

Landscape of Targetable Genetic Alterations in Advanced Cholangiocarcinoma among Patients in Southeast Asia

Bhavisha Patel^{1,*}, Balasankar Karavadi², Ravindrasinh M. Rajput³, Shivangi Gupta⁴, Rajashree Panigrahi⁵, P. Ravishankar⁶, Saksham Sood⁷ and Honganur Raju Manjunath⁸

¹Sumandeep Nursing College, Sumandeep Vidyapeeth (Deemed to be University), Vadodara, Gujarat, India

²Department of Bioinformatics, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India

³Faculty of Allied and Healthcare, Gokul Global University, Sidhpur, Gujarat, India

⁴Quantum University Research Center, Quantum University, Roorkee, Uttarakhand, 247667, India

⁵Department of Microbiology, IMS and SUM Hospital, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar, Odisha, India

⁶Department of General Surgery, Vinayaka Mission's Kirupananda Variyar Medical College & Hospitals Vinayaka Mission's Research Foundation (DU), Salem, Tamil Nadu, India

⁷Centre for Research Impact & Outcome, Chitkara University Institute of Engineering and Technology, Chitkara University, Rajpura, 140401, Punjab, India

⁸Department of Physics, Faculty of Engineering and Technology, JAIN (Deemed-to-be University), Bengaluru, Karnataka, India

Abstract: Cholangiocarcinoma (CCA) is a heterogeneous form of biliary tract cancer, for which treatment options remain limited; however, molecular profiling technology has helped in uncovering genomic mutations that can be used in precision oncology. This molecular profiling study has been carried out retrospectively in order to investigate the genomic alterations that are targetable in advanced cholangiocarcinomas of Southeast Asia. Samples from 126 patients were analyzed through targeted next generation sequencing in order to identify somatic mutations, copy number alterations, and gene fusions. Identified genomic alterations were characterized based on their clinical relevance and relation with biomarker-based treatment. A potentially actionable genomic alteration was observed in 93 patients (73.8%), while 28 patients (22.2%) had multiple genomic alterations. The commonest observed alterations consisted of mutations in TP53 (19.0%), fusion events of FGFR2 (16.7%), mutations in IDH1 (14.3%), mutations in KRAS (13.5%), and amplifications of ERBB2 (7.1%). This indicates the presence of considerable molecular diversity among patients with advanced cholangiocarcinoma and the existence of genomic subtypes that have clinical relevance in the Southeast Asian population. The detection of actionable genomic alterations among patients provides clear evidence on the need for genomic profiling as a strategy for molecular characterization and testing of biomarkers. Prospective multicentre studies will be necessary to confirm the results.

Keywords: Cholangiocarcinoma, Targetable Genetic Alterations, Next-Generation Sequencing (NGS), Precision Oncology, FGFR2 Fusion, IDH1/IDH2 Mutations, Southeast Asia.

1. INTRODUCTION

Cholangiocarcinoma (CCA) is a malignant neoplasm arising from the epithelial cells of the biliary tract and is one of the most aggressive hepatobiliary tumours. It is classified based on its anatomic origin into intrahepatic, perihilar, and distal types, each possessing its own pathological and molecular phenotypes. CCA has evolved greatly during the past decades in the world, particularly in regions where there is a high risk of chronic inflammation of the hepatobiliary system, infection with hepatitis virus, and parasitic infections like opisthorchiasis. The mechanisms of CCA tumorigenesis comprise the

interaction between genetic alterations, epigenomic alterations, and the microenvironment, which promote malignancy and tumor development. Molecular analysis has identified many alterations in genes that regulate cell proliferation and metabolism, such as TP53, KRAS, IDH1/2, and ARID1A. Gene structural alterations, for example, fusions in the FGFR2 gene, are common in intrahepatic CCA, leading to aberrant signaling related to tumor growth and survival. Such studies have contributed greatly to the understanding of molecular heterogeneity and pathobiology of cholangiocarcinoma [1-3].

Figure 1 describes the processes involved in precision oncology for identifying actionable genetic alterations in cholangiocarcinoma patients. The first step is the diagnosis of the patient. The next steps

*Address correspondence to this author at the Sumandeep Nursing College, Sumandeep Vidyapeeth (deemed to be University), Vadodara, Gujarat, India; E-mail: patelbhavisha30@gmail.com

involve the collection of tumor tissue samples, followed by aggressive tumor DNA extraction. The resulting purified genetic material is subjected to next-generation sequencing (NGS) to generate extensive genomic data. This is followed by bioinformatics analysis to identify and interpret genomic variants, which are used to identify clinically relevant and targetable mutations. The last step is the use of precision medicine, which involves selecting treatments based on the tumor's molecular profile, shaping treatment plans to support more individualized and potentially more successful treatment.

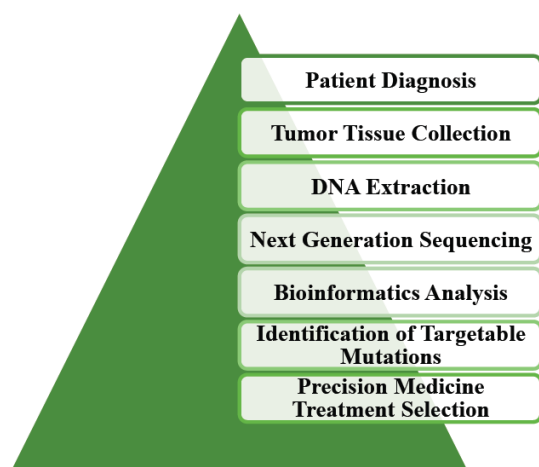


Figure 1: Conceptual Architecture of Precision Oncology Workflow.

The rapid development of genomic technologies has redefined the treatment of cholangiocarcinoma by enabling the identification of effective molecular changes. Next-generation sequencing (NGS) has revealed that a substantial proportion of cholangiocarcinoma tumours harbour potential targetable genomic events, including FGFR2 fusions, IDH1 mutations, HER2 amplifications, and BRAF mutations, which can serve as predictive biomarkers for targeted therapy. This has resulted in the creation of biomarker-oriented clinical trials and treatments, as well as molecularly targeted techniques, intended to improve patient outcomes. Moreover, chronic liver disease, biliary inflammation, and parasitic infection demonstrate the genomic landscape involvement of cholangiocarcinoma, driving regional mutation differences and tumor heterogeneity. Multi-regional sequencing studies have demonstrated high intratumoral heterogeneity, signifying the complexity of the tumor's evolution and resistance in this disease. Therefore, the spatial-temporal studies of the disease are essential to provide innovative precision oncology methodologies and more tailored cholangiocarcinoma treatment strategies [4]. The proposed paper will detail

advanced cholangiocarcinoma cases within the Southeast Asian demographic, highlighting the diversity of targetable genetic mutations as well as the varieties observed in the advanced stage of the disease. The study will be the first of its kind to employ robust genomic and molecular investigation in the Southeast Asian population to document, in Southeast Asia, the clinically actionable mutations toward precision treatment and personalization of treatment for advanced cases of the disease [5]. It further guides the empirical determination of novel biomarkers in targeted therapy development and clinical decision-making for cholangiocarcinoma [6, 7].

Given the high mortality rate associated with cholangiocarcinoma, along with a number of relatively tolerable treatment options that fail to address the disease effectively, the molecular variability of this cancer type implies the necessity of conducting the most accurate genomic studies for the purpose of diagnosis and targeted therapy. This study helps reveal the genomic alterations in the emerging cases of cholangiocarcinoma in Southeast Asia. The results of the study provide useful information about the molecular profiling and actionable mutations in the region.

2. BACKGROUND

The genetic complexity varies across the spectrum of cholangiocarcinoma. These factors are involved in the tumorigenesis, advancement, and metastasis of the tumor. The development of genomic profiling has identified recurrent somatic mutations and structural changes of important oncogenic signaling pathways. Oncogenic mutations such as FGFR2 gene fusions, mutations in IDH1/2, KRAS activation, and amplicon amplification of ERBB2 affect cellular growth and metabolic processes and apoptotic resistance. Dysregulation in transcriptional regulators and chromatin remodeling genes that promote malignant transformation and tumor heterogeneity has also been identified through functional genomic studies of cholangiocarcinoma cell lines [8, 9]. Moreover, genomic comparisons of the biliary tract malignancies found common molecular pathways between cholangiocarcinoma and gallbladder carcinoma, implying that common oncogenic pathways exist within the hepatobiliary malignancies [10, 11].

Precision medicine has greatly transformed the way cancer is treated by providing treatment options that are specific to the molecular properties of the tumor. The incorporation of genomic sequencing technologies

in clinical practice in cholangiocarcinoma has enabled the process of identifying actionable mutations and predictive biomarkers to inform the choice of targeted therapy. Selective agents targeting pathways like FGFR2, IDH1, and HER2 have been shown to be efficacious in any given subset of patients with a metastatic condition [12]. According to the recent reviews of therapeutic options, an individualized treatment strategy involving molecular diagnosis with targeted agents can enhance therapeutic response and slow down disease progression in patients with cholangiocarcinoma [13]. Additionally, the emergence of specific therapies has increased clinical management of advanced disease, providing new possibilities to enhance patient survival using precision oncology [14, 15].

Past studies have given a lot of information on the clinical importance of the targetable genetic changes in cholangiocarcinoma. Genomic analyses in large numbers have found molecular subgroups of the disease that are linked to different therapeutic and clinical responses [16]. Regional differences in mutation frequencies and response to treatment have also been found in population-based studies of cancer of the biliary tract, highlighting the relevance of genomic studies that are specific to a particular population [17]. Related research on malignancies of the biliary tract has reported actionable genetic changes common to cholangiocarcinoma and gallbladder cancer, which can have an impact on the development of therapeutics and the discovery of biomarkers [18]. Moreover, the introduction of genomic medicine in clinical trials within Southeast Asia has helped in the assessment of targeted therapies and biomarker-based treatment approaches in patients with cholangiocarcinoma [19, 20].

The existing literature shows that cholangiocarcinoma is a genomic disease with different alterations in various pathways involved in cancerogenesis. Molecular profiling, together with

precision medicine, has made it possible to identify actionable therapeutic targets and better stratify the treatment of patients with advanced disease. Nevertheless, it can be noticed that the regional genomic data of some populations are scarce, and it is important to conduct more studies that would help to define population-specific targetable mutations that can be used to develop personalized therapeutic approaches in cholangiocarcinoma.

3. METHODOLOGY

3.1. Population of Study and Data Collection

This retrospective molecular analysis investigated FFPE tumor tissue samples stored from 126 patients suffering from advanced-stage cholangiocarcinoma, which were collected from January 2020 to December 2024 in the oncology centers of Southeast Asia. For this study, existing diagnostic tissue samples and secondary clinical information were used; no new patients were recruited for this investigation.

The inclusion criteria were comprised of adults ≥18 years old at diagnosis with a histologic confirmation of locally advanced or metastatic intrahepatic, perihilar, or distal cholangiocarcinoma. Demographic and clinical data such as age, gender, tumor subtype, stage, treatment with systemic therapy, and follow-up information were acquired from the medical and pathology files. FFPE tissue blocks or pre-prepared DNA samples were collected from institutional pathology repositories. For samples having more than one tissue block, the block with high tumor nuclei (>20%) was chosen for NGS.

Table 1 shows the breakdown of the study subjects in the oncology clinics in Southeast Asia where the study was done. In total, 156 patients were screened, of which 126 patients were selected for genome analysis based on the inclusion criteria. The selected subjects were from five oncology centers in Thailand, Malaysia, Singapore, Indonesia, and Vietnam.

Table 1: Distribution of Study Cohort by Participating Center

Country	Screened Patients	Excluded Patients	Included Patients
Thailand	45	7	38
Malaysia	38	7	31
Singapore	32	5	27
Indonesia	24	5	19
Vietnam	17	6	11
Total	156	30	126

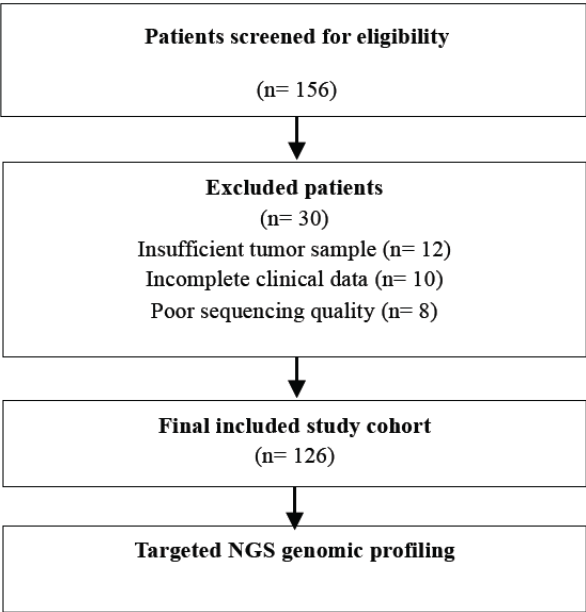


Figure 2: Patient Selection Flow Diagram.

Figure 2 depicts the patient selection process, which includes eligibility screening, exclusion criteria, final cohort selection, and the evolution of 126 patients towards genomic profiling through next-generation sequencing.

3.2. Ethical Approval and Data Privacy

This molecular profiling study was done in compliance with institutional guidelines and the Declaration of Helsinki. Approval from the Institutional Review Board/Ethics Committee of each respective institution was acquired. The fact that this study involved only the examination of archived and

anonymized FFPE samples of tissue specimens, along with secondary clinical data without any personal identification information, meant that the need for obtaining an informed consent from patients was waived by the ethics committee.

Figure 3 shows the gradual approach to the methodology employed by the study in genomic profiling of cholangiocarcinoma samples. The procedure starts with the enrolment of patient data and then proceeds to the collection of clinical data and processing of tumor samples to be ready to undergo a molecular examination. DNA extraction is then performed, followed by specific gene sequencing to create the genomic data. The sequencing results are processed by variant detection to determine the possible genetic alterations, which are then interpreted by variant annotation to establish the biological significance. Lastly, statistical analysis is performed to assess mutation patterns among the cohort so as to identify clinically actionable or targetable genetic changes that could be used to inform precision medicine-based treatment approaches.

Table 2 summarizes the baseline demographic and clinical characteristics of the 126 patients with advanced cholangiocarcinoma included in the study cohort.

3.2. Genetic Testing Methods Used

Genomic tumor profiling was done through targeted next-generation sequencing (NGS) of the FFPE tumor samples. DNA extraction from the tumors was carried

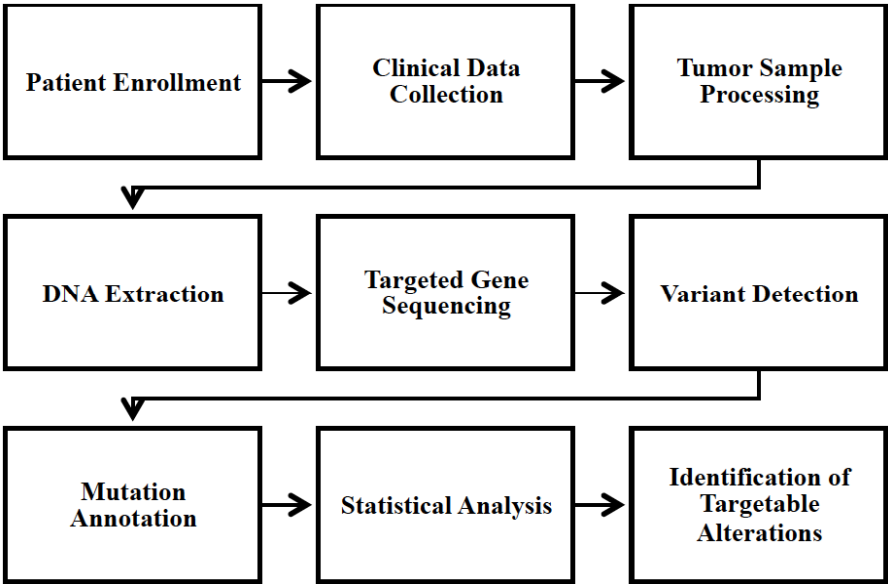


Figure 3: Methodological Workflow for Genomic Profiling and Identification of Targetable Alterations.

Table 2: Baseline Demographic and Clinical Characteristics of the Study Cohort (N = 126)

Clinical Variable	Parameter / Subgroup	Frequency (n)	Percentage (%)
Age (years)	Median (IQR)	58.5 (51–66)	—
	< 60	69	54.8%
	≥ 60	57	45.2%
Gender	Male	71	56.3%
	Female	55	43.7%
Tumor Subtype	Intrahepatic cholangiocarcinoma (iCCA)	78	61.9%
	Perihilar cholangiocarcinoma (pCCA)	31	24.6%
	Distal cholangiocarcinoma (dCCA)	17	13.5%
Disease Stage	Locally advanced (unresectable)	34	27.0%
	Metastatic	92	73.0%
Sample Type	Biopsy specimen	84	66.7%
	Surgical resection specimen	42	33.3%
Prior Systemic Therapy	Treatment-naïve	49	38.9%
	≥ 1 prior line of therapy	77	61.1%

out, followed by quality analysis and library preparation. The targeted cancer gene panel was used to screen for SNVs, indels, copy number variations, and gene fusions of cholangiocarcinoma that included 68 cancer-related genes. High-depth NGS sequencing was carried out to detect genomic variations. Alignment of the sequences against the human genome (GRCh38) was done, followed by variant calling and annotation.

The detection of variants was done through GATK, whereas the functional annotations were done using ANNOVAR. Statistical analysis was done with the help of R (version 4.3) and SPSS, and visualization was done using GraphPad Prism.

Table 3 defines the current molecular testing and sequencing parameters of genomic profiling applied in the study.

3.3. Analysis of Genetic Alterations

Cancer genome databases were used for the filtering and annotation of detected variants in order to establish the clinical relevance and potential implications of these variants. Pathogenic, likely pathogenic, and variants of unknown significance were the categories defined according to known criteria of clinical interpretation. Particular interest was devoted to the alterations of signaling pathways that were supposed to be involved in cholangiocarcinoma. Genetic alteration was considered on the basis of its prevalence in the studied cohort, calculated as the frequency of a particular genetic alteration in the population. The comparison of tumor subtypes was done in order to find out the differences in genomic patterns. Frequencies of mutations were summarized descriptively. The obtained genomic data were

Table 3: Parameters of Molecular Testing and Sequencing

Parameter	Description
Sample type	FFPE tumor tissue
Sequencing method	Targeted next-generation sequencing
Gene panel	68 cancer-associated genes
Variant types detected	SNVs, indels, copy number alterations, gene fusions
Reference genome	GRCh38
Variant calling	GATK
Variant annotation	ANNOVAR
Statistical tools	R v4.3 and SPSS

Table 4: Detection of the Identified Genetic Alterations

Alteration Type	Clinical Interpretation
Pathogenic mutation	Known driver mutation associated with cancer progression
Likely pathogenic mutation	Strong evidence suggesting oncogenic relevance
Variant of uncertain significance	Limited evidence for clinical impact
Copy number alteration	Gene amplification or deletion affecting expression
Gene fusion	Structural rearrangement leading to oncogenic signaling

combined with clinical variables to distinguish trends that may be used to inform individual treatment approaches and future biomarker-based therapeutic studies.

Table 4 shows the classification system, with the help of which the genomic alterations detected are interpreted. The framework assists in the classification of mutations based on their clinical importance and possible role in targeted treatment or biomarker-based treatment plans.

4. RESULTS

4.1. Prevalence of Targetable Genetic Alterations in Advanced Cholangiocarcinoma

In the genome evaluation, 126 advanced cholangiocarcinoma patients were subjected to targeted next-generation sequencing. Of the patients who participated in the genome evaluation, 93 patients (73.8%) were found to have one or more targetable

genomic alterations, whereas 33 patients (26.2%) did not have any targetable genomic alterations. In 28 patients (22.2%), more than one genomic alteration was detected.

Table 5 shows the total frequency of actionable genomic alterations in the study cohort, showing that most of the advanced cases of cholangiocarcinoma had at least one actionable genomic alteration.

4.2. Specific Genetic Alterations Identified

Extensive genomic studies found a number of recurrent mutations in oncogenic pathways of cell signaling, metabolism, and chromatin regulation. FGFR2 fusions, IDH1 mutations, KRAS mutations, TP53 alterations, and ERBB2 amplifications were the most common changes. These genetic events were observed in various tumor subtypes, but with different frequencies in different tumors based on their origin. Any mutations in receptor tyrosine kinase signaling pathways accounted for a large percentage of the observed changes. FGFR2 fusion was mostly detected

Table 5: Prevalence of Targetable Genetic Alterations

Category	Number of Patients	Percentage
Patients with ≥1 actionable mutation	93	73.8%
Patients without targetable alterations	33	26.2%
Patients with multiple alterations	28	22.2%
Total analyzed samples	126	100%

Table 6: Occurrence of Genetic Alterations

Gene Alteration	Number of Cases	Percentage
FGFR2 fusion	21	16.7%
IDH1 mutation	18	14.3%
KRAS mutation	17	13.5%
TP53 mutation	24	19.0%
ERBB2 amplification	9	7.1%

in intrahepatic cholangiocarcinoma cases. KRAS and TP53 mutations were, in contrast, equally distributed among intrahepatic and extrahepatic tumors.

Table 6 will give a summary of key genomic changes found in the study cohort. The data prove the heterogeneity of cholangiocarcinoma at the molecular level and underline the existence of several clinically actionable targets.

4.3. Treatment Protocols and Observed Response Patterns

Treatment and response information was available for 108 of 126 patients included in this study cohort. Patients received first- or second-line treatment based on regional clinical practice guidelines. Those receiving chemotherapy were treated with conventional chemotherapy with gemcitabine and cisplatin. Some patients with certain molecular alterations were treated with targeted therapies, such as FGFR inhibitors (pemigatinib or futibatinib) in case of FGFR2 fusion and IDH1 inhibitors (ivosidenib) in case of IDH1 mutation.

The response to radiotherapy was assessed retrospectively by means of sequential contrast-enhanced CT and MRI scans done at baseline and every 8 to 12 weeks during treatment, based on Response Evaluation Criteria in Solid Tumors (RECIST v1.1). Responses were categorized as partial response (PR), stable disease (SD) and progressive disease (PD).

Median follow-up in the cohort was 14.2 months (3.1-38.5 months). In patients harboring FGFR2 fusions receiving targeted FGFR inhibitors (n=21), there were 8 partial responses (38.1%), 7 stable diseases (33.3%), and 6 progressive diseases (28.6%). Patients with IDH1 mutations (n=18) receiving targeted therapies or protocol-based systemic treatments had 6 partial responses (33.3%), 8 stable diseases (44.4%), and 4 progressive diseases (22.2%). In contrast, patients with KRAS and TP53 mutations had higher progression under conventional cytotoxic therapies (52.9% and 58.3%, respectively). Because of the retrospective design of the current study and varied lines of

treatment, the results described above should be further confirmed in prospective and protocol-based clinical trials.

Treatment response patterns for patients with various genomic mutations have been presented in Table 7. Such findings serve as descriptive data and need to be verified through prospective clinical studies.

4.4. Performance Evaluation

The quality of the sequencing was assessed based on its coverage depth and reproducibility. The targeted regions of the genome received coverage depth exceeding 500x, which enabled accurate detection of genomic changes in the tested samples. Reproducible sequencing ensured identification of clinically significant genomic variants.

The distribution of targetable genomic alterations among patients with advanced cholangiocarcinoma is shown in Figure 4. Most patients harbored one or more targetable alterations; others had none or multiple genomic alterations.

Figure 5 shows the frequency distribution of the main genomic alterations that have been found in advanced cholangiocarcinoma. Genomic alterations included mutations in TP53, fusions in FGFR2, mutations in IDH1, mutations in KRAS, and amplifications in ERBB2.

4.5. Gene Panel Coverage Analysis

A gene panel coverage analysis was performed to evaluate the contribution of increasing panel size on the detection of actionable genomic alterations. Three hypothetical panel configurations containing 20, 40, and 68 genes were compared based on the number of detected actionable alterations within the study cohort. The analysis showed that increasing the number of analyzed genes improved the identification of potentially actionable genomic events, with the complete 68-gene panel providing the highest alteration detection coverage.

Table 7: Observed Treatment Response Patterns According to Genetic Alterations

Genetic Alteration	Partial Response	Stable Disease	Progressive Disease
FGFR2 fusion	8	7	6
IDH1 mutation	6	8	4
KRAS mutation	3	5	9
TP53 mutation	4	6	14

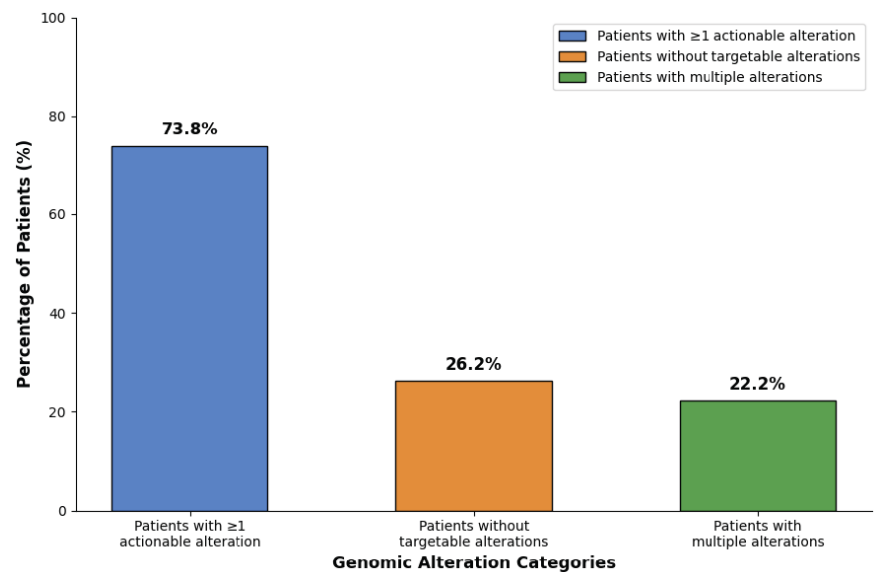


Figure 4: Distribution of Targetable Genetic Alterations in Advanced Cholangiocarcinoma.

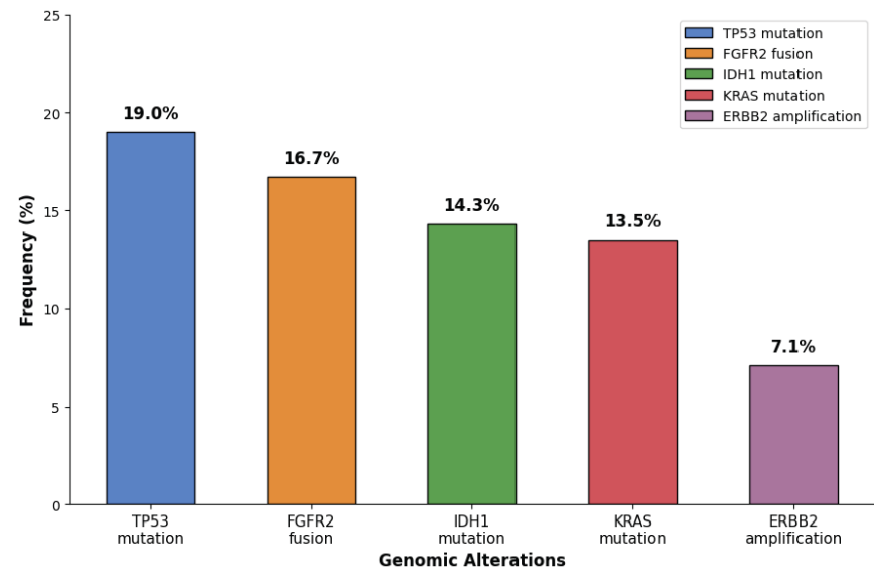


Figure 5: Frequency Distribution of Major Genomic Alterations in Advanced Cholangiocarcinoma.

Table 8: Gene Panel Coverage Analysis for Actionable Alteration Detection

Gene Panel Configuration	Genes Included	Detected Alterations	Detection Rate
Core panel	20	58	46.0%
Expanded panel	40	74	58.7%
Full panel	68	93	73.8%

From Table 8, which illustrates the evaluation of panel coverage, it is evident that there was an improvement in the detection of targetable changes as a result of increase in the size of the genomic panel. Although the bigger gene panel performed better in terms of sensitivity, more validation is required to determine the best panel design.

5. DISCUSSION

Cancers of the biliary tract, especially cholangiocarcinoma (CCA), exhibit unique regional and epidemiological differences around the world. Unlike in Western populations, where non-infectious causes such as primary sclerosing cholangitis and metabolic

liver diseases form the bulk of cases, Southeast Asians carry an additional risk of being exposed to liver flukes (*Opisthorchis viverrini*) and viral hepatitis infections. Even though they form some of the populations with high incidence rates of CCA, patients from Southeast Asia have largely been excluded from the important efforts at genomic research such as The Cancer Genome Atlas (TCGA) and the Memorial Sloan Kettering Cancer Center (MSKCC) IMPACT studies. This study directly addresses this critical literature gap by providing a multi-center genomic profile of advanced CCA across five Southeast Asian nations.

Among this population, 73.8% of patients had at least one actionable alteration in their genome, consistent with what is seen in large Western and East Asian populations for actionable alterations, which is 60%-78%. Nonetheless, the pattern of oncogenic drivers shows significant differences among these population studies:

- **FGFR2 Fusions and IDH1 Mutations:** The group identified fusions involving FGFR2 in 16.7% and mutations involving IDH1 in 14.3% of samples, which were enriched in intrahepatic CCA. These mutation rates are similar to those observed among Western intrahepatic CCA patients in previous work by Javle *et al.*, in which FGFR2 was found in ~13-15% of samples and IDH1 mutations in ~15-20% of samples, but significantly higher than previous work done in fluke-endemic East Asian samples, in which tumor suppressor mutations (TP53, ARID1A) were dominant. This demonstrates that targetable epigenetic and tyrosine kinase drivers remain highly relevant in Southeast Asian clinical practice.
- **TP53 and KRAS Mutations:** The top 2 mutations that occurred most frequently as non-actionable or challenging to target mutations were TP53 (19.0%) and KRAS (13.5%). Although there is a slight difference between the KRAS mutations in this patient cohort which have been reported to be slightly less than the 20-25% in Western distal CCA, the presence of KRAS and TP53 mutations was significantly associated with poor treatment outcome and fast progression of the disease on the use of the standard gemcitabine-based chemotherapy regimen.
- **ERBB2 Amplifications:** Amplifications in ERBB2 (HER2) were detected in 7.1% of cases.

Such rates are in agreement with the world-wide prevalence range of 5-10% in gallbladder and extrahepatic CCA variants, thus highlighting the relevance of applying dual HER2-directed treatment regimens (for example, trastuzumab and pertuzumab, or antibody drug-conjugates) in Southeast Asian population.

5.1. Bridging the Regional Gap and Clinical Implications

The past research in Southeast Asia was limited to single-center cohorts and those that have only considered transcriptomic analysis without any clinical applicability in precision oncology. The current study highlights that almost 75% of Southeast Asian advanced CCA patients harbor actionable mutations (like FGFR2 fusions treatable by pemigatinib and futibatinib and IDH1 mutations treated by ivosidenib), which offers empirical basis to advocate for implementation of targeted NGS panels early and routinely in regional clinical guidelines. In addition, based on this evaluation of panel coverage (see Table 8), study found that the comprehensive panels (>68 genes) detect much higher number of actionable mutations compared to core panels, which can be utilized as empirical basis for design of economical panels.

6. LIMITATIONS

There are several weaknesses in the present study. Firstly, the retrospective nature of the study, along with the use of data from the participating oncology centers, could result in selection bias and affect the applicability of the results to a wider population. Secondly, the size of the cohort was small (only 126 patients), and the frequencies of the genomic alterations need validation in larger multicenter cohorts. Thirdly, even though potentially actionable genomic alterations have been found, the treatment response data analysis is descriptive and was affected by heterogeneity of the treatment protocols and sizes of subgroups. Hence, prospective clinical trials are needed in order to validate the role of the biomarkers for prediction of the treatment response.

7. CONCLUSION

Southeast Asian advanced cholangiocarcinoma demonstrates extensive molecular heterogeneity, with many clinically relevant genomic aberrations present in a considerable proportion of patients. TP53 alterations (19.0%), FGFR2 fusions (16.7%), IDH1 mutations

(14.3%), KRAS mutations (13.5%), and ERBB2 amplifications (7.1%) form the core drivers in this cohort. Importantly, almost three-quarters of all advanced cases display at least one actionable genetic alteration, confirming the therapeutic importance of precision oncology for this cohort of patients, who traditionally suffer from unfavorable prognoses and have few therapeutic options available to them. Such observations stress the need for the implementation of next-generation sequencing for clinical purposes within the region. Biomarker identification helps clinicians make use of biomarker-based treatment options, enrollment in suitable clinical trials, and departure from conventional chemotherapy in favor of increased survival rates. Future studies should include multi-center clinical trials to validate genotype-specific treatments among heterogeneous groups of Asian patients. In addition, the implementation of the multi-omics approach, including transcriptomics and epigenomics, will be required to understand the mechanisms of developed resistance. Lastly, it is necessary to develop affordable genomic screening programs and regional collaboration strategies to provide equal opportunities for precision medicine in Southeast Asia.

ETHICS APPROVAL AND INFORMED CONSENT

Approved by institutional ethics committees; individual patient informed consent was waived for anonymized retrospective samples.

AUTHORS' CONTRIBUTIONS

All authors contributed to study design, data collection, genomic analysis, drafting, and final manuscript approval.

CONFLICTS OF INTEREST

The authors declare no competing financial interests or personal relationships relevant to this research article.

REFERENCES

- [1] Khosla D, Misra S, Chu PL, Guan P, Nada R, Gupta R, Hong JH. Cholangiocarcinoma: recent advances in molecular pathobiology and therapeutic approaches. *Cancers* 2024; 16(4): 801. <https://doi.org/10.3390/cancers16040801>
- [2] Casadio M, Biancaniello F, Overi D, Venere R, Carpino G, Gaudio E, Cardinale V. Molecular landscape and therapeutic strategies in cholangiocarcinoma: an integrated translational approach towards precision medicine. *International Journal of Molecular Sciences* 2021; 22(11): 5613. <https://doi.org/10.3390/ijms22115613>
- [3] Chiu P, Limoges J, Puddester R, Gretchev A, Carlsson L, Leslie K, Pike A. Developing policy infrastructure to guide genomics-informed oncology nursing in Canada: An interpretive descriptive study. *Canadian Journal of Nursing Research* 2024; 56(4): 363-376. <https://doi.org/10.1177/08445621241252615>
- [4] Oura K, Morishita A, Nakahara M, Tadokoro T, Fujita K, Tani J, Kobara H. Chronic liver disease-associated cholangiocarcinoma: genomic insights and precision therapeutic strategies. *Cancers* 2025; 17(18): 3052. <https://doi.org/10.3390/cancers17183052>
- [5] Tavolari S, Brandi G. Mutational landscape of cholangiocarcinoma according to different etiologies: a review. *Cells* 2023; 12(9): 1216. <https://doi.org/10.3390/cells12091216>
- [6] Cigliano A, Chen X, Calvisi DF. Current challenges to underpinning the genetic basis for cholangiocarcinoma. *Expert Review of Gastroenterology Hepatology* 2021; 15(5): 511-526. <https://doi.org/10.1080/17474124.2021.1915128>
- [7] Qu Z, Lau CW, Nguyen QV, Zhou Y, Catchpole DR. Visual analytics of genomic and cancer data: a systematic review. *Cancer Informatics* 2019; 18: 1176935119835546. <https://doi.org/10.1177/1176935119835546>
- [8] Jaidee R, Jusakul A, Pocasap P, Kukongviriyapan V, Senggunprai L, Prawan A, Kongpetch S. Establishment and genomic profiling of cholangiocarcinoma cells with functional characterization. *Scientific Reports* 2025; 15(1): 8621. <https://doi.org/10.1038/s41598-025-93192-1>
- [9] Busisiwe N, Seeley J, Strode A, Parker M. Beyond translations, perspectives for researchers to consider to enhance comprehension during consent processes for health research in sub-saharan Africa: a scoping review. *BMC Medical Ethics* 2023; 24(1): 43. <https://doi.org/10.1186/s12910-023-00920-1>
- [10] Choudhury S, Maiti A, Bandopadhyay A, Tripathi A, Sikdar N. Global gene expression landscape of gallbladder cancer and advances in targeted therapeutic strategies. *World Journal of Clinical Oncology* 2025; 16(10): 111028. <https://doi.org/10.5306/wjco.v16.i10.111028>
- [11] Warner JL, Jain SK, Levy MA. Integrating cancer genomic data into electronic health records. *Genome Medicine* 2016; 8(1): 113. <https://doi.org/10.1186/s13073-016-0371-3>
- [12] Moris D, Palta M, Kim C, Allen PJ, Morse MA, Lidsky ME. Advances in the treatment of intrahepatic cholangiocarcinoma: an overview of the current and future therapeutic landscape for clinicians. *CA: A Cancer Journal for Clinicians* 2023; 73(2): 198-222. <https://doi.org/10.3322/caac.21759>
- [13] Mahmood U, Abbass A, Khan K. Optimizing outcomes and personalizing care with targeted agents in advanced cholangiocarcinoma. *Cancer Treatment Reviews* 2024; 131: 102851. <https://doi.org/10.1016/j.ctrv.2024.102851>
- [14] Macarulla T, Neuzillet C, Prager GW, Rimassa L, Bridgewater J. Opportunities and Approaches to Optimising Advanced Cholangiocarcinoma Outcomes in the Era of Targeted Therapies: A Narrative Review. *Oncology and Therapy* 2025; 13(4): 939-962. <https://doi.org/10.1007/s40487-025-00370-2>
- [15] Brody JA, Morrison AC, Bis JC, O'Connell JR, Brown MR, Huffman JE, Cupples LA. Analysis commons, a team approach to discovery in a big-data environment for genetic epidemiology. *Nature Genetics* 2017; 49(11): 1560-1563. <https://doi.org/10.1038/ng.3968>
- [16] Cortiana V, Chorya H, Joshi M, Kannan S, Mahendru D, Vallabhaneni H, Park CH. Cholangiocarcinoma Insights: Established Foundations and Cutting-Edge Innovations from Dr. James Cleary's Pioneering Research. *Cancers* 2024; 16(3): 632. <https://doi.org/10.3390/cancers16030632>

- [17] Rimassa L, Khan S, Koerkamp BG, Roessler S, Andersen JB, Raggi C, Macias RI. Mapping the landscape of biliary tract cancer in Europe: challenges and controversies. *The Lancet Regional Health–Europe* 2025; 50. <https://doi.org/10.1016/j.lanepe.2024.101171>
- [18] Kuipers H, de Bitter TJ, de Boer MT, van der Post RS, Nijkamp MW, de Reuver PR, Hoogwater FJ. Gallbladder cancer: current insights in genetic alterations and their possible therapeutic implications. *Cancers* 2021; 13(21): 5257. <https://doi.org/10.3390/cancers13215257>
- [19] Thamlikitkul L, Parinyanitkul N, Sriuranpong V. Genomic medicine and cancer clinical trial in Thailand. *Cancer Biology Medicine* 2023; 21(1): 10. <https://doi.org/10.20892/j.issn.2095-3941.2023.0175>
- [20] Hong JH, Yong CH, Heng HL, Chan JY, Lau MC, Chen J, Teh BT. Integrative multiomics enhancer activity profiling identifies therapeutic vulnerabilities in cholangiocarcinoma of different etiologies. *Gut* 2024; 73(6): 966-984. <https://doi.org/10.1136/gutjnl-2023-330483>

Received on 04-08-2026

Accepted on 03-09-2026

Published on 30-09-2026

<https://doi.org/10.30683/1929-2279.2026.15.27>© 2026 Patel *et al.*; Licensee Neoplasia Research.

This is an open-access article licensed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the work is properly cited.