Clinical Medicine: Oncology

ORIGINAL RESEARCH

Adjuvant Gemcitabine-Oxaliplatin (GEMOX) after Curative Surgery in High-risk Patients with Cholangiocarcinoma

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Abstract

Background: There is no standard adjuvant chemotherapy to prevent recurrent cholangiocarcinoma (CCA), a rare cancer with poor prognosis. We assessed the efficacy and safety of GEMOX on intrahepatic and hilar CCA with high-risk factors after curative surgery.

Patients and Methods: Twenty two patients (mean age: 57 years old) with CCA received 6 cycles of GEMOX: gemcitabine 1,000 mg/m² on day 1 and oxaliplatin 85 mg/m² on day 2, q3w after a curative surgery.

Results: All patients completed 6 cycles of GEMOX. EGFR membranous expression was present in 20 CCA. The 5-year survival rate was 56% (CI 95%: 25.7–85.4); 2-year disease free survival rate was 28% (CI 95%: 3.4–52.6). Median time to progression was 15 months. The rate of recurrence after surgery and chemotherapy was 63% (14/22). Two patients died of disease progression. Twelve patients received cetuximab/GEMOX at the time of relapse. Six died after 12 months (9–48 months), three are still alive suggesting a clinical applicability of EGFR inhibitors in CCA.

Conclusion: Adjuvant chemotherapy with GEMOX alone seems ineffective in intrahepatic and hilar CCA with a high risk of relapse. Additional studies including targeted therapies to circumvent such poor chemosensitivity are needed.

Keywords: cholangiocarcinoma, liver, adjuvant chemotherapy, gemcitabine, oxaliplatin, epidermal growth factor receptor

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Introduction

Patients with cholangiocarcinoma (CAA) have extremely poor prognosis with an average 5-year survival rate of 5%–10%.[1] Among the clinicopathologic factors influencing the survival after surgical treatment, curative surgery, lymph node metastases, tumor size and cancer-free margin are the most predictive factors.[2–8] Recently, in a large series of CCA, multivariate analysis showed that EGFR expression was a risk factor for recurrence of intrahepatic CCA[9] with a five-year survival rate at around 20% and in hilar bile duct cancer, after curative resection, 40% of the patients had disease recurrence.[8] In these patients with high risk factors, adjuvant therapy may play a role in prolonging survival. The aim of this pilot study was to assess the efficacy and safety of adjuvant chemotherapy (GEMOX) in high-risk patients with intrahepatic and hilar CAA after curative surgery.

Patients and Methods

Patients and treatment

Patients were eligible for entry into this pilot study if they fulfilled the following criteria: age ≥18 years, Karnofsky Performance Status (KPS) ≥ 80%, histologically confirmed diagnosis of CCA tumor tissue available for immunohistochemical EGFR detection and curative surgery. Laboratory acceptance parameters included an absolute neutrophil count of ≥1,500 cells/μL, a platelet count of ≥100,000 cells/μL, a serum creatinine level < 130 μmol/L and a total serum bilirubin level < 3 × the upper normal limit. The protocol was approved by the institutional review board (Centre Hepato-Biliaire, Villejuif, France) and was conducted according to the principles of the Declaration of Helsinski and the rules of good clinical practices. Informed consent was obtained from all patients. Twenty two Caucasian patients (10 women, 12 men, mean age: 57 years old, range: 30–73, KPS > 80%) were treated in our institution for an intrahepatic (n = 10) or hilar CCA (n = 12). They had one or more dismal prognostic factors: lymph node involvement, positive histologic margins, pernervous and/or vascular tumoral embols. All patients received 6 cycles of GEMOX for 5 weeks after they had undergone curative surgery. All patients had histologically proven EGFR expressing CCA. Gemcitabine was given at an initial dose of 1000 mg/m² as a 10 mg/m²/min infusion on day 1 and oxaliplatin 85 mg/m² as a 4-h infusion on day 2. A prophylactic antiemetic treatment comprising 5-hydroxytryptamine type 3 receptor antagonists and dexamethasone was given. GEMOX was repeated every 3 weeks if the neutrophil count was >1500 cells/μL and the platelet count was >100 000/μL.

Immunohistochemistry

Immunohistochemical detection of EGFR was performed using EGFR antibodies (31G7 clone and/or 2-18C9 clone) on 4 μm thick deparaffinized tumor sections before any chemotherapy. Only cell membrane staining was considered to be specific. EGFR status was considered positive when > = 1% of tumor cells showed complete membranous staining. The percentage of tumor cells expressing EGF-R was semi-quantitatively assessed, and the intensity of staining was scored as follows: 0: no staining; 1+: weak, 2+: moderate; 3+: strong. When staining intensity was heterogeneous, the highest intensity was retained as the score. Hepatocytes and peripheral nerves served as positive internal controls and positive (HT29 cell line) and negative (CAM-1 cell line) external controls were included (Fig. 3).

Assessment of efficacy and toxicity

The primary endpoint of the study was disease free survival. The secondary endpoint was overall survival. CT scans were performed at base line and every three cycles. After the treatment, CT scans were performed every three months until progression. Toxicity was graded according to the National Cancer Institute-Common Toxicity Criteria (NCI CTC) (version 2.0) and evaluated at each cycle.

Statistical analysis

The Kaplan-Meier method was used to estimate overall and progression-free survival outcomes. Time to progression was calculated from the first day of GEMOX administration until the date when first progression of disease was observed. Overall survival was calculated from the first day of GEMOX administration until the date of death or last follow-up.

Results

Efficacy

Between 2000 and 2005, 22 patients with histologically confirmed CCA underwent curative surgery and

lymphadenectomy. Patient characteristics are summarized in Table 1. The different surgical procedures included 7 right hepatectomies extended to segments 4 and 1; 8 left hepatectomies extended to segment 1; 5 left hepatectomies extended to segments 4 and 1; 1 right hepatectomy extended to segments 4; 1 left hepatectomy extended to segment 1, 4. Hepatic resection was considered as curative (R0) when there was no evidence of microscopic disease (8 patients). Resection was R1 (only microscopic disease) for 14 patients.

The men/women ratio was 12/10. The median age of the patients was 57 years (range 30–73). The median follow-up period was 30 months (range 8–66). Immunohistochemical results were available for 21 of the 22 patients according to the results of positive controls (Table 2). For the latest tumor, positive internal controls were always negative in spite of several tests.

Table 1. Patient characteristics.

Characteristics	
Number of patients	22
Age (years)	
Median (range)	57 (30–73)
Gender	
Men/Women	12/10
Performance status (Karnofsky %)	90
Cholangiocarcinoma	
Extra hepatic (Klatskin)	12
Intrahepatic	10
TNM stage	
pT2	9
pT3	9
pT4	4
Surgical treatment	
Partial hepatectomy right/left	10/12
Indications of adjuvant treatment	
Positive margins	14
Negative margins	8
N+	7
Tumoral embol: perinervous/vascular	10/10
Complete response	8
Progression disease	14

One patient could have more than one indication for adjuvant treatment.

Table 2. EGFR protein expression.

Patient N°	**EGFR protein expression (% of positive tumor cells/intensity)**	**Grade of differentiation**
1	50%/2+	Moderate
2	20%/2+	Moderate
3	100%/2+	Poor
4	30%/1+	Poor
5	40%/2+	Poor
6	0%	Moderate
7	100%/2+	Well
8	100%/2+	Poor
9	5%/1+	Poor
10	70%/2+	Well
11	1%/2+	Moderate
12	5%/1+	Moderate
13	1%/1+	Well
14	1%/2+	Well
15	30%/1+	Moderate
16	20%/2+	Moderate
17	70%/2+	Moderate
18	30%/3+	Poor
19	70%/2+	Poor
20	NE	Moderate
21	30%/2+	Poor
22	30%/2+	Well

Abbreviation: NE, not evaluable.

EGFR membranous expression was present in 20 CCA (95%) and one tumor was negative. If a 10% cut-off was chosen, 15 tumors out of 21 (76%) were EGFR-positive. EGFR was mainly overexpressed in the moderately and/or poorly differentiated tumors, whereas only two well-differentiated cases showed EGFR overexpression.

All 22 patients completed 6 cycles of adjuvant GEMOX. The 5-year survival rate was 56% (CI 95%: 25.7–85.4) (Fig. 1) and the 2-year disease free survival rate was 28% (CI 95%: 3.4–52.6) (Fig. 2). The median time to progression was 15 months: 10 months for hilar CCA (n = 12), and 15 months for intrahepatic CCA (n = 10). Eight patients (hilar = 4, intrahepatic n = 4) had no progression of the disease. These 8 patients

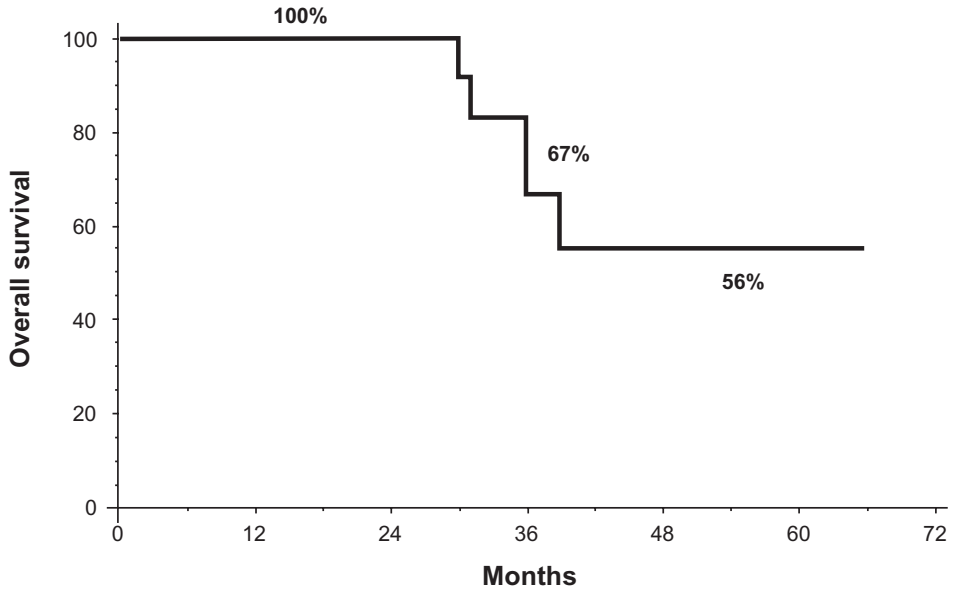


Figure 1. Overall survival in high risk patients.

had no lymph node metastases but 5 had a positive histologic margin (R1). The rate of recurrence after surgery and chemotherapy was 63% (14/22). The recurrences occurred in the liver, peritoneum and metastatic lymph nodes (hilar and celiac lymph nodes). Two patients died 13 and 29 months after the beginning of the treatment due to disease progression. After relapse, a second line of chemotherapy adding cetuximab to GEMOX was performed for 12 patients. Six patients died after 12 months on average (range: 9–48 months) after cetuximab onset. A third line of chemotherapy was administered to 6 patients, 3 of whom are still alive. (Table 3).

Safety

All 22 patients completed 6 cycles of GEMOX. Treatment was well tolerated. No reductions in gemcitabine and oxaliplatin doses or treatment delays were

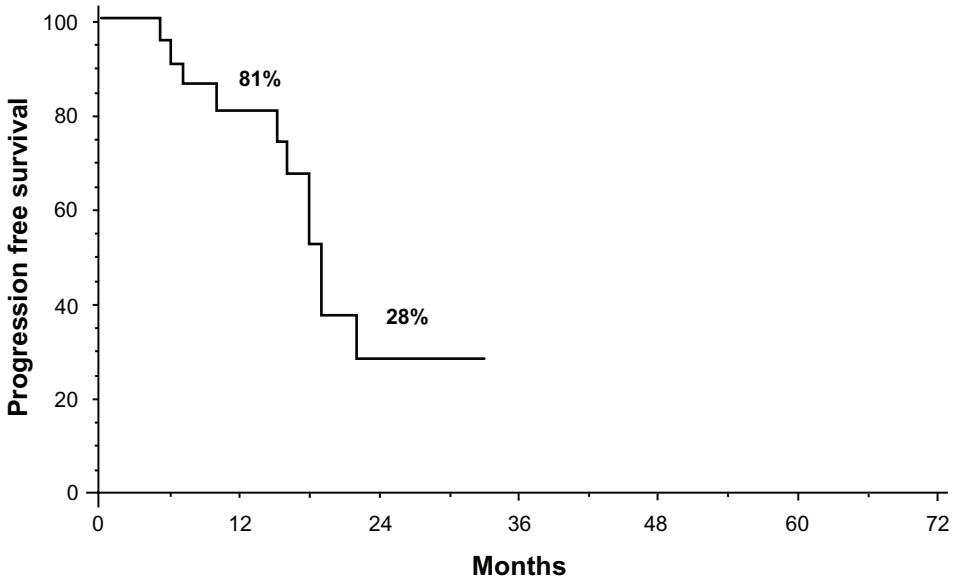
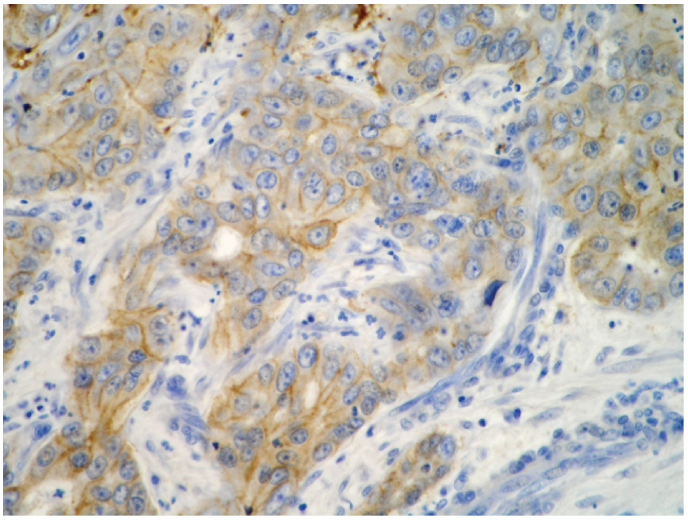


Figure 2. Progression free survival in high risk patients.



Anti-EGFR

Figure 3. Anti-EGFR immunostaining (31G7 monoclonal antibody—LSAB technique) with +++ positivity × 400.

necessary. All patients were assessable for toxicity. There was no treatment-related death, anemia, neutropenia, thrombocytopenia, or neurotoxicity. Acne-like rash was observed in all patients treated with cetuximab and occurred within the first three weeks of the treatment.

Discussion

Our study analyses the impact of adjuvant chemotherapy in hilar and intrahepatic CCA after curative surgery. All patients had 1 or more factors of poor prognosis such as positive margin, lymph node metastases, lymphatic vessel invasion or perineural invasion and 95% of tumors had membranous expression of EGFR. In our study, we used GEMOX after curative surgery since regimens including gemcitabine were found to be active and safe for the patients with advanced biliary tract carcinoma.[10–15] Even if well tolerated, the adjuvant GEMOX regimen alone does not seem to be very effective in our high-risk patients reaching a 2-year progression free survival rate of 28%. In comparison, a 3-year disease-free survival rate of 43% has been reported in resected patients with hilar or intrahepatic CCA[4,8] and the five-year survival rate for intrahepatic CCA patients with EGFR-positive tumors was low at around 20%.[9] A possible limited benefit of GEMOX may be due to EGFR expression that has been associated with increasing resistance to chemotherapy.[16,17] However, there were 8 patients (4 intrahepatic and 4 hilar CCA) with no tumor progression, probably due to the fact that these patients had no lymph node metastasis which is a significant risk factor of tumor recurrence[7] Furthermore, in hilar CCA without lymph node involvement, major hepatectomy can offer long term survival even in the case of R1 resection.[8]

Table 3. Outcome of the patients.

Disease related death	8
Number of recurrent case	14
Liver	7
Peritoneum	1
Lymph node	6
Alive after second and third line of chemotherapy	3

The median survival is not reached in our study because 12 patients received additional treatment with cetuximab/GEMOX at the time of relapse. Since adding cetuximab circumvents tumor resistance to chemotherapy, the tumor may respond to a therapy on which it had previously progressed. This mechanism has previously been documented in metastatic colorectal cancer or in recurrent head and neck cancer.[16,18] In human CCA cell lines, the EGFR kinase inhibitors AG1478 or ZD1839 significantly suppress CCA cell growth.[19] Recently, cetuximab or inhibitors of tyrosine kinase have been used clinically in intrahepatic CCA.[20,21] Some case reports have been reported[22,23] and in patients with refractory advanced intra hepatic CCA, adding cetuximab to GEMOX circumvented tumor resistance to chemotherapy in some patients.[21] These studies suggest a clinical applicability of EGFR inhibitors in CCA. For improving the use of adjuvant GEMOX in EGFR positive CCA, drugs that target tumor cell—associated receptor tyrosine kinase might be useful in patients classified as high-risk.

Conclusion

Given the results of the present study, we consider that six cycles of adjuvant GEMOX is not the optimal chemotherapy in patients with high risk factors. The limited benefit of GEMOX is possibly due to EGFR expression, because EGFR overexpression has been associated with more aggressive disease and increased resistance to chemotherapy. Our data may indicate the

need for additional studies regarding the role of the target therapy in adjuvant treatment among certain subsets of CCA including those with EGFR-positive tumors.

Disclosures

The authors report no conflicts of interest.

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