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GI Cancers and Hepatobilliary Malignancies

Genomic Landscape and Targeted Treatment of Gallbladder Cancer: Results of a First Ongoing Prospective Study

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Abstract

Background Prognosis of gallbladder cancer (GBC) has not changed in the past 20 years. Comprehensive genomic profiling (CGP) carries potential to determine the actionability for multiple targets, including *ERBB2*, *ERBB3*, *MET*, *ROSI*, *FGFR*, and *PIK3*. This study evaluates the role of CGP and targeted therapies.

Methods This is a multicenter, prospective, single-arm study. All consecutive patients of unresectable and/or metastatic GBC of age ≥18 years were enrolled. Hybrid capture-based CGP was performed by Foundation Medicine CDx. All patients received first-line chemotherapy with gemcitabine–cisplatin regimen. Patients with *ERBB2/3* amplification received trastuzumab with capecitabine or nab-paclitaxel, and patients with *MET* amplification were treated with crizotinib. For *ERBB2/3* mutations, lapatinib plus capecitabine regimen was used.

Results Fifty patients were studied with a median age of 56 years (range 26–83) and a male-to-female ratio of 1:1.6. *ERBB2* and *ERBB3* amplification was seen in 9 (18%) and 2 (4%) patients, respectively. Four patients with ERBB2 amplification received trastuzumab and/or lapatinib, showed partial response, and maintained response beyond 12 weeks. One patient had mixed response, whereas two patients progressed on trastuzumab and lapatinib. Three patients with *ERBB3* mutations showed response to lapatinib–capecitabine. One patient with *MET* amplification responded to crizotinib for 4 weeks. *PIK3* mutations were present in 14% of cases and were independent of *ERBB* aberrations.

Conclusion GBC is enriched in 28% of patients with *ERBB2* and *ERBB3* amplifications and/or mutations. Responses are seen with lapatinib in concurrent *ERBB2* mutation and amplification. *ERBB3* mutation showed response to lapatinib. *MET* and *PIK3* are new findings in GBC, which may be targeted.

Keywords
- comprehensive genomic profiling
- *ERBB2/3*
- gallbladder cancer
- targeted therapy

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Introduction

Gallbladder cancer (GBC) has peculiar geographic distribution; although it is rare in Northern America, it is one of the most common malignancies in North India, Pakistan, Bangladesh, Chile, Ecuador, Japan, and Korea.[1-3] It is more common in females, and its anatomical location, presentation with obstructive jaundice, and chemotherapy refractoriness make it one of the aggressive malignancies, limiting median overall survival to 3 months in metastatic setting in untreated patients. Gemcitabine–cisplatin is the standard of care as per the meta-analysis of ABC02 and BT22 trials of advanced biliary cancers in the first-line setting, wherein majority of patients had cholangiocarcinoma.[4] Survival in GBC has not improved over the past 20 years,[5] highlighting the importance of newer therapies. In the era of personalized and precision medicine, we felt the need to conduct a prospective study of comprehensive genomic profiling (CGP) in advanced GBC to find tumor- and site-specific genomic alterations. The purpose was to find out driver mutation and amplification in GBC and treat these patients with available therapies in the absence of standard of care in the second-line setting.

Methods

Patients

The study was conducted at two tertiary care centers of Armed Forces. All consecutive patients of locally advanced unresectable and/or metastatic GBC were included. Fifty patients were enrolled from August 2018 after approval of the Institutional Ethical Committee. Diagnosis of GBC was made on the basis of imaging findings and was confirmed with biopsy and/or fine needle aspiration cytology. Patients aged ≥18 years were eligible. For staging work-up, contrast-enhanced computed tomography (CT) of the chest, abdomen, and pelvis, or positron emission tomography CT of the whole body was performed. Patients were analyzed for baseline complete blood count, liver function tests, renal function tests, carbohydrate antigen (CA) 19–9 levels, and carcinoembryonic antigen (CEA) levels. Patients with serum bilirubin ≤3 mg/dL and aspartate transaminase/alanine aminotransferase up to three times of normal were permitted for enrollment. Patients of Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) 3 and 4 were excluded. Demographic profile information was collected as per the prespecified protocol. Patients were excluded if they had intrahepatic or extrahepatic cholangiocarcinoma. ECOG method was used for assessing PS.[6]

Biopsy

Biopsies were performed under ultrasound guidance for all patients before start of chemotherapy. Laparoscopic biopsies were performed for patients who could not undergo ultrasound-guided biopsies. Laparoscopic biopsies were taken from local lesion and/or peritoneal deposits. Biopsy sample was preserved in 10% neutral-buffered formalin for 24 hours, and, subsequently, formalin-fixed paraffin-embedded blocks were made. These blocks were centrally collected and transported to a central facility in the United States. Biopsies were permitted after any lines of therapy if patient ECOG PS was 0 or 1.

Comprehensive Genomic Profiling

CGP was performed by Foundation Medicine CDx technology. The complete panel of genes analyzed in this study is shown in ►**Supplement Table S1** (online only). The turnaround time was 3 weeks. The analysis also included PDL1 expression by immunohistochemistry (Dako 22C3 platform). Microsatellite instability (MSI) was evaluated by genome-wide analysis of 95 microsatellite loci. This assay detected alterations in a total of 324 genes, using the Illumina HiSeq 4000 platform. Hybrid-capture-selected libraries were sequenced to high uniform depth (targeting >500X median coverage with >99% of exons at coverage > ×100). Sequence data were processed using a customized analysis pipeline designed to detect all classes of genomic alterations, including base substitutions, indels, copy number alterations (amplifications and homozygous deletions), and selected genomic rearrangements (e.g., gene fusions) (►**Supplement Table S1**, online only).

Treatment

Patients were treated with the first-line chemotherapy: gemcitabine 1,000 mg/m^2 and cisplatin 25 mg/m^2 on day 1 and 8 every 3 weeks for three cycles, and response assessment was performed. For patients presenting with obstructive jaundice, obstruction was relieved by endoscopic retrograde cholangiopancreatography and stenting or percutaneous transhepatic biliary drainage wherever feasible. For responding patients (partial response or stable disease), three more cycles of the same regimen were given. In the second-line and subsequent-line settings, wherever target was detected, the patient received targeted therapy. Molecular targets were *ERBB2*, EBBB3, amplification and/or mutations, MET amplification, and *PIK3* mutations. Toxicity data were collected, and response to therapy was assessed by clinical benefit and/or imaging and serological markers (CA19–9 and CEA). As per protocol, targeted therapy was allowed only in the second and subsequent line of therapy. Patients who had *ERBB2* amplification received trastuzumab plus chemotherapy (nab-paclitaxel or FOLFOX). On trastuzumab progression, eligible patients received lapatinib and capecitabine. Patients with *ERBB3* amplification received trastuzumab plus lapatinib, and *ERBB3*-mutated patients received lapatinib. MET amplification was treated with crizotinib 250 mg BD. Everolimus was used in *PIK3*-mutated patients. Immunotherapy was not administered.

Statistics

Descriptive statistics was used to analyze the variables. To assess the association between variables, chi-square test and Fisher's exact test were used wherever needed. Survival analysis was performed using the Kaplan–Meier method. Log-rank test was used to evaluate the outcome differences between groups of patients. Cox regression analysis was used

for univariate analysis. The significant univariate variables of value up to $p < 0.10$ were considered for multivariate analysis using Cox regression proportional hazard analysis.

Results

A total of 50 patients were studied, and CGP information was available for all patients. Median age was 56.5 years (range: 26–83 years), with a male-to-female ratio of 1:1.6. Three patients underwent laparoscopic biopsies. Biopsy for liver lesion was performed in rest of the patients, and no major complication was observed. Two patients required observation for persistent pain for 24 hours. No fistula formation was observed. The targeted genomic landscape with percentages is shown in ►**Fig. 1**. The targeted findings (*ERBB2*, *ERBB3*, MET, and *PIK3* aberrations) were not frequent in patients aged <60 years as compared with those aged >60 years ($p = 0.78$). Details of patients, disease status, and response to treatment are given in ►**Table 1**.

ERBB2 amplification was observed in nine (18%) of the cases. *ERBB3* aberrations were seen in five (10%) cases, including two amplifications and three point mutations. MET amplification was seen in three patients. It was co-amplified with *ERBB2* in one patient, and another had *ERBB3* mutation along with MET amplification. Four patients received trastuzumab and chemotherapy in various lines of therapy. One patient showed partial response, two patients had progressive disease, and one patient had mixed response. One patient died before the next-generation sequencing (NGS) report was available, and one patient had sudden death not related to malignancy before the start of therapy.

Concurrent mutation and amplifications were seen at high rate. Of seven patients, two patients had concurrent *ERBB2* mutation and amplification. These mutations were S310F and V777L. One patient with S310F mutation did respond to lapatinib and continued with the same regimen. The point mutations in *ERBB3* domain were V104L, G284R, and R426W. One patient of *ERBB3* mutation maintained response to lapatinib beyond 12 weeks.

PIK3 mutations were seen in seven (14%) cases. No concurrent *PIK3* mutations were seen with *ERBB2* and *ERBB3* alterations. Three patients had *PIK3*CA H1047R mutation and two patients had *PIK3*CA-E545K mutation, highlighting recurrent genomic alterations in these domains.

TP53 gene abnormalities were present in 85% of cases. However, there were no recurrent genomic signatures in *TP53*. *NF* mutations were seen in five (10%) patients, *NF1* mutations in three patients, and *NF2* mutations in two patients. Only one patient had fibroblast growth factor receptor (*FGFR*) 2 mutation, and one had *FGFR3* amplification. Two patients were screened for germline mutation for *PTEN* loss and *NF1* mutations by NGS. Both patients did not have germline abnormality.

PDL1 expression data were available for 35 patients, and it was > = 1% in 31% of cases. *PDL1* expression ranged from 1 to 100% and did not show prognostic significance at a cutoff of 1%. Tumor mutational burden (TMB) data were available for 43 patients. Median TMB was 5 mut/Mb, with a range of 1 to 14 mut/Mb. MSI ($n = 43$) was stable in all cases.

Discussion

A prospective study of NGS has not been conducted in GBC previously. Various retrospective series have been published in the GBC evaluating the targets by NGS. Biliary tract cancers comprise intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma, and GBCs. In this study, only GBC subset was studied, strengthening the literature for this subsite. Across all age ranges, actionability was found.

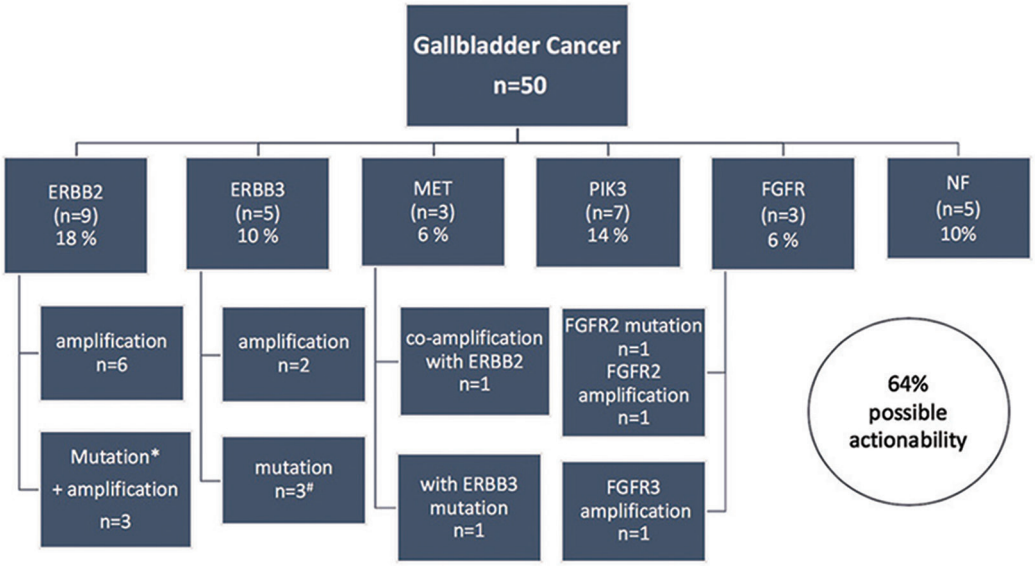

Fig. 1 Targeted genomic landscape in 50 patients of gallbladder cancer. **ERBB2* S310F, L869R, and V777L mutations were concurrently present with amplification. **ERBB3* mutations were V104L, G284R, and R426W.

Table 1 Response to ERBB2 and ERBB3 directed therapy in various lines of therapy TFI-3 (treatment free interval of 3 months), SD- Stable disease

Age/sex	ERBB	Metastatic sites	Prior therapies	Targeted therapy	Response	Duration of response on targeted therapy	Remarks
39 y/ male	*ERBB2* amplification	Both lobes of the liver abdominal wall deposits	Gem-Cis 3[a] (PD) CapOX 6[a] (PD) FOLFIRI2[a] (PD)	T + Nab-paclitaxel 6[a] PD Lap-Cap (6[a])	PR	8 mo	Presently on Lap+T (post 6[a] Lap-Cap)
50 y/female	*ERBB2*, S310F amplification	Liver, periportal peripancreatic lymph nodes	Gem-Cis 2[a] (PD)	T + Gem-Cap (3[a] PD), Lap-Cap	PR	4 mo	Presently on Lap-Cap
70 y/female	*ERBB2* amplification	Peritoneal deposits, ascites, adnexa, liver, lung, omentum, LNs, abdominal wall deposits	Gem-Ox 6[a] (PD)	T + Nab-paclitaxel 3[a] (PD) Lap-Cap	PR	6 mo	Presently on Lap-cap; pain free
34 y/ female	*ERBB2* amplification	Liver, LNs, Lung	Gem-Cis 6[a] (PD) Irinotecan 6[a] (PD)	T + Nab-paclitaxel	MR	3 mo	Comorbidity - Rheumatic heart disease; showed mixed response to Nab-paclitaxel and T
54 y/ male	*ERBB2* amplification	Liver, LNs regional and RPLC, omentum	–	–	–	–	Underwent ERCP and stenting; sudden death at home before commencing targeted therapy
44 y/female	*ERBB2* amplification	Liver, periportal and portacaval LNs	Gem-Cis	–	–	–	Presently on Gem-Cis protocol as prespecified protocol
72 y/male	*ERBB2* V777L, amplification	Large GB mass with liver infiltration, liver metastases	–	–	–	–	Rapid clinical worsening, no systemic therapy received; CA19–9 > 500 U/mL, CEA 2,076.02 ng/mL
47 y/male	*ERBB2* L869R amplification	Local mass, LNs, cerebellar, bone	Gem-Cap 1[a] (PD)	–	–	–	PD on first line and died before commencing targeted therapy
70 y/male	*ERBB2* amplification	Liver, LNs	BSC	–	–	–	Did not receive systemic chemotherapy; rapid clinical worsening
68 y/female	*ERBB3* G284R mutation	Liver, omental, mesenteric deposits, ascites	Gem-Cap (adjt) Gem-Cis (first line)	Lap-Cap	PR (ascites subsided)	2.6 mo	Died due to acute coronary event; off pain medicines for duration of response
62 y/ female	*ERBB3* mutation V104L	LNs mesenteric, RPLN, Lt SCLNs anterior abdominal wall deposit	Gem-Cis (6[a] PD)	Lap-Cap	CB	1 mo	Response evaluation awaited; CB present at 1 m; treatment is ongoing
31 y/female	*ERBB3* amplification	Liver, LNs	Gem-Cis 3[a] (PD)	Lap-Cap	PR	1.8 mo	Pregnancy associated Gall bladder cancer; rapid progression after 2 mo of Lap-Cap; developed ascites, pleural effusion
34 y/male	*ERBB3* mutation R426W	Both lobes of the liver, RPLN, periportal LNs	Gem-Cis 6[a] SD TFI-3 mo Gem-Cap 4[a] PD FOLFIRI 3[a]	–	–	–	Biopsy was performed after second line of therapy; presently on FOLFIRI
46 y/female	*ERBB3* amplification	Liver, omentum, ascites	Gem-Cap 2[a] PD	T+ Gem-Cis	–	–	Received 1[a]

Abbreviations: adjt, adjuvant; CA 19–9, carbohydrate antigen 19–9; CB, clinical benefit present; CEA, carcinoembryonic antigen; ERCP, endoscopic retrograde cholangiopancreatography; Gem-Cis, gemcitabine + cisplatin; Lap-Cap, lapatinib + capecitabine; LN, lymph nodes; PD, progressive disease; PR, partial response; RPLN, retroperitoneal lymph nodes; T, trastuzumab. FOLFIRI, Fluoro-uracil,Leucovirin & Irinotecan
[a]Cycle of chemotherapy.

In a study by Li et al of whole-exome and targeted-gene sequencing in GBC, *ERBB* pathway was extensively mutated in 36.8% tissue samples.[7] In this study, 16% had *ERBB2* amplification. This study was limited by the absence of clinical data.

ERBB2, S310F, L869R, and V777L mutations were seen in three patients. These mutations are reported in other solid malignancies. They may pose resistance to trastuzumab.[8-10] The patients with S310F mutation responded to lapatinib. In this ongoing study, we found promising responses to trastuzumab in the second-line therapy. The work on *ERBB2*-targeted therapy in biliary tract cancer was published by Javle et al.[11] In this retrospective series, six cases of GBC were treated with trastuzumab, and responses were seen for short duration.

ERBB3 mutations were seen in three (7%) patients, in whom encouraging responses to lapatinib (a pan-*ERBB* inhibitor) were seen. Li et al studied *ERBB2*/*ERBB3* mutation and *PDL1* expression in cell lines. *ERBB2* and *ERBB3* mutations were seen in 7 to 8% of GBC samples.[12] We also found similar rates of mutations.

Two patients of *ERBB2* amplification had coamplification with *MET*, which is not reported before in GBC. One patient with *MET* amplification received crizotinib; however, response lasted for 4 weeks. This highlights the importance of deeper understanding of the role of molecular pathogenesis of GBC and mechanisms of resistance for these pathways. Ratio of *MET*/CEP7 > 2.2 was suggested for the effectiveness of crizotinib in lung cancer trials.[13] Future studies are warranted to explore the correlation between NGS and *MET*/CEP7 ratio on FISH (fluorescence in situ hybridization).

FGFR2 mutations, amplifications, and fusions have been reported in intrahepatic cholangiocarcinoma in 15% of patients with characteristics of indolent clinical course.[14-16] Our study highlights the importance of being site-specific for biliary tract cancers. In this study, *FGFR2* mutation–amplification was seen in one patient each. One patient had *FGFR3* amplification. Recently, *FGFR* inhibitor was approved in urinary bladder carcinoma, and it is being studied in intrahepatic cholangiocarcinoma with early promising results.[17,18]

PIK3 mutations H1047R (three cases) and E545K (two cases) have not been reported before in GBC in prospective setting. These are possible targets for alpelisib, which received approval for metastatic breast cancer. The genomic signatures were similar to breast cancer.[19,20]

Previously, the percentage of actionability has been reported[21]; however, these are retrospective in nature and carried bias. *PDL1* expression > 1% was seen in 31% of cases in our study. *PDL1* expression by microarray technique is studied by Neyaz et al.[22]

TMB as a biomarker for immunotherapy has been studied in various malignancies, such as lung cancer, renal cell carcinoma, and head and neck cancer. TMB was analyzed by Yang et al in tissue samples of GBC, and reported mutational burden in 17%,[23] with a median TMB of 5 mu/Mb, which is very much similar to our data. Their study was limited by nonavailability of clinical data, retrospective design, and two different cohorts of Chinese and American patients. We used the Foundation Medicine CDx platform, which is a validated tool for the use of immunotherapy.

MSI (n = 43) was stable in all cases. As the prevalence is <5% for MSI,[24] the use of it as an immunotherapy biomarker is of limited utility and requires future larger data.

Strength of the Study

The main strengths of this study are that GBC as a subset of biliary tract malignancy was studied and the role of precision therapy was explored in the second-line settings.

Limitation of the Study

A limitation of this study was that as a pilot work, the numbers of patients were less. The survival data and correlation with baseline variables will be published once data mature.

Conclusion

Role of personalized and precision medicine by CGP has expanded in GBC. Percentage of driver mutations differs by site in biliary tract malignancies. GBC is characterized preponderance of *ERBB* alterations (26%), including *ERBB2* and *ERBB3* amplification and mutations. *ERBB3* domain mutation can be targeted with lapatinib. Coamplifications and comutations are possible mechanisms of resistance in GBC for trastuzumab. Future studies on combination therapies with lapatinib or pertuzumab are needed. *FGFR2* mutation and amplification are rare as compared with intrahepatic cholangiocarcinoma. *PIK3* mutations were present in 14% of cases with recurrent genomic signatures. Outcomes with targeted therapies in the second-line setting will be published once data mature.

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Conflicts of Interest
There are no conflicts of interest.

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