Clinical and Genomic Characterization of *ERBB2*-Altered Gallbladder Cancer: Exploring Differences Between an American and a Chilean Cohort

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ABSTRACT

PURPOSE Gallbladder cancer (GBC) is a biliary tract malignancy characterized by its high lethality. Although the incidence of GBC is low in most countries, specific areas such as Chile display a high incidence. Our collaborative study sought to compare clinical and molecular features of GBC cohorts from Chile and the United States with a focus on *ERBB2* alterations.

METHODS Patients were accrued at Memorial Sloan Kettering Cancer Center (MSK) or the Pontificia Universidad Católica de Chile (PUC). Clinical information was retrieved from medical records. Genomic analysis was performed by the next-generation sequencing platform MSK-Integrated Mutation Profiling of Actionable Cancer Targets.

RESULTS A total of 260 patients with GBC were included, 237 from MSK and 23 from PUC. There were no significant differences in the clinical characteristics between the patients identified at MSK and at PUC except in terms of lithiasis prevalence which was significantly higher in the PUC cohort (85% *v* 44%; $P = .0003$). The prevalence of *ERBB2* alterations was comparable between the two cohorts (15% *v* 9%; $P = .42$). Overall, *ERBB2* alterations were present in 14% of patients (8% with *ERBB2* amplification, 4% *ERBB2* mutation, 1.5% concurrent amplification and mutation, and 0.4% *ERBB2* fusion). Notably, patients with GBC that harbored *ERBB2* alterations had better overall survival (OS) versus their *ERBB2*-wild type counterparts (22.3 months *v* 11.8 months; $P = .024$).

CONCLUSION The prevalence of lithiasis seems to be higher in Chilean versus US patients with GBC. A similar prevalence of *ERBB2* alterations of overall 14% and better OS suggests that a proportion of them could benefit from human epidermal growth factor receptor type 2–targeted therapies. The smaller cohort of Chile, where the disease prevalence is higher, is a reminder and invitation for the need of more robust next-generation sequencing analyses globally.

ACCOMPANYING CONTENT

Appendix

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INTRODUCTION

Gallbladder cancer (GBC) is a highly lethal disease and the most common biliary tract malignancy. High mortality rates can be attributed to several factors, including advanced stage at diagnosis and the limited efficacy of treatments in advanced-stage patients.[1,2] Although GBC is considered a rare disease in most countries, its incidence displays a remarkable geographical variability. For example, GBC incidence in the United States is about 1.13 cases per 100,000. In sharp contrast, incidence rates in southern Chile or northern India can reach up to 27 cases or 21.5 cases per 100,000, respectively.[3,4]

Therapeutic options for patients with GBC are very limited. However, the search for therapeutic targets during the past decade have made significant progress in characterizing the molecular landscape of biliary tract cancers (BTCs), including GBC. Indeed, studies have reported that alterations in the *ERBB2* gene (that encodes human epidermal growth factor receptor type 2 [HER2]) are prevalent among patients with GBC[4,5] and therefore have postulated HER2 as an actionable target. Previous studies in breast and gastric cancers have demonstrated the efficacy of anti–HER2-targeted therapies in patients that harbor *ERBB2*-amplified tumors.[6-8] Preliminary evidence in patients with *ERBB2*-amplified BTC shows encouraging activity of the combination of the anti-HER2

CONTEXT

Key Objective

Are there differences in the prevalence of *ERBB2* alterations and other relevant features between American and Chilean patients with gallbladder cancer?

Knowledge Generated

The prevalence of *ERBB2* alterations was comparable between the two cohorts (15% *v* 9%), and the prevalence of other potentially targetable alterations was very low. Lithiasis significantly was more frequent in the Chilean cohort.

Relevance

Our findings show similar genomic features in these two different populations and underscore the relevance of *ERBB2* as a prevalent and universal targetable alteration in gallbladder cancer. Lithiasis was very frequent in Chilean gallbladder patients which as a high-risk area reinforce the need to treat this risk factor proactively.

antibodies trastuzumab and pertuzumab,[9] the anti-HER2 antibody-drug conjugate trastuzumab deruxtecan, and the bispecific anti-HER2 antibody zanidatamab.[10-12] Moreover, a recent report derived from the phase II SGNTUC-019 basket trial included 30 patients with HER2-positive metastatic BTC and demonstrated good tolerability and clinically significant antitumor activity using the combination of trastuzumab and tucatinib.[13]

TABLE 1. Baseline Characteristics of Patients With GBC by Site of Origin

Variable	Site of Origin (cohort)		*P*
	MSK (n = 237)	PUC (n = 23)	
Age, years, median (range)	65.2	66.1	.36
Sex, No. (%)			
Male	73 (31)	8 (35)	.69
Female	164 (69)	15 (65)	
Stage at diagnosis, No. (%)			
I	12 (5)	3 (13)	.29
II	52 (23)	3 (13)	
III	43 (19)	6 (26)	
IV	121 (53)	11 (48)	
Unknown	9 (4)	0	
Race, No. (%)			
White	158 (67)	22 (96)	NA
Asian	27 (11)	0	
Black	26 (11)	0	
Other	8 (3)	1 (4)	
Unknown	18 (8)	0	
Lithiasis,[a] No. (%)			
Yes	73 (44)	18 (85)	.0003
No	94 (56)	3 (15)	

Abbreviations: GBC, gallbladder cancer; MSK, Memorial Sloan Kettering Cancer Center; NA, not applicable; PUC, Pontificia Universidad Católica de Chile.

[a]Twenty-seven percent of missing data.

Although studies have described several recurrent somatic mutations in the extracellular, transmembrane, and kinase domains of *ERBB2*,[14] the frequency of these alterations rarely exceeds 10% in any single tumor, which makes the assessment of their clinical significance difficult. Recently, the SUMMIT trial evaluated the efficacy of neratinib (a pan-HER tyrosine kinase inhibitor) in multiple tumor types harboring *ERBB2* mutations. This study included a small subset of 25 patients with *ERBB2*-mutant BTC. Neratinib in these patients was associated with a response rate of 16% and a progression-free survival of 2.8 months.[15,16]

As pointed earlier, GBC incidence is characterized by its geographical variability. Similarly, actionable targets such as BRCA1/2 or ARID1A are more prevalent in specific geographical areas or populations.[17] Herein, we aimed to comprehensively characterize the clinical and molecular features of *ERBB2*-driven GBC and to explore the biologic differences between an American (US) and a Chilean cohort. This work is part of a collaborative tumor profiling initiative between the Memorial Sloan Kettering Cancer Center (MSK) and the Pontificia Universidad Católica de Chile (PUC).

METHODS

Patients and Data Collection

Patients with GBC were evaluated at MSK or PUC for genomic tumor profiling between 2014 and 2021. At MSK, patients were consented for prospective tumor genomic profiling using the MSK-Integrated Mutation Profiling of Actionable Cancer Targets (IMPACT) assay (ClinicalTrials.gov identifier: NCT01775072). At PUC, patients were consented via the institutional biobank protocol. Both protocols allowed the collection of clinical data and were approved by their respective institutional review boards. Clinical data were

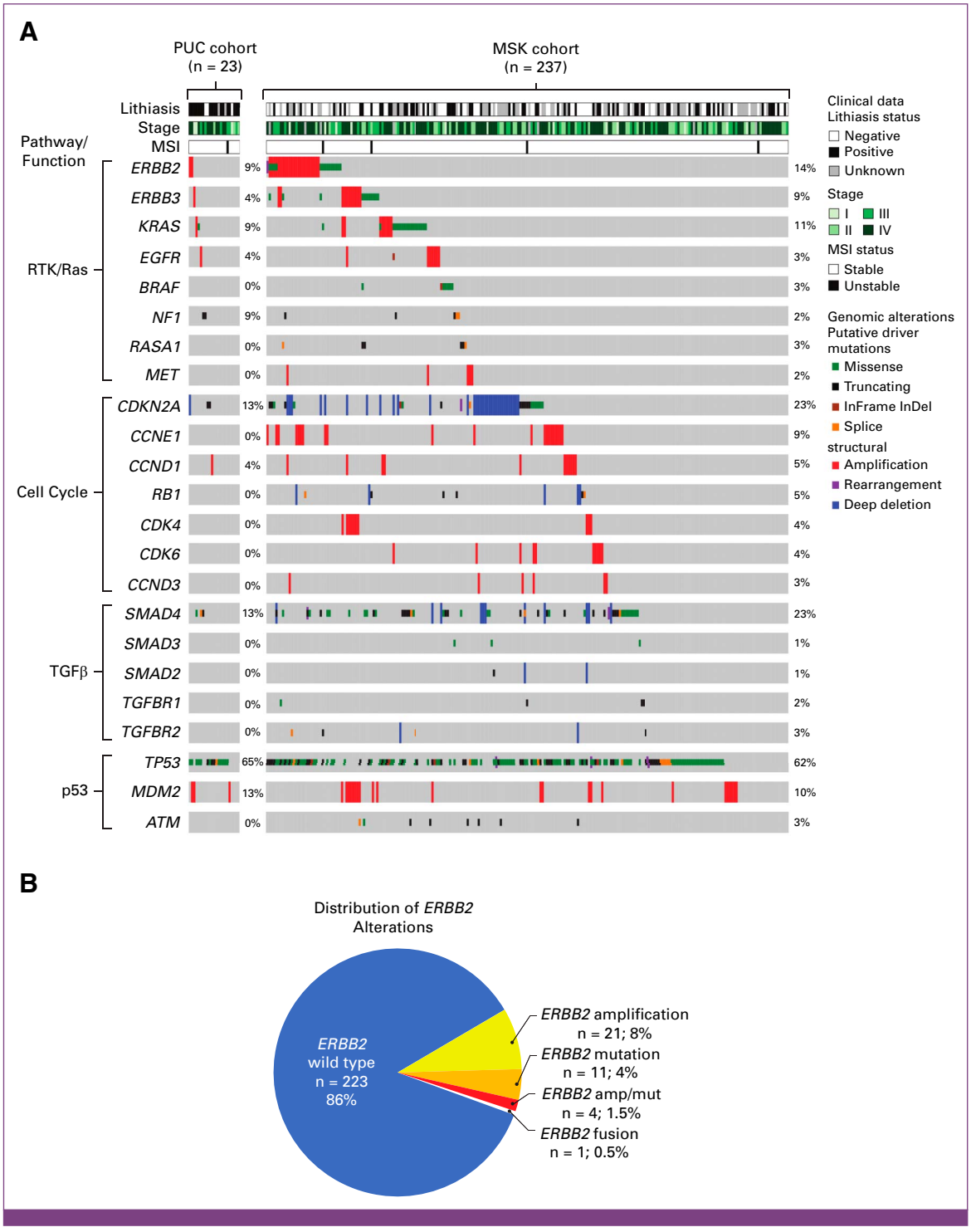

FIG 1. Clinical and genomic characteristics of patients with gallbladder cancer. (A) Oncoprint plot comparing lithiasis, stage at diagnosis, MSI status, and genomic features in Chilean (n = 23) and US (n = 237) GBC cohorts. (B) Distribution of *ERBB2* alterations. GBC, gallbladder cancer; MSI, microsatellite instability; MSK, Memorial Sloan Kettering Cancer Center; PUC, Pontificia Universidad Católica de Chile.

obtained from electronic medical records. Collected information included age, sex, race, stage, presence of lithiasis, systemic treatments received, and clinical outcomes. All tumor specimens were prospectively reviewed to confirm histology and to estimate purity.

Molecular Profiling

MSK-IMPACT was performed as described at the MSK Center of Molecular Oncology.[18] This assay includes up to 505 cancer-associated genes covering exons, selected

introns, and noncoding regions. MSK-IMPACT can detect mutations, small insertions and deletions, copy number alterations, and specific structural rearrangements. Genomic alterations were filtered for driver variants using OncoKB.[19] Microsatellite instability (MSI) was determined using the MSIsensor algorithm.[20] Genes were classified as amplified if they had a fold change ≥2. High concordance for *ERBB2* amplification with immunohistochemistry and fluorescence in situ hybridization (98.4%) has been established in a validation set of 252 patients.[21] Genes were grouped into pathways using curated templates from The Cancer Genome Atlas PanCancer analysis.[22] The FACETS algorithm[23] and the FACETS-suite package were used to correct copy number segmentation data for tumor purity.

Statistical Analysis

Descriptive statistics were used to summarize the characteristics of these cohorts. For the analysis of the prevalence of molecular alterations, a point estimate of the percentage of patients along with an exact 95% CI was reported. A two-tailed Fisher's exact test was used to identify significant associations between specific *ERBB2* alterations and specific clinical characteristics and to compare MSK and PUC cohorts. Median overall survival (OS) was calculated from the time of diagnosis using the Kaplan-Meier method.

Ethics Approval and Consent for Publication

Research was approved by the Memorial Sloan-Kettering Institutional Review and Privacy Board (institutional review board approval protocol ID 19-082, dated June 3, 2019) and by the Ethics and Scientific Committee for Health Sciences at the PUC, project ID 180807011, dated February 5, 2019. Patients signed the 12-225 form for the sequencing of solid tumors and/or consent forms for publication.

RESULTS

A total of 260 patients with GBC were included in our study. Of these, 237 were enrolled at MSK and 25 patient samples were obtained at PUC and were shipped for tumor profiling at MSK. Two samples did not pass quality controls and therefore were not analyzed. Except for the prevalence of lithiasis which was higher in the PUC compared with the MSK cohort (85% *v* 44%; $P = .0003$), baseline characteristics were similar between the two cohorts (Table 1; Fig 1A). Regarding our genomic analysis, the Oncoprint plot in Figure 1A shows the frequency of alterations in a set of 23 cancer genes involved in the RTK/RAS pathway, cell cycle genes, and the TGFβ and p53 pathways, and Appendix Figure A1 shows the frequency of genomic alterations across different clinical stages. In Appendix Figure A2, we describe the copy number alteration profile and the pathway-level analysis between the two cohorts. The frequency of alterations in most evaluated genes was similar between these two cohorts including *ERBB2* (9% *v* 14%; $P = .42$). In the merged cohort, 37 of 260 (14%) patients displayed *ERBB2* alterations (95% CI, 10 to 19), 8% had gene amplification (95% CI, 5 to 12), 4.2% had *ERBB2* mutations (95% CI, 2 to 7), 1.5% had concurrent amplification and mutation (95% CI, 0.5 to 4), and 0.4% had *ERBB2* fusion (95% CI, 0.05 to 2.7; Fig 1B). Table 2 shows that age ($P = .61$), sex ($P = .33$), stage at diagnosis ($P = .93$), and lithiasis ($P = .09$) did not display significant differences by *ERBB2* status. Our analyses confirmed that most *ERBB2* mutations were missense and located within the extracellular (44%) or the kinase domain (39%). Commonly observed mutations included S310F/Y (n = 8), R678Q (n = 2), L755S (n = 2), and D769Y (n = 2; Appendix Fig A3). Within the mutated group, the most common alteration was missense mutations (39%). In addition, four patients had two concurrent *ERBB2* mutations. Patients with GBC that harbored *ERBB2*-altered tumors had a distinct genomic profile with a trend toward lower concurrent *KRAS* alterations (3% *v* 12%; $P = .14$) and higher prevalence of *TP53* alterations (81% *v* 59%; $P = .016$). After excluding MSI-high (MSI-H) tumors, concurrent *KRAS* alterations were significantly lower in *ERBB2*-altered tumors ($P = .032$; Appendix Fig A2). Tumor mutational burden (TMB) was higher in the *ERBB2*-altered group compared with the wild-type (WT) group (median, 5.3 *v* 3.5; $P = .007$). This difference remained even after excluding

TABLE 2. Baseline Characteristics of Patients With GBC by *ERBB2* Status

Variable	*ERBB2* Status		*P*
	Wild Type (n = 223)	Altered (n = 37)	
Age, years, median (range)	66 (37-91)	64.5 (39-86)	.61
Sex, No. (%)			
Male	72 (32)	9 (25)	.33
Female	151 (68)	27 (75)	
Race, No. (%)			
White	159 (71)	21 (57)	.29
Asian	23 (10)	4 (11)	
Black	19 (9)	7 (19)	
Other	7 (3)	2 (5)	
Unknown	15 (7)	3 (8)	
Stage at diagnosis, No. (%)			
I	12 (5)	3 (8)	.93
II	53 (23)	2 (5)	
III	40 (18)	9 (24)	
IV	112 (50)	20 (54)	
Unknown	6 (2)	3 (8)	
Lithiasis,[a] No. (%)			
Yes	82 (51)	9 (33)	.09
No	79 (49)	18 (67)	

Abbreviation: GBC, gallbladder cancer.
[a]27% of missing data.

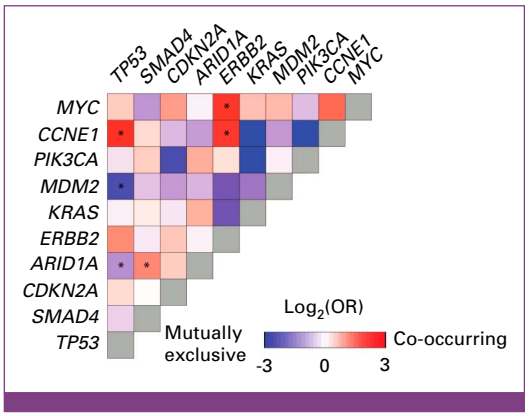

FIG 2. Co-occurring and mutually exclusive mutations in patients with GBC. GBC, gallbladder cancer; OR, odds ratio. *Corrected $P < .05$.

MSI-H tumors ($P = .008$). By contrast, the prevalence of MSI-H tumors did not show statistically significant differences between *ERBB2* WT and *ERBB2*-altered ($P = .53$; Appendix Fig A4). A single case of *ERBB2-CDK12* fusion was identified. This rearrangement is a duplication that results in a fusion of *ERBB2* exons 1-2 to *CDK12* exons 3-14. The fusion does not include the kinase domain of *ERBB2* and includes the kinase domain of *CDK12*. Interestingly, this tumor also harbored a *RET* fusion. The prevalence of targetable *FGFR2* fusions and *IDH1* mutations including the two cohorts was 0% and 0.3%, respectively.

Subsequently, we performed an analysis of genes with tendency toward co-occurrence or mutual exclusivity in the merged cohort. As shown in Figure 2, *ERBB2* amplification or mutation was mutually exclusive with *MYC* and *CCNE1* alterations with a corrected $P < .05$. In addition, *TP53* alteration co-occurred with *CCNE1* alteration but was mutually exclusive with *ARID1A* alterations.

With a median follow-up of 24 months, the median OS was similar between the MSK and PUC cohorts (12 months *v* 17 months; hazard ratio [HR], 0.81 [95% CI, 0.45 to 1.47]; $P = .49$). By contrast, after adjusting relevant covariates, patients who harbored *ERBB2* alterations displayed better OS compared with *ERBB2* WT (22.3 months *v* 11.8 months; HR, 0.53 [95% CI, 0.29 to 0.96]; $P = .024$; Figs 3A, 3B, and Table 3). It is noteworthy that 27% of *ERBB2*-altered patients received anti–HER2-targeted therapies, predominantly antibody drug conjugates (60%).

DISCUSSION

Our study analyzed and compared clinical and genomic characteristics of Chilean and US patients with GBC. Although most evaluated features did not show significant differences, we found a higher prevalence of lithiasis in Chilean patients. Previous studies have reported a high prevalence of gallstone disease in Chilean patients, especially among Chileans with Mapuche ancestry.[24-26] Studies suggest that this could be attributed to an increase in bile acid synthesis associated with increased synthesis of liver cholesterol, especially in females.[27]

In line with previous reports, our genomic analysis confirmed *ERBB2* as the most frequently altered potentially actionable gene (including amplifications and mutations) in GBC.[28,29] Indeed, previous GBC studies have reported a prevalence of *ERBB2* alterations that ranged from 2% to 31% (Appendix Table A1). Furthermore, a large study that included 760 GBC specimens reported that *ERBB2* genomic alterations and EGFR/ERBB pathway alterations were present in 13.9% and 22.9% of cases, respectively.[30] Investigators also found that 14.2% of patients displayed alterations in DNA repair genes; however, these were not associated with *ERBB2* alterations. When we compared clinical characteristics of *ERBB2* WT patients versus those of patients who harbored *ERBB2* alterations, we observed a

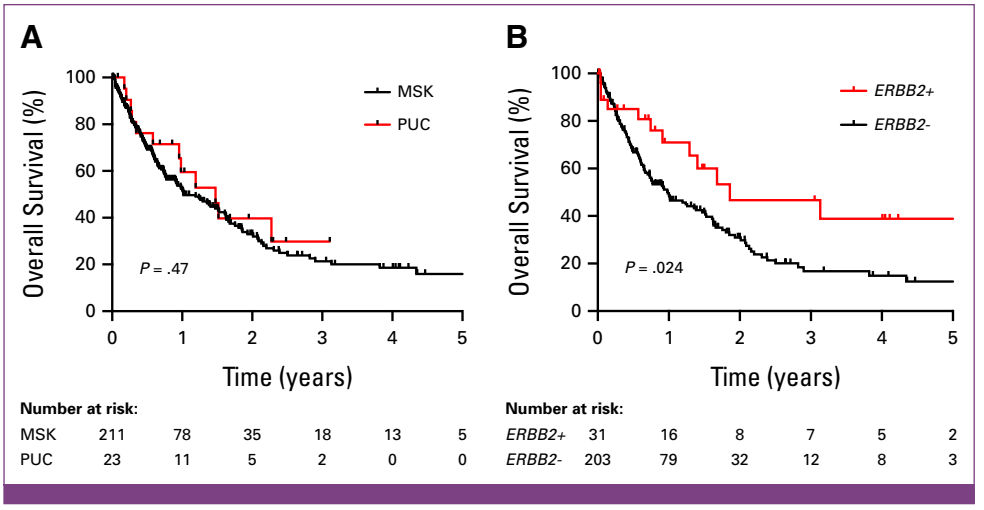

FIG 3. Overall survival of patients with gallbladder cancer (A) by site of origin and (B) by *ERBB2* status. MSK, Memorial Sloan Kettering Cancer Center; PUC, Pontificia Universidad Católica de Chile.

TABLE 3. Multivariable Cox Proportional Hazards Model

Variable	HR (95% CI)	*P*
Age	1.01 (0.99 to 1.02)	.18
Sex		
Male	Ref	
Female	1.027 (0.7 to 1.49)	.88
ERBB2 status		
Negative (WT)	Ref	.017
Positive	0.49 (0.27 to 0.88)	
Stage at diagnosis		
I/II/III	Ref	.0001
IV	2.6 (1.82 to 3.76)	

Abbreviations: HR, hazard ratio; Ref, reference; WT, wild type.

lower incidence of lithiasis in *ERBB2*-altered that did not reach statistical significance. Previous reports have shown that gallstones are a risk factor for GBC associated with chronic inflammation and early *TP53* mutations.[31-33] Notably, *ERBB2* alterations were associated with higher TMB in our cohort even after excluding MSI-high patients (5.3 mut/Mb *v* 3.5 mut/Mb; $P = .007$). This association was previously reported by others[28] and opens the possibility for immunotherapy with anti-PD1/PDL1 immune checkpoint inhibitors in this subset. Indeed, the phase III TOPAZ-1 trial demonstrated a significant improvement in OS by the addition of the anti-PDL1 monoclonal antibody durvalumab to gemcitabine plus cisplatin in metastatic or locally advanced nonresectable BTC.[34] Similarly, the KEYNOTE-966, another phase III trial, demonstrated clinically meaningful improvements in OS by the anti–PD-1 monoclonal antibody pembrolizumab plus gemcitabine and cisplatin, also in metastatic or locally advanced nonresectable BTC.[35] *ERBB2* status in these trials is not reported. Other biomarkers such as PD-L1 status have not demonstrated predictive features of clinical benefit in this context.

Unexpectedly, patients who harbored *ERBB2* alterations in our study displayed better OS rates versus *ERBB2* WT counterparts. By contrast, previous studies have reported poorer prognosis for this subset. A study by Li et al[36] performed whole-exome sequencing in a group of 157 patients with GBC and found that *ERBB2*/*ERBB3* mutations were associated with poorer prognoses and shorter OS (6.5 months *v* 11 months in *ERBB2*/*ERBB3*-negative [$P = .009$]). Unfortunately, the authors do not provide details on the treatments received by *ERBB2*-mutant patients. A second study demonstrated that HER2 overexpression was associated with poorer disease-free survival and OS in resected BTCs. Investigators report that 50% of patients received adjuvant treatment that included gemcitabine or capecitabine and 4% received radiotherapy.[37] It is unclear if the better OS rates in our cohort could be explained by better access to anti-HER2 therapies in a proportion of patients who harbored *ERBB2* alterations.

Global GBC incidence rates and the prevalence of *ERBB2* alterations in these tumors are low. Thus, confirming a potential association between risk factors (such as lithiasis) and *ERBB2* alterations will demand collaborative analyses of clinically annotated databases or collaborative prospective studies. This adds to other limitations of the study discussed herein. First, sample sizes in our compared cohorts from Chile (PUC; n = 23, 9%) and United States (MSK; n = 237, 91%) are not balanced, which limits a fair comparison between these cohorts. Our research team in Chile recently established an in-house next-generation sequencing program, and we expect to increase the number of analyzed samples soon. Regardless, this is a reminder for the need of increased awareness and education globally of the value of next-generation sequencing as part of cancer care. Second, we were unable to analyze HER2 protein expression by immunohistochemistry, which would enhance accessibility and efficiency. While this is the current standard to establish HER2 overexpression, it is also the standard for anti-HER2 therapies. This is relevant considering that even patients with breast cancer categorized as HER2-low can benefit from anti-HER2 treatments.[38] Finally, our merged cohort is highly heterogeneous in terms of stage and received treatments, which precludes us from drawing conclusions in a relatively small subgroup such as *ERBB2*-altered tumors. Our results at this point do not support the routine use of NGS to detect *ERBB2* alterations given its significantly higher cost compared with HER2 immunohistochemistry and the low prevalence of other potentially targetable alterations in GBC.

In summary, the Chilean GBC cohort reported herein displayed a higher proportion of lithiasis. The similar *ERBB2* amplifications and mutations in the two cohorts were the most frequent potentially actionable alterations in our merged GBC cohort (14%) and were associated with better patient OS. The smaller cohort of Chile where the disease prevalence is higher is a reminder and invitation for the need of more robust next-generation sequencing analyses globally. Our findings warrant further investigation in an expanded sample of Chilean GBCs. Future studies should also assess the epigenetic makeup and the metabolomic landscape of GBC.

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DATA SHARING STATEMENT

S.M. and G.K.A.-A. had full access to the data in the study. Data requests may be submitted to the corresponding author (G.K.A.-A.), which will be submitted for ethical approval (abou-alg@mskcc.org).

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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APPENDIX

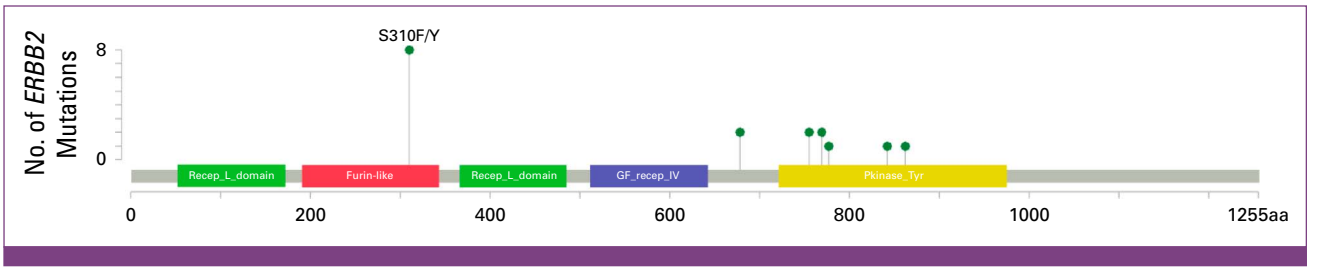

FIG A1. Lollipop plot of *ERBB2*-coding variants detected by MSK-IMPACT. IMPACT, Integrated Mutation Profiling of Actionable Cancer Targets; MSK, Memorial Sloan Kettering Cancer Center.

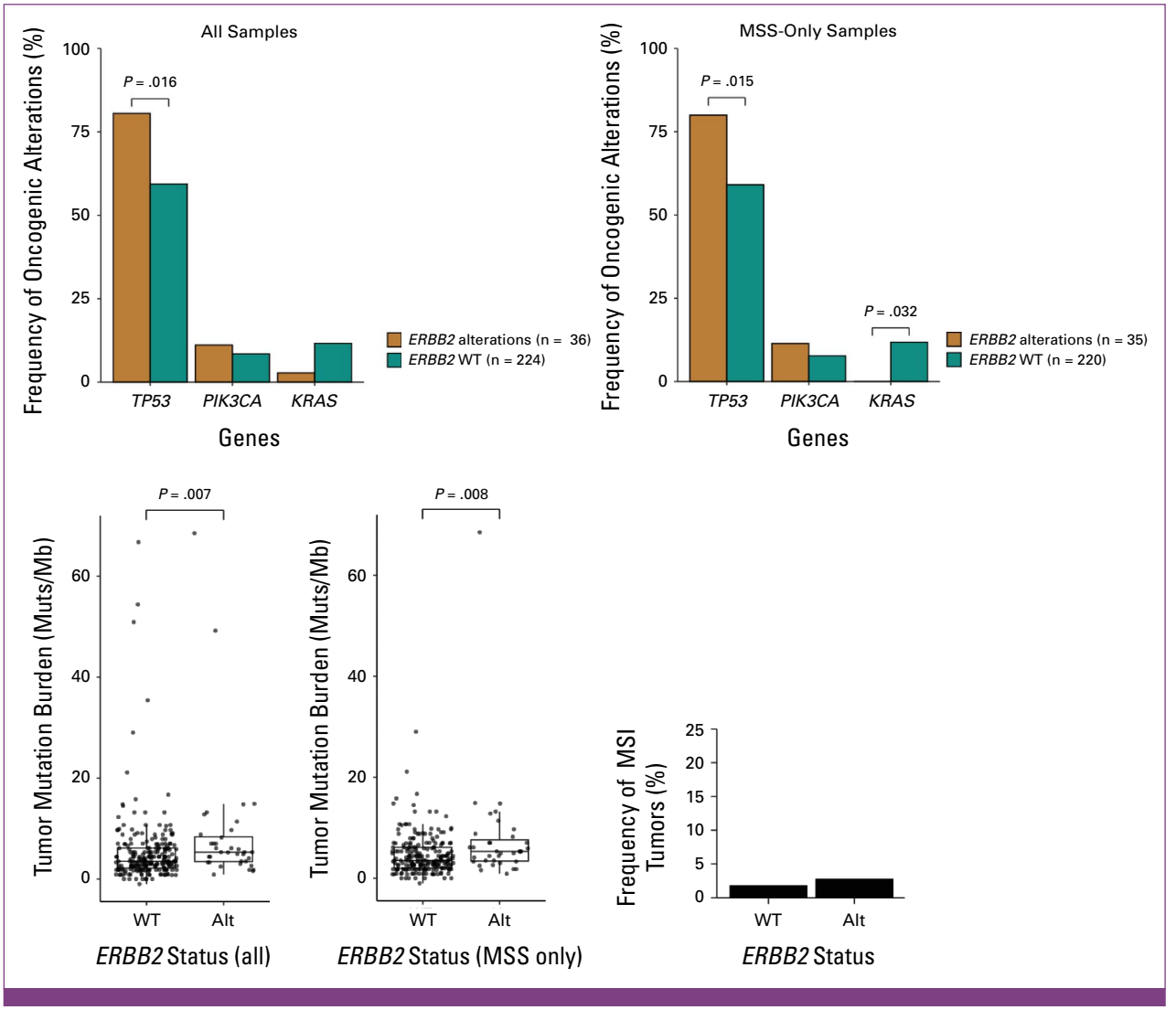

FIG A2. Comparison of genomic alterations and tumor mutation burden in *ERBB2*-altered and WT tumors. MSI, microsatellite instability; MSS, microsatellite stable; WT, wild type.

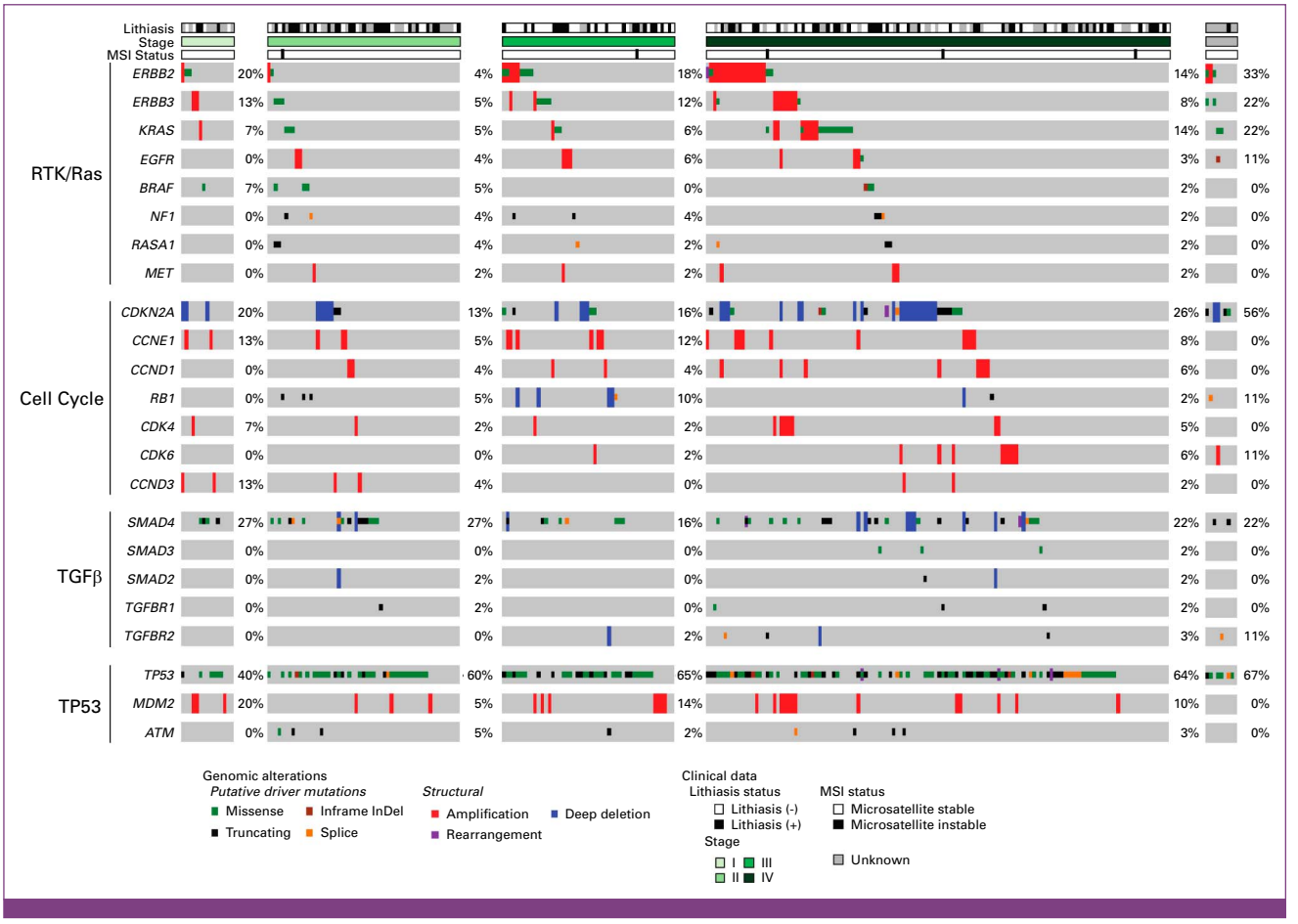

FIG A3. Genomic alterations across different stages. MSI, microsatellite instability.

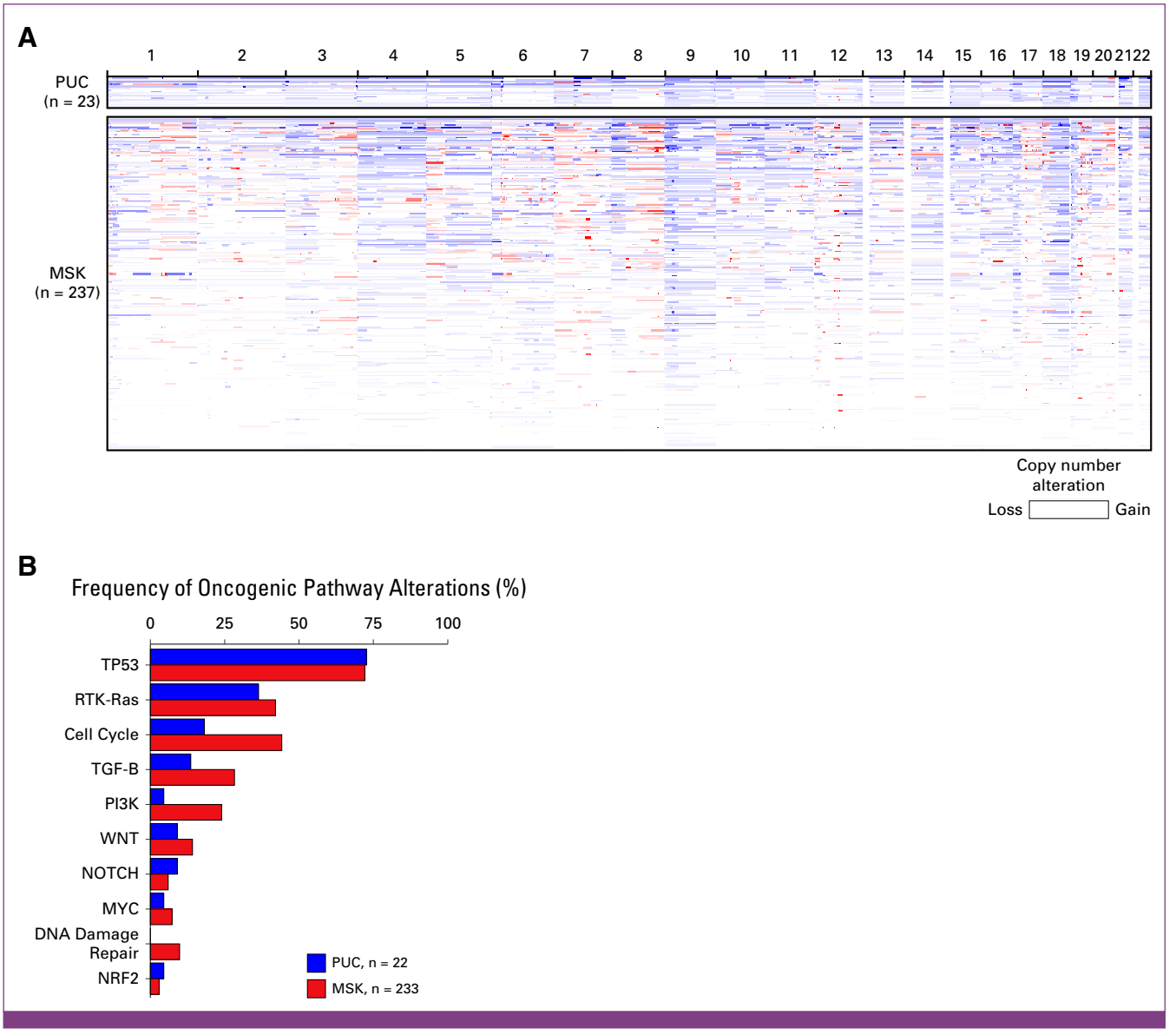

FIG A4. (A) Copy number alteration profile between MSK and PUC cohorts. (B) Frequency of oncogenic pathway alterations in MSK and PUC cohorts. Only microsatellite stable tumors are included. MSK, Memorial Sloan Kettering Cancer Center; PUC, Pontificia Universidad Católica de Chile.

TABLE A1. Previous NGS Studies Conducted in Gallbladder Cancer and *ERBB2* Data

Reference	Sample Size	Female, %	Mean/Median Age, Years	Country	Sample Site, %				Alteration Frequency		
					Primary Tumor	Liver Metastasis	Lymph Nodes	Other Sites	*ERBB2* Amplification, %	*ERBB2* Mutations, %	*ERBB2* MUT Position
Li et al[39]	57	59.6	60.7/MI	China	No data	No data	No data	No data	No data	9.20	E235K; G262R; V566I
Nakamura et al[40]	28	53.7	Missing	Japan (11)	100	0	0	0	No data	No data	No data
Wardell et al[41]	46	45.7	69.2/70.5	Italy (26); Japan (20)	No data	No data	No data	No data	No data	No data	No data
Li et al[36]	157	65.0	62/MI	China	No data	No data	No data	No data	2	8	>4
Narayan et al[24]	81	63.0	MI/64.9	Chile (21) Japan (11); United States (49)	100	0	0	0	6.90	2.90	L1098M
Abdel-Wahab et al[30]	760	69.0	MI/64.0	United States	48.0	21.7	6.2	24.1	9.3	4.3	No data
Pandey et al[42]	160	61.6	59.2/60.5	India (60); Korea (91); Chile (9)	100	0	0	0	13.3	11.3	>4
Nepal et al[43]	60	71.0	MI/64	China (52); Chile (8)	100	0	0	0	No data	9.6	>4
Lin et al[44]	11	81.8	72/MI	China	100	0	0	0	31.5	No data	p.S310Y; p.Q893*
MSK/PUC Study, 2023	260	69.1	64.1/66.0	Mixed	47.3	22.8	5.1	24.8	9.6	5.8	>4

NOTE. In cells marked as missing or no data, articles did not publish data nor supplementary material to infer the information.
Abbreviations: NGS, next generation sequencing; MI, missing information; MSK, Memorial Sloan Kettering Cancer Center; PUC, Pontificia Universidad Católica de Chile.