# Expression of Kit and platelet-derived growth factor receptors α and β in cholangiocarcinoma, and case report of therapy with imatinib mesylate (STI571)

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**We have evaluated the expression of Kit, a receptor encoded by the c-*kit* protooncogene, and platelet-derived growth factor-receptors (PDGF-R) α and β in cholangiocarcinoma specimens from 13 separate patients, and provide a case report of a therapeutic trial of imatinib mesylate in one patient. Archived pathologic samples from 13 patients with cholangiocarcinoma were obtained. Tissue sections were hybridized with anti-Kit, anti-PDGF-Rα and anti-PDGF-Rβ monoclonal antibodies. Kit, PDGF-Rα and PDGF-Rβ expression was seen in 31, 69 and 46% of samples, respectively. All patients with PDGF-Rβ expression also expressed PDGF-Rα. Three out of four patients with Kit expression did not express either PDGF receptor and only one patient exhibiting expression of PDGF expressed Kit. Cohen's κ statistic demonstrated that PDGF and Kit expression were inversely correlated with borderline significance ($p=0.052$; $\kappa=-0.4742$, 95% confidence interval −0.9821 to 0.03364). In one case, strong Kit expression was noted in a tumor from a metastatic lymph node, but was absent in the primary tumor, suggesting that Kit may be related to invasive or metastatic potential. Given the high level of expression defined in this study, a prospective clinical trial incorporating imatinib mesylate, alone or in combination with cytotoxic chemotherapy, and especially in chemotherapy-naive patients, should be considered for patients with cholangiocarcinoma. *Anti-Cancer Drugs* 14:651–657 © 2003 Lippincott Williams & Wilkins.**

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## Introduction

Cholangiocarcinoma arising from the intrahepatic biliary tract has a poor prognosis and limited survival if not amenable to surgical resection [1,2]. Patients with unresectable or metastatic disease have a life expectancy of less than 1 year and there is no standard effective, systemic therapy available. The therapeutic use of a specific tyrosine kinase inhibitor, imatinib mesylate (STI571), has not been previously reported in a patient with cholangiocarcinoma. There is substantial evidence for the presence of mutations or for overexpression of the p53 tumor suppressor gene [3–5], K-*ras* [3,5], MDM2 [3] and the anti-apoptotic protein BCL-2 [6] in cholangiocarcinoma. Aberrant expression of the tyrosine kinase c-ERB-B2 has also been described with expression reported ranging from low levels [7] up to 70% [8]. However, minimal information is available regarding the expression of Kit, a cell-surface receptor encoded by the c-*kit* protooncogene, and the platelet-derived growth factor receptors (PDGF-R) in biliary tract malignancies. Stem cell factor, the ligand for Kit, is expressed by biliary epithelial cells and is thought to bind to mast cell Kit [9]. Two reports suggest that Kit itself may be expressed on the bile duct precursors in embryonic liver and on adult biliary tract epithelial cells [10,11]. PDGF-β is synthesized by cholangiocytes during chronic cholestasis in rats, although expression of the receptor, PDGF-Rβ, appears to be localized to hepatic stellate cells [12]. PDGF has also been found to be secreted in the bile in patients with cholangiocarcinoma at rates above those seen in benign and other malignant abnormalities of the biliary tract [13].

Imatinib mesylate represents a new class of therapeutic agents in oncology designed specifically for inhibition of the Bcr–Abl tyrosine kinase constitutively activated in chronic myelogenous leukemia (CML) [14]. This agent has shown significant activity in CML [15,16] and also in gastrointestinal stromal tumors (GIST) [17,18]. GISTs are characterized by an activating gain-of-function mutation in c-*kit* [19] which is specifically inhibited by imatinib mesylate [20,21]. This drug binds to the ATP-binding site of the target kinase and prevents transfer of phosphate from ATP to the tyrosine residues of various substrates [22]. In addition to suppressing kinase activity of Bcr–Abl and Kit, imatinib mesylate inhibits signaling through native PDGF-Rα and PDGF-Rβ [23,24] as well as tel-PDGF-R fusion

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**Fig. 1**

Evaluation of tyrosine kinase receptor expression in the patient described in the case report. (A) H & E stain of primary cholangiocarcinoma ( $\times$300). Insert depicts same tumor at low power ( $\times$ 75). (B) Immunoperoxidase staining for Kit showing weakly positive expression (brown coloration). (C) Positive staining for PDGF-Rα expression. (D) Positive staining for PDGF-Rβ expression. (B)–(D) at $\times$ 300 magnification.

constructs seen in hematologic malignancies such as chronic myelomonocytic leukemia [25,26]. PDGF provides autocrine stimulation of cancer growth in many solid tumor models and can also act as a paracrine factor inducing changes in adjacent stroma and local angiogenesis. PDGF-Rs are expressed on a variety of solid tumors and are therefore attractive targets for therapeutic intervention.

In this study, we report on the frequency of expression of Kit, and PDGFα and PDGFβ in tumor samples from patients with cholangiocarcinoma. We also present a case report of a therapeutic trial of imatinib mesylate in a patient with $Kit^+$ cholangiocarcinoma.

## Materials and methods

### Tumor tissue samples

Archived pathologic specimens from patients with a prior diagnosis of cholangiocarcinoma were obtained according to Institutional guidelines. Specimens from a total of 13 different patients were retrieved representing surgical resections or core biopsies performed at the University of California, Irvine Medical Center over the period of time from 1999 to April 2002.

### Immunoperoxidase staining

Paraffin-embedded samples were deparaffinized with xylene/ethanol and incubated with primary antibody (2 μg/ml) for 30 min at 25°C. The following antibodies were utilized for this study: anti-Kit (anti-CD117; Dako, Carpinteria, CA), anti-PDGF-Rα (clone C-20; Santa Cruz Biotechnology, Santa Cruz, CA) and anti-PDGF-Rβ (clone P-20; Santa Cruz Biotechnology). After incubation with the primary antibody, slides were incubated with biotinylated donkey anti-rabbit secondary antibody (1 μg/ml) for 30 min. Sections were then exposed to avidin–horseradish peroxidase (HRP) for another 30 min and DAB chromogen. Donkey serum was utilized as a blocking agent to reduce background staining. Negative controls included slides hybridized with secondary antibody and HRP/DAB chromagen, but without primary antibody. Positive controls performed in concert with each experiment included staining of paraffin-embedded tissue samples known to express the relevant cell surface receptor protein. For Kit, a GIST was utilized as a positive control and a Ventana Medical System (Ventana, AZ) detection kit was utilized.

### Statistical analysis

The Cohen's κ statistic was computed to estimate the agreement between the protein (Kit, PDGF-Rα and PDGF-Rβ) expression variables, in pairs. This statistic is traditionally used when two or more 'observers' classify an object using a nominal scale (0, 1; +/–). The null hypothesis is $\kappa = 0$, i.e. no agreement. The κ statistic is a measure of correlation. It has a maximum of 1.0 when agreement is perfect. A value of 0 indicates no agreement greater than chance. A positive κ statistic implies that the agreement (correlation) is positive and a negative value implies an inverse correlation.

The Fisher's exact statistic was also computed to estimate the degree of association between the proteins, with respect to expression. The null hypothesis is that the proteins are independent. Both tests use the $\chi^2$ distribution to compute $p$ values.

### Case report

A 63-year-old woman was diagnosed with inoperable, stage IV cholangiocarcinoma in July 1999. She presented with abdominal discomfort and bloating, and was found to have involvement of the left and right lobes of the liver as well as impingement of the right hepatic vein. She received gemcitabine on a weekly or every-other-week schedule from September 1999 through February 2001 with relatively stable disease. Sight progression of liver disease was noted at that time, although gemcitabine was continued. In May 2001, the patient's clinical performance status began to decline and computed tomography (CT) scan of the abdomen revealed clearly progressive disease in the liver. During a family discussion about the limited options for therapy available, family members requested that the tumor be tested for c-*kit* expression to determine whether treatment with imatinib mesylate could be contemplated. Because the tumor tested positive for Kit protein, the patient was begun on a therapeutic trial with imatinib mesylate.

Imatinib mesylate was initiated at a daily dose of 400 mg on 15 July 2001. Initially it was well tolerated. After 1

**Fig. 2**

Immunoperoxidase staining of cholangiocarcinomas from different patients depicting positive expression of PDGF-Rα (A), positive expression of PDGF-Rβ (B), lack of expression of PDGF-Rα (C) and lack of expression of PDGF-Rβ (D). All images recorded at $\times$ 300 magnification.

**Fig. 1**

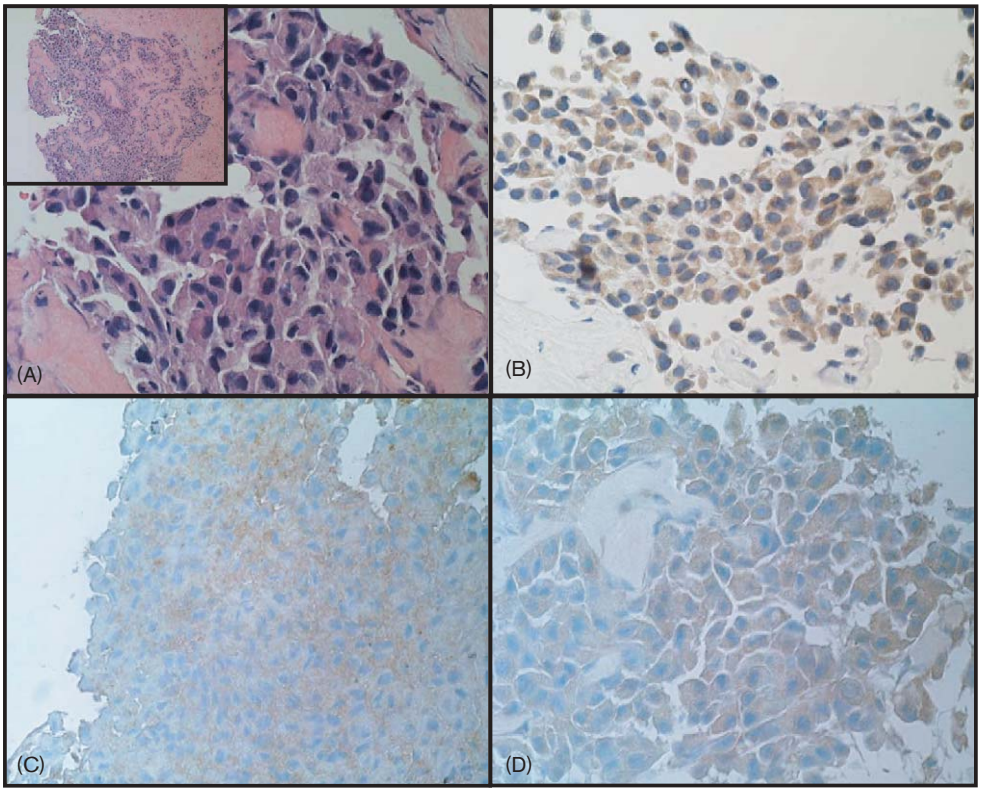
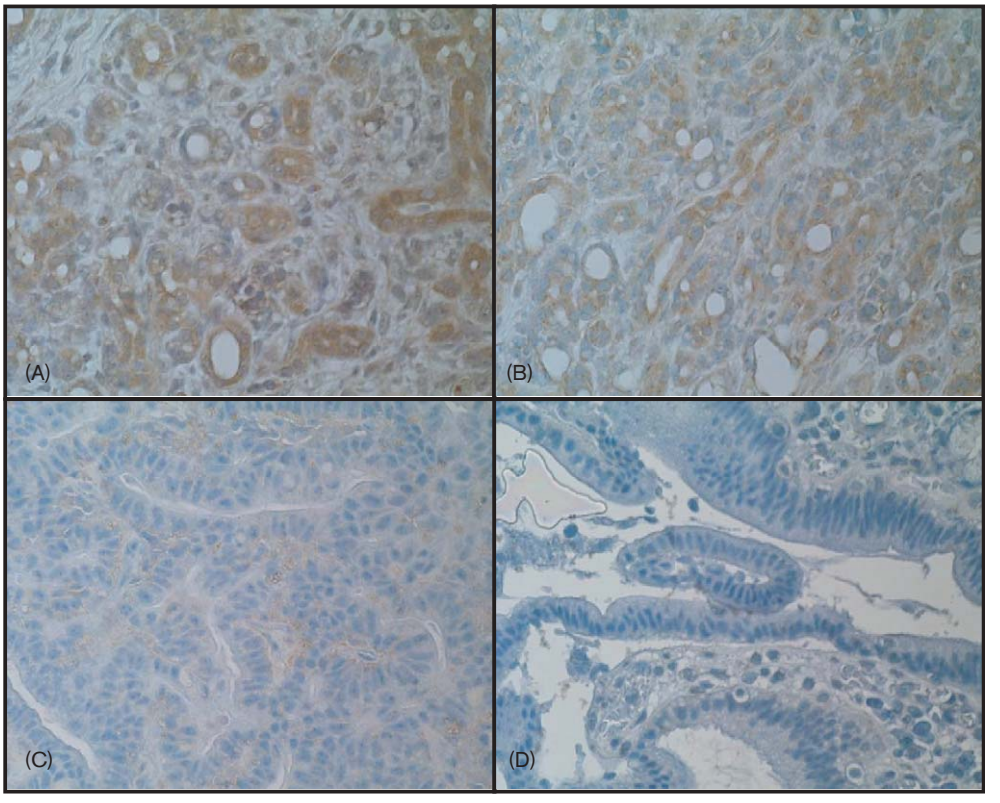



**Fig. 2**



week, a faint skin rash on the trunk and upper extremities had appeared. This was treated with antihistamines and topical steroid cream. After 2 weeks, the cutaneous eruption had become intensely pruritic, markedly erythematous, and now involved the entire trunk and both upper and lower extremities (grade III). Imatinib mesylate was continued at 400 mg/day and the patient was treated with systemic steroids (prednisone 40 mg/day for 1 week followed by a rapid taper over 10 days) with resolution of the cutaneous rash. By 11 September 2001, 2 months after initiation of imatinib mesylate therapy, the patient developed edema of the eyes, hands and abdomen (grade III) which progressed over a period of 5–7 days. This necessitated discontinuation of imatinib mesylate and institution of diuretic therapy. Other toxicities encountered during the treatment course included frequent bowel movements (grade I) and transient hypertension (169/94; grade I). No hematologic toxicity was noted.

Pre-treatment CA19-9 level was 49 U/ml (normal $< 37$ U/ml). This remained relatively constant throughout imatinib mesylate therapy and was recorded at 52 U/ml following the treatment course. Measurement of tumor masses by CT scans done prior to therapy and after discontinuation of therapy revealed: left lobe liver mass $= 8 \times 5$ to $8 \times 9$ cm, right lobe liver lesions $= 0.8 \times 0.8$ to $1.2 \times 1.2$ cm, $1.4 \times 1.4$ to $2.3 \times 2.5$ cm, $1.1 \times 1.1$ to $1.5 \times 1.5$ cm. Two new subcentimeter lesions were noted on the post-treatment CT scan. Positron emission tomography scan in early October 2001 confirmed the presence of metabolically active disease within the liver. The overall increase in the sum of the perpendicular diameters of the measurable lesions was 86% (43.8–81.4 $cm^2$).

Following the 8-week course of imatinib mesylate, the patient elected not to receive additional therapy. She expired in December 2001.

## Results

Expression of each of the imatinib mesylate-sensitive tyrosine kinase receptors, Kit, PDGF-Rα and PDGF-Rβ, was seen in the tumor from the patient described in the case report (Fig. 1). Kit expression was weak, but clearly evident, in the tumor specimen. Overall, every cholangiocarcinoma tested, except for a single case, expressed at least one of these receptors (Table 1). PDGF-Rβ was expressed in 46% of cases, although this expression was restricted to tumors that also expressed PDGF-Rα. The latter was detected in 69% of cases. Expression, and lack of expression, of the receptors was easily distinguished by immunohistochemistry on the paraffin embedded sections (Fig. 2). The Kit receptor was expressed in 31% of cases.

Data were analyzed with the Cohen's κ statistic to estimate the agreement between protein expression variables, in pairs. The null hypothesis is $\kappa = 0$. A κ value of 1.0 indicates perfect correlation. A value of 0 indicates no agreement greater than chance. A negative κ value indicates an inverse correlation. The data were further analyzed using the Fisher's exact statistic to estimate the degree of association between the expression of different proteins. This latter evaluation resulted in identical $p$ values to those obtained with the Cohen's κ statistic.

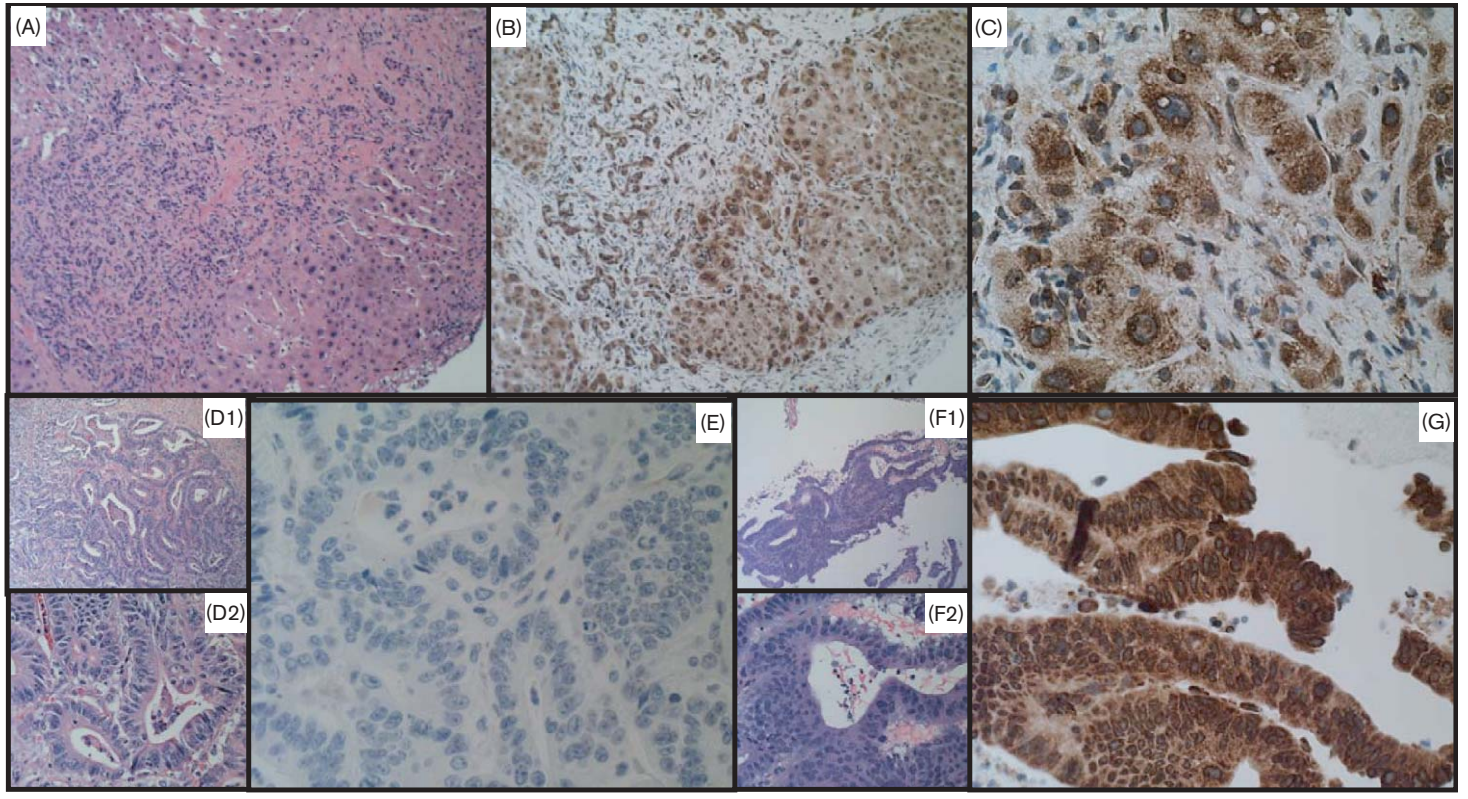
There was a positive correlation between PDGF-Rα and PDGF-Rβ expression which reached marginal significance. The κ value was 0.5517 [95% confidence interval (CI) 0.1540 to 0.9494), with a two-tailed asymptotic $p$ value of $p = 0.026$ and a two-tailed exact $p$ value of $p = 0.069$. There was an inverse correlation between PDGF-R and Kit expression with a κ value of $-0.4742$ (95% CI $-0.9821$ to 0.0336). The two-tailed asymptotic $p$ value was $p = 0.021$ and the two-tailed exact $p$ value was $p = 0.051$. Analysis of correlation between PDGF-Rα expression and Kit expression was identical to that for PDGF-R expression overall. PDGF-Rβ expression alone

**Table 1. Expression of Kit, PDGF-Rα and PDGF-Rβ in 13 patients with cholangiocarcinoma**

| Patient no. | PDGF-Rα | PDGF-Rβ | Kit |
|---|---|---|---|
| 1 | – | – | weak + |
| 2[a] | + | + | weak + |
| 3 | + | – | – |
| 4 | + | – | – |
| 5 | + | – | – |
| 6 | strong + | trace + | – |
| 7 | + | + | – |
| 8 | + | trace + | – |
| 9 | – | – | – |
| 10 | + | + | – |
| 11 | + | + | – |
| 12 | – | – | + |
| 13 | – | – | + (lymph node) |
| Total positive | 9/13 (69%) | 4/13 (31%); 2/13 trace (15%); overall 6/13 (46%) | 4/13 (31%) |

[a]This represents the patient described in the clinical case report.

**Fig. 3**




(A–C) Strongest Kit staining in primary cholangiocarcinoma tumors among all patient samples tested (patient 12). (A) H & E, ×400 magnification. (B) Immunoperoxidase with anti-Kit, ×100 magnification. (C) Immunoperoxidase with anti-KIT, ×400 magnification. (D–G) Immunoperoxidase staining for Kit expression from patient 13. (D1 and D2) H & E of the primary cholangiocarcinoma tumor, ×100 and ×400 magnification, respectively. (E) Lack of Kit expression in the primary tumor, ×400 magnification. (F1 and F2) H & E of a regional lymph node with metastatic cholangiocarcinoma, ×100 and ×400 magnification, respectively. (G) Strong Kit expression in the tumor cells metastatic to the regional lymph node, ×400 magnification.

was not statistically correlated, in either a positive or negative direction, with Kit expression.

Some of the tumor samples, though not those from the patient described in the case report, exhibited very high levels of Kit expression. One example depicting intense Kit expression is shown in Figure 3(A–C). In another case (patient 13), the primary tumor did not react positively with the anti-Kit antibody. However, an adjacent lymph node containing metastatic cholangiocarcinoma exhibited extremely strong Kit expression (Fig. 3D–G). This was the only case in our series for which a metastatic site was available for testing.

## Discussion

Kit expression has been documented in a wide variety of human malignancies, most notably gastrointestinal stromal tumor, but also thyroid cancer, small cell lung cancer, breast cancer, seminoma and acute myelogenous leukemia [27]. This is the first report describing the expression of Kit and PDGF-R cell-surface tyrosine kinases in cholangiocarcinoma. Detection of these receptors was easily accomplished utilizing standard immunohistochemical techniques and a high frequency of expression in cholangiocarcinoma specimens was found. Interestingly, Kit and PDGF-R expression were inversely correlated, being mutually exclusive in 11 out of the 13 cases analyzed.

Expression of Kit and/or PDGF-R does not indicate increased tyrosine kinase activity, and does not necessarily mean that the growth of the cholangiocarcinoma cells are dependent upon, or even influenced by, signaling through these receptors. For example, we do not know if any gain-of-function mutation in the c-*kit* gene, such as the those arising in exons 9, 11 or 13 in GISTs [28,29], has occurred in these cholangiocarcinomas. Signaling through either Kit or PDGF-R may be just one of many mechanisms utilized by this type of cancer to enhance proliferative capacity.

In one patient, strong Kit expression was seen in a lymph node with metastatic cancer cells, but was absent in the primary tumor. This suggests that upregulation of Kit may be related to the metastatic potential in this disease. It also implies that the overexpression of Kit is likely an epigenetic phenomenon and is not related to mutational events at the c-*kit* locus occurring in the primary tumor. Molecular analysis of the c-*kit* gene of primary tumor and tumor cells from a metastatic site, with special attention to codons known to be affected by mutation in other tumors, will be necessary to confirm this assumption.

The availability of a tyrosine kinase inhibitor with specificity for Kit, PDGF-Rα and PDFG-Rβ makes this an attractive therapeutic option for patients with unresectable cholangiocarcinoma. This report describes one patient treated with imatinib mesylate who did not have a favorable response. However, this patient had a chemotherapy-resistant tumor following a prolonged course of chemotherapy, received imatinib mesylate as a single agent and had only weak expression of the Kit receptor. The response rate to this agent in chemotherapy-naive, Kit or PDGF-R positive cholangiocarcinoma patients, and the optimal dose for these patients, will need to be defined, as will the response rate when it is combined with chemotherapy. Nonetheless, given the relatively high frequency of expression of Kit, PDGF-Rα and PDGF-Rβ found in this survey of cholangiocarcinomas, use of imatinib mesylate as initial therapy, alone or in combination with cytotoxic chemotherapy, should be further explored through a prospective clinical trial.

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