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Systematic Review

Gallbladder Cancer: Current Insights in Genetic Alterations and Their Possible Therapeutic Implications

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Simple Summary: Knowledge of genetic alterations in gallbladder cancer (GBC) continues to increase. This systematic review provides an overview of frequently occurring genetic alterations in GBC and describes their possible therapeutic implications. We detected three frequently (>5%) altered genes (*ATM*, *ERBB2* and *PIK3CA*) for which targeted therapies are available in other cancer types. For solid cancers with microsatellite instability or a high tumor mutational burden pembrolizumab is FDA-approved. Altogether, these five biomarkers might be used in future molecular panels to enable precision medicine for patients with GBC. We found only nine clinical trials evaluating targeted therapies in GBC directed at frequently altered genes (*ERBB2*, *ARID1A*, *ATM* and *KRAS*). This underlines the challenges to perform such clinical trials in this rare, heterogeneous cancer type and emphasizes the need for multicenter clinical trials.

Abstract: Due to the fast progression in molecular technologies such as next-generation sequencing, knowledge of genetic alterations in gallbladder cancer (GBC) increases. This systematic review provides an overview of frequently occurring genetic alterations occurring in GBC and their possible therapeutic implications. A literature search was performed utilizing PubMed, EMBASE, Cochrane Library, and Web of Science. Only studies reporting genetic alterations in human GBC were included. In total, data were extracted from 62 articles, describing a total of 3893 GBC samples. Frequently detected genetic alterations (>5% in >5 samples across all studies) in GBC for which targeted therapies are available in other cancer types included mutations in *ATM*, *ERBB2*, and *PIK3CA*, and *ERBB2* amplifications. High tumor mutational burden (TMB-H) and microsatellite instability (MSI-H) were infrequently observed in GBC (1.7% and 3.5%, respectively). For solid cancers with TMB-H or MSI-H pembrolizumab is FDA-approved and shows an objective response rates of 50% for TMB-H GBC and 41% for MSI-H biliary tract cancer. Only nine clinical trials evaluated targeted therapies in GBC directed at frequently altered genes (*ERBB2*, *ARID1A*, *ATM*, and *KRAS*). This underlines the challenges to perform such clinical trials in this rare, heterogeneous cancer type and emphasizes the need for multicenter clinical trials.

Keywords: gallbladder cancer; gene mutations; genetic alterations; tumor mutational burden; microsatellite instability; targeted therapy

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1. Introduction

Gallbladder cancer (GBC) is a relatively uncommon malignancy, with a worldwide incidence of less than 2 per 100,000 [1]. However, GBC shows a broad geographical and ethnic distribution, with low incidence rates in developed countries and higher incidence rates in South American countries, India, Pakistan, Japan and Korea. Higher incidence rates are also observed among Mexican Americans, Indian Americas, and Eastern Europeans [1–3]. Well-established risk factors include age, obesity, female gender, family history, cholelithiasis, and anomalous junction of the pancreatobiliary duct [4–6].

At present, radical resection by cholecystectomy with lymphadenectomy of the hepatoduodenal ligament and wedge or segment resection of the liver is the only treatment with curative intent [7]. Unfortunately, most patients present at an advanced stage and are unresectable [8,9]. Even after resection, the five-year survival rate ranges from 18% to 34% [10,11]. Palliative systemic chemotherapy for patients with GBC has shown limited efficacy. In patients with locally advanced or metastatic biliary tract cancer (BTC), including GBC, the ABC-02 trial reported a median overall survival of 11.7 months in the BTC group treated with gemcitabine plus cisplatin and 8.1 months in the BTC group treated with gemcitabine monotherapy [12].

With the rapid developments in next-generation sequencing (NGS), our knowledge of genetic alterations occurring in GBC has increased over the past decade [13]. Consequently, the number of preclinical studies and clinical trials evaluating therapies targeting these genetic alterations is slowly increasing [14]. Several reviews on genetic alterations in biliary tract cancer have been published. However, a systematic review specifically focusing on genetic alterations in GBC and their therapeutic implications is lacking.

2. Materials and Methods

2.1. Literature Search

The protocol for this systematic review was prospectively registered in the PROSPERO registry (CRD42021265246). The study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [15].

A literature search was performed until 26 March 2021 in PubMed, EMBASE, Cochrane Library, and Web of Science. Keywords or medical subject headings (MeSH) used for the search are provided in Table A1. Only full-text articles published in English in 2000 or afterwards were selected.

2.2. Study Selection

Citations were deduplicated by using tools in Endnote and Rayyan (rayyan.ai). Subsequently, all duplications were manually verified. Titles and abstracts of all retrieved records were then independently screened for eligibility by two investigators (H.K. and T.J.J.d.B.) using the Rayyan platform for systematic reviews. Discordance was re-evaluated by both investigators and resolved by consensus after discussion. Full papers were obtained for records that were considered potentially eligible by both investigators. In the case of studies with overlapping data, the article reporting the largest cohort was selected.

Only studies were included that identified somatic genetic alterations in human GBC with Polymerase Chain Reaction (PCR) with Sanger sequencing, NGS (targeted, whole-exome, and whole-genome sequencing), MassArray, or SNaPshot. Studies using immunohistochemistry or FISH techniques were excluded. Case reports, retracted articles, and preliminary results were excluded, as well as studies focusing on gene expression, genetic alterations in cell lines, cell-free DNA from serum, and mitochondrial DNA.

This review focused on adenocarcinoma of the gallbladder. Other histologies, i.e., adenosquamous, squamous, neuroendocrine, and sarcomatoid carcinoma, were excluded. In case no histological type was reported, tumors were assumed to represent adenocarcinomas since only 5% of GBC have histology other than adenocarcinoma [16,17].

2.3. Data Extraction

Data extracted included first author, year of publication, study population, sequencing technique, number of samples, and reported genetic alterations and their frequencies. Included alterations were non-synonymous mutations and copy number aberrations (DNA amplifications and deletions). In addition, frequencies of high tumor mutational burden (TMB) and the presence of microsatellite instability (MSI) were extracted.

Only frequently occurring genetic alterations (>5% [18,19] across all studies and in >5 of all included GBC samples) were included. Per genetic alteration, weighted average of reported frequencies was calculated by using the number of samples analyzed in a study as the weight.

A column scatter plot was constructed for all frequently occurring genetic alterations. Bars displayed the minimum and maximum reported frequency. Dots represented the frequency per study. Diamonds represented the weighted averages.

No risk of bias was assessed since no methods are available that could assess confounders such as inter-population diversity and variations in DNA techniques [20,21]. Nevertheless, to facilitate the reader to assess the studies' quality and risk of bias, details of each included study were displayed in tables.

2.4. Therapeutic Implications

Actionable alterations were identified by comparing the frequently altered genes detected in this study with the actionable genes of the OncoKB database (accessed 21 July 2021) [22]. Only actionable genes with level 1 and 2 therapeutic evidence in solid tumors were analyzed. Level 1 evidence was defined as a U.S. Food and Drug Administration (FDA)-recognized biomarker predictive of response to FDA-approved drugs for a specific indication and level 2 as a standard care biomarker predictive of response to FDA-approved drugs for another indication. Data extracted included targetable alteration, drug, level of evidence, and cancer type.

Second, clinicaltrials.gov and clinicaltrialsregister.eu were examined for clinical trials targeting frequently occurring genetic alterations detected in this study in patients with GBC or BTC (accessed 26 July 2021).

3. Results

3.1. Literature Search and Study Selection

In total, 4324 records were retrieved from all databases. After removing duplicate records, 2159 records were screened for eligibility based on title and abstract (Figure 1). A total of 92 articles underwent full-text reading. Out of 92 articles, 30 were excluded due to: no data for adenocarcinoma of the gallbladder available (N = 12), overlapping cohorts (N = 8), the wrong type of article (N = 5), no genetic alteration frequency data available (N = 3), wrong study population (N = 1), and not written in English (N = 1). A total of 62 articles were included for final data extraction, describing a total of 3893 GBC samples from individual patients [18,19,23–82].

3.2. Most Frequently Mutated Genes

Figure 2A represents all frequently occurring gene mutations in GBC. Table A2 shows the results for all frequent gene mutations. *TP53* was the most frequently mutated gene in GBC (weighted average 57%), followed by *SHH* (weighted average 20%), *ELF3* (weighted average 19%), *ARID1A* (weighted average 14%), and *SMAD4* (weighted average 13%).

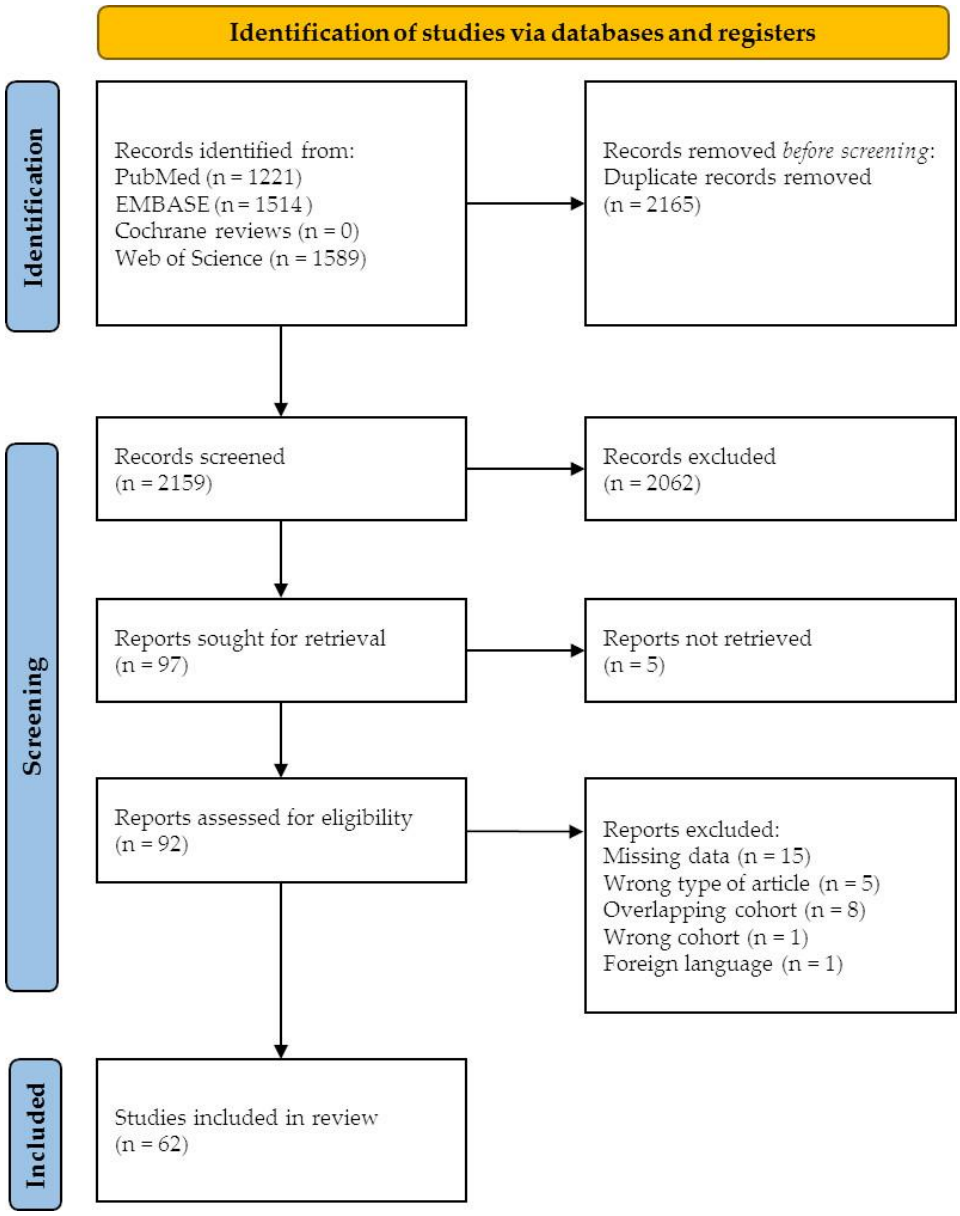

Figure 1. Identification of studies via databases.

3.3. Amplifications and Deletions

Ten studies reported frequencies of copy number aberrations occurring in GBC (Figure 2B). Frequently occurring copy number alterations included amplifications of the oncogenes *CCNE1*, *CDK4*, *ERBB2*, *FRS2*, *KRAS*, *MDM2*, and *MYC* (Table 1). Deletions were observed in *CDKN2A* and *CDKN2B*.

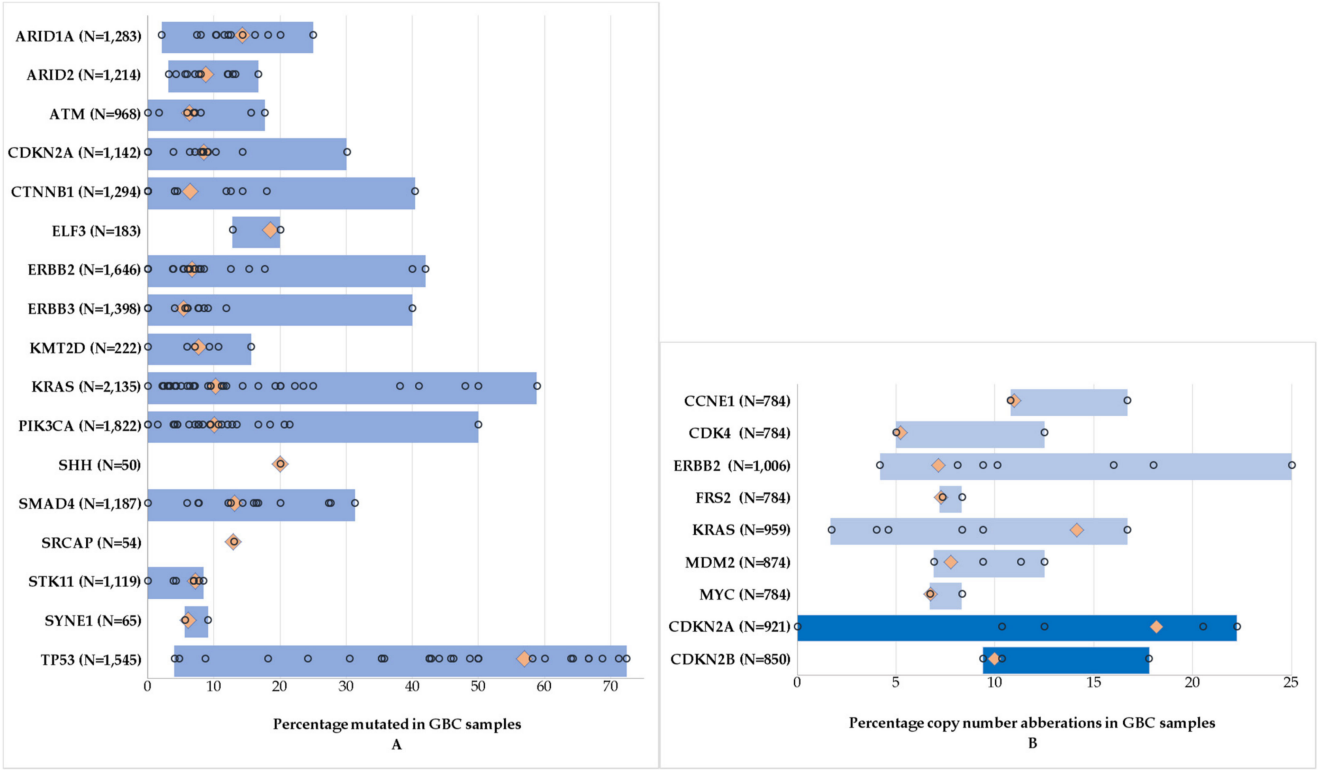

Figure 2. Frequently occurring genetic alterations (>5% across all studies and in >5 of all included samples) in gallbladder cancer. Numbers represent the total number of samples tested for this gene. Blue bars represent the minimum and maximum reported alteration frequencies. Dots represent the mutation frequency per study. Orange diamonds represent the weighted average of all frequencies. (**A**) Gene mutations. (**B**) Gene amplifications (light blue) and gene deletions (dark blue).

3.4. Tumor Mutational Burden (TMB)

The reported frequencies of TMB ranged between 2.6 and 7.03 mutations/Megabase (Mb) in GBC samples (Table 2). Among 864 patients with known TMB status, 15 patients (1.7%) had high TMB (TMB-H). However, the threshold to define TMB-H varied between studies.

3.5. Microsatellite Instability (MSI)

Table 3 outlines all studies (N = 13) reporting on MSI status in 1162 patients with GBC. This table shows that a high diversity of marker panels and MSI definitions were used. A broad range of MSI-high frequencies were reported, although most studies reported a low incidence. The weighted average for MSI-high frequency was 3.5%.

Table 1. Copy number aberrations in gallbladder cancer.

Gene	WA	N	Frequency	Methods	Histology	Population	Author	Year	Ref.
CCNE1	11.0%	82/760	11%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
		4/24	17%	NGS	N.A.	America	Okamura	2021	[27]
CDK4	5.2%	38/760	5%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
		3/24	13%	NGS	N.A.	America	Okamura	2021	[27]
CDKN2A	18.2%	156/760	21%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
		6/58	10%	ultra-deep targeted NGS	AC	China	Lin	2019	[70]
		4/32	13%	targeted NGS	AC	Chile, Japan	Narayan	2019	[73]
		0/25	0%	targeted exome sequencing	N.A.	Korea	Chae	2019	[79]
		10/45	22%	real-time PCR	AC	Japan	Tadokoro	2007	[83]
CDKN2B	10.0%	135/760	18%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
		6/58	10%	ultra-deep targeted NGS	AC	China	Lin	2019	[70]
		3/32	9%	targeted NGS	AC	Chile, Japan	Narayan	2019	[73]
ERBB2	7.1%	77/760	10%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
		1/24	4%	NGS	N.A.	America	Okamura	2021	[27]
		1/4	25%	TS	AC	Korea	Yoo	2016	[37]
		9/111	8%	NGS	N.A.	America	Mondaca	2019	[38]
		9/50	18%	NGS	N.A.	India	Patel	2020	[39]
		3/32	9%	targeted NGS	AC	Chile, Japan	Narayan	2019	[73]
		4/25	16%	targeted exome sequencing	N.A.	Korea	Chae	2019	[79]
FRS2	7.3%	55/760	7%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
		2/24	8%	NGS	N.A.	America	Okamura	2021	[27]
KRAS	14.1%	35/760	5%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
		2/24	8%	NGS	N.A.	America	Okamura	2021	[27]
		10/60	17%	PCR + DS	AC	Taiwan	Huang	2017	[67]
		1/58	2%	ultra-deep targeted NGS	AC	China	Lin	2019	[70]
		3/32	9%	targeted NGS	AC	Chile, Japan	Narayan	2019	[73]
		1/25	4%	targeted exome sequencing	N.A.	Korea	Chae	2019	[79]
MDM2	7.8%	86/760	11%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
		3/24	13%	NGS	N.A.	America	Okamura	2021	[27]
		4/58	7%	ultra-deep targeted NGS	AC	China	Lin	2019	[70]
		3/32	9%	targeted NGS	AC	Chile, Japan	Narayan	2019	[73]
MYC	6.8%	51/760	7%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
		2/24	8%	NGS	N.A.	America	Okamura	2021	[27]

WA: weighted average (calculated by using the number of samples analyzed in a study as the weight); N.A.: not available; AC: adenocarcinoma; NGS: next-generation sequencing; PCR: polymerase chain reaction; DS: direct sequencing; TS: targeted sequencing.

Table 2. Tumor mutational burden in gallbladder cancer.

Author	Origin	Histology	N	TMB (Mut/Mb [Range])	TMB-H Definition	TMB-H	Ref.
Patel	India	N.A.	43	5 (1–14)	-	-	[39]
Weinberg	America	N.A	104	-	≥17 mut/Mb	6/104 (5.8%)	[59]
Li	China	N.A.	12	7.03	-	-	[47]
Abdel-Wahab	America	N.A.	760	2.6 (0–403)	≥19.5 mut/Mb	9/760 (1.2%)	[19]

TMB: tumor mutational burden; TMB-H: high tumor mutational burden; mut: mutations; Mb: megabase.

Table 3. Microsatellite instability in gallbladder cancer.

Author	Origin	Histology	MSI Markers	MSI Definition	MSI	Ref.
Nagai	Japan	N.A.	D2S97, D6S477, D8S339, D9S131, D10S197, D17S796, D18S36, TP53 (17p12), DCC (18q21), APC (5q21)	Shifts in ≥30% of markers	7/17 (41%)	[24]
Kim	Korea	N.A.	3p12-22 (D3S1274, D3S4103, D3S1766) 5q11-23 (D5S107, D5S409, IRF1) 8p22-23 (D8S254, D8S261) 9p22 (IFNA, D9S126, D9S104) 13q13-14 (D13S118 and D13S133) 17p11-13 (D17S786, D17S796,TP53) 18q12-21 (D18S34)	Shifts in ≥1 marker	3/15 (20%)	[31]
Nagahashi	Japan, Hungary	AC	NCI: BAT-25, BAT-26, D2S123, D5S346, D17S250	Shifts in ≥2 markers	9/34 (27%)	[35]
Patel	India	N.A.	Genome-wide analysis of 95 loci	N.A.	0/43 (0%)	[39]
Abdel-Wahab	U.S.	N.A.	114 loci	Shifts in ≥2 markers	3/551 (1%)	[19]
Wistuba	Chile	AC	81 loci on 3p, 8p, 9q and 22q	Shifts in ≥1 marker	6/12 (50%)	[43]
Pandey	Chile, Korea, India	AC	Exome-wide analysis	MSI score > 0.35	3/152 (2%)	[46]
Li	China	N.A.	NGS	N.A.	0/12 (0%)	[47]
Rashid	China	AC	NCI (BAT-25, BAT-26, D2S123, D5S346, D17S250) and TGFβRII	Shifts in ≥40% of D2S123, D5S346, D17S25, or alteration of BAT-25, BAT-26 or TGFβRII	2/64 (3%)	[49]
Goeppert	Germany	AC	BAT25, BAT26, and CAT25	Shifts in ≥2 markers	1/69 (1%)	[52]
Yoshida	Japan	AC	p53, APC, DCC, NM23-H1, D2S123, D3S1029, D5S107, D17S261, D18S34	Shifts in ≥33% of markers	0/30 (0%)	[54]
Roa	Chile	AC	NCI: BAT25, BAT26, D2S123, D5S346, D17S250 and BAT40, D3S1067, D3S1286, D3S1262, D3S1478, D12S1638, D12S347, D16S265	Shifts in >30% of markers	6/59 (10%)	[55]
Weinberg	U.S.	N.A.	Targeted NGS over 7000 loci	N.A.	1/104 (1%)	[59]

MSI: microsatellite instability; N.A.: not available; AC: adenocarcinoma; NCI: National Cancer Institute recommendation; NGS: next-generation sequencing.

3.6. Possible Therapeutic Implications

3.6.1. Targetable Alterations in Other Malignancies

Currently, no FDA-approved therapies targeting gene mutations or copy number aberrations are available for GBC. However, several targeted therapies directed at actionable alterations that were also frequently observed in GBC are FDA-approved in other cancers (Table 4). These alterations include mutations in *ATM*, *PIK3CA* and *ERBB2*, and amplifications in *ERBB2*. For solid cancers with MSI-H or TMB-H, pembrolizumab is approved by the FDA.

3.6.2. Clinical Trials

Table 5 presents all clinical trials that include GBC patients harboring frequently occurring genetic alterations. No completed studies were identified; all studies are currently recruiting participants.

Table 4. FDA-approved drugs targeting genetic alterations in other malignancies.

Target	Level	Malignancy	Agent	ORR	Ref.
ATM ¥	1	Prostate cancer	Olaparib	*BRCA1*, *BRCA2*, or *ATM:* 28/84 (33%)	[84]
ERBB2 *	1	Esophagogastric cancer	Pembrolizumab + trastuzumab + chemotherapy	32/35 (91%)	[85]
			Trastuzumab + chemotherapy	139/294 (47%)	[86]
	1	Breast cancer	Trastuzumab deruxtecan	61/119 (51%)	[87]
			Ado-trastuzumab emtansine	173/397 (44%)	[88]
			Lapatinib + letrozole	31/111 (28%)	[89]
			Lapatinib + capecitabine	36/163 (22%)	[90]
			Margetuximab + chemotherapy	67/266 (25%)	[91]
			Neratinib	34/117 (29%)	[92]
			Trastuzumab	30/114 (26%)	[93]
			Trastuzumab + pertuzumab + chemotherapy	275/343 (80%)	[94]
			Trastuzumab + tucatinib + capecitabine	138/340 (41%)	[95]
			Trastuzumab deruxtecan	112/184 (61%)	[96]
	2	Colorectal cancer	Lapatinib + trastuzumab	9/32 (28%)	[97]
			Trastuzumab + pertuzumab	18/57 (32%)	[98]
			Trastuzumab deruxtecan	24/53 (45%)	[99]
ERBB2 *	2	Uterine serous carcinoma	Trastuzumab + carboplatin-taxol	4/9 (44%)	[100]
ERBB2 ¥	2	NSCLC	Ado-trastuzumab emtansine	8/18 (44%)	[101]
			Trastuzumab deruxtecan	50/91 (55%)	[102]
PIK3CA ¥	1	Breast cancer	Alpelisib + fulvestrant	21/121 (17%	[103]
TMB-H	1	Solid tumors	Pembrolizumab	30/102 (29%)	[104]
MSI-H	1	Solid tumors	Pembrolizumab	59/149 (40%)	[105]
MSI-H	1	Colorectal cancer	Nivolumab	23/74 (31%)	[106]
			Ipilumab + nivolumab	65/119 (55%)	[107]

¥: mutation; *: amplification. ORR: objective response rate; NSCLC: non-small cell lung cancer; MSI-H: high microsatellite instability; TMB-H: high tumor mutational burden.

Table 5. Ongoing clinical trials targeting genetic alterations in GBC.

Target	Phase	Agent	Country	Trial ID
ERBB2 signal pathway components	2	FORFIRINOX + (cetuximab, trastuzumab, gefitinib, lapatinib, everolimus, sorafenib, or crizotinib)	China	NCT03768375
ERBB2 signal pathway components	2	GEMOX + afatinib	China	NCT04183712
ERBB2 overexpression/ amplification	2	Trastuzumab + pertuzumab	U.S.	NCT02091141
ERBB2 overexpression/ amplification	1,2	Tucatinib + trastuzumab + (FOLFOX or CAPOX)	U.S.	NCT04430738
ERBB2 overexpression/ amplification or mutations	2, basket	Tucatinib + trastuzumab	U.S., Japan, Belgium	NCT04579380
ERBB2 amplification	2	Zanidatamab	U.S., Canada, Chile, China, France, Italy, Korea, Spain, U.K.	NCT04466891
KRAS (or *NRAS*) mutation	1	ELI-002 immunotherapy	U.S.	NCT04853017
DNA repair gene mutations (including *ARID1A*, *ATM*, and others)	2	Olaparib	U.S.	NCT04042831
TMB $\geq$ 10 mutations/Mb	2	Atezolizumab	U.S.	NCT02091141

U.S.: United States; U.K.: United Kingdom; TMB: tumor mutational burden.

Most studies evaluate targeted therapies directed at *ERBB2* alterations and related signal pathway components. One trial assesses the safety and efficacy of ELI-002 immunotherapy, a novel amphiphile therapeutic vaccine targeting KRAS-driven cancers, for patients with *KRAS* or *NRAS* mutations (G12D or G12R) in various solid tumor types including GBC (NCT04853017). Moreover, a phase 2 trial analyzes the efficacy and toxicity of olaparib in patients with metastatic BTC with an DNA repair gene mutation, including *ARID1A* and *ATM* (NCT04042831). The NCT02091141 trial evaluates six treatment regimens based on molecular testing in patients with advanced solid tumors, including BTC. For example, patients with tumors demonstrating elevated TMB (≥10 mutations/Mb) will receive atezolizumab.

4. Discussion

In this systematic review of 62 articles assessing genetic alterations in GBC, 3893 GBC samples were analyzed. Frequently occurring genetic alterations included mutations in 17 genes and amplifications/deletions in nine genes. Since no targeted therapy is currently available for GBC, frequent genetic alterations detected in this study were matched to actionable genetic alterations in other solid tumors. Only mutations in *ATM*, *ERBB2* and *PIK3CA* and amplifications in *ERBB2* are currently targetable with FDA-approved drugs in other solid tumors. Therefore, these alterations are potential targets in GBC and might be included in future molecular testing panels for personalized treatment decisions.

In GBC, the prevalence of TMB-H was low. Previous studies have shown that TMB-H is correlated with clinical response to immunotherapy in several tumors, including BTC, and that patients with TMB-H tumors can benefit from immune checkpoint inhibitors such as pembrolizumab [108–110]. In patients with GBC receiving immunotherapy, a higher objective response rate was observed for TMB-high tumors compared to TMB-low tumors (50% vs. 25%) [47]. Pembrolizumab has been FDA-approved for patients with TMB-H advanced solid cancers.

Another biomarker associated with response to immune checkpoint inhibitors is MSI, which was observed in 3.5% of patients in the present study. For patients with MSI-H cancers, pembrolizumab is also approved by the FDA, as well as by the Pharmaceuticals and Medical Devices Agency (PMDA, Japan). The European Medicines Agency (EMA, European Union) and the National Medical Products Administration (NMPA, China) have not yet given approval for this agent in patients with MSI-H (or TMB-H). In patients with cholangiocarcinoma who received pembrolizumab, an objective response rate of 41% was seen [111]. Therefore, although only beneficial in a small proportion of patients, both TMB-H and MSI-H are interesting biomarkers in precision medicine for GBC.

A broad range was observed in genetic alteration frequencies in GBC across all studies. In part, this could be attributed to differences in sequencing technologies and assessed gene regions. For example, NGS has a slightly lower sensitivity for mutation detection compared to some other technologies like real-time PCR testing, although detecting low-frequency genetic alterations through (ultra-) deep sequencing with a higher coverage can partly overcome this issue [112–114]. Moreover, geographical differences in mutation patterns might be present, as previous studies have shown [35,46,73]. However, no one-to-one regional comparisons of genetic alteration frequencies could be made due to different sequencing techniques used throughout all studies. Substantial differences were also observed regarding the frequency of MSI. A large variation in marker panels and MSI definitions, and some missing MSI definitions, complicated comparison of all MSI frequencies. Although a weighted average of MSI frequency was calculated to minimalize the influence of sample size on the average frequency, our results should be taken with caution due to the heterogeneity in MSI panels and definitions. Usage of the National Cancer Institute (NCI) panel of microsatellites and definitions in future research would facilitate MSI comparison among studies [115].

Since some genetic alterations might impede the efficacy of therapies targeting other genetic alterations, co-existing alterations might be an important determinant for therapeu-

tic sensitivity and resistance. For example, patients with solid stage IV cancer types harboring amplification in the *MDM2* family or *EGFR* aberrations who received anti-PD1/PDL1 immunotherapy showed increased tumor growth [116]. Unfortunately, co-existing alterations could not be assessed in this study due to differences in sequencing techniques and missing data in some studies.

Only nine clinical trials which evaluated targeted therapies directed at frequently occurring genetic alterations in GBC were identified. Though we assessed only trials targeting these genetic alterations specifically, these limited results underline that GBC remains a relatively under-investigated cancer type regarding therapeutic agents. Unfortunately, initiation of clinical trials including patients with GBC is logistically challenging since GBC is a rare cancer type in most Western countries. The large inter-tumor heterogeneity of GBC poses another challenge for targeted therapies. However, basket trials, a clinical trial that tests agents in different cancer types with the same genetic alteration, could provide a solution to this challenge in future studies.

This study has several limitations. First, histological types and subtypes of GBC samples could not always be retrieved. It is important to report histological (sub)types to be able to determine whether differences in genetic alterations are associated with different histology. Second, a publication bias might exist. Therefore, actual overall alteration frequencies might be over- or underestimated.

5. Conclusions

In conclusion, GBC has a diverse mutational landscape. Frequently occurring genetic alterations in GBC that are actionable in other solid tumors are rare, including mutations in *ATM*, *ERBB2*, *PIK3CA* and amplifications in *ERBB2.* For all solid tumors with MSI-high or TMB-high, including GBC, the immune checkpoint inhibitor pembrolizumab is FDA-approved. Few clinical trials targeting the frequently altered genes in GBC are performed, emphasizing the need for multicenter clinical basket trials.

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Appendix A

Table A1. Keywords and subject headings used in the literature search.

Database			
PubMed	**EMBASE**	**Cochrane Reviews**	**Web of Science**
(“Gallbladder Neoplasms”[Mesh] OR (gallbladder[tiab] AND (neoplasm * [tiab] OR cancer * [tiab] OR lesion * [tiab] OR tumor* [tiab] OR tumour * [tiab] OR carcinom * [tiab] OR malignan * [tiab]))) AND (“Mutation”[Mesh] OR “Epigenesis, Genetic”[Mesh] OR “Genome”[Mesh] OR molecular[tiab] OR gene[tiab] OR genes[tiab] OR genetic * [tiab] OR genom * [tiab] OR sequenc * [tiab] OR mutation*[tiab] OR exome * [tiab])	(‘gallbladder cancer’/exp OR (gallbladder AND (neoplasm * OR cancer* OR tumor * OR tumour * OR carcinom * OR malignan *)):ab,ti,kw) AND (‘gene mutation’/exp OR ‘gene sequence’/exp OR (exome * OR molecular OR gene OR genes OR genetic * OR genom * OR sequenc * OR mutation *):ab,ti,kw)	(gallbladder AND (neoplasm * OR cancer * OR tumor * OR tumour * OR carcinom * OR malignan *)) AND (molecular OR gene OR genes OR genetic * OR genom * OR sequenc * OR mutation * OR exome *)	(gallbladder AND (neoplasm * OR cancer * OR tumor * OR tumour * OR carcinom * OR malignan *)) AND (exome * OR molecular OR gene OR genes OR genetic * OR genom * OR sequenc * OR mutation *)

*: show keywords in a search strategy.

Table A2. Studies analyzing frequently mutated genes in gallbladder cancer.

Gene	WA	N	Frequency	Methods	Histology	Population	Author	Year	Ref.
ARID1A	14.3%	1/5	20%	WES	AC	Japan	Akita	2019	[48]
		2/14	14%	TS	N.A.	China	Li	2017	[44]
		2/47	4%	WES	AC	China	Yang	2021	[56]
		2/25	8%	targeted exome sequencing	N.A.	Korea	Chae	2019	[79]
		3/24	13%	NGS	N.A.	America	Okamura	2021	[27]
		3/26	12%	NGS	N.A.	Italy	Simbolo	2014	[61]
		4/54	7%	NGS	AC	Greece	Papadopoulou	2018	[40]
		4/39	10%	WES/WGS	N.A.	Japan	Ebata	2021	[57]
		15/144	10%	WES	AC	India, Korea, Chile	Pandey	2020	[46]
		7/58	12%	ultra-deep targeted NGS	AC	China	Lin	2019	[70]
		8/32	25%	targeted NGS	AC	Chile, Japan	Narayan	2019	[73]
		10/55	18%	NGS	N.A.	America	Javle	2016	[23]
		123/760	16%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
ARID2	8.8%	1/32	3%	targeted NGS	AC	Chile, Japan	Narayan	2019	[73]
		1/17	6%	WES	N.A.	India	Iyer	2019	[29]
		1/14	7%	TS	N.A.	China	Li	2017	[44]
		1/24	4%	NGS	N.A.	America	Okamura	2021	[27]
		3/25	12%	targeted exome sequencing	N.A.	Korea	Chae	2019	[79]
		3/54	6%	NGS	AC	Greece	Papadopoulou	2018	[40]
		3/39	8%	WES/WGS	N.A.	Japan	Ebata	2021	[57]
		19/144	13%	WES	AC	India, Korea, Chile	Pandey	2020	[46]
		2/12	17%	NGS	N.A.	China	Li	2020	[47]
		7/58	12%	ultra-deep targeted NGS	AC	China	Lin	2019	[70]
		8/47	17%	WES	AC	China	Yang	2021	[56]
		61/760	8%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
ATM	6.3%	0/4	0%	TS	AC	Korea	Yoo	2016	[37]
		1/14	7%	NGS	N.A.	Japan	Noguchi	2017	[32]
		1/58	2%	NGS	AC	America	Maynard	2020	[41]
		2/25	8%	targeted exome sequencing	N.A.	Korea	Chae	2019	[79]
		5/32	16%	targeted NGS	AC	Chile, Japan	Narayan	2019	[73]
		3/17	18%	WES	N.A.	India	Iyer	2019	[29]
		4/58	7%	ultra-deep targeted NGS	AC	China	Lin	2019	[70]
		45/760	6%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
CDKN2A	8.5%	0/24	0%	NGS	N.A.	America	Okamura	2021	[27]
		0/5	0%	WES	AC	Japan	Akita	2019	[48]
		0/4	0%	TS	AC	Korea	Yoo	2016	[37]
		1/11	9%	ultra-deep targeted NGS	N.A.	India	Yadav	2017	[77]
		1/26	4%	NGS	N.A.	Italy	Simbolo	2014	[61]
		1/14	7%	NGS	N.A.	Japan	Noguchi	2017	[32]
		13/144	9%	WES	AC	India, Korea, Chile	Pandey	2020	[46]
		1/12	8%	NGS	N.A.	China	Li	2020	[47]
		2/25	8%	targeted exome sequencing	N.A.	Korea	Chae	2019	[79]
		2/32	6%	targeted NGS	AC	Chile, Japan	Narayan	2019	[73]
		2/14	14%	TS	N.A.	China	Li	2017	[44]
		4/13	31%	PCR-SSCP + DS	N.A.	Korea	Kim	2001	[31]
		6/58	10%	ultra-deep targeted NGS	AC	China	Lin	2019	[70]

Table A2. *Cont.*

Gene	WA	N	Frequency	Methods	Histology	Population	Author	Year	Ref.
		64/760	8%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
CTNNB1	6.4%	0/21	0%	SNaPshot	AC	America	Moy	2015	[53]
		0/68	0%	WES + Sanger seq	AC	Japan	Akita	2019	[48]
		0/26	0%	NGS	N.A.	Italy	Simbolo	2014	[61]
		0/4	0%	TS	AC	Korea	Yoo	2016	[37]
		0/14	0%	NGS	N.A.	Japan	Noguchi	2017	[32]
		0/58	0%	ultra-deep targeted NGS	AC	China	Lin	2019	[70]
		1/25	4%	SNaPshot	N.A.	America	Borger	2012	[30]
		2/46	4%	mass array + seq	AC	India	Kumari	2014	[68]
		2/14	14%	TS	N.A.	China	Li	2017	[44]
		2/17	12%	WES	N.A.	India	Iyer	2019	[29]
		18/144	13%	WES	AC	India, Korea, Chile	Pandey	2020	[46]
		9/50	18%	PCR-SSCP + seq	AC	India	Dixit	2020	[36]
		19/47	40%	NGS	AC	Greece	Papadopoulou	2018	[40]
		30/760	4%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
ELF3	18.6%	29/144	20%	WES	AC	India, Korea, Chile	Pandey	2020	[46]
		5/39	13%	WES/WGS	N.A.	Japan	Ebata	2021	[57]
ERBB2	6.7%	0/21	0%	SNaPshot	AC	America	Moy	2015	[53]
		0/46	0%	mass array	AC	India	Kumari	2014	[68]
		0/4	0%	TS	AC	Korea	Yoo	2016	[37]
		0/58	0%	ultra-deep targeted NGS	AC	China	Lin	2019	[70]
		1/26	4%	NGS	N.A.	Italy	Simbolo	2014	[61]
		2/32	6%	targeted NGS	AC	Chile, Japan	Narayan	2019	[73]
		1/14	7%	TS	N.A.	China	Li	2017	[44]
		2/54	4%	NGS	AC	Greece	Papadopoulou	2018	[40]
		2/5	40%	WES	AC	Japan	Akita	2019	[48]
		2/25	8%	targeted exome sequencing	N.A.	Korea	Chae	2019	[79]
		3/50	6%	NGS	N.A.	India	Patel	2020	[39]
		3/17	18%	WES	N.A.	India	Iyer	2019	[29]
		3/24	13%	NGS	N.A.	America	Okamura	2021	[27]
		3/39	8%	WES/WGS	N.A.	Japan	Ebata	2021	[57]
		4/47	9%	WES	AC	China	Yang	2021	[56]
		22/144	15%	WES	AC	India, Korea, Chile	Pandey	2020	[46]
		6/111	5%	NGS	N.A.	America	Mondaca	2019	[38]
		5/12	42%	NGS	N.A.	China	Li	2020	[47]
		11/157	7%	WES	N.A.	China	Li	2019	[18]
		40/760	5%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
ERBB3	5.4%	2/5	40%	WES	AC	Japan	Akita	2019	[48]
		1/11	9%	ultra-deep targeted NGS	N.A.	India	Yadav	2017	[77]
		1/17	6%	WES	N.A.	India	Iyer	2019	[29]
		0/24	0%	NGS	N.A.	America	Okamura	2021	[27]
		0/32	0%	targeted NGS	AC	Chile, Japan	Narayan	2019	[73]
		3/39	8%	WES/WGS	N.A.	Japan	Ebata	2021	[57]
		4/47	9%	WES	AC	China	Yang	2021	[56]
		3/50	6%	NGS	N.A.	India	Patel	2020	[39]
		3/54	6%	NGS	AC	Greece	Papadopoulou	2018	[40]
		0/58	0%	ultra-deep targeted NGS	AC	China	Lin	2019	[70]
		17/144	12%	WES	AC	India, Korea, Chile	Pandey	2020	[46]

Table A2. *Cont.*

Gene	WA	N	Frequency	Methods	Histology	Population	Author	Year	Ref.
		12/157	8%	WES	N.A.	China	Li	2019	[18]
		30/760	4%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
KMT2D	7.7%	0/58	0%	ultra-deep targeted NGS	AC	China	Lin	2019	[70]
		1/17	6%	WES	N.A.	India	Iyer	2019	[29]
		1/14	7%	TS	N.A.	China	Li	2017	[44]
		5/32	16%	targeted NGS	AC	Chile, Japan	Narayan	2019	[73]
		5/54	9%	NGS	AC	Greece	Papadopoulou	2018	[40]
		5/47	11%	WES	AC	China	Yang	2021	[56]
KRAS	10.3%	1/42	2%	nested PCR, PCR-RFLP + DS	AC	Japan, Hungary	Nagahashi	2008	[35]
		0/27	0%	Oncomap	AC	America	Deshpande	2011	[66]
		0/29	0%	nested PCR, PCR-RFLP + DS	AC	Peru	Vidaurre	2019	[82]
		1/35	3%	PCR + seq	AC	Bolivia	Asai	2014	[42]
		1/25	4%	SNaPshot	N.A.	America	Borger	2012	[30]
		1/46	2%	mass array+ seq	AC	India	Kumari	2014	[68]
		1/4	25%	TS	AC	Korea	Yoo	2016	[37]
		1/24	4%	NGS	N.A.	America	Okamura	2021	[27]
		1/14	7%	TS	N.A.	China	Li	2017	[44]
		2/9	22%	PCR + seq	AC	Japan	Shibata	2008	[33]
		2/64	3%	Seq	AC	China	Rashid	2002	[49]
		2/29	7%	PCR	AC	America	Pai	2011	[62]
		2/60	3%	PCR + DS	AC	Taiwan	Huang	2017	[67]
		2/14	14%	NGS	N.A.	Japan	Noguchi	2017	[32]
		2/21	10%	SNaPshot	AC	America	Moy	2015	[53]
		2/12	17%	NGS	N.A.	China	Li	2020	[47]
		2/32	6%	targeted NGS	AC	Chile, Japan	Narayan	2019	[73]
		3/15	20%	PCR-RFLP + DS	N.A.	Korea	Kim	2001	[31]
		4/35	11%	DS	N.A.	Taiwan	Chang	2013	[25]
		4/34	12%	PCR	N.A.	Korea	Kim	2015	[60]
		4/68	6%	WES and Sanger seq	AC	Japan	Akita	2019	[48]
		6/144	4%	WES	AC	India, Korea, Chile	Pandey	2020	[46]
		4/58	7%	ultra-deep targeted NGS	AC	China	Lin	2019	[70]
		5/55	9%	NGS	N.A.	America	Javle	2016	[23]
		5/26	19%	NGS	N.A.	Italy	Simbolo	2014	[61]
		5/25	20%	targeted exome sequencing	N.A.	Korea	Chae	2019	[79]
		5/157	3%	WES	N.A.	China	Li	2019	[18]
		6/54	11%	NGS	AC	Greece	Papadopoulou	2018	[40]
		8/21	38%	PCR-RFLP	AC	India	Singh	2004	[63]
		8/34	24%	Seq	AC	India	Sharma	2017	[65]
		9/81	11%	PCR	N.A.	Japan	Tomioka	2019	[45]
		10/17	59%	PCR	N.A.	Japan	Nagai	2002	[24]
		10/20	50%	PCR + RFLP and DS	N.A.	Korea	Kim	2000	[50]
		12/25	48%	PCR-RFLP	AC	India	Shukla	2020	[34]
		16/39	41%	PCR-RFLP	N.A.	India	Kazmi	2013	[71]
		72/760	9%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
PIK3CA	10.0%	0/34	0%	PCR	N.A.	Korea	Kim	2015	[60]
		0/58	0%	ultra-deep targeted NGS	AC	China	Lin	2019	[70]
		1/23	4%	PCR+ seq	N.A.	Switzerland	Riener	2008	[72]
		1/68	1%	WES + Sanger seq	AC	Japan	Akita	2019	[48]

Table A2. *Cont.*

Gene	WA	N	Frequency	Methods	Histology	Population	Author	Year	Ref.
		1/14	7%	TS	N.A.	China	Li	2017	[44]
		1/25	4%	targeted exome sequencing	N.A.	Korea	Chae	2019	[79]
		2/46	4%	mass array + seq	AC	India	Kumari	2014	[68]
		2/4	50%	TS	AC	Korea	Yoo	2016	[37]
		2/21	10%	SNaPshot	AC	America	Moy	2015	[53]
		2/24	8%	NGS	N.A.	America	Okamura	2021	[27]
		2/26	8%	NGS	N.A	Italy	Simbolo	2014	[61]
		3/27	11%	Oncomap	AC	America	Deshpande	2011	[66]
		3/25	12%	SNaPshot	N.A.	America	Borger	2012	[30]
		3/14	21%	NGS	N.A.	Japan	Noguchi	2017	[32]
		3/21	14%	targeted NGS	AC	Chile, Japan	Narayan	2019	[73]
		11/144	8%	WES	AC	India, Korea, Chile	Pandey	2020	[46]
		2/12	17%	NGS	N.A.	China	Li	2020	[47]
		5/47	11%	WES	AC	China	Yang	2021	[56]
		6/157	4%	WES	N.A.	China	Li	2019	[18]
		7/55	13%	NGS	N.A.	America	Javle	2016	[23]
		7/34	21%	Seq	AC	India	Sharma	2017	[65]
		8/130	6%	TS	N.A.	China	Zhao	2016	[78]
		10/54	19%	NGS	AC	Greece	Papadopoulou	2018	[40]
		102/760	13%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
SHH	20.0%	10/50	20%	PCR + SSCP + seq	AC	India	Dixit	2017	[26]
SMAD4	13.1%	0/4	0%	TS	AC	Korea	Yoo	2016	[37]
		1/17	6%	WES	N.A.	India	Iyer	2019	[29]
		1/5	20%	WES	AC	Japan	Akita	2019	[48]
		2/12	17%	NGS	N.A.	China	Li	2020	[47]
		2/26	8%	NGS	N.A.	Italy	Simbolo	2014	[61]
		2/14	14%	NGS	N.A.	Japan	Noguchi	2017	[32]
		3/24	13%	NGS	N.A.	America	Okamura	2021	[27]
		11/144	8%	WES	AC	India, Korea, Chile	Pandey	2020	[46]
		3/11	27%	ultra-deep targeted NGS	N.A.	India	Yadav	2017	[77]
		4/25	16%	targeted exome sequencing	N.A.	Korea	Chae	2019	[79]
		10/32	33%	targeted NGS	AC	Chile, Japan	Narayan	2019	[73]
		9/55	16%	NGS	N.A.	America	Javle	2016	[23]
		16/58	28%	ultra-deep targeted NGS	AC	China	Lin	2019	[70]
		92/760	12%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
SRCAP	13.0%	7/54	13%	NGS	AC	Greece	Papadopoulou	2018	[40]
STK11	7.2%	0/68	0%	WES and Sanger seq	AC	Japan	Akita	2019	[48]
		1/24	4%	NGS	N.A.	America	Okamura	2021	[27]
		1/26	4%	NGS	N.A.	Italy	Simbolo	2014	[61]
		10/144	7%	WES	AC	India, Korea, Chile	Pandey	2020	[46]
		3/39	8%	WES/WGS	N.A.	Japan	Ebata	2021	[57]
		4/58	7%	ultra-deep targeted NGS	AC	China	Lin	2019	[70]
		64/760	8%	NGS	N.A.	America	Abdel-Wahab	2020	[19]
SYNE1	6.2%	3/54	6%	NGS	AC	Greece	Papadopoulou	2018	[40]
		1/11	9%	ultra-deep targetedNGS	N.A.	India	Yadav	2017	[77]
TP53	57.0%	1/21	5%	SNaPshot	AC	America	Moy	2015	[53]
		1/25	4%	SNaPshot	N.A.	America	Borger	2012	[30]

Table A2. *Cont.*

Gene	WA	N	Frequency	Methods	Histology	Population	Author	Year	Ref.
		2/4	50%	TS	AC	Korea	Yoo	2016	[37]
		2/11	18%	ultra-deep targeted NGS	N.A.	India	Yadav	2017	[77]
		3/5	60%	WES	AC	Japan	Akita	2019	[48]
		4/46	9%	mass array + seq	AC	India	Kumari	2014	[34]
		5/14	36%	PCR-SSCP + DS	N.A.	Korea	Kim	2001	[31]
		6/17	36%	PCR-SSCP + seq	N.A.	Japan	Nagai	2002	[24]
		6/17	36%	WES	N.A.	India	Iyer	2019	[29]
		7/14	50%	TS	N.A.	China	Li	2017	[44]
		7/29	24%	nested PCR, PCR-RFLP + DS	AC	Peru	Vidaurre	2019	[82]
		8/12	67%	NGS	N.A.	China	Li	2020	[47]
		9/14	64%	NGS	N.A.	Japan	Noguchi	2017	[32]
		17/40	43%	nested PCR +DS	AC	Japan, Hungary	Nagahashi	2008	[35]
		11/25	44%	PCR-SSCP	AC	India	Shukla	2020	[34]
		11/24	46%	NGS	N.A.	America	Okamura	2021	[27]
		12/26	46%	NGS	N.A.	Italy	Simbolo	2014	[61]
		1535	43%	PCR + seq	AC	Bolivia	Asai	2014	[42]
		16/25	64%	targeted exome sequencing	N.A.	Korea	Chae	2019	[79]
		22/32	69%	targeted NGS	AC	Chile, Japan	Narayan	2019	[73]
		18/59	31%	Seq	AC	Austria	Puhalla	2004	[69]
		72/144	50%	WES	AC	India, Korea, Chile	Pandey	2020	[46]
		19/39	49%	WES/WGS	N.A.	Japan	Ebata	2021	[57]
		20/30	67%	PCR + DS	AC	Chile	Moreno	2005	[81]
		23/54	43%	NGS	AC	Greece	Papadopoulou	2018	[40]
		32/55	58%	NGS	N.A.	America	Javle	2016	[23]
		42/58	72%	ultra-deep targeted NGS	AC	China	Lin	2019	[70]
		541/760	71%	NGS	N.A.	America	Abdel-Wahab	2020	[19]

WA: weighted average (calculated by using the number of samples analyzed in a study as the weight); N.A.: not available; AC: adenocarcinoma; NGS: next-generation sequencing; PCR: polymerase chain reaction; Seq: sequencing; DS: direct sequencing; WES: whole-exome sequencing; WGS: whole-genome sequencing; SSCP: single-strand conformation polymorphism; RFLP: restriction fragment length polymorphism; TS: targeted sequencing.

References

1. Stinton, L.M.; Shaffer, E.A. Epidemiology of gallbladder disease: Cholelithiasis and cancer. *Gut Liver* **2012**, *6*, 172–187. [CrossRef]
2. Lazcano-Ponce, E.C.; Miquel, J.F.; Munoz, N.; Herrero, R.; Ferrecio, C.; Wistuba, I.I.; Alonso de Ruiz, P.; Aristi Urista, G.; Nervi, F. Epidemiology and Molecular Pathology of Gallbladder Cancer. *CA Cancer J. Clin.* **2001**, *51*, 349–364. [CrossRef]
3. Are, C.; Ahmad, H.; Ravipati, A.; Croo, D.; Clarey, D.; Smith, L.; Price, R.R.; Butte, J.M.; Gupta, S.; Chaturvedi, A.; et al. Global epidemiological trends and variations in the burden of gallbladder cancer. *J. Surg. Oncol.* **2017**, *115*, 580–590. [CrossRef]
4. Larsson, S.C.; Wolk, A. Obesity and the risk of gallbladder cancer: A meta-analysis. *Br. J. Cancer* **2007**, *96*, 1457–1461. [CrossRef]
5. Sharma, A.; Sharma, K.L.; Gupta, A.; Yadav, A.; Kumar, A. Gallbladder cancer epidemiology, pathogenesis and molecular genetics: Recent update. *World J. Gastroenterol.* **2017**, *23*, 3978–3998. [CrossRef]
6. Campbell, P.T.; Newton, C.C.; Kitahara, C.M.; Patel, A.V.; Hartge, P.; Koshiol, J.; McGlynn, K.A.; Adami, H.O.; De Gonzalez, A.B.; Freeman, L.E.B.; et al. Body size indicators and risk of gallbladder cancer: Pooled analysis of individual-level data from 19 prospective cohort studies. *Cancer Epidemiol. Biomarkers Prev.* **2017**, *26*, 597–606. [CrossRef] [PubMed]
7. Aloia, T.A.; Járufe, N.; Javle, M.; Maithel, S.K.; Roa, J.C.; Adsay, V.; Coimbra, F.J.F.; Jarnagin, W.R. Gallbladder Cancer: Expert consensus statement. *HPB* **2015**, *17*, 681–690. [CrossRef] [PubMed]
8. Misra, S.; Chaturvedi, A.; Misra, N.C.; Sharma, I.D. Carcinoma of the gallbladder. *Lancet Oncol.* **2003**, *4*, 167–176. [CrossRef]
9. Duffy, A.; Capanu, M.; Abou-Alfa, G.K.; Huitzil, D.; Jarnagin, W.; Fong, Y.; D'Angelica, M.; Dematteo, R.P.; Blumgart, L.H.; O'Reilly, E.M. Gallbladder cancer (GBC): 10-Year experience at Memorial Sloan-Kettering Cancer Centre (MSKCC). *J. Surg. Oncol.* **2008**, *98*, 485–489. [CrossRef]

10. Lau, C.S.M.; Zywot, A.; Mahendraraj, K.; Chamberlain, R.S. Gallbladder Carcinoma in the United States: A Population Based Clinical Outcomes Study Involving 22,343 Patients from the Surveillance, Epidemiology, and End Result Database (1973–2013). *HPB Surg.* **2017**, *2017*, 1532835. [CrossRef]
11. Lohman, E.D.S.; De Bitter, T.; Verhoeven, R.; Van Der Geest, L.; Hagendoorn, J.; Mohammad, N.H.; Daams, F.; Klümpen, H.-J.; Van Gulik, T.; Erdmann, J.; et al. Trends in Treatment and Survival of Gallbladder Cancer in the Netherlands; Identifying Gaps and Opportunities from a Nation-Wide Cohort. *Cancers* **2020**, *12*, 918. [CrossRef] [PubMed]
12. Valle, J.; Wasan, H.; Palmer, D.H.; Cunningham, D.; Anthoney, A.; Maraveyas, A.; Madhusudan, S.; Iveson, T.; Hughes, S.; Pereira, S.P.; et al. Cisplatin plus Gemcitabine versus Gemcitabine for Biliary Tract Cancer. *N. Engl. J. Med.* **2010**, *362*, 1273–1281. [CrossRef] [PubMed]
13. Montalvo-Jave, E.E.; Rahnemai- Azar, A.A.; Papaconstantinou, D.; Deloiza, M.E.; Tsilimigras, D.I.; Moris, D.; Mendoza-Barrera, G.E.; Weber, S.M.; Pawlik, T.M. Molecular pathways and potential biomarkers in gallbladder cancer: A comprehensive review. *Surg. Oncol.* **2019**, *31*, 83–89. [CrossRef] [PubMed]
14. De Lorenzo, S.; Garajova, I.; Stefanini, B.; Tovoli, F. Targeted therapies for gallbladder cancer: An overview of agents in preclinical and clinical development. *Expert Opin. Investig. Drugs* **2021**, *30*, 759–772. [CrossRef] [PubMed]
15. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D.G. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *BMJ* **2009**, *339*, 332–336. [CrossRef]
16. Samuel, S.; Mukherjee, S.; Ammannagari, N.; Pokuri, V.K.; Kuvshinoff, B.; Groman, A.; LeVea, C.M.; Iyer, R. Clinicopathological characteristics and outcomes of rare histologic subtypes of gallbladder cancer over two decades: A population-based study. *PLoS ONE* **2018**, *13*, e0198809. [CrossRef]
17. Niu, C.; Wang, S.; Guan, Q.; Ren, X.; Ji, B.; Liu, Y. Neuroendocrine tumors of the gallbladder (Review). *Oncol. Lett.* **2020**, *19*, 3381–3388. [CrossRef]
18. Li, M.; Liu, F.; Zhang, F.; Zhou, W.; Jiang, X.; Yang, Y.; Qu, K.; Wang, Y.; Ma, Q.; Wang, T.; et al. Genomic ERBB2 / ERBB3 mutations promote PD-L1-mediated immune escape in gallbladder cancer: A whole-exome sequencing analysis. *Gut* **2019**, *68*, 1024–1033. [CrossRef]
19. Abdel-Wahab, R.; Yap, T.A.; Madison, R.; Pant, S.; Cooke, M.; Wang, K.; Zhao, H.; Bekaii-Saab, T.; Karatas, E.; Kwong, L.N.; et al. Genomic profiling reveals high frequency of DNA repair genetic aberrations in gallbladder cancer. *Sci. Rep.* **2020**, *10*, 22087. [CrossRef]
20. Roos, E.; Soer, E.C.; Klompmaker, S.; Meijer, L.L.; Besselink, M.G.; Giovannetti, E.; Heger, M.; Kazemier, G.; Klümpen, H.J.; Takkenberg, R.B.; et al. Crossing borders: A systematic review with quantitative analysis of genetic mutations of carcinomas of the biliary tract. *Crit. Rev. Oncol. Hematol.* **2019**, *140*, 8–16. [CrossRef]
21. Sohani, Z.N.; Sarma, S.; Alyass, A.; De Souza, R.J.; Robiou-Du-Pont, S.; Li, A.; Mayhew, A.; Yazdi, F.; Reddon, H.; Lamri, A.; et al. Empirical evaluation of the Q-Genie tool: A protocol for assessment of effectiveness. *BMJ Open* **2016**, *6*, e010403. [CrossRef]
22. Chakravarty, D.; Gao, J.; Phillips, S.M.; Kundra, R.; Zhang, H.; Wang, J.; Rudolph, J.E.; Yaeger, R.; Soumerai, T.; Nissan, M.H.; et al. OncoKB: A Precision Oncology Knowledge Base. *JCO Precis. Oncol.* **2017**, *1*, 1–16. [CrossRef]
23. Javle, M.; Bekaii-Saab, T.; Jain, A.; Wang, Y.; Kelley, R.K.; Wang, K.; Kang, H.C.; Catenacci, D.; Ali, S.; Krishnan, S.; et al. Biliary cancer: Utility of next-generation sequencing for clinical management. *Cancer* **2016**, *122*, 3838–3847. [CrossRef]
24. Nagai, M.; Watanabe, M.; Iwase, T.; Yamao, K.; Isaji, S. Clinical and genetic analysis of noncancerous and cancerous biliary epithelium in patients with pancreaticobiliary maljunction. *World J. Surg.* **2002**, *26*, 91–98. [CrossRef] [PubMed]
25. Chang, Y.T.; Chang, M.C.; Huang, K.W.; Tung, C.C.; Hsu, C.; Wong, J.M. Clinicopathological and prognostic significances of EGFR, KRAS and BRAF mutations in biliary tract carcinomas in Taiwan. *J. Gastroenterol. Hepatol.* **2014**, *29*, 1119–1125. [CrossRef] [PubMed]
26. Dixit, R.; Pandey, M.; Tripathi, S.K.; Dwivedi, A.N.D.; Shukla, V.K. Comparative Analysis of Mutational Profile of Sonic hedgehog Gene in Gallbladder Cancer. *Dig. Dis. Sci.* **2017**, *62*, 708–714. [CrossRef] [PubMed]
27. Okamura, R.; Kurzrock, R.; Mallory, R.J.; Fanta, P.T.; Burgoyne, A.M.; Clary, B.M.; Kato, S.; Sicklick, J.K. Comprehensive genomic landscape and precision therapeutic approach in biliary tract cancers. *Int. J. Cancer* **2021**, *148*, 702–712. [CrossRef] [PubMed]
28. Ali, A.; Mishra, P.K.; Sharma, S.; Arora, A.; Saluja, S.S. Effects of PTEN gene alteration in patients with gallbladder cancer. *Cancer Genet.* **2015**, *208*, 587–594. [CrossRef] [PubMed]
29. Iyer, P.; Shrikhande, S.V.; Ranjan, M.; Joshi, A.; Gardi, N.; Prasad, R.; Dharavath, B.; Thorat, R.; Salunkhe, S.; Sahoo, B.; et al. ERBB2 and KRAS alterations mediate response to EGFR inhibitors in early stage gallbladder cancer. *Int. J. Cancer* **2019**, *144*, 2008–2019. [CrossRef]
30. Borger, D.R.; Tanabe, K.K.; Fan, K.C.; Lopez, H.U.; Fantin, V.R.; Straley, K.S.; Schenkein, D.P.; Hezel, A.F.; Ancukiewicz, M.; Liebman, H.M.; et al. Frequent Mutation of Isocitrate Dehydrogenase (IDH)1 and IDH2 in Cholangiocarcinoma Identified Through Broad-Based Tumor Genotyping. *Oncologist* **2012**, *17*, 72–79. [CrossRef]
31. Kim, Y.T.; Kim, J.; Jang, Y.H.; Lee, W.J.; Ryu, J.K.; Park, Y.K.; Kim, S.W.; Kim, W.H.; Yoon, Y.B.; Kim, C.Y. Genetic alterations in gallbladder adenoma, dysplasia and carcinoma. *Cancer Lett.* **2001**, *169*, 59–68. [CrossRef]
32. Noguchi, R.; Yamaguchi, K.; Ikenoue, T.; Terakado, Y.; Ohta, Y.; Yamashita, N.; Kainuma, O.; Yokoi, S.; Maru, Y.; Nagase, H.; et al. Genetic alterations in Japanese extrahepatic biliary tract cancer. *Oncol. Lett.* **2017**, *14*, 877–884. [CrossRef] [PubMed]

33. Shibata, T.; Kokubu, A.; Gotoh, M.; Ojima, H.; Ohta, T.; Yamamoto, M.; Hirohashi, S. Genetic Alteration of Keap1 Confers Constitutive Nrf2 Activation and Resistance to Chemotherapy in Gallbladder Cancer. *Gastroenterology* **2008**, *135*, 1358–1368. [CrossRef] [PubMed]
34. Shukla, S.K.; Singh, G.; Shahi, K.S.; Pant, P. Genetic Changes of P 53 and Kras in Gallbladder Carcinoma in Kumaon Region of Uttarakhand. *J. Gastrointest. Cancer* **2020**, *51*, 552–559. [CrossRef]
35. Nagahashi, M.; Ajioka, Y.; Lang, I.; Szentirmay, Z.; Kasler, M.; Nakadaira, H.; Yokoyama, N.; Watanabe, G.; Nishikura, K.; Wakai, T.; et al. Genetic changes of p53, K-ras, and microsatellite instability in gallbladder carcinoma in high-incidence areas of Japan and Hungary. *World J. Gastroenterol.* **2008**, *14*, 70–75. [CrossRef]
36. Dixit, R.; Pandey, M.; Tripathi, S.K.; Dwivedi, A.N.D.; Shukla, V.K. Genetic mutational analysis of β-catenin gene affecting GSK-3β phosphorylation plays a role in gallbladder carcinogenesis: Results from a case control study. *Cancer Treat. Res. Commun.* **2020**, *23*, 100173. [CrossRef]
37. Yoo, K.H.; Kim, N.K.D.; Kwon, W.I.; Lee, C.; Kim, S.Y.; Jang, J.; Ahn, J.; Kang, M.; Jang, H.; Kim, S.T.; et al. Genomic alterations in biliary tract cancer using targeted sequencing. *Transl. Oncol.* **2016**, *9*, 173–178. [CrossRef]
38. Mondaca, S.; Razavi, P.; Xu, C.; Offin, M.; Myers, M.; Scaltriti, M.; Hechtman, J.F.; Bradley, M.; O'Reilly, E.M.; Berger, M.F.; et al. Genomic Characterization of ERBB2 -Driven Biliary Cancer and a Case of Response to Ado-Trastuzumab Emtansine. *JCO Precis. Oncol.* **2019**, *3*, 1–9. [CrossRef]
39. Patel, A.; Soneji, D.; Singh, H.P.; Kumar, M.; Bandyopadhyay, A.; Mathur, A.; Sharma, A.; Gahlot, G.P.S.; MS, S.; Guleria, B.; et al. Genomic Landscape and Targeted Treatment of Gallbladder Cancer: Results of a First Ongoing Prospective Study. *South Asian J. Cancer* **2020**, *9*, 074–079. [CrossRef]
40. Papadopoulou, K.; Murray, S.; Manousou, K.; Tikas, I.; Dervenis, C.; Sgouros, J.; Rontogianni, D.; Lakis, S.; Poulios, C.; Pervana, S.; et al. Genotyping and mRNA profiling reveal actionable targets in biliary tract cancers. *Ann. Oncol.* **2017**, *28*, v246. [CrossRef]
41. Maynard, H.; Stadler, Z.K.; Berger, M.F.; Solit, D.B.; Ly, M.; Lowery, M.A.; Mandelker, D.; Zhang, L.; Jordan, E.; El Dika, I.; et al. Germline alterations in patients with biliary tract cancers: A spectrum of significant and previously underappreciated findings. *Cancer* **2020**, *126*, 1995–2002. [CrossRef]
42. Asai, T.; Loza, E.; Roig, G.V.G.; Ajioka, Y.; Tsuchiya, Y.; Yamamoto, M.; Nakamura, K. High frequency of TP53 but not K-ras gene mutations in bolivian patients with gallbladder cancer. *Asian Pac. J. Cancer Prev.* **2014**, *15*, 5449–5454. [CrossRef]
43. Wistuba, I.I.; Maitra, A.; Carrasco, R.; Tang, M.; Troncoso, P.; Minna, J.D.; Gazdar, A.F. High resolution chromosome 3p, 8p, 9q and 22q allelotyping analysis in the pathogenesis of gallbladder carcinoma. *Br. J. Cancer* **2002**, *87*, 432–440. [CrossRef] [PubMed]
44. Li, M.; Chen, L.; Qu, Y.; Sui, F.; Yang, Q.; Ji, M.; Shi, B.; Chen, M.; Hou, P. Identification of MAP kinase pathways as therapeutic targets in gallbladder carcinoma using targeted parallel sequencing. *Oncotarget* **2017**, *8*, 36319–36330. [CrossRef] [PubMed]
45. Tomioka, Y.; Sung, Y.N.; Sawada, R.; Hong, S.M.; Akita, M.; Itoh, T.; Ajiki, T.; Fukumoto, T.; Zen, Y. IL-33 overexpression in gallbladder cancers associated with pancreatobiliary maljunction. *Histopathology* **2019**, *75*, 365–375. [CrossRef] [PubMed]
46. Pandey, A.; Stawiski, E.W.; Durinck, S.; Gowda, H.; Goldstein, L.D.; Barbhuiya, M.A.; Schröder, M.S.; Sreenivasamurthy, S.K.; Kim, S.W.; Phalke, S.; et al. Integrated genomic analysis reveals mutated ELF3 as a potential gallbladder cancer vaccine candidate. *Nat. Commun.* **2020**, *11*, 4225. [CrossRef] [PubMed]
47. Li, J.; Wei, Q.; Wu, X.; Sima, J.; Xu, Q.; Wu, M.; Wang, F.; Mou, H.; Hu, H.; Zhao, J.; et al. Integrative clinical and molecular analysis of advanced biliary tract cancers on immune checkpoint blockade reveals potential markers of response. *Clin. Transl. Med.* **2020**, *10*, e118. [CrossRef]
48. Akita, M.; Fujikura, K.; Ajiki, T.; Fukumoto, T.; Otani, K.; Hirose, T.; Tominaga, M.; Itoh, T.; Zen, Y. Intracholecystic Papillary Neoplasms Are Distinct from Papillary Gallbladder Cancers: A Clinicopathologic and Exome-sequencing Study. *Am. J. Surg. Pathol.* **2019**, *43*, 783–791. [CrossRef]
49. Rashid, A.; Ueki, T.; Gao, Y.T.; Houlihan, P.S.; Wallace, C.; Wang, B.S.; Shen, M.C.; Deng, J.; Hsing, A.W. K-ras mutation, p53 overexpression, and microsatellite instability in biliary tract cancers: A population-based study in China. *Clin. Cancer Res.* **2002**, *8*, 3156–3163.
50. Kim, S.W.; Her, K.H.; Jang, J.Y.; Kim, W.H.; Kim, Y.T.; Park, Y.H. K-ras oncogene mutation in cancer and precancerous lesions of the gallbladder. *J. Surg. Oncol.* **2000**, *75*, 246–251. [CrossRef]
51. Hirosawa, T.; Ishida, M.; Ishii, K.; Kanehara, K.; Kudo, K.; Ohnuma, S.; Kamei, T.; Motoi, F.; Naitoh, T.; Selaru, F.M.; et al. Loss of BAP1 expression is associated with genetic mutation and can predict outcomes in gallbladder cancer. *PLoS ONE* **2018**, *13*, e0206643. [CrossRef]
52. Goeppert, B.; Roessler, S.; Renner, M.; Loeffler, M.; Singer, S.; Rausch, M.; Albrecht, T.; Mehrabi, A.; Vogel, M.N.; Pathil, A.; et al. Low frequency of mismatch repair deficiency in gallbladder cancer. *Diagn. Pathol.* **2019**, *14*, 36. [CrossRef]
53. Moy, A.P.; Shahid, M.; Ferrone, C.R.; Borger, D.R.; Zhu, A.X.; Ting, D.; Deshpande, V. Microsatellite instability in gallbladder carcinoma. *Virchows Arch.* **2015**, *466*, 393–402. [CrossRef]
54. Yoshida, T.; Sugai, T.; Habano, W.; Nakamura, S.I.; Uesugi, N.; Funato, O.; Saito, K. Microsatellite instability in gallbladder carcinoma: Two independent genetic pathways of gallbladder carcinogenesis. *J. Gastroenterol.* **2000**, *35*, 768–774. [CrossRef]
55. Roa, J.C.; Roa, I.; Correa, P.; Vo, Q.; Araya, J.C.; Villaseca, M.; Guzmán, P.; Schneider, B.G. Microsatellite instability in preneoplastic and neoplastic lesions of the gallbladder. *J. Gastroenterol.* **2005**, *40*, 79–86. [CrossRef]
56. Yang, D.; Chen, T.; Zhan, M.; Xu, S.; Yin, X.; Liu, Q.; Chen, W.; Zhang, Y.; Liu, D.; Yan, J.; et al. Modulation of mTOR and epigenetic pathways as therapeutics in gallbladder cancer. *Mol. Ther. Oncolytics* **2021**, *20*, 59–70. [CrossRef]

57. Ebata, N.; Fujita, M.; Sasagawa, S.; Maejima, K.; Okawa, Y.; Hatanaka, Y.; Mitsuhashi, T.; Oosawa-tatsuguchi, A.; Tanaka, H.; Miyano, S.; et al. Molecular classification and tumor microenvironment characterization of gallbladder cancer by comprehensive genomic and transcriptomic analysis. *Cancers* **2021**, *13*, 733. [CrossRef] [PubMed]
58. Spizzo, G.; Puccini, A.; Xiu, J.; Goldberg, R.M.; Grothey, A.; Shields, A.F.; Arora, S.P.; Khushmann, M.; Salem, M.E.; Battaglin, F.; et al. Molecular profile of BRCA-mutated biliary tract cancers. *ESMO Open* **2020**, *5*, e000682. [CrossRef] [PubMed]
59. Weinberg, B.A.; Xiu, J.; Lindberg, M.R.; Shields, A.F.; Hwang, J.J.; Poorman, K.; Salem, M.E.; Pishvaian, M.J.; Holcombe, R.F.; Marshall, J.L.; et al. Molecular profiling of biliary cancers reveals distinct molecular alterations and potential therapeutic targets. *J. Gastrointest. Oncol.* **2019**, *10*, 652–662. [CrossRef] [PubMed]
60. Kim, S.T.; Jang, K.T.; Lee, J.; Jang, H.M.; Choi, H.J.; Jang, H.L.; Park, S.H.; Park, Y.S.; Lim, H.Y.; Kang, W.K.; et al. Molecular subgroup analysis of clinical outcomes in a phase 3 study of gemcitabine and oxaliplatin with or without erlotinib in advanced biliary tract cancer. *Transl. Oncol.* **2015**, *8*, 40–46. [CrossRef] [PubMed]
61. Simbolo, M.; Fassan, M.; Ruzzenente, A.; Mafficini, A.; Wood, L.D.; Corbo, V.; Melisi, D.; Malleo, G.; Vicentini, C.; Malpeli, G.; et al. Multigene mutational profiling of cholangiocarcinomas identifies actionable molecular subgroups. *Oncotarget* **2014**, *5*, 2839–2852. [CrossRef] [PubMed]
62. Pai, R.K.; Pai, R.K.; Mojtahed, K. Mutations in the RAS/RAF/MAP kinase pathway commonly occur in gallbladder adenomas but are uncommon in gallbladder adenocarcinomas. *Appl. Immunohistochem. Mol. Morphol.* **2011**, *19*, 133–140. [CrossRef] [PubMed]
63. Singh, M.K.; Chetri, K.; Pandey, U.B.; Kapoor, V.K.; Mittal, B.; Choudhuri, G. Mutational spectrum of K-ras oncogene among Indian patients with gallbladder cancer. *J. Gastroenterol. Hepatol.* **2004**, *19*, 916–921. [CrossRef] [PubMed]
64. Saetta, A.A.; Papanastasiou, P.; Michalopoulos, N.V.; Gigelou, F.; Korkolopoulou, P.; Bei, T.; Patsouris, E. Mutational analysis of BRAF in gallbladder carcinomas in association with K-ras and p53 mutations and microsatellite instability. *Virchows Arch.* **2004**, *445*, 179–182. [CrossRef] [PubMed]
65. Sharma, A.; Kumar, A.; Kumari, N.; Krishnani, N.; Rastogi, N. Mutational frequency of KRAS, NRAS, IDH2, PIK3CA, and EGFR in North Indian gallbladder cancer patients. *Ecancermedicalscience* **2017**, *11*, 757. [CrossRef]
66. Deshpande, V.; Nduaguba, A.; Zimmerman, S.M.; Kehoe, S.M.; MacConaill, L.E.; Lauwers, G.Y.; Ferrone, C.; Bardeesy, N.; Zhu, A.X.; Hezel, A.F. Mutational profiling reveals PIK3CA mutations in gallbladder carcinoma. *BMC Cancer* **2011**, *11*, 60. [CrossRef]
67. Huang, W.C.; Tsai, C.C.; Chan, C.C. Mutation analysis and copy number changes of KRAS and BRAF genes in Taiwanese cases of biliary tract cholangiocarcinoma. *J. Formos. Med. Assoc.* **2017**, *116*, 464–468. [CrossRef]
68. Kumari, N.; Corless, C.L.; Warrick, A.; Beadling, C.; Nelson, D.; Neff, T.; Krishnani, N.; Kapoor, V.K. Mutation profiling in gallbladder cancer in Indian population. *Indian J. Pathol. Microbiol.* **2014**, *57*, 9–12. [CrossRef]
69. Puhalla, H.; Kandioler, D.; Ludwig, C.; Filipits, M.; Wrba, F.; Laengle, F.; Jakesz, R.; Gruenberger, T. p53 Analysis in Gallbladder Cancer: Comparison of Gene Analysis Versus Immunohistochemistry. *Anticancer Res.* **2004**, *24*, 1201–1206.
70. Lin, J.; Dong, K.; Bai, Y.; Zhao, S.; Dong, Y.; Shi, J.; Shi, W.; Long, J.; Yang, X.; Wang, D.; et al. Precision oncology for gallbladder cancer: Insights from genetic alterations and clinical practice. *Ann. Transl. Med.* **2019**, *7*, 467. [CrossRef]
71. Kazmi, H.R.; Chandra, A.; Nigam, J.; Noushif, M.; Parmar, D.; Gupta, V. Prognostic significance of k-ras Codon 12 mutation in patients with Resected gallbladder cancer. *Dig. Surg.* **2013**, *30*, 233–239. [CrossRef] [PubMed]
72. Riener, M.O.; Bawohl, M.; Clavien, P.A.; Jochum, W. Rare PIK3CA hotspot mutations in carcinomas of the biliary tract. *Genes Chromosom. Cancer* **2008**, *47*, 363–367. [CrossRef] [PubMed]
73. Narayan, R.R.; Creasy, J.M.; Goldman, D.A.; Gönen, M.; Kandoth, C.; Kundra, R.; Solit, D.B.; Askan, G.; Klimstra, D.S.; Basturk, O.; et al. Regional differences in gallbladder cancer pathogenesis: Insights from a multi-institutional comparison of tumor mutations. *Cancer* **2019**, *125*, 575–585. [CrossRef] [PubMed]
74. Peraldo Neia, C.; Cavalloni, G.; Balsamo, A.; Venesio, T.; Napoli, F.; Sassi, F.; Martin, V.; Frattini, M.; Aglietta, M.; Leone, F. Screening for the FIG-ROS1 fusion in biliary tract carcinomas by nested PCR. *Genes Chromosom. Cancer* **2014**, *53*, 1033–1040. [CrossRef]
75. Leone, F.; Cavalloni, G.; Pignochino, Y.; Sarotto, I.; Ferraris, R.; Piacibello, W.; Venesio, T.; Capussotti, L.; Risio, M.; Aglietta, M. Somatic mutations of epidermal growth factor receptor in bile duct and gallbladder carcinoma. *Clin. Cancer Res.* **2006**, *12*, 1680–1685. [CrossRef]
76. Roa, I.; Garcia, H.; Game, A.; De Toro, G.; De Aretxabala, X.; Javle, M. Somatic Mutations of PI3K in Early and Advanced Gallbladder Cancer: Additional Options for an Orphan Cancer. *J. Mol. Diagn.* **2016**, *18*, 388–394. [CrossRef]
77. Yadav, S.; DE Sarkar, N.; Kumari, N.; Krishnani, N.; Kumar, A.; Mittal, B. Targeted Gene Sequencing of Gallbladder Carcinoma Identifies High-impact Somatic and Rare Germline Mutations. *Cancer Genom. Proteom.* **2017**, *14*, 495–506. [CrossRef]
78. Zhao, S.; Cao, Y.; Liu, S.B.; Wang, X.A.; Bao, R.F.; Shu, Y.J.; Hu, Y.P.; Zhang, Y.J.; Jiang, L.; Zhang, F.; et al. The E545K mutation of PIK3CA promotes gallbladder carcinoma progression through enhanced binding to EGFR. *J. Exp. Clin. Cancer Res.* **2016**, *35*. [CrossRef]
79. Chae, H.; Kim, D.; Yoo, C.; Kim, K.-P.; Jeong, J.H.; Chang, H.-M.; Lee, S.S.; Park, D.H.; Song, T.J.; Hwang, S.; et al. Therapeutic relevance of targeted sequencing in management of patients with advanced biliary tract cancer: DNA damage repair gene mutations as a predictive biomarker. *Eur. J. Cancer* **2019**, *120*, 31–39. [CrossRef]
80. Goldenberg, D.; Rosenbaum, E.; Argani, P.; Wistuba, I.I.; Sidransky, D.; Thuluvath, P.J.; Hidalgo, M.; Califano, J.; Maitra, A. The V599E BRAF mutation is uncommon in biliary tract cancers. *Mod. Pathol.* **2004**, *17*, 1386–1391. [CrossRef]

81. Moreno, M.; Pimentel, F.; Gazdar, A.F.; Wistuba, I.I.; Miquel, J.F. TP53 abnormalities are frequent and early events in the sequential pathogenesis of gallbladder carcinoma. *Ann. Hepatol. Off. J. Mex. Assoc. Hepatol.* **2005**, *4*, 192–199. [CrossRef]
82. Vidaurre, T.; Casavilca, S.; Montenegro, P.; Gomez, H.; Calderón, M.; Navarro, J.; Aramburu, J.; Poquioma, E.; Tsuchiya, Y.; Asai, T.; et al. Tumor protein p53 and K-ras gene mutations in Peruvian patients with gallbladder cancer. *Asian Pac. J. Cancer Prev.* **2019**, *20*, 289–294. [CrossRef]
83. Tadokoro, H.; Shigihara, T.; Ikeda, T.; Takase, M.; Suyama, M. Two distinct pathways of p16 gene inactivation in gallbladder cancer. *World J. Gastroenterol.* **2007**, *13*, 6396–6403. [CrossRef]
84. de Bono, J.; Mateo, J.; Fizazi, K.; Saad, F.; Shore, N.; Sandhu, S.; Chi, K.N.; Sartor, O.; Agarwal, N.; Olmos, D.; et al. Olaparib for Metastatic Castration-Resistant Prostate Cancer. *N. Engl. J. Med.* **2020**, *382*, 2091–2102. [CrossRef] [PubMed]
85. Janjigian, Y.Y.; Maron, S.B.; Chatila, W.K.; Millang, B.; Chavan, S.S.; Alterman, C.; Chou, J.F.; Segal, M.F.; Simmons, M.Z.; Momtaz, P.; et al. First-line pembrolizumab and trastuzumab in HER2-positive oesophageal, gastric, or gastro-oesophageal junction cancer: An open-label, single-arm, phase 2 trial. *Lancet Oncol.* **2020**, *21*, 821–831. [CrossRef]
86. Bang, Y.J.; Van Cutsem, E.; Feyereislova, A.; Chung, H.C.; Shen, L.; Sawaki, A.; Lordick, F.; Ohtsu, A.; Omuro, Y.; Satoh, T.; et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): A phase 3, open-label, randomised controlled trial. *Lancet* **2010**, *376*, 687–697. [CrossRef]
87. Shitara, K.; Bang, Y.-J.; Iwasa, S.; Sugimoto, N.; Ryu, M.-H.; Sakai, D.; Chung, H.-C.; Kawakami, H.; Yabusaki, H.; Lee, J.; et al. Trastuzumab Deruxtecan in Previously Treated HER2-Positive Gastric Cancer. *N. Engl. J. Med.* **2020**, *382*, 2419–2430. [CrossRef]
88. Verma, S.; Miles, D.; Gianni, L.; Krop, I.E.; Welslau, M.; Baselga, J.; Pegram, M.; Oh, D.-Y.; Diéras, V.; Guardino, E.; et al. Trastuzumab Emtansine for HER2-Positive Advanced Breast Cancer. *N. Engl. J. Med.* **2012**, *367*, 1783–1791. [CrossRef]
89. Johnston, S.; Pippen, J.; Pivot, X.; Lichinitser, M.; Sadeghi, S.; Dieras, V.; Gomez, H.L.; Romieu, G.; Manikhas, A.; Kennedy, M.J.; et al. Lapatinib combined with letrozole versus letrozole and placebo as first-line therapy for postmenopausal hormone receptor-Positive metastatic breast cancer. *J. Clin. Oncol.* **2009**, *27*, 5538–5546. [CrossRef] [PubMed]
90. Geyer, C.E.; Forster, J.; Lindquist, D.; Chan, S.; Romieu, C.G.; Pienkowski, T.; Jagiello-Gruszfeld, A.; Crown, J.; Chan, A.; Kaufman, B.; et al. Lapatinib plus Capecitabine for HER2-Positive Advanced Breast Cancer. *N. Engl. J. Med.* **2006**, *355*, 2733–2743. [CrossRef]
91. Rugo, H.S.; Im, S.A.; Cardoso, F.; Cortés, J.; Curigliano, G.; Musolino, A.; Pegram, M.D.; Wright, G.S.; Saura, C.; Escrivá-De-Romaní, S.; et al. Efficacy of Margetuximab vs Trastuzumab in Patients with Pretreated ERBB2-Positive Advanced Breast Cancer: A Phase 3 Randomized Clinical Trial. *JAMA Oncol.* **2021**, *7*, 573–584. [CrossRef] [PubMed]
92. Martin, M.; Bonneterre, J.; Geyer, C.E.; Ito, Y.; Ro, J.; Lang, I.; Kim, S.B.; Germa, C.; Vermette, J.; Wang, K.; et al. A phase two randomised trial of neratinib monotherapy versus lapatinib plus capecitabine combination therapy in patients with HER2+ advanced breast cancer. *Eur. J. Cancer* **2013**, *49*, 3763–3772. [CrossRef]
93. Vogel, C.L.; Cobleigh, M.A.; Tripathy, D.; Gutheil, J.C.; Harris, L.N.; Fehrenbacher, L.; Slamon, D.J.; Murphy, M.; Novotny, W.F.; Burchmore, M.; et al. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J. Clin. Oncol.* **2002**, *20*, 719–726. [CrossRef] [PubMed]
94. Baselga, J.; Cortés, J.; Kim, S.-B.; Im, S.-A.; Hegg, R.; Im, Y.-H.; Roman, L.; Pedrini, J.L.; Pienkowski, T.; Knott, A.; et al. Pertuzumab plus Trastuzumab plus Docetaxel for Metastatic Breast Cancer. *N. Engl. J. Med.* **2012**, *366*, 109–119. [CrossRef]
95. Murthy, R.K.; Loi, S.; Okines, A.; Paplomata, E.; Hamilton, E.; Hurvitz, S.A.; Lin, N.U.; Borges, V.; Abramson, V.; Anders, C.; et al. Tucatinib, Trastuzumab, and Capecitabine for HER2-Positive Metastatic Breast Cancer. *N. Engl. J. Med.* **2020**, *382*, 597–609. [CrossRef] [PubMed]
96. Modi, S.; Saura, C.; Yamashita, T.; Park, Y.H.; Kim, S.-B.; Tamura, K.; Andre, F.; Iwata, H.; Ito, Y.; Tsurutani, J.; et al. Trastuzumab Deruxtecan in Previously Treated HER2-Positive Breast Cancer. *N. Engl. J. Med.* **2020**, *382*, 610–621. [CrossRef] [PubMed]
97. Tosi, F.; Sartore-Bianchi, A.; Lonardi, S.; Amatu, A.; Leone, F.; Ghezzi, S.; Martino, C.; Bencardino, K.; Bonazzina, E.; Bergamo, F.; et al. Long-term Clinical Outcome of Trastuzumab and Lapatinib for HER2-positive Metastatic Colorectal Cancer. *Clin. Colorectal Cancer* **2020**, *19*, 256–262.e2. [CrossRef] [PubMed]
98. Meric-Bernstam, F.; Hurwitz, H.; Raghav, K.P.S.; McWilliams, R.R.; Fakih, M.; VanderWalde, A.; Swanton, C.; Kurzrock, R.; Burris, H.; Sweeney, C.; et al. Pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer (MyPathway): An updated report from a multicentre, open-label, phase 2a, multiple basket study. *Lancet Oncol.* **2019**, *20*, 518–530. [CrossRef]
99. Siena, S.; Di Bartolomeo, M.; Raghav, K.; Masuishi, T.; Loupakis, F.; Kawakami, H.; Yamaguchi, K.; Nishina, T.; Fakih, M.; Elez, E.; et al. Trastuzumab deruxtecan (DS-8201) in patients with HER2-expressing metastatic colorectal cancer (DESTINY-CRC01): A multicentre, open-label, phase 2 trial. *Lancet Oncol.* **2021**, *22*, 779–789. [CrossRef]
100. Fader, A.N.; Roque, D.M.; Siegel, E.; Buza, N.; Hui, P.; Abdelghany, O.; Chambers, S.K.; Secord, A.A.; Havrilesky, L.; O'Malley, D.M.; et al. Randomized Phase II trial of carboplatin-paclitaxel versus carboplatin-paclitaxel-trastuzumab in uterine serous carcinomas that overexpress human epidermal growth factor receptor 2/neu. *J. Clin. Oncol.* **2018**, *36*, 2044–2051. [CrossRef] [PubMed]
101. Li, B.T.; Shen, R.; Buonocore, D.; Olah, Z.T.; Ni, A.; Ginsberg, M.S.; Ulaner, G.A.; Offin, M.; Feldman, D.; Hembrough, T.; et al. Ado-trastuzumab emtansine for patients with HER2-mutant lung cancers: Results from a phase II basket trial. *J. Clin. Oncol.* **2018**, *36*, 2532–2537. [CrossRef] [PubMed]
102. Li, B.T.; Smit, E.F.; Goto, Y.; Nakagawa, K.; Udagawa, H. Trastuzumab Deruxtecan in HER2-Mutant Non–Small-Cell Lung Cancer. *N. Engl. J. Med.* **2021**. [CrossRef] [PubMed]

103. Rugo, H.S.; Lerebours, F.; Ciruelos, E.; Drullinsky, P.; Ruiz-Borrego, M.; Neven, P.; Park, Y.H.; Prat, A.; Bachelot, T.; Juric, D.; et al. Alpelisib plus fulvestrant in PIK3CA-mutated, hormone receptor-positive advanced breast cancer after a CDK4/6 inhibitor (BYLieve): One cohort of a phase 2, multicentre, open-label, non-comparative study. *Lancet Oncol.* **2021**, *22*, 489–498. [CrossRef]
104. Marabelle, A.; Fakih, M.; Lopez, J.; Shah, M.; Shapira-Frommer, R.; Nakagawa, K.; Chung, H.C.; Kindler, H.L.; Lopez-Martin, J.A.; Miller, W.H.; et al. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: Prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. *Lancet Oncol.* **2020**, *21*, 1353–1365. [CrossRef]
105. Marcus, L.; Lemery, S.J.; Keegan, P.; Pazdur, R. FDA approval summary: Pembrolizumab for the treatment of microsatellite instability-high solid tumors. *Clin. Cancer Res.* **2019**, *25*, 3753–3758. [CrossRef] [PubMed]
106. Overman, M.J.; McDermott, R.; Leach, J.L.; Lonardi, S.; Lenz, H.J.; Morse, M.A.; Desai, J.; Hill, A.; Axelson, M.; Moss, R.A.; et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): An open-label, multicentre, phase 2 study. *Lancet Oncol.* **2017**, *18*, 1182–1191. [CrossRef]
107. Overman, M.J.; Lonardi, S.; Wong, K.Y.M.; Lenz, H.J.; Gelsomino, F.; Aglietta, M.; Morse, M.A.; Van Cutsem, E.; McDermott, R.; Hill, A.; et al. Durable clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair-deficient/microsatellite instability-high metastatic colorectal cancer. *J. Clin. Oncol.* **2018**, *36*, 773–779. [CrossRef]
108. Goodman, A.M.; Kato, S.; Bazhenova, L.; Patel, S.P.; Frampton, G.M.; Miller, V.; Stephens, P.J.; Daniels, G.A.; Kurzrock, R. Tumor mutational burden as an independent predictor of response to immunotherapy in diverse cancers. *Mol. Cancer Ther.* **2017**, *16*, 2598–2608. [CrossRef]
109. Yarchoan, M.; Hopkins, A.; Jaffee, E.M. Tumor Mutational Burden and Response Rate to PD-1 Inhibition. *N. Engl. J. Med.* **2017**, *377*, 2500–2501. [CrossRef]
110. Ott, P.A.; Bang, Y.J.; Piha-Paul, S.A.; Abdul Razak, A.R.; Bennouna, J.; Soria, J.C.; Rugo, H.S.; Cohen, R.B.; O'Neil, B.H.; Mehnert, J.M.; et al. T-cell–inflamed gene-expression profile, programmed death ligand 1 expression, and tumor mutational burden predict efficacy in patients treated with pembrolizumab across 20 cancers: KEYNOTE-028. *J. Clin. Oncol.* **2019**, *37*, 318–327. [CrossRef]
111. Marabelle, A.; Le, D.T.; Ascierto, P.A.; Di Giacomo, A.M.; de Jesus-Acosta, A.; Delord, J.P.; Geva, R.; Gottfried, M.; Penel, N.; Hansen, A.R.; et al. Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/ mismatch repair–deficient cancer: Results from the phase II KEYNOTE-158 study. *J. Clin. Oncol.* **2020**, *38*, 1–10. [CrossRef] [PubMed]
112. Singh, R.R. Next-Generation Sequencing in High-Sensitive Detection of Mutations in Tumors: Challenges, Advances, and Applications. *J. Mol. Diagn.* **2020**, *22*, 994–1007. [CrossRef] [PubMed]
113. Ma, X.; Shao, Y.; Tian, L.; Flasch, D.A.; Mulder, H.L.; Edmonson, M.N.; Liu, Y.; Chen, X.; Newman, S.; Nakitandwe, J.; et al. Analysis of error profiles in deep next-generation sequencing data. *Genome Biol.* **2019**, *20*. [CrossRef] [PubMed]
114. Berger, M.F.; Mardis, E.R. The emerging clinical relevance of genomics in cancer medicine. *Nat. Rev. Clin. Oncol.* **2018**, *15*, 353–365. [CrossRef]
115. Boland, C.R.; Thibodeau, S.N.; Hamilton, S.R.; Sidransky, D.; Eshleman, J.R.; Burt, R.W.; Meltzer, S.J.; Rodriguez-Bigas, M.A.; Fodde, R.; Ranzani, G.N.; et al. A National Cancer Institute workshop on microsatellite instability for cancer detection and familial predisposition: Development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res.* **1998**, *58*, 5248–5257.
116. Kato, S.; Goodman, A.; Walavalkar, V.; Barkauskas, D.A.; Sharabi, A.; Kurzrock, R. Hyperprogressors after immunotherapy: Analysis of genomic alterations associated with accelerated growth rate. *Clin. Cancer Res.* **2017**, *23*, 4242–4250. [CrossRef] [PubMed]