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Molecular Characterization of ERBB2-Altered Biliary Tract Cancer

Erdem Kolemen
Kocaeli University Faculty of Medicine
Gökhan Uygun
Ohio State University Hospital
Devrim Çabuk
Kocaeli University Faculty of Medicine
Tarik Demir
Tarik.Demir@osumc.edu

Ohio State University Hospital

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Abstract

Purpose

ERBB2-targeted therapies have demonstrated clinical activity in ERBB2-amplified biliary tract cancers (BTCs), highlighting the need to clarify molecular determinants of response.

Methods

We characterized the prevalence, anatomical distribution, and co-alteration patterns of ERBB2 amplifications and mutations using the AACR Project GENIE v19.0-public cohort. Activating ERBB2 alterations were defined by OncoKB annotations.

Results

Among 1,483 patients with BTC who underwent next-generation sequencing, 90 (6.1%) harbored ERBB2 alterations: amplification in 56 (3.77%), mutation in 25 (1.68%), and concurrent amplification and mutation in 9 (0.6%). Alterations were enriched in gallbladder adenocarcinoma (17.24%) compared with extrahepatic (10.09%) and intrahepatic cholangiocarcinoma (3.17%; $p < 0.001$). Extracellular-domain mutations predominated (53.8%), mainly involving the S310 hotspot. ERBB2-altered tumors frequently co-harbored TP53 (65.6%), CDK12 (40.0%), SMAD4 (23.3%), and actionable CCNE1 amplifications (17.8%), and showed significantly higher tumor mutational burden than wild-type tumors.

Conclusion

ERBB2 alterations define a distinct BTC subset characterized by recurrent extracellular-domain mutations and specific co-alteration networks, supporting comprehensive genomic profiling for biomarker development and resistance prediction in ERBB2-targeted treatment strategies. These data may help refine patient selection and inform rational combination approaches in future clinical trials of HER2-directed agents.

1. INTRODUCTION

Biliary tract cancers (BTCs), including intrahepatic cholangiocarcinoma (ICC), extrahepatic cholangiocarcinoma (ECC), and gallbladder adenocarcinoma (GBA), are highly aggressive malignancies associated with a dismal prognosis[1, 2]. Although the recent integration of immune checkpoint inhibitors (such as durvalumab or pembrolizumab) with standard first-line gemcitabine and cisplatin chemotherapy has significantly improved overall survival, the absolute clinical benefit remains modest[3, 4]. The urgent need for more effective systemic interventions has catalyzed a paradigm shift toward

precision oncology, driven by the discovery that a substantial subset of BTCs harbors targetable genomic alterations[5].

Among these actionable targets, the epidermal growth factor receptor family member *ERBB2* (HER2) has emerged as a crucial oncogenic driver. *ERBB2* alterations—encompassing gene amplifications, activating somatic mutations, or their concurrent occurrence—are detected across BTC subtypes, with the highest prevalence consistently observed in gallbladder cancer[6]. The clinical validation of HER2 as a therapeutic target has accelerated the investigation of several targeted strategies, including antibody–drug conjugates (e.g., trastuzumab deruxtecan), bispecific antibodies (e.g., zanidatamab), and dual-targeted combinations, all of which have demonstrated compelling antitumor activity in HER2-positive biliary tract cancer and are reshaping the therapeutic landscape[7–9].

Despite these advances, the clinical efficacy of HER2-directed monotherapy is often limited by primary or acquired resistance. The mechanisms driving therapeutic resistance to HER2 blockade are multifactorial and often governed by the broader genomic context of the tumor[10]. Evidence indicates that co-occurring genomic aberrations can provide alternative survival signals that effectively bypass HER2 inhibition[11]. Because BTC is characterized by profound inter- and intra-tumoral heterogeneity, single-biomarker assessments of HER2 status are often insufficient to reliably predict treatment outcomes, making a comprehensive delineation of the co-mutational landscape imperative to identify resistance nodes[12, 13].

To address these complex resistance mechanisms and accurately map the co-mutational landscape of *ERBB2*-driven BTC, large multicenter datasets that capture rare molecular events are essential. In this study, we leverage The American Association for Cancer Research (AACR) Project Genomics Evidence Neoplasia Information Exchange (GENIE) database to comprehensively characterize the genomic landscape of *ERBB2*-altered BTC, mapping the prevalence, anatomical distribution, and distinct co-alteration patterns of *ERBB2* amplifications and mutations to inform future biomarker development and combinatorial precision medicine strategies.

2. MATERIALS AND METHODS

2.1. Study Design and Patient Population

We conducted a retrospective, multi-institutional analysis using the AACR Project GENIE database (Cohort v19.0-Public) (RRID:SCR_026217) [14]. Clinical and genomic data were extracted from patients with pathologically confirmed BTC, anatomically categorized as ICC, ECC, or GBA. Because the AACR Project GENIE database consists of publicly available, deidentified data, this study was exempt from institutional review board approval and from the requirement for informed patient consent [14].

From an initial cohort of 2,117 patients with BTC, we applied strict exclusion criteria: 634 patients without comprehensive *ERBB2* profiling and 67 patients with *ERBB2* variants of unknown significance were excluded to focus on definitive oncogenic alterations. For patients with multiple sequenced

samples, only the earliest available sample was retained. The final eligible cohort comprised 1,483 patients.

2.2. Genomic Profiling and Annotation

Comprehensive genomic profiling of tumor specimens had been performed as part of routine clinical care at participating AACR Project GENIE institutions using center-specific, clinically validated next-generation sequencing (NGS) assays; details of the sequencing platforms, variant-calling pipelines, and data harmonization procedures are provided in the AACR Project GENIE 19.0-public Data Guide (https://www.aacr.org/wp-content/uploads/2026/02/19.0_public_data_guide.pdf). We analyzed the mutational landscape with a primary focus on activating *ERBB2* alterations, encompassing gene amplifications, oncogenic somatic mutations, and their concurrent presence. The oncogenic effect and clinical actionability of detected *ERBB2* alterations and co-occurring genetic events were annotated using the OncoKB precision oncology knowledge base (RRID:SCR_014782)[15]. We systematically mapped the prevalence and distribution of these alterations across the three BTC anatomical subtypes and evaluated the broader genomic context, including tumor mutational burden (TMB) and co-occurring alterations in critical oncogenic and tumor suppressor genes (e.g., *TP53*, *SMAD4*, *CDKN2A*, *CCNE1*, and *PIK3CA*).

2.3. Statistical Analysis

Baseline clinical characteristics and genomic distributions were compared between the *ERBB2*-altered and *ERBB2*-wild-type cohorts. Continuous variables were assessed using the Mann–Whitney U test or the Kruskal–Wallis test, while categorical variables were evaluated using Pearson's chi-square test or two-sided Fisher's exact test. p-values were adjusted for multiple comparisons using the Benjamini–Hochberg false discovery rate (FDR) method, with an FDR-corrected q-value of < 0.05 defining statistical significance. All statistical analyses were conducted utilizing IBM SPSS Statistics for Windows, Version 23.0, and the cBioPortal for Cancer Genomics.

3. RESULTS

3.1. Patient Characteristics

A total of 1,483 patients with BTC were included, of whom 90 (6.1%) harbored *ERBB2* alterations and 1,393 (93.9%) were *ERBB2* wild type. The median age was consistent at 64 years across both the *ERBB2*-altered and wild-type cohorts ($p = 0.839$), and no significant differences were observed regarding sex ($p = 0.647$). Baseline characteristics of patients were listed in Table 1.

Table 1. Baseline Characteristics of the Study Cohort Stratified by *ERBB2* Alteration Status

Characteristic		ERBB2 Altered (n=90)	ERBB2 Wild-Type (n=1393)	p Value	q Value
Age, median (range), y		64 (20–83)	64 (19–88)	.839	.886
Sex	**Male**	41 (45.6%)	607 (43.6%)	.647	.723
	Female	39 (43.3%)	659 (47.3%)		
	Unknown	10 (11.1%)	127 (9.1%)		
Ethnicity	**Non-Hispanic**	61 (67.8%)	1099 (78.9%)	**<.005**	**.0179**
	Hispanic	19 (21.1%)	233 (16.7%)		
	Unknown	10 (11.1%)	61 (4.4%)		
Race	**White**	47 (52.2%)	960 (68.9%)	**<.005**	**.0179**
	Asian	15 (16.7%)	109 (7.8%)		
	Black	12 (13.3%)	75 (5.4%)		
	Other	4 (4.4%)	31 (2.2%)		
	Native American	1 (1.1%)	3 (0.2%)		
	Unknown	11 (12.2%)	215 (15.4%)		
Center	**MSK**	75 (83.3%)	1046 (75.1%)	.097	0.253
	Duke	8 (8.9%)	108 (7.8%)		
	DFCI	4 (4.4%)	189 (13.6%)		
	VICC	3 (3.3%)	50 (3.6%)		
Sample Type	**Primary tumor**	62 (68.9%)	1055 (75.7%)	.364	.493
	Metastatic	27 (30.0%)	311 (22.3%)		
	NOS or NA	1 (1.1%)	27 (2%)		
TMB	**Median (range), mut/Mb**	4.44	2.56	<.001	
	≥10 mut/Mb	13 (14.3%)	57 (4.1%)	<.001	
	≥20 mut/Mb	3 (3.3%)	15 (1.1%)	.093	

Abbreviations: BTC, biliary tract cancer; TMB, tumor mutational burden; NOS, Not otherwise specified; NA, Not applicable; MSK, Memorial Sloan Kettering Cancer Center; DUKE, Duke University; DFCI, Dana-Farber Cancer Institute; VICC, Vanderbilt-Ingram Cancer Center.

Baseline demographic and clinicopathologic characteristics of patients stratified by *ERBB2* alteration status. Continuous variables are presented as median (range), and categorical variables as counts. Comparisons between groups were performed using appropriate statistical tests. P values represent unadjusted comparisons, and q values indicate false discovery rate–adjusted P values for multiple testing.

Ethnicity was categorized as non-Hispanic or Hispanic. Race categories were defined as reported in the dataset. Sample type reflects the source of tumor material used for genomic analysis. Tumor mutational burden (TMB) is reported as median values and stratified thresholds (≥10 and ≥20 mutations per megabase).

However, significant differences were identified in the demographic distribution of *ERBB2* alterations. Ethnicity was significantly associated with *ERBB2* status (p = 0.0046, q = 0.0179), with the *ERBB2*-altered cohort showing a higher proportion of **Hispanic** patients (21.1% vs. 16.7%) and a lower proportion of non-Hispanic patients (67.7% vs. 78.8%) compared to the wild-type group.

Similarly, racial distribution varied significantly (p = 0.0047, q = 0.0179). **Asian** (16.6% vs. 7.8%) and **Black** (13.3% vs. 5.3%) patients were disproportionately represented in the *ERBB2*-altered cohort compared to the wild-type cohort. Conversely, White patients constituted a smaller fraction of the *ERBB2*-altered group (52.2% vs. 68.9%). These findings suggest a potential association between ancestry-related genetic factors or environmental exposures and the prevalence of *ERBB2* drivers in BTC.

No statistically significant differences were identified with respect to sequencing center (p=0.886), sample type (primary vs metastatic; p=0.364), or sample class (tumor vs cfDNA; p=0.442), indicating a balanced distribution of technical and sampling variables across groups.

3.2. Association of *ERBB2* Alterations and Mutational Burden

TMB was significantly higher in *ERBB2*-altered tumors compared to *ERBB2*-wild-type tumors (median: 4.44 vs 2.56 mut/Mb; Mann–Whitney U test, p < .001) (Table 1).

The proportion of TMB-high tumors (≥20 mut/Mb) did not significantly differ between *ERBB2*-altered and *ERBB2*-wild-type groups (3.3% vs 1.1%; Fisher's exact test, p = 0.093). In logistic regression analysis, *ERBB2* alterations were associated with a higher likelihood of TMB-high status (≥20 mut/Mb), although this did not reach statistical significance odds ratio (OR) = 3.13, 95% confidence interval (CI): 0.89–11.02, p = 0.075.

The proportion of TMB-high tumors (≥10 mut/Mb) was significantly higher in *ERBB2*-altered tumors compared to *ERBB2*-wild-type tumors (14.3% vs 4.1%; p < .001). In logistic regression analysis, *ERBB2* alterations were independently associated with increased odds of TMB-high status (OR: 3.91, 95% CI: 2.05–7.44; p < .001). As microsatellite instability-high (MSI-H) status unavailable, TMB associations are limited.

3.3. Prevalence and Distribution of *ERBB2* Alterations

Table 2 summarizes the distribution of *ERBB2* alterations across BTC subtypes. The prevalence of *ERBB2* alterations varied markedly across anatomical subtypes. *ERBB2* alterations were significantly enriched in GBA, occurring in 35 of 203 cases (17.2%), followed by ECC in 21 of 208 cases (10.1%), and ICC in 34 of 1,072 cases (3.2%; p<.001).

Table 2. Distribution of *ERBB2* Alterations by Tumor Subtype

Alteration Type	**ICC** (n=1072)	**ECC** (n=208)	**GBA** (n=203)	(N=1483)
Any *ERBB2* alteration	34 (3.2%)	21 (10.1%)	35 (17.2%)	90 (6.1%)
Amplification	20 (1.9%)	13 (6.3%)	23 (11.3%)	56 (3.8%)
Mutation	11 (1.0%)	6 (2.9%)	8 (3.9%)	25 (1.7%)
Amplification with Mutation	3 (0.3%)	2 (1.0%)	4 (2.0%)	9 (0.6%)

Abbreviations: *ERBB2*, erb-b2 receptor tyrosine kinase 2; GBA, gallbladder adenocarcinoma; ECC, extrahepatic cholangiocarcinoma; ICC, intrahepatic cholangiocarcinoma. Distribution of *ERBB2* alterations across biliary tract cancer subtypes, including GBA, ECC, and ICC. Data are presented as counts, with percentages calculated within each tumor subtype. Overall *ERBB2* alteration frequency in the cohort was 6.1% (90/1483). Among altered cases, gene amplification was the most frequent alteration, followed by mutation and concurrent mutation with amplification.

When stratified by alteration type, *ERBB2* amplifications were the dominant event across all subtypes, particularly in GBA (11.3%) and ECC (6.2%), compared with ICC (1.9%). *ERBB2* mutations were less frequent, observed in 3.9% of GBA, 2.8% of ECC, and 1.0% of ICC. Concurrent amplification and mutation events were rare across all subtypes (<1%). (Figure 1)

These findings highlight a subtype-specific gradient of *ERBB2* alterations, with GBA showing the highest burden, driven primarily by gene amplification.

3.4. Mutation landscape of *ERBB2* alterations in BTC

The mutational landscape of *ERBB2* alterations in BTC revealed a total of 39 mutations identified across 34 patients, with 5 patients harboring multiple mutations. *ERBB2*-mutant tumors, the most frequent mutated domain was the extracellular domain (53.8%) with the most common alterations were in the

Furin-like domain exon 8 (51.28% [20/39]). The most frequent hotspot mutations were S310F/Y (51.28% [20/39]), followed by R678Q (20.51% [8/39]).Notably, all detected *ERBB2* alterations were missense mutations, indicating amino acid substitutions that are likely to impact receptor structure and function. Additionally, concurrent *ERBB2* amplification was observed in 9 patients with *ERBB2* mutations (ICC: 3, ECC: 2, GBA: 4), highlighting a subset of tumors with dual oncogenic activation mechanisms. (Figure 2)

3.5. **Coalterations:**

Next, we evaluated co-occurring genetic alterations associated with *ERBB2* alterations in BTC. Compared with *ERBB2* wild-type tumors, *ERBB2*-altered tumors showed significantly higher frequencies of *TP53* (65.56% vs 28.14%; q < .001), *SMAD4* (23.33% vs 7.39%; q < .001), and *CDKN2A* (14.44% vs 5.31%; q = 0.03) mutations (Figure 3).

Statistically significant co-occurring copy number gains in *ERBB2*-altered tumors were observed in *CDK12* (40.0% vs 0%), *CCNE1* (17.78% vs 1.36%; q < .001), *RARA* (16.67% vs 0%), and *SMARCE1* (17.5% vs 0%). While *CDK12*, *RARA*, and *SMARCE1* co-amplifications likely reflect regional chromosome 17q amplification events, *CCNE1* amplification represents a biologically relevant co-alteration, suggesting additional cell cycle dysregulation in *ERBB2*-driven tumors.

Next we stratified the patients amplification-only and mutation-only cohorts. study showed that *TP53* (79.63% vs 33.33%) mutation and *CDK12* (53.70% vs 0%), *CCNE1* (22.22% vs 8.33%; q < .001), *RARA* (20.37% vs 0%), and *SMARCE1* (17.86% vs 0%) co-occurring copy number gains more frequently seen in amplification only cohort. (Figure 3)

Subsequently, we performed an analysis of genes with tendency toward co-occurrence or mutual exclusivity in the cohort. *ERBB2* amplification or mutation was co-occurred with *TP53* and *SMAD4* alterations with a corrected p < .05. In addition, *TP53* alteration was mutually exclusive with *ARID1A, PBRM1* and *MDM2* alterations. (Figure 4)

Our co-occurrence and mutual exclusivity analysis revealed distinct genomic interaction patterns. *TP53* demonstrated strong co-occurrence with *ERBB2*, *MYC*, and *SMAD4*, supporting its central role in tumor progression and genomic instability. In contrast, significant mutual exclusivity was observed between *TP53* and *MDM2*, as well as *ARID1A* and *PBRM1*, suggesting functional redundancy in key tumor suppressor pathways. These findings highlight the presence of alternative oncogenic routes and cooperative genomic events shaping tumor biology.

3.6. Potential Cognate-Targeted Therapies for Genomic Co-Alterations Associated *ERBB2* Alterations

Among 90 patients with *ERBB2 alterations*, the median number of alterations per patient was five (range, one to 77); Analysis of clinically actionable alterations (OncoKB Level 1–2) co-occurring with *ERBB2* alterations revealed a limited overlap with other targetable genomic events. Notably, tumor TMB-H was identified in 13 cases, representing the most frequent actionable co-alteration, while *IDH1* mutations

were detected in only 3 cases. In contrast, no co-occurring alterations were observed in other established targetable drivers, including *FGFR2* fusions, *BRAF V600E*, *RET* fusions, and *NTRK1–3* fusions. (Supplementary Table 1)

The AACR project GENIE does not currently contain data on dMMR/MSI-H tumors. Data on microsatellite instability–high (MSI-H) status were unavailable.

Sixteen of 90 patients (17.8%) of patients had one or more standard-of-care (OncoKB Level 1–2) co-alterations. An additional 64.4% (58/90) of patients had one or more co-alterations targetable with investigational agents (OncoKB Level 3–4). Overall, 67.8% (61/90) of patients had one or more genomic co-alterations actionable with either standard-of-care or investigational agents.

Overall, these findings demonstrate that *ERBB2*-altered BTCs exhibit minimal co-occurrence with other major actionable genomic alterations, supporting a pattern of mutual exclusivity among major targetable drivers.

We further evaluated the prevalence of genomic alterations previously reported to influence response to HER2-directed therapies, including monoclonal antibodies (mAbs), tyrosine kinase inhibitors (TKIs), and antibody-drug conjugates (ADCs), within *ERBB2*-altered BTCs.

Among these, *TP53* alterations were the most frequent (66%), followed by *CCNE1* (18%), *PIK3CA* (6%), and *ERBB3* (6%). Less common alterations included *RB1* (2%), *PTEN* (1%), and *MET* (1%), while *EGFR* and *IGF1R* alterations were not observed. (Table 3)

Table 3. Prevalence of genomic alterations previously reported to influence response to HER2-directed therapies

GENE	Drug Resistance	N = 90 (%)	References
TP53	mAb	59 (66)	Liu [16], Fedorova [17]
PIK3CA	mAb, TKI	5 (6)	Kim[18], Berns[19]
CCNE1	mAb, TKI	16 (18)	Lee[20],Kim[18], Scalriti[21]
RB1	ADC	2 (2)	Gupta[22]
PTEN	mAb	1 (1)	Yokoyama[23], Berns[19], Nagata[24]
ERBB3	mAb, TKI, ADC	5 (6)	Hanker[25], Smyth[26], Schwarz[27], Ritter[28]
EGFR	mAb, ADC	0 (0)	Schwarz[27], Ritter[28]
MET	mAb, TKI	1 (1)	Kim[18], Paulson[29], Shattuck[30]
IGF1R	mAb	0	Nahta[31], Nahta[32]

Abbreviations: mAb, monoclonal antibody; TKI, tyrosine kinase inhibitor; ADC, antibody–drug conjugate.

4. DISCUSSION

BTCs harboring actionable genomic alterations represent a distinct and highly complex molecular subgroup. Our comprehensive analysis of the AACR Project GENIE database-one of the largest genomic cohorts of BTCs to date- reveals that *ERBB2* alterations occur in approximately 6.1% of patients with BTC. A marked anatomical gradient demonstrates significant enrichment in GBA (17.2%) compared with ECC (10.1%) and ICC (3.2%), confirming that GBA is the primary niche for HER2-targeted exploration in biliary malignancies. The mutual exclusivity observed between *ERBB2* and other established actionable alterations, such as *FGFR2* fusions and *IDH1* mutations, reinforces *ERBB2*'s status as a primary oncogenic driver.

The clinical interpretation of *ERBB2* alterations is complicated by the multilayered co-occurrence of genomic events that likely govern innate and acquired therapeutic resistance. We found that *ERBB2*-altered BTCs are characterized by high rates of concurrent mutations in critical tumor suppressors, notably *TP53* (66%) and *SMAD4* (23%) [16, 17]. Furthermore, we identified actionable co-amplifications in cell-cycle regulators such as *CCNE1* (18%) and alterations in the PI3K pathway (*PIK3CA*, 6%). Amplification of *CCNE1* and activation of PI3K-AKT signaling are established mechanisms that confer resistance to both monoclonal antibodies and TKIs by providing alternative, HER2-independent survival signals [18–21]. The finding that nearly 20% of *ERBB2*-altered BTCs harbor *CCNE1* gains suggests that a substantial subset of patients may be predisposed to poor outcomes with traditional HER2-targeted monotherapy, underscoring the potential utility of rational combinatorial strategies, such as pairing HER2 blockade with cell-cycle or PI3K inhibitors[22, 28].

Interestingly, although gene amplification was the dominant mechanism of activation, we identified a significant proportion of extracellular domain missense mutations, most notably the S310 hotspot. The prevalence of exon 8 Furin-like domain mutations in BTC suggests a specific oncogenic requirement for receptor dimerization in this tissue type [11]. The clinical implications of this distinction are profound: while the pan-HER TKI neratinib yielded a modest 16% objective response rate in the SUMMIT trial for patients with *ERBB2*-mutant BTC, next-generation ADCs like trastuzumab deruxtecan (T-DXd) have demonstrated robust activity in HER2-expressing and amplified BTCs[7, 33]. Emerging evidence from other solid tumors suggests that activating ERBB2 mutations may increase receptor ubiquitination and ADC internalization, raising the possibility that T-DXd could retain activity in some *ERBB2*-mutant, non-amplified biliary tract cancers[34].

Furthermore, our analysis demonstrated that *ERBB2*-altered BTCs have a significantly higher median TMB. High TMB is a well-established predictive biomarker for response to immune checkpoint inhibitors. Given that the addition of durvalumab or pembrolizumab to first-line chemotherapy is now the standard of care for advanced BTC, the elevated TMB in *ERBB2*-altered tumors provides a compelling biological

rationale for exploring the synergy of HER2-targeted agents with PD-1/PD-L1 blockade in future prospective trials[3, 4].

This study is subject to several limitations inherent to the AACR Project GENIE registry, including potential referral bias and variability in sequencing platforms across institutions. The database's lack of detailed longitudinal treatment and clinical outcome data precludes a definitive evaluation of the prognostic and predictive significance of the identified co-mutations. Furthermore, these findings should not be interpreted as supporting the routine use of NGS solely for the detection of ERBB2 alterations, particularly given its greater cost and more limited accessibility compared with HER2 immunohistochemistry. Nevertheless, NGS may offer additional value by simultaneously identifying ERBB2 mutations, amplifications, and potential co-alterations associated with resistance to targeted therapies. Prospective studies are needed to determine the clinical utility of this broader genomic approach.

5. CONCLUSION

ERBB2-altered BTC constitutes a molecularly distinct subset characterized by significant anatomical heterogeneity, elevated TMB, and a complex co-mutational landscape. While the mutual exclusivity of *ERBB2* with other major drivers supports its role as a primary therapeutic target, the frequent co-occurrence of resistance-modifying alterations such as *TP53* mutations and *CCNE1* amplifications may challenge the efficacy of monotherapy approaches.

Declarations

6. **ACKNOWLEDMENTS**

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Authors' Contributions

E. Kolemen: Formal analysis, visualization, writing–original draft, Data curation **G. Uygun:** visualization, Investigation **D. Çabuk:** Supervision, writing–review and editing **T. Demir:** Conceptualization, supervision, project administration, writing–review and editing.

Data availability

The data and analytics tools used in this study are publicly available at: (The AACR Project GENIE Consortium et al., 2024 - https://www.aacr.org/professionals/research/aacr-project-genie/aacr-project-genie-data/).

Ethical approval

This study was exempt from institutional review board approval and informed consent because deidentified GENIE data are publicly available to the entire scientific community.

Informed consent to participate

Not applicable. This was a retrospective database study.

Consent to publish

Not applicable. The study did not include any identifiable individual details, images, or videos.

Clinical trial number

Not applicable

Competing Interests

The author(s) declare (s) no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Figures

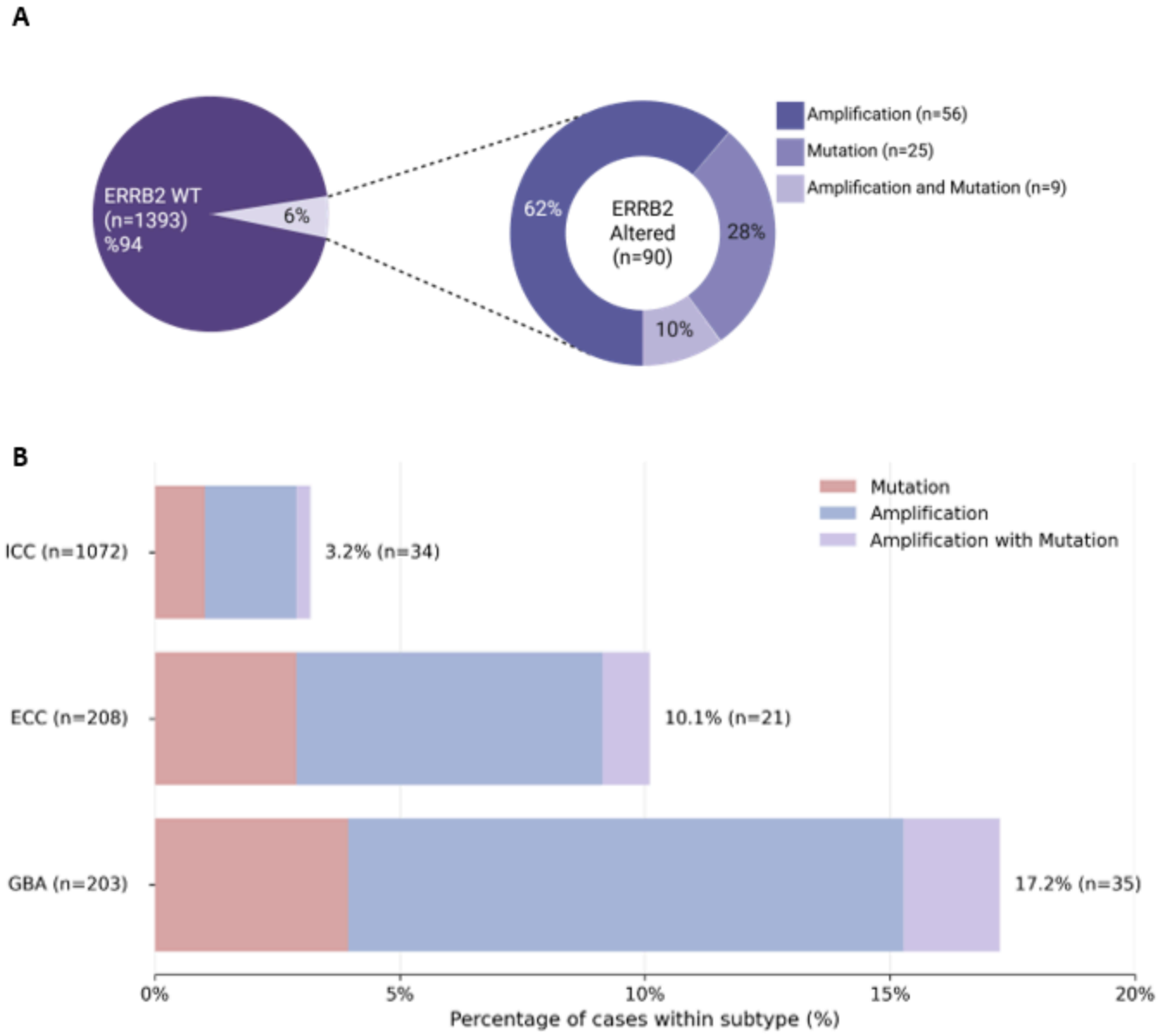


Figure 1

Distribution of *ERBB2* Alterations

1A: Distribution of *ERBB2* Alterations **1B**: *ERBB2*Alteration Frequency Across Biliary Tract Cancer Subtypes. Abbreviations: *ERBB2*, erb-b2 receptor tyrosine kinase 2; WT, Wild type; GBA, gallbladder adenocarcinoma; ECC, extrahepatic cholangiocarcinoma; ICC, intrahepatic cholangiocarcinoma.

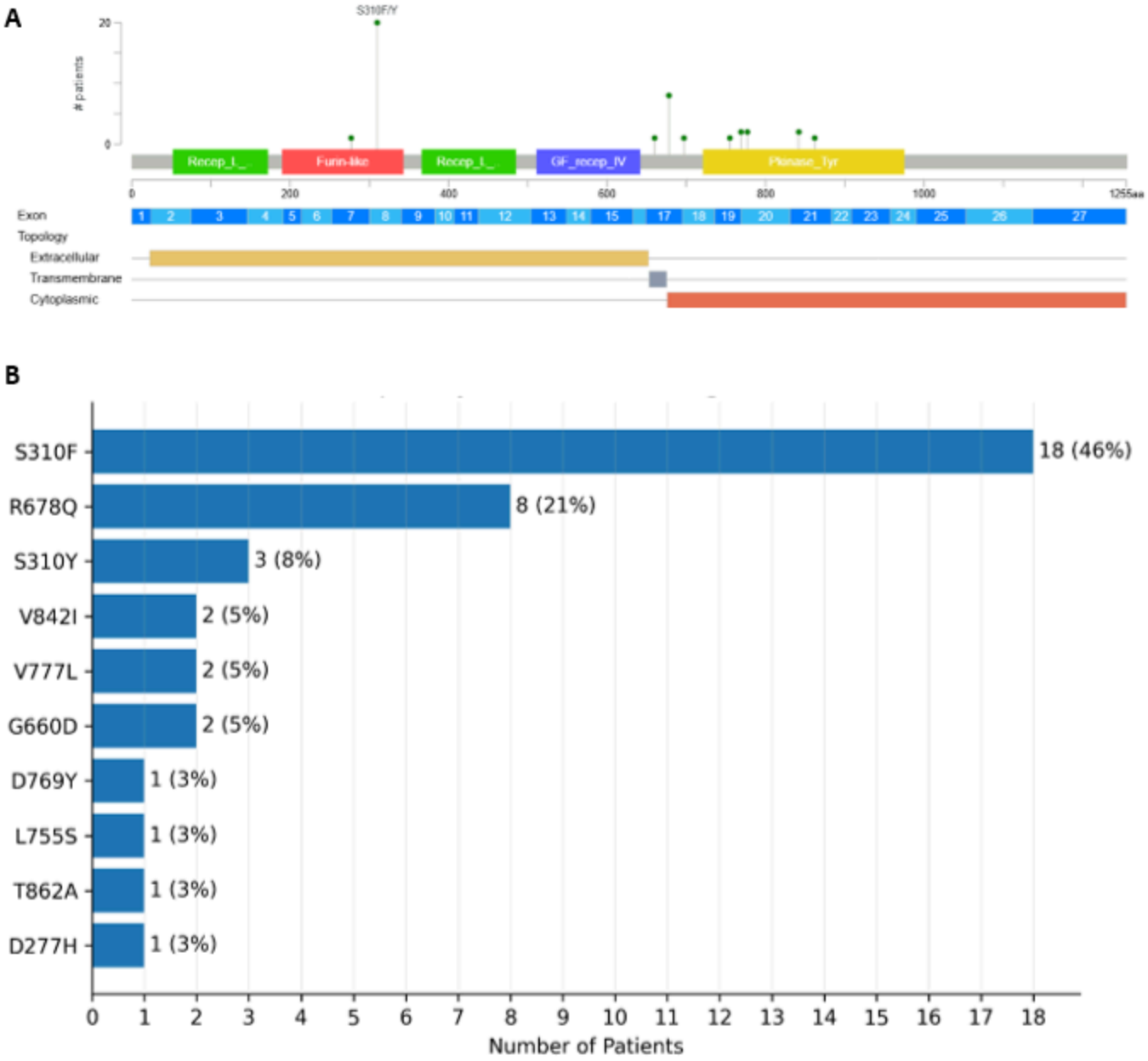


Figure 2

Mutation landscape of *ERBB2* alterations in BTC

2A: Lollipop Plot of *ERBB2* Coding Variants **2B:**Frequency of *ERBB2* Coding Variants Abbreviations: aa, amino acid; GF receptor IV, growth factor receptor domain IV; kinase Tyr, protein tyrosine kinase domain.



Figure 3

Oncoprint of Genomic Alterations in Biliary Tract Cancer

Oncoprint illustrating the genomic landscape of biliary tract cancer. Each column represents an individual tumor sample, and each row represents a gene. Colored bars indicate the type of genomic alteration, including missense mutations, truncating mutations, splice site alterations, amplifications,

deep deletions, and structural variants. The top panels summarize clinical and molecular features, including age at sequencing, mutation burden, mutation spectrum, sample type, and sex. Frequencies of alterations for each gene are indicated on the left.

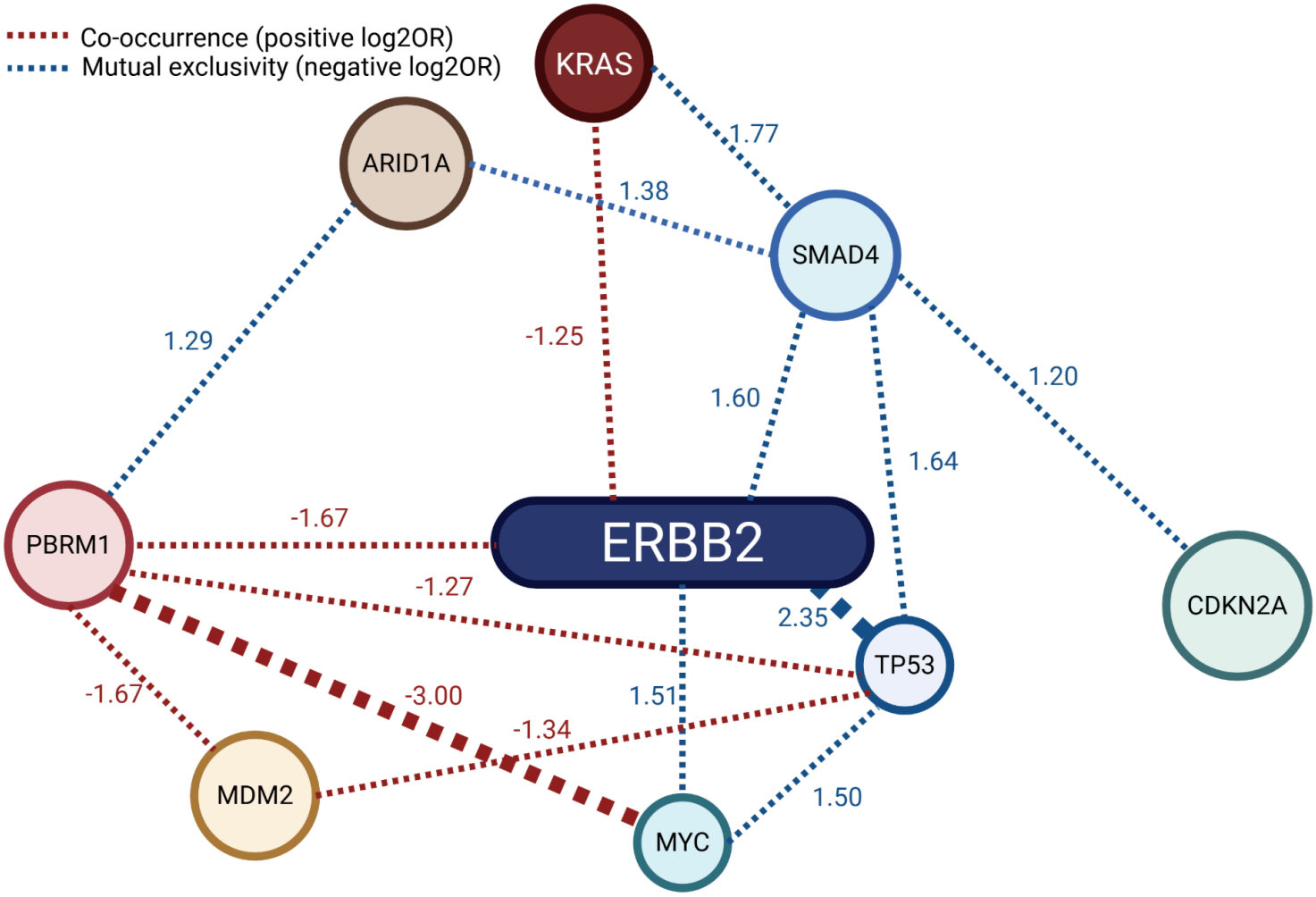


Figure 4

Network of Genomic Co-occurrence and Mutual Exclusivity in BTC

Network representation of pairwise genomic associations centered on *ERBB2*. Nodes represent recurrently altered genes, and edges represent pairwise relationships quantified by log2 odds ratios (ORs). Blue edges indicate co-occurrence (positive log2OR), whereas red edges indicate mutual exclusivity (negative log2OR). Edge thickness is proportional to the magnitude of association. Numeric labels denote log2OR values for each gene pair.

Supplementary Files

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- Supplementarydata.docx