

Biliary tract carcinomas: From chemotherapy to targeted therapy

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Contents

1. Introduction	136
2. Molecular, genetic and epigenetic events in BTC	137
3. Surgery and adjuvant treatment	139
4. Systemic therapy in advanced disease	139
4.1. Chemotherapy	139
4.1.1. Effective combinations: FPD-platinum compounds; GEM-platinum compounds; FPD–GEM	139
4.1.2. GEM–cisplatin as a new standard of care	141
4.2. Targeted therapies	142
5. Conclusion	143
Conflict of interest	144
Reviewers	144
Acknowledgments	144
References	144
Biographies	148

Abstract

Biliary tract carcinomas (BTC) are a group of tumours arising from the epithelial cells of intra- and extra-hepatic biliary ducts and the gallbladder, characterised by a poor prognosis.

Surgery is the only curative procedure, but the risk of recurrence is high and furthermore, the majority of patients present with unresectable disease at the time of diagnosis. Systemic therapy is the mainstay of treatment for patients who present recurrent or metastatic disease. Progress has been made in the last decade to identify the most effective chemotherapy regimens, with the recent recommendation of the combination of gemcitabine–cisplatin as the standard schedule.

Comprehension of the molecular basis of cholangiocarcinogenesis and tumour progression has recently led to the experimentation of targeted therapies in patients with BTC, demonstrating promising results.

In this review we will discuss the clinical experience with systemic treatment for BTC, focusing on future directions with targeted therapies.

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1. Introduction

Biliary tract carcinomas are a group of tumours arising from the epithelial cells of intra- and extra-hepatic biliary ducts and the gallbladder. They can be divided in gallbladder carcinomas (GBC) and cholangiocarcinomas

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(CC). The latter includes extrahepatic cholangiocarcinomas (EHC), intrahepatic cholangiocarcinomas (IHC) and Klatskin tumour, a CC occurring at the junction of the right and left hepatic ducts.

Histologically, more than 90% of BTC are well-differentiated and fall into the category of mucin-producing adenocarcinomas; other types, such as squamous cell carcinoma and small cell carcinoma are less common.

Even though BTC is the 2nd second most common primary hepatic tumour, after hepatocellular carcinoma (HCC), it is still considered to be a rare disease in the Western world, with an incidence of 1–2 cases/100,000. On the contrary, these neoplasms are more common in Eastern countries and South America, with up to 96 cases/100,000 [1].

Prognosis for advanced BTC, which is defined as metastatic or surgically unresectable, is very poor, as median overall survival (OS) is generally less than 1 year following diagnosis [2].

In the majority of cases there is no familial predisposition or specific genetic mutation. Hereditary forms, especially for GBC, have been associated with specific syndromes, such as Gardner Syndrome, Hereditary non-polyposis colorectal cancer (HNPCC) and Neurofibromatosis.

However, a number of environmental and pathologic conditions have been identified as probable risk factors. Biliary diseases such as primary sclerosing cholangitis (PSC) [3], cirrhosis, hepato/chole/choledocholithiasis, chronic cholecystitis, chronic non-alcoholic liver disease, and Hepatic C Virus (HCV) infection can all promote neoplastic transformation [4]. In Eastern countries, infection by liver flukes, such as *Clonorchis sinensis* or *Opistorchis viverrini*, has proven to be the strongest risk factor.

CC is more common in the 7th decade, with a slight prevalence for men, whereas GBC tends to mainly affect women with a median age of onset at 65 years. This gender difference might be explained with the different prevalence of certain risk factors (e.g. cholelithiasis is more common in women).

2. Molecular, genetic and epigenetic events in BTC

BTC is the result of malignant transformation of cholangiocytes, in which genetic and epigenetic changes are required for transformation, promotion, and progression [5].

In this section we will illustrate the main molecular pathways that are related to cancerous transformation, such as NO, COX2 and EGFR. We also report the incidence of specific, key role gene mutations in BTC. Finally we provide an outlook on the newest perspectives in molecular research.

Fig. 1 summarises the most important molecular events involved in carcinogenesis. Chronic inflammation is the main risk factor that contributes to the pathogenesis of this kind of neoplasm, as it induces cholangiocytes to produce chemokines and cytokines. This signal cascade results in promotion of growth and survival advantages: the subsequent activation of nitric oxide (NO) or cyclooxygenase-2

(COX2) pathways causes damage in the DNA mismatch repair machinery. The resultant DNA damage leads to accumulation of mutations and alteration of genes involved in cell growth, inhibition of apoptosis and promotion of angiogenesis, such as K-RAS, p53, mdm2, waf-1, p16INK4a, DPC4/Smad4 and APC [6–13].

A close relationship exists between COX-2 and Epithelial Growth Factor Receptor (EGFR) family members. In mice models, constitutive expression of ErbB2 and EGFR in gallbladder and biliary tree epithelia results in elevated COX-2 and subsequent development of BTC. Activation of the EGFR pathway may occur via various different mechanisms. It has been demonstrated that TGF- α , commonly contained in bile acids stimulates the activation of EGFR and its downstream pathways [14,15]. These include, among others, enhancement of COX-2 expression and prostaglandin E2 (PGE2) production that, through the PGE2/EP1 receptor, induces transactivation of EGFR. This signalling is, in part, enhanced by Src [16], a tyrosine kinase (TK) implicated in tumour cell proliferation, adhesion and metastasis [17]. Src is also an important mediator of many downstream effects of EGFR [18].

The EGFR pathway regulates the synthesis and secretion of several angiogenic growth factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and Interleukine 8 (IL-8) [19].

Acquired genetic mutations in the EGFR pathway may be responsible for the activation of carcinogenesis. EGFR-activating mutations in the TK domain are found in about 15% of cases [20,21], and EGFR gene amplifications are detected in 6% of BTC [22].

Other members of the EGFR family, such as ErbB2, may also be intricately involved; for example, overexpression of ErbB2 which is detected in hepatolithiasis and PCS [23,24], has been reported in EHC [25,26], IHC [27,28] and CC in general [29].

The mutational status of K-RAS has been evaluated in several clinical and preclinical studies that are summarised in Table 1. We recently demonstrated that the incidence of K-RAS mutations in Italian patients was low (6.1%) [25]: this is in accordance with other Western studies [30,31]. However, the highest percentage of K-RAS mutations was found in Eastern countries (38–52%) suggesting that geographical differences in aetiology or genetics might explain this variability [32–36].

B-RAF was found to be mutated in 22% of GBC and 33% of European IHC patients [37,38]. In our experience we observed B-RAF mutations in 8.1% of patients, which is generally lower than other reports [25].

Mutational analysis of PI3KCA revealed that hotspot mutations within exons 9 and 20 are rare in BTCs and the frequency ranges from 4% to 9%. Mutations in PTEN were only found in 4% of CC without loss of protein expression [25,39].

The aberrant expression of specific microRNAs (miRNAs), important mediators of posttranscriptional regulation

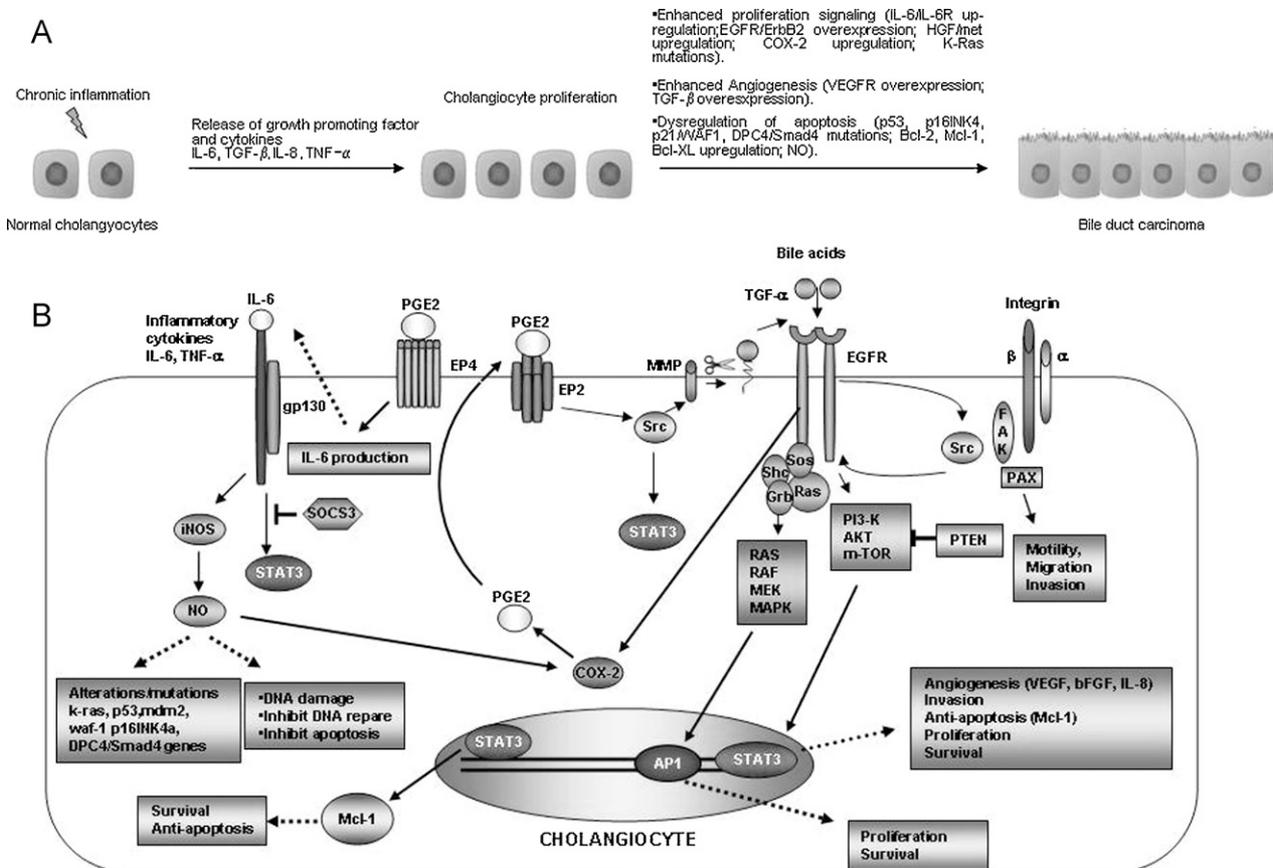


Fig. 1. (A) Multistep pathogenesis of bile duct carcinoma. (B) Molecular events in bile duct carcinogenesis: Pro-inflammatory cytokines induce inducible nitric oxide synthase (iNOS) and COX2. Both iNOS and COX2 induce DNA damage (p53, p16INK4, p21/WAF1, DPC4/Smad4 mutations). Activation of EGFR by TGF- α stimulates MAPK activity, resulting in induction of COX-2 transcription and enhanced synthesis of PGE2. PGE2 can also activate EGFR by an EP2 receptor-dependent mechanism, via Src, by stimulating the release of TGF- α . EGFR ligands up-regulate VEGF and other growth factors, which stimulate angiogenesis through the activation of COX-2 and MAPK pathways. Integrins can promote EGFR Src-mediated phosphorylation in the absence of growth factors. The binding of ligands to EGFR results in the direct activation of Src, which might be enhanced in the presence of integrin–FAK–Src complexes. IL6 receptor binds IL6 through gp130 surface molecules. This activation causes dimerization and translocation of Signal Transducer and Activator of Transcription (STAT3) into the nucleus, with consecutive induction of anti-apoptotic genes such as Bcl2 and BclXL. STAT3 also induces transcription of its natural inhibitor SOCS3.

of mRNA, is the most recent development in preclinical research. Several studies are focused on validating the role of miRNAs in BTC. In particular, among the mechanisms of tumour growth sustained by Interleukin 6 (IL-6) [40–44], there is recent evidence for a role of the involvement of miR-

148a, miR-152 [45] and miR-370 [46]. Other studies have shown implications of miRNAs in key-role processes of carcinogenesis; miR-21 [47], miR-29b [48] are implicated in inhibition of apoptosis through the modulation of PDCD4, TIMP3 and Mcl-1. miR-141 and miR-200b are overexpressed

Table 1

The incidence of K-RAS mutation in clinical and preclinical studies of BTC.

Author	Year	Country	Site	Percentage of K-RAS mutation	Enrolled patients
Kang JK [33]	1999	Korea	IHC	22.5%	40
Saetta AA [38]	2004	Greece	GBC	25%	21
Suto T [34]	2000	Japan	EHC	9.6%	52
Tsuda H [32]	1992	Japan	CC	56%	9
Boberg KM [3]	2000	Norway	CC	33%	33
Isa T [35]	2002	Japan	CC	39.1%	23
Xu RF [36]	2011	China	CC	38.2%	34
Gruenberger B [30]	2010	Austria	BTC	10%	30
Pignochino Y [25]	2010	Italy	BTC	6.1%	49
Bekaii-Saab T [31]	2011	USA	BTC	8%	28

CC, cholangiocarcinomas; IHC, intrahepatic cholangiocarcinomas; EHC, extrahepatic cholangiocarcinomas; GBC, gallbladder carcinomas; BTC, Biliary tract carcinomas.

in tumour cholangiocytes. In particular, miR-200b dysregulates the protein tyrosine phosphatase non-receptor type 12 (PTPN12), contributing to tumour cell survival, proliferation and response to therapy [49].

3. Surgery and adjuvant treatment

An evaluation of surgical indications and procedures goes beyond the purpose of this review, but it is generally accepted that surgery offers the only chance for cure in both CC and GBC, and should be performed when primary disease is considered resectable; unfortunately the risk of recurrence, even after radical resection is high, with 5-year survival rates in the range of 20–40% of patients [50–52].

Strategies to improve progression free survival (PFS) include both Radiotherapy (RT) and Chemotherapy (CT), which have been investigated alone or in combination in the adjuvant setting. Their role, however, is still undefined, due to the limited number of patients evaluated, the prevalence of retrospective trials and the heterogeneity of stages and types studied. In clinical practice and according to international guidelines, a concurrent chemoradiation treatment with 5-fluorouracil (5FU) or adjuvant CT with 5FU or gemcitabine (GEM) should be considered [53].

4. Systemic therapy in advanced disease

4.1. Chemotherapy

Because of the relatively low incidence of these tumours compared to other more common malignancies, in the past years clinical practice has only been based on small Phase II trials. Many of these have included heterogeneous population of patients, such as pancreatic carcinomas or HCC in addition to BTC, which have made the formulation of a standard of care particularly difficult.

Following Glimelius' randomised trial [54], the first published study that demonstrated a clear benefit of CT over best supportive care (BSC) in pancreatic and biliary cancer, systemic CT has become the mainstay of the treatment plan in patients with unresectable or metastatic disease, as it improves both Quality of Life (QoL) and OS. Some other studies have also confirmed this outcome [55,56].

5FU and other fluoropyrimidines (FPD) have been the backbone of therapy of CC and GBC through the 90s: 5FU, as a single agent or in combination with leucovorin, yields variable Response Rates (RRs) [56–59].

Since the late 90s GEM has been extensively investigated as an effective drug in different cancers; in particular it has demonstrated efficacy both in pancreatic and BTC, pathologies in which it has become of central importance. Phase II clinical trials using single agent GEM in CC and GBC have generally shown satisfactory RRs as well as a good safety profile. Even though these studies have only included a small

number of patients, and occasionally different cancer types such as pancreatic and HCC, we can assume that GEM alone yields RRs in about 20% of patients, with an Overall Disease Control Rate (DCR) in approximately two-thirds of the patient population. Indeed, OS is around 8 months, significantly higher than the OS reported in the literature for BSC [60–71].

Combination therapy often included combinations with doxorubicin, mitomycin and, as it will be further elucidated, platinum compounds [72–77].

The use of triplets, and multi-drug therapy in general, has recently proven to be a feasible strategy for fit patients in metastatic pancreatic cancer [78]. Similarly in BTC, the combination treatments GFP (GEM, 5FU, cisplatin), GFO (GEM, 5FU, oxaliplatin), ECF (epirubicin, cisplatin and 5FU) and PEFG (GEM, 5FU, cisplatin, epirubicin) have all been used, with positive outcomes [79–83].

Results of the most representative studies cited above are shown in Table 2.

4.1.1. Effective combinations: FPD-platinum compounds; GEM-platinum compounds; FPD–GEM

Therefore, GEM, FPD and platinum derivatives have all been tested in different combinations. Results are summarised in Table 3. Here we will briefly discuss the results of the most relevant.

The randomised controlled trial (RCT) of Ducreux et al. in 2005 [59] may resume outcomes from fluoropyrimidine and platinum compounds therapy [84–95]: 58 patients were randomised to either receive high-dose 5FU or 5FU, folinic acid and cisplatin. RRs and OS in the combination arm were higher compared to single 5FU therapy (18.5% vs. 7.1% and 8 vs. 5 months) but these results were hampered by higher haematological and gastrointestinal toxicity. The authors concluded that, because of the occurrence of severe side effects in patients with a poor life expectancy, the 5FU-cisplatin combination did not warrant further investigation in a Phase III RCT.

The phase II trial reported by Riechelmann et al. evaluated the combination therapy of gemcitabine and capecitabine [96]: objective response (OR) was observed in 29% of the 75 patients, with 3 patients having a complete response (CR); average OS was 12.7 months. No unexpected or dose-limiting toxicities were evident. Similar results have been observed in other studies combining GEM with capecitabine [97,98] or other FPD [99–104].

Gemcitabine has also been evaluated for combination therapy with either cisplatin, oxaliplatin or, to a lesser extent, carboplatin, with similar RRs in all cases. From greater than 20 Phase II trials we can deduce that on average, DCR is about 55% with platinum combinations, with OS in the range of 8–10 months. Haematological toxicity was a common finding, with variable incidence of anaemia, thrombocytopenia and neutropenia; as predictable, peripheral sensory neuropathy was exclusively noticed in patients treated with

Table 2
Overview on studies of systemic treatment in BTC.

Study		Patients		Drugs	Results	
Author	Year				OR	OS
Sanz-Altamira PM	2001	25		IRI	8%	10 ms
Kubicka S	2001	43	CC 23 HCC 20	GEM GEM	5% 30%	NA NA
Lin MH	2003	24		GEM	12.5%	7.2 ms
Tsavaris N	2004	30		GEM	30.0%	17.1 ms in GBC 11.4 ms in BTC
Park JS	2005	23		GEM	26.1%	13.1 ms
Okusaka T	2006	40		GEM	17.5%	7.6 ms
Taal BG	1993	30		MMC	10%	NA
Androulakis N	2006	29		OXALI	20.6%	7 ms
Papakostas P	2001	25		TXT	20%	8 ms
Malik IA	2003	30		5FU + FA	7%	14.8 ms
Kornek GV	2004	51	Arm A 26 Arm B 25	MMC + CAPE MMC + GEM	31% 20%	9.25 ms 6.7 ms
Furuse J	2006	24		U + DOX	12.5%	7.6 ms
Harvey JH	1984	17		5-FU + MMC + DOX	31.0%	NA
Lee S	2009	31		5FU + DOX + MMC	12.9%	6.7 ms
Glimelius B	1996	90	Arm A Arm B	5FU + LV + ETP + BSC or 5FU + LV + BSC (elderly & poor PS pts) BSC	NA NA	6 ms 2.5 ms
Takada T	1996	83	Arm A 42 Arm B 41	5-Fu + DOX + MMC BSC	7.2% NA	NA NA
Raderer M	1999	39	Arm A 20 Arm B 19	5FU + LV + MMC GEM	25% 16%	9.5 ms 6.5 ms
Rao S	2005	54	Arm A 27 Arm B 27	5FU + ETP + LV EPR + CDDP + 5FU	19.2% 15%	9.02 ms 12.03 ms
Kruth J	2010	28		CAPE + TXT + MMC	21.4%	6.8 ms
Feisthammel J	2007	30		IRI + 5FU + FA	10%	166 days in ICC 273 days in GBC
Park SH	2006	43		EPR + CDDP + CAPE	40%	8 ms
Ellis PA	1995	32		EPR + CDDP + 5FU	40% in BTC 29% in HCC	NA
Takada T	1998	83	Arm A 42 Arm B 41	FU + DOX + MMC BSC	7.2% NA	NA NA
Sharma A	2010	81	Arm A 27 Arm B 28 Arm C 26	BSC FU + FA GEM + OXALI	0% 14.3% 30.8%	4.5 ms 4.6 ms 9.5 ms
Yamashita Y	2006	8		GEM + 5FU + CDDP	37.5%	23.5 ms
Yamashita Y	2010	21		GEM + 5FU + CDDP	33.3%	18.8 ms
Plyzos A	1996	13		MMC + 5 FU + FA	23%	22 ws
Cereda S	2010	37		CDDP + EPR + 5FU + GEM	43%	12.1 ms
Eckel F	2000	30		CTX + LV + 5FU + TAM	0%	7.3 ms
Park KH	2005	40		EPR + CDDP + U + LV	22.5%	34 ws
Kajanti M	1994	22		EPR + MTX + 5FU + LV	0%	9 ms
Patt YZ	2001	41		CDDP + IFN α -2b + DOX + 5FU	21%	14 ms

OR, overall response; OS, overall survival; CC, cholangiocarcinomas; HCC, hepatocellular carcinoma; 5-FU, 5-fluorouracil; BSC, best supportive care; CAPE, capecitabine; CDDP, cisplatin; IRI, irinotecan; CTX, cyclophosphamide; DOX, doxorubicin; EPR, epirubicin; ETP, etoposide; FA, folic acid; FU, fluorouracil; GEM, gemcitabine; IFN α -2b, interferon alpha 2-b; L-OHP, oxaliplatin; LV, leucovorin; MMC, mitomycin C; MTX, methotrexate; TXT, docetaxel; U, uracil; TAM, tamoxifen.

Table 3
Overview on trials investigating platinum-based regimens in BTC.

Study		Patients		Drugs	Results					
Author	Year				CR	PR	OR	SD	PD	OS
Kobayashi K	2006	42		5-FU + CDDP	0%	42.9%	42.9%	30.9%	26.2%	NA
Chatni SS	2008	65		5-FU + CDDP	7.69%	26.15%	33.84%	13.85%	32.30%	5.7 ms
Ducreux M	1998	25		5-FU + CDDP	0%	24%	24%	NA	NA	NA
Kim TW	2003	42		CAPE + CDDP	2.40%	19%	21.4%	NA	NA	9.1 ms
Cho JY	2005	44		CAPE + GEM	0%	32%	34%	NA	NA	14 ms
Knox JJ	2005	45		GEM + CAPE	NA	NA	31%	42%	NA	14 ms
Murad AM	2003	26		GEM + 5-FU	3.8%	26.9%	30.7%	NA	NA	9 ms
Malik IA	2003	11	Arm A 8 Arm B 3	GEM + CDDP GEM	9%	55%	64%	NA	NA	42 ws
Ducreux M	2005	58	Arm A 29 Arm B 29	5FU 5FU, FA + CDDP	0% 4%	7% 15%	7.1% 19%	46% 44%	NA NA	5.0 ms 8.0 ms
Doval DC	2004	30		GEM + CDDP	13.3%	23.3%	36.60%	23.3%	13.2%	20 ws
Thongprasert S	2005	43		GEM + CDDP	0%	27.5%	27.5%	32.5%	NA	36 ws
Giuliani F	2006	38		GEM + CDDP	3%	29%	32%	21%	47%	8+ ms
Kim ST	2006	29		GEM + CDDP	0%	34.5%	34.5%	13.8%	44.8%	11 ms
Lee GW	2006	24		GEM + CDDP	0%	21%	21%	50%	29%	9.3 ms
Park BK	2006	27		GEM + CDDP	NA	33.3%	33.3%	25.9%	NA	10.0 ms
Charoentum C	2007	42		GEM + CDDP	0%	21%	21%	26%	31%	10.8 ms
Lee J	2008	39		GEM + CDDP	NA	17.1%	17.1%	28.6%	45.7%	8.6 ms
Meyerhardt JA	2008	30		GEM + CDDP	0%	21%	21%	36%	NA	9.7 ms
Goldstein D	2011	50		GEM + CDDP	0%	26%	26%	24%	NA	6.8 ms
Valle JW	2009	86	Arm A 44 Arm B 42	GEM GEM + CDDP	0% 0%	22.6% 27.8%	22.6% 27.8%	35.5% 47.1%	NA NA	NA NA
Valle J	2010	410	Arm A 204 Arm B 206	CDDP + GEM GEM	NA NA	NA NA	NA NA	NA NA	NA NA	11.7 8.1
André T	2004	56	Arm A (33) PS 0–2 bilirubin <2.5× normal GEMOX as first-line chemotherapy Arm B (23) PS >2+/ or bilirubin >2.5× normal +/or prior chemotherapy	GEM + OXALI GEM + OXALI	NA NA	NA NA	36% 22%	26% 30%	39% 48%	15.4 ms 7.6 ms
Wagner AD	2009	72	BTC 37 GBC 35	GEM, OXALI + 5-FU GEM, OXALI + 5-FU	NA NA	NA NA	19% 23%	NA NA	NA NA	10.0 ms 9.9 ms
Harder J	2006	31		GEM + OXALI	0%	26%	26%	45%	29%	11 ms
Verderame F	2006	24		GEM + OXALI	4%	46%	50%	NA	NA	12 ms
Manziona L	2007	34		GEM + OXALI	6%	35%	41%	NA	NA	10 ms
André T	2008	67		GEM + OXALI	0%	14.9%	14.9%	35.8%	NA	8.8 ms
Jang JS	2010	53		GEM + OXALI	1.9%	17%	18.90%	50.9%	NA	8.3 ms
Kim HJ	2009	40		GEM + OXALI	NA	NA	15%	37.2	NA	8.5 ms
Li J	2010	34		GEM + OXALI	3.7%	14.8%	18.5%	66.7%	14.8%	11.6 ms
Sharma A	2010	50		GEM + OXALI	6.2%	15%	21.20%	35.4%	36%	7.5 ms
Williams KJ	2010	48		GEM + CBDCA	NA	NA	31.1%	NA	NA	10.6 ms
Julka PK	2006	20		GEM + CBDCA	21%	15.7%	36.7%	NA	NA	NA

CR, complete response; OR, overall response; OS, overall survival; PD, progression disease; PR, partial response; PS, performance status; SD, stable disease; BTC, biliary tract carcinomas; GBC, gallbladder carcinomas; 5-FU, 5-fluorouracil; CAPE, capecitabine; CBDCA, carboplatin; CDDP, cisplatin; FA, folinic acid; FU, fluorouracil; GEM, gemcitabine; OXALI, oxaliplatin.

oxaliplatin, and liver and renal toxicities were more frequently observed in the case of cisplatin [105–126].

4.1.2. GEM–cisplatin as a new standard of care

Undoubtedly, from this melting-pot of small studies, it has been difficult to determine the optimal treatment for clinical practice.

Pooled analysis by Eckel's et al. [127] evaluated data from 104 trials, with 2810 patients being treated. Analysis of patients who experienced OR or stable disease (SD) pointed to the combination of GEM-platinum compounds as the most effective in terms of RR, DCR, and OS.

These considerations are supported by a recent, small Phase III study by Sharma et al. [56] who randomised 99

patients affected by unresectable GBC to receive: arm A – BSC; arm B – 5FU and folinic acid (FUFa); arm C – modified GEM–oxaliplatin (mGEMOX). Results show significant differences in OR of both CT groups over BSC, with ORs of 0% in arm A, 14.3% in arm B and 37% in arm C ($p=0.003$). The combination arm was the only treatment to significantly impact on life expectancy: after a median follow-up of 9 months, the FUFa regimen did not prolong OS when compared with BSC (4.5 months vs. 4.6 months), whereas the mGEMOX treatment proved to have a significant benefit, with an OS of 9.5 months ($p=0.039$).

Suggestions have been turned into a standard of care by recent RCTs. The randomised Phase II ABC-01 trial suggested that the addition of cisplatin to GEM could improve DCR (58.0% for single GEM arm vs. 75.0% of the GEM–cisplatin arm) [128]. Given these results, Valle et al. extended and powered this study to a Phase III trial, the ABC-02 [129]. Eligible patients were affected by metastatic, unresectable or recurrent BTC. Four hundred and ten patients were randomly assigned to receive GEM (1000 mg/m² days 1, 8, 15 q 28) or GEM cisplatin (1000 mg/m² + 25 mg/m² days 1, 8 q 21) for up to 24 weeks of treatment. Primary endpoint was OS. Consistent with previous clinical and preclinical data, the ABC-02 trial confirmed the advantage of combination therapy over GEM alone. Patients who received GEM and cisplatin had an improvement in PFS of 3 months (8.0 months vs. 5.0 months; $p<0.001$). A clear benefit was also seen for life expectancy, with a median OS of 11.7 months, as compared to 8.1 months for the single agent group ($p<0.001$); the analysis of pre-specified baseline factors was consistent with these data, regardless of the subgroup taken into account. No significant increase of toxicity was observed between the groups, except for abnormal liver function, which was more frequently noticed in the single agent arm, most likely due to inferior disease control.

The importance of this trial is that it can eventually provide a definite standard regimen for a disease that has been “orphaned” for too long.

Oxaliplatin is widely used in clinical practice instead of cisplatin: the safety profile of the GEMOX regimen and the good RRs discussed above strongly suggest that this is not a suboptimal treatment when compared to the standard schedule with cisplatin.

4.2. Targeted therapies

In recent years we have entered the era of targeted therapies: advances in the comprehension of molecular alterations that promote the development of neoplastic cells have led to new therapeutic modalities that have also recently involved the treatment of BTC.

EGFR family pathway dysregulation has a key role in the development of many types of human cancer, such as pulmonary, breast, colorectal and, as described above, BTC; these alterations may consist of receptor overexpression,

amplification, activating mutations in the TK domain, or activation of autocrine growth factor loops.

Different strategies targeting EGFR have been developed such as tyrosine kinase inhibitors (TKIs) or monoclonal antibodies (mAbs) directed against the extracellular domain of the receptor, used alone or in association with CT.

Erlotinib, a small-molecule inhibiting the EGFR TK domain, was approved by the Food and Drug Administration (FDA), in combination with GEM, for the treatment of pancreatic cancer on the basis of a Phase III trial that provided a small, but statistically significant favourable outcome [130]. Its efficacy as a single agent in BTC was evaluated in a Phase II study [131] in which 42 patients received 150 mg oral erlotinib daily. The majority (57%) of patients had already received a first line treatment for metastatic or locally advanced BTC. Three patients (8%) had a confirmed partial response (PR), whereas 17 (43%) achieved a SD for a median of 4.4 months (range 2–20 months). OS was 7.5 months (52% of patients alive after 6 months; 15% at 18 months) and median Time to Progression (TTP) was 2.6 months.

More recently, at the latest American Society of Clinical Oncology (ASCO) meeting, Lim et al. presented a Phase III randomised trial in which GEMOX alone (Arm A), or in combination with erlotinib (Arm B) was evaluated in 268 Korean patients with BTC, also including ampullary carcinomas. Even though no difference in OS and PFS was observed in the whole population, subgroup analysis showed a benefit on PFS of the combination with erlotinib in CC (5.9 months vs. 3.0 months of the GEMOX arm, $p=0.049$) [132].

Patients were not screened for mutational status of EGFR or KRAS; it is reasonable to believe that results would have been more significant in some of these patient subpopulations.

Lapatinib, a dual EGFR1 and ErbB2 inhibitor, registered for the treatment of HER2 positive breast cancer, has been tested in a Phase II trial including both BTC and HCC, but failed to be efficient [133]: in particular, results for the BTC group are dismal, with a RR of 0%, a median PFS of 1.8 months and median OS of 5.2 months.

Cetuximab, an mAb directed against EGFR appears to be one of the most promising new drugs that could soon be introduced into clinical practice for BTC. A small case series on 5 patients demonstrated excellent responses (1 CR, 3 RP and 1 SD) and correlated with EGFR expression [134].

Gruenberger et al. recently presented a Phase II trial in which cetuximab, combined with GEM and oxaliplatin, was given every 2 weeks for 12 cycles [30]. Among the 30 patients enrolled, OR was achieved in 19 (63%) cases, with 3 CR and 16 PR. The authors underline that 9 patients in the responders group were converted to operable status by treatment, and could thus undergo potentially curative resection, with a striking benefit when compared to the inoperable patients (median PFS was 21.2 months versus 6.8 months). In clinical practice though, conversion rate is not well-defined, because of the significant inter-surgeon and inter-centre variability.

Hopefully, on the basis of these studies, neoadjuvant treatment in BTC is likely to be explored, as well as in colon cancer [135].

Furthermore, molecular analysis revealed that KRAS mutation was a rare finding (10% of patients), which was not directly correlated with failure to treatment as, out of the 3 mutated patients, 2 had a PR (and liver resection in one case) and 1 had SD. On the contrary, a significant correlation was observed between skin toxicity and the response to treatment, with all the patients having grade 2 or 3 skin rash achieving CR or PR, whereas all patients with progression disease (PD) having no rash or grade 1 rash.

The BINGO trial, whose interim results were presented at the ASCO annual meeting in 2009, is a phase II trial in which patients were randomised for receiving GEMOX alone or in combination with cetuximab. In 36 of the 101 patients enrolled, combination therapy seemed to improve PFS rate at 4 months from 44% to 61%, with tolerable toxicity profile [136].

Combination therapy of traditional CT regimens and mAbs against EGFR will be investigated in further trials; according to *Clinicaltrials.gov* six more studies, one of which is chaired by our Institution, will soon yield results of association therapy, not only with cetuximab, but also with panitumumab, a fully humanized mAb targeting EGFR.

Targeting the VEGF pathway, either at the ligand or receptor level, is a consolidated strategy in many human cancers. Bevacizumab, sorafenib and sunitinib are the most common new generation drugs that inhibit this specific signalling.

Zhu et al. presented the results of a Phase II trial [137] in which bevacizumab, administered at as dose of 10 mg/kg biweekly, was added to the GEMOX regimen. Of the 35 patients enrolled, 14 achieved a PR (RR 40%) and 10 an SD. Median OS was 12.7 months and median PFS was 7 months. A reduction of the maximum standardised uptake value (mSUV) assessed by (18F)FDG-PET scan after 2 cycles of therapy correlated with an increased PFS and OS.

The role of bevacizumab still remains unclear because of the lack of a direct comparison with a standard GEMOX arm: data are not strongly superior to those reported in literature for the association of GEM and oxaliplatin without mAbs.

So far, bevacizumab has been used in association with CT. Recently, attempts have been made to combine it with EGFR inhibitors, aiming to produce a synergistic antitumor effect.

This has led to the design of a study using erlotinib (150 mg once daily orally, days 1–28) and bevacizumab (5 mg/kg intravenously every 2 weeks, days 1, 15) [138]. PR was achieved in nine of the 49 evaluable patients, and in six cases (12%) response was prolonged with a median duration of 8.4 months; 25 patients (51%) had SD. Median OS (9.9 months) and TTP (4.4 months) were superior to those expected for erlotinib monotherapy.

Unlike bevacizumab, which binds free VEGF, sorafenib is a small multi-kinase inhibitor with anti-angiogenic activity, as it competitively inhibits VEGFR family (VEGFR 1, 2, 3), and other targets such as platelet-derived growth factor

receptor family (PDGFR-b), stem-cell growth factor receptor (c-KIT), Fms-like tyrosine kinase 3 (Flt-3), and the receptor encoded by the ret protooncogene (RET).

In preclinical trials, sorafenib was demonstrated to have anticancer activity in murine models of CC, and occasionally in some case reports [25,139,140]. Despite these good preclinical data, the first Phase II trial of sorafenib monotherapy in advanced BTC has shown low activity profile [141]. Forty-six patients received 400 mg twice a day; 26 of them had already been treated with one or more CT lines. Only 1 patient (2.2%) achieved PR, whereas 14 (30.4%) had SD. Overall, median PFS and OS were dismal, being 2.3 and 4.4 months, respectively.

Further studies will evaluate if the addition of sorafenib to GEM or capecitabine/oxaliplatin can improve outcome. Furthermore, sorafenib will be evaluated in association with erlotinib. Preliminary data of a randomised Phase II trial of GEM plus sorafenib or placebo have been presented at the latest ASCO meeting: the combination therapy shows DCR of 70% (7% PR, 63% SD), with manageable and predictable toxicities [142].

Relatively newer VEGFR inhibitors are being investigated as well: cediranib is a potent anti-angiogenic TKI that has been previously evaluated in different types of cancer, such as glioblastoma, colorectal, lung, renal and prostate with ambiguous results [143–145]. According to *Clinicaltrials.gov* its role in treating BTC is being evaluated in at least two Phase II-III trials in association with cisplatin and GEM.

Similarly, vandetanib is a multi-targeted receptor TKI that inhibits, among others, two key signalling pathways, VEGFR-2 and EGFR. Clinical evaluation of this molecule is being conducted not only for BTC (the VANGOUGH trial is already recruiting) but also for other malignancies [146–148].

Targeting the RAS/RAF/MEK/ERK pathway maybe, in the not-too-distant future, a beneficial strategy. A recent publication has shown an interesting role for selumetinib, an inhibitor of MEK1/2 in BTC [31]. Selumetinib was used in pretreated patients in 39% of cases. Even though data are limited by the small number of patients evaluated, only 28 patients (12%) achieved a PR and 68% a SD, which was durable in 56% of cases.

In the literature we also found other studies, some already published, others only recruiting that are testing the efficacy of well-known targeted drugs, such as everolimus or imatinib in BTC. The lack of preclinical bases certainly makes these strategies less attractive than those we have previously outlined [149].

5. Conclusion

We have overviewed the medical treatment of BTC from standard CT to targeted therapies: undoubted progress has been made in understanding the mechanisms of cancer

growth and in detecting effective agents against this type of cancer, especially in the last decade.

Distinguishing BTC from other hepato-pancreatic malignancies has been the first step of this process. As a further clarification, we believe that at least CC and GBC should be considered as different entities: subgroup analysis in many studies suggests that patients affected by GBC have a generally worse outcome. The pattern of recurrence after radical resection is also different, with local relapse being more common in hilar CC and distant in GBC [150]. Eventually, the comprehension of these slight, but significant, differences might be useful for selection of the most suitable treatment for a specific disease. Similarly, biological analysis may help in pointing out molecular differences among populations, which might be due both to different genetics or aetiology. It is likely that a thorough molecular analysis may further drive studies with drugs with a specific molecular target, both in the advanced and adjuvant setting.

A standard, evidence-based regimen, is now fully recognised; GEM and cisplatin are, nowadays, the only established treatment whose efficacy has proven to be applicable to both Western and Eastern patients [151].

Some issues however still remain unsolved. Firstly, the equivalency between GEM–cisplatin and GEM–oxaliplatin, already accepted in clinical practice, has not been validated in RCT.

The comprehension of the molecular basis of cholangiocarcinogenesis, and the results of preclinical studies should stringently drive clinical research. However, the diffusion of targeted therapies in gastrointestinal malignancies and the availability of new, effective molecules have facilitated their direct clinical development.

EGFR and VEGF are the principal pathways involved in cholangiocarcinogenesis, already being tested in the clinical setting. In our opinion, EGFR pathway is the most likely to give positive clinical results; first of all, preclinical bases for EGFR in BTC are more consolidated than those for VEGF. Preliminary clinical results show a certain activity that needs to be confirmed. Moreover, the presence of already validated predictive factors of response/resistance to anti EGFR is certainly useful to select a potentially responsive population.

VEGF signalling may have a secondary role in cholangiocarcinogenesis. Thus, the availability of a large number of effective inhibitors may justify their direct clinical development in a disease that does not have many therapeutic options,

Patients with BTC should then be invited to participate in clinical trials, as this is the only method to answer unsolved enigmas.

Conflict of interest

Actual or potential conflicts of interest do not exist.

Reviewers

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