if *EGFR*-targeting agents prove beneficial in *KRAS* wild-type patients, we will need to have trials available for the many patients with *KRAS* and/or *BRAF* mutations. The potential benefits of bevacizumab or other antiangiogenic agents when added to chemotherapy should be further examined in randomized studies. Will MEK inhibitors have a role in *KRAS*-mutant BTC, possibly in combination with PI3K inhibitors, as was shown to be beneficial in a mouse model of NSCLC?⁹¹ Proving the value of enriching for specific populations of BTC for targeted therapy will prompt us to expedite the routine use of genetic screening in these patients. This is already occurring in selected trials (Table 5). Given the smaller number of patients with BTC compared with other common solid tumors, coordination of trials among institutions and cooperative groups, both nationally and internationally, will be the key to progress.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory**

Role: Aram F. Hezel, Bayer (C), Andrew X. Zhu, Genentech (C), Bayer (C), sanofi-aventis (C) **Stock Ownership:** None **Honoraria:** None

Research Funding: None **Expert Testimony:** None **Other**

Remuneration: None

AUTHOR CONTRIBUTIONS

Conception and design: Aram F. Hezel, Vikram Deshpande, Andrew X. Zhu

Collection and assembly of data: Aram F. Hezel, Vikram Deshpande, Andrew X. Zhu

Data analysis and interpretation: Aram F. Hezel, Vikram Deshpande, Andrew X. Zhu

Manuscript writing: Aram F. Hezel, Vikram Deshpande, Andrew X. Zhu

Final approval of manuscript: Aram F. Hezel, Vikram Deshpande, Andrew X. Zhu

REFERENCES

- Shai Y, El-Serag HB: The epidemiology of cholangiocarcinoma. *Semin Liver Dis* 24:115-125, 2004
- Lee CH, Chang CJ, Lin YJ, et al: Viral hepatitis-associated intrahepatic cholangiocarcinoma shares common disease processes with hepatocellular carcinoma. *Br J Cancer* 100:1765-1770, 2009
- El-Serag HB, Engels EA, Landgren O, et al: Risk of hepatobiliary and pancreatic cancers after hepatitis C virus infection: A population-based study of U.S. veterans. *Hepatology* 49:116-123, 2009
- Eckel F, Schmid RM: Chemotherapy in advanced biliary tract carcinoma: A pooled analysis of clinical trials. *Br J Cancer* 96:896-902, 2007
- Jarnagin WR, Ruo L, Little SA, et al: Patterns of initial disease recurrence after resection of gallbladder carcinoma and hilar cholangiocarcinoma: Implications for adjuvant therapeutic strategies. *Cancer* 98:1689-1700, 2003
- Mosconi S, Beretta GD, Labianca R, et al: Cholangiocarcinoma. *Crit Rev Oncol Hematol* 69:259-270, 2009
- Wistuba II, Gazdar AF: Gallbladder cancer: Lessons from a rare tumour. *Nat Rev Cancer* 4:695-706, 2004
- Khan SA, Thomas HC, Davidson BR, et al: Cholangiocarcinoma. *Lancet* 366:1303-1314, 2005
- Thomas MB: Biological characteristics of cancers in the gallbladder and biliary tract and targeted therapy. *Crit Rev Oncol Hematol* 61:44-51, 2007
- Olnes MJ, Erlich R: A review and update on cholangiocarcinoma. *Oncology* 66:167-179, 2004
- Anderson CD, Pinson CW, Berlin J, et al: Diagnosis and treatment of cholangiocarcinoma. *Oncologist* 9:43-57, 2004
- Hezel AF, Zhu AX: Systemic therapy for biliary tract cancers. *Oncologist* 13:415-423, 2008
- Maitra A, Hruban RH: Pancreatic cancer. *Annu Rev Pathol* 3:157-188, 2008
- Vogelstein B, Fearon ER, Hamilton SR, et al: Genetic alterations during colorectal-tumor development. *N Engl J Med* 319:525-532, 1988
- Zen Y, Adsay NV, Bardadin K, et al: Biliary intraepithelial neoplasia: An international interobserver agreement study and proposal for diagnostic criteria. *Mod Pathol* 20:701-709, 2007
- Zen Y, Sasaki M, Fujii T, et al: Different expression patterns of mucin core proteins and cytokeratins during intrahepatic cholangiocarcinogenesis from biliary intraepithelial neoplasia and intraductal papillary neoplasm of the bile duct: An immunohistochemical study of 110 cases of hepatolithiasis. *J Hepatol* 44:350-358, 2006
- Abraham SC, Lee JH, Hruban RH, et al: Molecular and immunohistochemical analysis of intraductal papillary neoplasms of the biliary tract. *Hum Pathol* 34:902-910, 2003
- Klöppel G, Kosmahl M: Is the intraductal papillary mucinous neoplasia of the biliary tract a counterpart of pancreatic papillary mucinous neoplasm? *J Hepatol* 44:249-250, 2006
- Zen Y, Fujii T, Itatsu K, et al: Biliary papillary tumors share pathological features with intraductal papillary mucinous neoplasm of the pancreas. *Hepatology* 44:1333-1343, 2006
- Nakanishi Y, Zen Y, Kondo S, et al: Expression of cell cycle-related molecules in biliary premalignant lesions: Biliary intraepithelial neoplasia and biliary intraductal papillary neoplasm. *Hum Pathol* 39:1153-1161, 2008
- Hoang MP, Murakata LA, Katabi N, et al: Invasive papillary carcinomas of the extrahepatic bile ducts: A clinicopathologic and immunohistochemical study of 13 cases. *Mod Pathol* 15:1251-1258, 2002
- Yamagiwa H: Mucosal dysplasia of gallbladder: Isolated and adjacent lesions to carcinoma. *Jpn J Cancer Res* 80:238-243, 1989
- Roa JC, Anabalón L, Roa I, et al: Promoter methylation profile in gallbladder cancer. *J Gastroenterol* 41:269-275, 2006
- Duarte I, Llanos O, Domke H, et al: Metaplasia and precursor lesions of gallbladder carcinoma: Frequency, distribution, and probability of detection in routine histologic samples. *Cancer* 72:1878-1884, 1993
- Albores-Saavedra J, Alcántara-Vazquez A, Cruz-Ortiz H, et al: The precursor lesions of invasive gallbladder carcinoma: Hyperplasia, atypical hyperplasia and carcinoma in situ. *Cancer* 45:919-927, 1980
- Roa I, de Aretxabala X, Araya JC, et al: Preneoplastic lesions in gallbladder cancer. *J Surg Oncol* 93:615-623, 2006
- Hanada K, Tsuchida A, Iwao T, et al: Gene mutations of K-ras in gallbladder mucosae and gallbladder carcinoma with an anomalous junction of the pancreaticobiliary duct. *Am J Gastroenterol* 94:1638-1642, 1999
- Yanagisawa N, Mikami T, Saegusa M, et al: More frequent beta-catenin exon 3 mutations in gallbladder adenomas than in carcinomas indicate different lineages. *Cancer Res* 61:19-22, 2001
- Rashid A, Gao YT, Bhakta S, et al: Beta-catenin mutations in biliary tract cancers: A population-based study in China. *Cancer Res* 61:3406-3409, 2001
- Chang HJ, Jee CD, Kim WH: Mutation and altered expression of beta-catenin during gallbladder carcinogenesis. *Am J Surg Pathol* 26:758-766, 2002
- Kim YT, Kim J, Jang YH, et al: Genetic alterations in gallbladder adenoma, dysplasia and carcinoma. *Cancer Lett* 169:59-68, 2001
- Watanabe H, Date K, Itoi T, et al: Histological and genetic changes in malignant transformation of gallbladder adenoma. *Ann Oncol* 10:136-139, 1999 (suppl 4)
- Wistuba II, Miquel JF, Gazdar AF, et al: Gallbladder adenomas have molecular abnormalities different from those present in gallbladder carcinomas. *Hum Pathol* 30:21-25, 1999
- Shimotake T, Aoi S, Tomiyama H, et al: DPC-4 (Smad-4) and K-ras gene mutations in biliary tract epithelium in children with anomalous pancreaticobiliary ductal union. *J Pediatr Surg* 38:694-697, 2003
- Tannapfel A, Sommerer F, Benicke M, et al: Mutations of the BRAF gene in cholangiocarcinoma but not in hepatocellular carcinoma. *Gut* 52:706-712, 2003
- Rashid A, Ueki T, Gao YT, et al: K-ras mutation, p53 overexpression, and microsatellite instability in biliary tract cancers: A population-based study in China. *Clin Cancer Res* 8:3156-3163, 2002
- Suto T, Habano W, Sugai T, et al: Aberrations of the K-ras, p53, and APC genes in extrahepatic bile duct cancer. *J Surg Oncol* 73:158-163, 2000
- Tannapfel A, Benicke M, Katalinic A, et al: Frequency of p16(INK4A) alterations and K-ras mutations in intrahepatic cholangiocarcinoma of the liver. *Gut* 47:721-727, 2000
- Ohashi K, Nakajima Y, Kanehiro H, et al: Ki-ras mutations and p53 protein expressions in intrahepatic cholangiocarcinomas: Relation to gross tumor morphology. *Gastroenterology* 109:1612-1617, 1995
- 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* 445:179-182, 2004
- Goldenberg D, Rosenbaum E, Argani P, et al: The V599E BRAF mutation is uncommon in biliary tract cancers. *Mod Pathol* 17:1386-1391, 2004
- Leone F, Cavalloni G, Pignochino Y, et al: Somatic mutations of epidermal growth factor receptor in bile duct and gallbladder carcinoma. *Clin Cancer Res* 12:1680-1685, 2006
- Gwak GY, Yoon JH, Shin CM, et al: Detection of response-predicting mutations in the kinase domain of the epidermal growth factor receptor gene in cholangiocarcinomas. *J Cancer Res Clin Oncol* 131:649-652, 2005
- 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* 206:356-365, 2005
- Riener MO, Bawohl M, Clavien PA, et al: Rare PIK3CA hotspot mutations in carcinomas of the biliary tract. *Genes Chromosomes Cancer* 47:363-367, 2008
- Malumbres M, Barbacid M: RAS oncogenes: The first 30 years. *Nat Rev Cancer* 3:459-465, 2003
- Levi S, Urbano-Ispizua A, Gill R, et al: Multiple K-ras codon 12 mutations in cholangiocarcinomas demonstrated with a sensitive polymerase chain reaction technique. *Cancer Res* 51:3497-3502, 1991
- Kiguchi K, Carbajal S, Chan K, et al: Constitutive expression of ErbB-2 in gallbladder epithelium results in development of adenocarcinoma. *Cancer Res* 61:6971-6976, 2001
- Hansel DE, Rahman A, Hidalgo M, et al: Identification of novel cellular targets in biliary tract cancers using global gene expression technology. *Am J Pathol* 163:217-229, 2003
- Kornprat P, Rehak P, Ruschoff J, et al: Expression of IGF-I, IGF-II, and IGF-IR in gallbladder carcinoma: A systematic analysis including primary and corresponding metastatic tumours. *J Clin Pathol* 59:202-206, 2006
- Alvaro D, Barbaro B, Franchitto A, et al: Estrogens and insulin-like growth factor 1 modulate neoplastic cell growth in human cholangiocarcinoma. *Am J Pathol* 169:877-888, 2006
- Wolf S, Lorenz J, Mössner J, et al: Treatment of biliary tract cancer with NVP-AEW541: Mechanisms of action and resistance. *World J Gastroenterol* 16:156-166, 2010
- Ueki T, Hsing AW, Gao YT, et al: Alterations of p16 and prognosis in biliary tract cancers from a population-based study in China. *Clin Cancer Res* 10:1717-1725, 2004

54. Tannapfel A, Weinans L, Geissler F, et al: Mutations of p53 tumor suppressor gene, apoptosis, and proliferation in intrahepatic cholangiocellular carcinoma of the liver. *Dig Dis Sci* 45:317-324, 2000
55. Hahn SA, Bartsch D, Schroers A, et al: Mutations of the DPC4/Smad4 gene in biliary tract carcinoma. *Cancer Res* 58:1124-1126, 1998
56. Argani P, Shaikat A, Kaushal M, et al: Differing rates of loss of DPC4 expression and of p53 overexpression among carcinomas of the proximal and distal bile ducts. *Cancer* 91:1332-1341, 2001
57. Su GH, Hruban RH, Bansal RK, et al: Germ-line and somatic mutations of the STK11/LKB1 Peutz-Jeghers gene in pancreatic and biliary cancers. *Am J Pathol* 154:1835-1840, 1999
58. Kim WY, Sharpless NE: The regulation of INK4/ARF in cancer and aging. *Cell* 127:265-275, 2006
59. Levine AJ, Finlay CA, Hinds PW: P53 is a tumor suppressor gene. *Cell* 116:S67-S69, 2004
60. Massagué J: TGFbeta in cancer. *Cell* 134:215-230, 2008
61. Yoshida S, Todoroki T, Ichikawa Y, et al: Mutations of p16Ink4/CDKN2 and p15Ink4B/MTS2 genes in biliary tract cancers. *Cancer Res* 55:2756-2760, 1995
62. Xu X, Kobayashi S, Qiao W, et al: Induction of intrahepatic cholangiocellular carcinoma by liver-specific disruption of Smad4 and Pten in mice. *J Clin Invest* 116:1843-1852, 2006
63. Farazi PA, Zeisberg M, Glickman J, et al: Chronic bile duct injury associated with fibrotic matrix microenvironment provokes cholangiocarcinoma in p53-deficient mice. *Cancer Res* 66:6622-6627, 2006
64. Hezel AF, Bardeesy N: LKB1; linking cell structure and tumor suppression. *Oncogene* 27:6908-6919, 2008
65. Ji H, Ramsey MR, Hayes DN, et al: LKB1 modulates lung cancer differentiation and metastasis. *Nature* 448:807-810, 2007
66. Shiraishi K, Okita K, Harada T, et al: Comparative genomic hybridization analysis of genetic aberrations associated with development and progression of biliary tract carcinomas. *Cancer* 91:570-577, 2001
67. Gorunova L, Parada LA, Limon J, et al: Non-random chromosomal aberrations and cytogenetic heterogeneity in gallbladder carcinomas. *Genes Chromosomes Cancer* 26:312-321, 1999
68. Miller G, Socci ND, Dhall D, et al: Genome wide analysis and clinical correlation of chromosomal and transcriptional mutations in cancers of the biliary tract. *J Exp Clin Cancer Res* 28:62, 2009
69. Ooi A, Suzuki S, Nakazawa K, et al: Gene amplification of Myc and its coamplification with ERBB2 and EGFR in gallbladder adenocarcinoma. *Anticancer Res* 29:19-26, 2009
70. Shibata T, Kokubu A, Gotoh M, et al: Genetic alteration of Keap1 confers constitutive Nrf2 activation and resistance to chemotherapy in gallbladder cancer. *Gastroenterology* 135:1358-1368, 2008
71. Fava G, Marzoni M, Benedetti A, et al: Molecular pathology of biliary tract cancers. *Cancer Lett* 250:155-167, 2007
72. Okabe H, Beppu T, Hayashi H, et al: Hepatic stellate cells may relate to progression of intrahepatic cholangiocarcinoma. *Ann Surg Oncol* 16:2555-2564, 2009
73. Chuaysri C, Thuwajit P, Paupairoj A, et al: Alpha-smooth muscle actin-positive fibroblasts promote biliary cell proliferation and correlate with poor survival in cholangiocarcinoma. *Oncol Rep* 21:957-969, 2009
74. Kobayashi N, Hiraoka N, Yamagami W, et al: FOXP3+ regulatory T cells affect the development and progression of hepatocarcinogenesis. *Clin Cancer Res* 13:902-911, 2007
75. Terada T, Matsunaga Y: Increased mast cells in hepatocellular carcinoma and intrahepatic cholangiocarcinoma. *J Hepatol* 33:961-966, 2000
76. Zen Y, Harada K, Sasaki M, et al: Intrahepatic cholangiocarcinoma escapes from growth inhibitory effect of transforming growth factor-beta1 by overexpression of cyclin D1. *Lab Invest* 85:572-581, 2005
77. Benckert C, Jonas S, Cramer T, et al: Transforming growth factor beta 1 stimulates vascular endothelial growth factor gene transcription in human cholangiocellular carcinoma cells. *Cancer Res* 63:1083-1092, 2003
78. Sirica AE, Dumur CI, Campbell DJ, et al: Intrahepatic cholangiocarcinoma progression: Prognostic factors and basic mechanisms. *Clin Gastroenterol Hepatol* 7:S68-S78, 2009
79. Yoshikawa D, Ojima H, Iwasaki M, et al: Clinicopathological and prognostic significance of EGFR, VEGF, and HER2 expression in cholangiocarcinoma. *Br J Cancer* 98:418-425, 2008
80. Giatromanolaki A, Sivridis E, Simopoulos C, et al: Hypoxia inducible factors 1alpha and 2alpha are associated with VEGF expression and angiogenesis in gallbladder carcinomas. *J Surg Oncol* 94:242-247, 2006
81. Park BK, Paik YH, Park JY, et al: The clinicopathologic significance of the expression of vascular endothelial growth factor-C in intrahepatic cholangiocarcinoma. *Am J Clin Oncol* 29:138-142, 2006
82. Hida Y, Morita T, Fujita M, et al: Vascular endothelial growth factor expression is an independent negative predictor in extrahepatic biliary tract carcinomas. *Anticancer Res* 19:2257-2260, 1999
83. Elmore LW, Sirica AE: Phenotypic characterization of metaplastic intestinal glands and ductular hepatocytes in cholangiofibrotic lesions rapidly induced in the caudate liver lobe of rats treated with furan. *Cancer Res* 51:5752-5759, 1991
84. Elmore LW, Sirica AE: "Intestinal-type" of adenocarcinoma preferentially induced in right/caudate liver lobes of rats treated with furan. *Cancer Res* 53:254-259, 1993
85. Sirica AE: Biliary proliferation and adaptation in furan-induced rat liver injury and carcinogenesis. *Toxicol Pathol* 24:90-99, 1996
86. Radaeva S, Ferreira-Gonzalez A, Sirica AE: Overexpression of C-NEU and C-MET during rat liver cholangiocarcinogenesis: A link between biliary intestinal metaplasia and mucin-producing cholangiocarcinoma. *Hepatology* 29:1453-1462, 1999
87. Lai GH, Sirica AE: Establishment of a novel rat cholangiocarcinoma cell culture model. *Carcinogenesis* 20:2335-2340, 1999
88. Lai GH, Zhang Z, Shen XN, et al: ErbB-2/neu transformed rat cholangiocytes recapitulate key cellular and molecular features of human bile duct cancer. *Gastroenterology* 129:2047-2057, 2005
89. Sirica AE, Zhang Z, Lai GH, et al: A novel "patient-like" model of cholangiocarcinoma progression based on bile duct inoculation of tumorigenic rat cholangiocyte cell lines. *Hepatology* 47:1178-1190, 2008
90. Olive KP, Jacobetz MA, Davidson CJ, et al: Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science* 324:1457-1461, 2009
91. Engelman JA, Chen L, Tan X, et al: Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med* 14:1351-1356, 2008
92. Sharpless NE, Depinho RA: The mighty mouse: Genetically engineered mouse models in cancer drug development. *Nat Rev Drug Discov* 5:741-754, 2006
93. Wu Q, Kiguchi K, Kawamoto T, et al: Therapeutic effect of rapamycin on gallbladder cancer in a transgenic mouse model. *Cancer Res* 67:3794-3800, 2007
94. Kuper H, Ye W, Broomé U, et al: The risk of liver and bile duct cancer in patients with chronic viral hepatitis, alcoholism, or cirrhosis. *Hepatology* 34:714-718, 2001
95. Shaib YH, El-Serag HB, Davila JA, et al: Risk factors of intrahepatic cholangiocarcinoma in the United States: A case-control study. *Gastroenterology* 128:620-626, 2005
96. Alpini G, Elias I, Glaser SS, et al: Gamma-interferon inhibits secretin-induced cholestasis and cholangiocyte proliferation in a murine model of cirrhosis. *J Hepatol* 27:371-380, 1997
97. Valle J, Wasan H, Palmer DH, et al: Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *NEJM* 362:1273-1281, 2010
98. 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:XXXX, 2009 (suppl 15s; abstr 4520)
99. Philip PA, Mahoney MR, Allmer C, et al: Phase II study of erlotinib in patients with advanced biliary cancer. *J Clin Oncol* 24:3069-3074, 2006
100. Ramanathan RK, Belani CP, Singh DA, et al: A phase II study of lapatinib in patients with advanced biliary tree and hepatocellular cancer. *Cancer Chemother Pharmacol* 64:777-783, 2009
101. Bekaii-Saab TMP, Xiaobai L, Saji M, et al: A multi-institutional study of AZD6244 (ARRY-142886) in patients with advanced biliary cancers. 100th Annual Meeting of the American Association for Cancer Research, Denver, CO, April 18-22, 2009 (abstr LB-129)
102. Bengala C, Bertolini F, Malavasi N, et al: Sorafenib in patients with advanced biliary tract carcinoma: A phase II trial. *Br J Cancer* 102:68-72, 2010
103. 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* 25:XXXX, 2007 (suppl 18s; abstr 4639)
104. Zhu AX, Meyerhardt JA, Blaszkowsky LS, et al: Efficacy and safety of gemcitabine, oxaliplatin, and bevacizumab in patients with advanced biliary tract cancers and correlation of FDG-PET changes with clinical outcome: A phase II study. *Lancet Oncol* 11:48-54, 2010
105. Lynch TJ, Bell DW, Sordella R, et al: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 350:2129-2139, 2004
106. Tsao MS, Sakurada A, Cutz JC, et al: Erlotinib in lung cancer: Molecular and clinical predictors of outcome. *N Engl J Med* 353:133-144, 2005

107. Paule B, Herelle MO, Rage E, et al: Cetuximab plus gemcitabine-oxaliplatin (GEMOX) in patients with refractory advanced intrahepatic cholangiocarcinomas. *Oncology* 72:105-110, 2007

108. Sprinzl MF, Schimanski CC, Moehler M, et al: Gemcitabine in combination with EGF-receptor antibody (Cetuximab) as a treatment of cholangiocarcinoma: A case report. *BMC Cancer* 6:190, 2006

109. Holen KD, Mahoney MR, LoConte NX, et al: Efficacy report of a multicenter phase II trial testing a biologic-only combination of biweekly bevacizumab and daily erlotinib in patients with unresectable biliary cancer (BC): A Phase II Consortium (P2C) study. *J Clin Oncol* 26:XXXX, 2008 (suppl; abstr 4522)

110. Cho JY, Paik YH, Chang YS, et al: Capecitabine combined with gemcitabine (CapGem) as first-line treatment in patients with advanced/metastatic biliary tract carcinoma. *Cancer* 104:2753-2758, 2005

111. Iyer RV, Gibbs J, Kuvshinov B, et al: A phase II study of gemcitabine and capecitabine in advanced cholangiocarcinoma and carcinoma of the gallbladder: A single-institution prospective study. *Ann Surg Oncol* 14:3202-3209, 2007

112. 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* 23:2332-2338, 2005

113. Riechmann RP, Townsley CA, Chin SN, et al: Expanded phase II trial of gemcitabine and capecitabine for advanced biliary cancer. *Cancer* 110:1307-1312, 2007

114. Harder J, Riecken B, Kummer O, et al: Out-patient chemotherapy with gemcitabine and oxaliplatin in patients with biliary tract cancer. *Br J Cancer* 95:848-852, 2006

115. Gebbia N, Verderame F, Di Leo, et al: A phase II study of oxaliplatin (O) and gemcitabine (G) first line chemotherapy in patients with advanced biliary tract cancers. *J Clin Oncol* 23:XXXX, 2005 (suppl 16S; abstr 4132)

116. André T, Tournigand C, Rosmorduc O, et al: Gemcitabine combined with oxaliplatin (GEMOX) in advanced biliary tract adenocarcinoma: A GERCOR study. *Ann Oncol* 15:1339-1343, 2004

Not an ASCO Member? Subscribe to *Journal of Oncology Practice*

Journal of Oncology Practice (JOP) is ASCO's bimonthly forum for providing its subscribers with information, news, and tools to enhance practice efficiency and promote a high standard of quality for patient care in your practice.

Every issue of *JOP* includes important features on cancer policy issues and their practical effect on cancer care, methods for enhancing the quality of patient care, and tools for improving practice management.

Whether you are in an office or hospital setting, a community or academic environment, *JOP* provides practical information and advice that oncologists and other oncology professionals can apply immediately to their practice. Key features include:

- Published for all members of the practice—physicians, nurses, and administrators
- Timely and relevant information to help practices succeed
- Focus on improving practice efficiency and quality of care
- Covers legal, financial, technology, and personnel issues

Subscribe today at www.jop.ascopubs.org

American Society of Clinical Oncology