Overexpression of the *HER2/neu* Gene: A New Therapeutic Possibility for Patients With Advanced Gallbladder Cancer

Iván Roa,[1] Gonzalo de Toro,[2] Kurt Schalper,[3] Xabier de Aretxabala,[4] Chaitanya Churi,[5] Milind Javle[5]

ABSTRACT

BACKGROUND: The *HER2/neu* gene is a proto-oncogene that can predict the response to treatment with trastuzumab, pertuzumab, and lapatinib. This study was conducted to determine the frequency of *HER2/neu* overexpression and to identify a subgroup of patients with gallbladder cancer who would benefit from targeted therapy.

METHODS: Patients with gallbladder cancer (n = 187; 165 women and 22 men) with a recorded follow-up of at least 5 years were included, along with control subjects (n = 75). An automated immunohistochemical technique was used with an anti-ErbB2 antibody. Scoring was conducted according to the CAP/ASCO (College of American Pathologists/American Society of Clinical Oncology) criteria for breast cancer.

RESULTS: Overexpression of HER2/neu was observed in 12.8% of the cases. Of those, 0% were mucosal, 14.3% muscular, 12.8% subserosal, and 10.6% serosal. In 20% of the cases, equivocal staining was observed. Overexpression was more frequent in the advanced cancers and in the better differentiated tumors (13.8% and 17.4%, respectively), but the difference was nonsignificant. The patients with overexpression of HER2/neu had a worse overall survival, when compared with those who had no expression at 5 years (34% vs. 41%).

CONCLUSION: This is the single largest study of HER2/neu expression in gallbladder cancer to use commonly accepted scoring criteria. The results indicate that HER2/neu overexpression occurred in 14% of the advanced gallbladder cancer cases. This subgroup may benefit from inhibitors of the HER2/neu pathway.

Gastrointest Cancer Res 7:42–48.

[1]*Creative Bioscience*
Santiago, Chile

[2]*Pathology Service*
Puerto Montt Hospital
Puerto Montt, Chile

[3]*Pathology Department*
Faculty of Medicine
Yale University
New Haven, CT

[4]*Surgery Department*
Clínica Alemana
Santiago, Chile

[5]*Gastrointestinal Medical Oncology*
UT-MD Anderson Cancer Center
Houston, TX

This study was supported by Fondecyt Project 1120208

Submitted: September 23, 2013
Accepted: October 14, 2013

Mortality secondary to gallbladder cancer continues to be an important public health concern in Chile.[1] Among the causes of cancer mortality in Chile, gallbladder cancer is the second most common in women and the sixth most common in men, with an incidence of 26.2 and 9.4/100,000 and a mortality rate of 15.6 and 6.0/100,000, respectively.[2,3]

The *HER2/neu* gene is a transforming gene homologous to the *V-ERB* gene of avian erythroblastosis. The subsequent cloning of two other related human genes identified a four-member receptor family referred to as EGFr (Her1, erbB1), HER2 (erbB2, HER2/neu), HER3 (erbB3), and HER4 (erbB4).[4,5] These receptors cooperate in the regulation of different processes, including cell proliferation, differentiation, and survival.[6,7] The *HER2/neu* gene is located on the 17q12-q21 chromosomal region and acts as an oncogene. Its amplification mainly translates into protein overexpression.[8] The HER2/neu receptors undergo dimerization and transphosphorylation of their intracellular domains, which transactivate numerous intracellular signaling molecules and adaptor proteins (Shc), which in turn lead to the activation of a large variety of downstream pathways, such as RAS-RAF-MEK-ERK1/2 and PI3k-AKT-mTOR, with various biological effects.[9,10] The overexpression of the HER2/neu protein, either as the product of gene amplification or transcriptional deregulation, is observed in approximately 20–30% of breast and ovarian cancers.[11] Breast cancers can have up to 25–50 copies of the *HER2* gene, and up to a 40–100-fold increase in HER2 protein, resulting in 2 million receptors expressed at the tumor cell surface.[12,13] This deregulation gives the tumor cell a proliferative advantage, a capacity that it can maintain in most cases during the progression of the disease from the invasive phase and results in distant metastases.[14,15] The overexpression and amplification of *HER2/neu* has also been shown in other cancers, including gastric,

Address correspondence to: Iván Roa, Avenida Del Valle Norte 857, Oficina 102, Ciudad Empresarial, Huechuraba Santiago, Chile, Código Postal 8580702, Phone: +56 9203107; Fax: (713) 745-8620; E-mail: ivanroaesterio@gmail.com

esophageal, and endometrial, and in those tumors, it is also associated with a worse prognosis.[16–19]

In patients with advanced breast and gastric cancer, which are known to overexpress or amplify the *HER2/neu* gene, HER2/neu receptor blockers have already been incorporated into conventional chemotherapy, using monoclonal antibodies, such as trastuzumab and pertuzumab, or tyrosine kinase inhibitors associated with EGFr or HER2/neu, such as lapatinib.[16,20,21] In most studies, a favorable, albeit limited, response to the treatment has been shown.[22,23] These results have created new expectations for the use of molecularly targeted agents for colon and lung cancer.[17,24,25] In gallbladder cancer, there have been few reports regarding the overexpression and amplification of *HER2/neu*. However, small case numbers limited these studies, and the results are difficult to interpret because of the variety of criteria used to determine protein overexpression.[26–38]

The overexpression of HER2/neu has been described by using immunohistochemical scores, and various protocols have been developed to measure the intensity of the staining and/or the number of immunostained cells. In breast[39] and gastric[40] cancers, the HER2/neu scoring system is relatively standardized; however, this is not the case with other HER2/neu-expressing cancers. Immunohistochemical overexpression has been restricted in breast cancer to those cases with complete membrane positivity in at least 30% of the tumor cells of the infiltrating component[39]; however, the HER2/neu scoring criteria used in gastric cancer have caused greater ambiguity.[41,42] Considering the significance of gallbladder cancer in Chile and the potential therapeutic impact of targeted therapy for patients with advanced gallbladder cancer, a comprehensive assessment of HER2/neu overexpression in this population is needed.

MATERIALS AND METHODS

Cases and Controls

Included in the study were 187 patients with primary gallbladder adenocarcinomas diagnosed at the Temuco Regional Hospital, the Puerto Montt Regional Hospital, and the Diagnostic Center of Temuco in Chile. From these centers, a surgical pathologist selected formalin-fixed, paraffin-embedded tumor samples. As a control arm, 75 nontumorous gallbladders were used, obtained from elective cholecystectomies for gallstones or other surgical procedures. All pathology samples were anonymized. The study was approved by the Ethics Committee of the sponsoring institution and the Ethics Advisory Committee of the National Council of Science and Technology (CONICYT).

Preparation of Tissue Microarrays

Three areas of the tumor were selected from each of the samples and marked in sections previously stained with hematoxylin and eosin (H&E) with a Nikon object marker. For the preparation of the tissue microarrays (TMAs), the Semiautomated Tissue MicroArrayer (Pathology Devices, Inc.) was used with 2-mm needles. The tissue cores were extracted from the donor block and placed on the receiving block. The inclusions were processed at 55°C for 30 minutes, to obtain adhesion between the samples and the receiving block.

Immunohistochemical Technique

A standard technique for fixing tissue in formalin and embedding it in paraffin was used. The 4-μm-thick histologic sections obtained from the TMAs were deparaffinized and hydrated in decreasing alcohol concentrations. Antigens were recovered by exposure to microwaves in citrate buffer (pH 6.0) and washed in PBS (pH 7.4). The monoclonal antibody anti-ErbB2 (NCL-CB11; Novocastra) was used at a dilution of 1:40. The primary antibody was incubated at room temperature for 60 minutes and then incubated with the complex Super Picture Polymer Detection Kit (Zymed) in a Dako autostainer.

Methods for Measuring Positivity

Positivity was measured according to the CAP/ASCO (College of American Pathologists/American Society of Clinical Oncology) guidelines summarized in Table 1. We used the same immunohistochemical criteria for HER2/Neu positivity as has been used in breast cancer, given their widespread acceptance in the scientific literature.[39]

Statistical Analysis

Statistical comparisons were performed with χ^2 tests. Fisher's exact test was used for the analysis of contingency tables and analysis of variance for the averages. Correlations with survival and prognosis were examined with Kaplan-Meier actuarial survival curves and log rank tests. Statistical significance was set at $P < .05$.

RESULTS

The group with cancer comprised 187 cases; the patients' characteristics are summarized in Table 2. The majority (88%) were women, with an average age of 61.6 years (SD $\pm$13.5); the average age of the men was 69.0 years (SD $\pm$14.3) (Table 2A). The difference between the ages of the men and women was statistically significant ($P = .02$). All the cases involved adenocarcinomas. Sixteen percent of the cases involved early tumors (14 mucosal carcinomas and 14 with muscular involvement only) and 84% were more advanced (125 subserosal and 34 serosal carcinomas) (Table 2B). Deeper tumor infiltration occurred at a higher age than did purely mucosal involvement of the cancer that occurred at an earlier age ($P < .001$). Only 12% of the tumors were well differentiated, with the great majority being moderately (67%) or poorly (21%) differentiated (Table 2C).

The immunohistochemical staining score for HER2/neu in the 75 control subjects was 0 in 40 (53.3%) cases, 1+ in 28 (37.3%), and 2+ in 7 (9.3%) (Table 3). In the control group, no case was observed that could have been considered unequivocally positive (3+). Of the gallbladder cancer specimens, 90 (48.1%) stained negative, 35 (18.7%) were 1+, 38 (20.3%) were 2+, and 24 (12.8%) were considered positive (3+) for overexpression of HER2/neu. Table 3 contains a summary of the intensity of the HER2/neu lesion in relation to the level of tumor infiltration in the gallbladder wall. Positivity in the mucosal carcinomas was not observed; however, 14.3% with muscular involvement only, 12.8% of the subserosal, and 17.6% of the serosal carcinomas showed 3+ positivity, with a frequency of 12.8% for the entire group and 13.8% for the more advanced (subserosal and serosal) carcinoma groups. Overexpression of HER2/neu was more frequently found in the well-differentiated (17.4%) tumors than in those that were poorly differentiated (10.3%); nevertheless, the difference was

Table 1. HER2/neu scoring guidelines

Breast cancer[39]

Score		HER2 Overexpression
0	Negative/no staining: staining in <10% of tumor cells	Negative
1	Negative/faint: barely perceptible incomplete membrane staining in >10% of tumor cells	Negative
2	Equivocal/weak to moderate: complete membrane staining in >10% of tumor cells	Equivocal
3	Positive/strong: complete membrane staining in >30% of tumor cells	Positive

Gastric cancer[40]

Score	Surgical specimen staining pattern	Biopsy specimen staining pattern	HER2 Overexpression
0	No reactivity or membranous reactivity in <10% of tumor cells	No reactivity in any tumor cell	Negative
1	Faint or barely perceptible membranous reactivity in 10% or more of tumor cells; cells are reactive in only part of the membrane	Tumor cell cluster with faint or barely perceptible membranous reactivity, irrespective of percentage of tumor cells stained	Negative
2	Weak to moderate, complete basolateral or lateral membranous reactivity in 10% or more of tumor cells	Tumor cell cluster with weak to moderate, complete basolateral or lateral membranous reactivity, irrespective of percentage of tumor cells stained	Equivocal
3	Strong, complete basolateral or lateral membranous reactivity in 10% or more of tumor cells	Tumor cell cluster with strong, complete basolateral or lateral membranous reactivity, irrespective of percentage of tumor cells stained	Positive

Table 2. Patients' information and histologic data

A. Gender distribution

Gender	*n* (total)	Mean age	SD
Female	165	61.6	13.5
Male	22	69.0	14.3
Total	187	62.5	14.4

B. Tumor infiltration level in relation to gender and age

Depth	*n* (total)	Female	Mean age
Mucosal	14	14	51.1
Muscular	14	12	63.4
Subserosal	125	110	63.2
Serosal	34	29	64.1

C. Histological differentiation and gender

Grade	*n* (total)	Female	Male
Well	23	20	3
Moderate	125	110	15
Poor	39	35	4

Table 3. Infiltration level and HER2/neu score

	0	1	2	3	Total	% (3+)
Tumor infiltration						
Mucosal	5	7	2	0	14	0
Muscular	4	4	4	2	14	14.3
Subserosal	67	18	24	16	125	12.8
Serosal	14	6	8	6	34	17.6
Total	90	35	45	24	187	12.8
Chronic cholecystitis	40	28	7	0	75	0

not significant ($P = .3$). The early carcinomas (mucosal and muscular) had a lower incidence of HER2/neu overexpression, compared with the advanced-stage carcinomas (subserosal and serosal) (7.1% vs. 13.8%); however, this difference was not statistically significant, given the small sample of patients. Actuarial survival at 5 years (Figure 1) was 100% in the patients with mucosal carcinomas, 93% in those with muscular involvement only, and 32% and 5% in those with subserosal and serosal carcinomas, respectively. At 70 months of follow-up, all the patients with serosal carcinomas had died. The patients with tumors that overexpressed HER2/neu showed an actuarial survival of 34% at 5 years, in comparison with 41% of those who had negative tumors (Figure 2). This difference in survival of 7% at 5 years was also not statistically significant.

DISCUSSION

Our results showed overexpression of HER2/neu in 12.8% of the patients with gallbladder cancer and in 13.8% of patients with the deeper infiltrating cancers, who could benefit from HER2/neu-directed monoclonal antibodies, such as trastuzumab and pertuzumab, or tyrosine kinase inhibitors such as lapatinib. Although HER2/neu expression in this disease has been reported by others, our

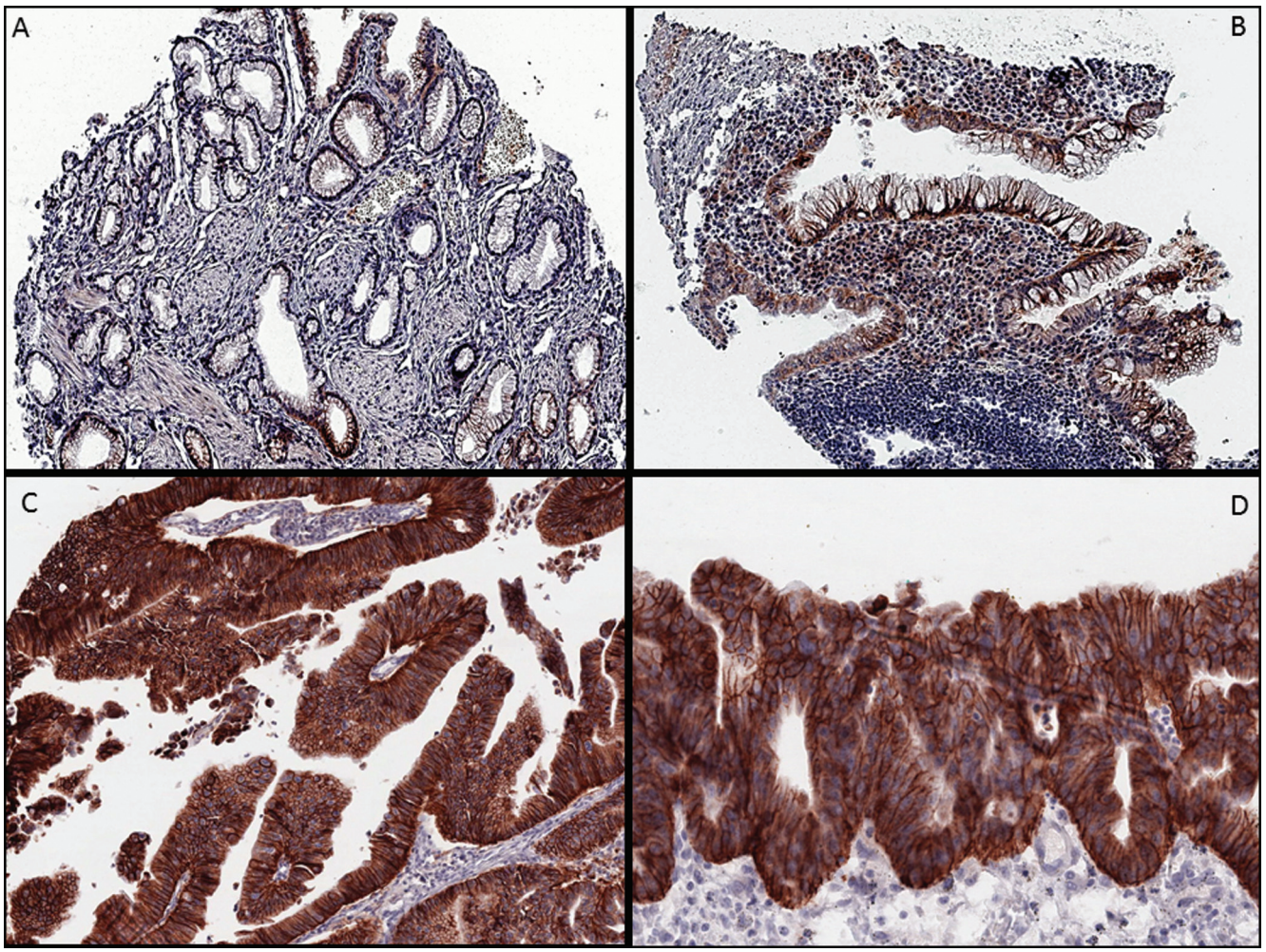

Figure 1. HER2/neu immunohistochemical staining: (A) focal, weak (1+) positivity in the superficial area of the mucosa in pyloric metaplasia; (B) positive basolateral membrane staining (2+) in intestinal metaplasia; and (C, D) gallbladder adenocarcinoma with strong membrane-positive staining (+3) in more than 30% of the tumor cells.

stringent criteria for HER2/neu positivity as well as our large sample adds to the strength of our findings. Our results are difficult to compare with those of other published series, considering the variation in criteria used for the evaluation of HER2/neu positivity in the literature.[26–38] Some of these studies have reported extremely contradictory data that are difficult to interpret, such as those of Toledo et al,[32] with positivity in 91.7% of nontumorous gallbladder mucosa in the areas of intestinal metaplasia, 90% of carcinomas in situ, and 33% of advanced carcinomas. In this study, despite these remarkable statistics, gene amplification with hybridization techniques was not successfully demonstrated in any of the cases, which raises questions about the interpretation of the positivity and specificity of the antibody used—variations of which have already been reported.[43] Other investigators have reported HER2/neu positivity between 2% and 33%, but, as we indicated, with positivity evaluation criteria that cannot be compared and with those used for a small number of observations.[26–31] Table 4 summarizes the published results of HER2/neu and gallbladder cancer. The criteria used to establish positivity or overexpression in cases have varied despite the use of a similar immunohistochemical platform. The use of different primary antibodies and the absence of processing and interpretation guidelines have facilitated the disparity in results. Puhalla et al[29] observed a greater degree of overexpression in gallbladder tumors with better differentiation and deeper tumor infiltration. Patients with tumors that overexpressed HER2/neu had a 7% lower survival at 5 years than those whose tumors did not. Although these differences are not statistically significant, they are similar to those described for other cancers, where the tumors associated with HER2/neu overexpression exhibited more aggressive behavior.[4,42,44,45]

Our preliminary results may justify conducting controlled clinical trials of targeted therapy in patients with advanced gallbladder cancer with overexpression of HER2/neu, to demonstrate the value of such therapy in this group of patients who currently have few therapeutic options and a terrible prognosis. In addition, it is important to determine the correlation between overexpression of the protein and the degree of *HER2/neu* gene amplification by means of hybridization in situ, as noted in breast and stomach cancer, wherein 3+ immunohistochemical positivity correlates with gene amplification in more than 85% of cases.[40,46,47] However, in gallbladder cancer, this consistency has not yet been demonstrated. It must also be

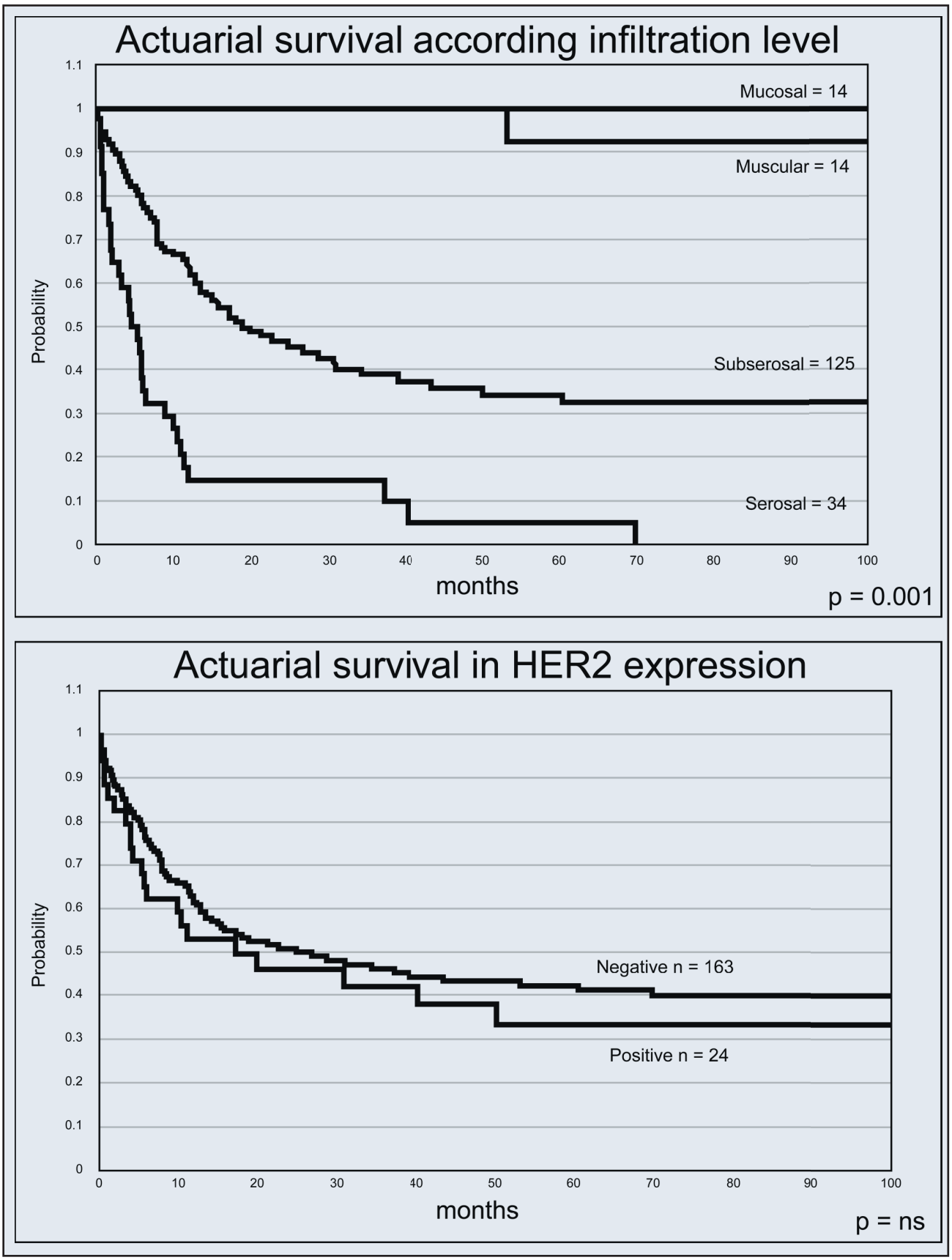

Figure 2. Top: actuarial survival of the total group according to the level of tumor infiltration of the gallbladder wall; bottom: survival curves in patients with (positive) and without (negative) HER2 overexpression. Patients with overexpression had a worse survival rate; however, the difference was not significant.

Table 4. Summary of published results of Her2/neu and gallbladder cancer

Authors	IHC platform	GBC cases (*n*)	HER-2/Neu Scoring	Fish ±	Results	Comments
Matsuyama et al[34]	DAKO	43	Intensity of membrane staining (1+ to 3+)	ND	1+: 2.3% 2+: 4.7% 3+: 4.7%	No correlation with grade
Kim et al[33]	DAKO	71	Percentage of staining cells (1+: 5-33%; 2+: 34-66%; 3+:>67%)	ND	1+: 64% 2+: 27% 3+: 9%	High expression correlated with poor survival
Nakazawa et al[35]	Nichirei	89	Intensity of staining:cytoplasmic (1+) or membrane (1+ to 3+)	Yes	2+: 9%, 3+: 6.7%	All 3+ IHCs were FISH+; 5 cases (56%) 2+ were FISH+
Harder et al[26]	DAKO	34	Membrane (1+ to 3+) only when 10% or more positive cells	Yes	1+: 21% 2+: 18% 3+: 3%	All 3+ confirmed on FISH
Puhalla et al[29]	DAKO	55	membrane staining (+ or −)	ND	12%	correlated with advanced T stages
Chaube et al[36]	NCL-CerbB2 clone CB11	78	Cytoplasm/ membrane staining cells	ND	+: 25%	Inverse correlation with grade
Kumari et al[37]	DAKO	97	Complete membrane staining >10% cells: 3+; incomplete membrane: 2+	ND	3+: 10% 2+: 4%	Xanthogranulomatous inflammation was inversely correlated with Her-2 over-expression
Kawamoto et al[38]	DAKO	77	Complete membrane staining > 10%:3+	Yes	3+and 2+: 31,2%	strong correlation between HER-2/neu IHC and FISH positivity
Toledo et al[32]	Novocastra NCLCBE-356	22	Membrane staining >30% of the gallbladder epithelium	Yes	33% AC 90% CIS 91.7 IM	All cases FISH negative

AC, advanced carcinoma; CIS, carcinoma in situ; ND, not done; FISH, fluorescence in situ hybridization; GBC, gallbladder cancer; IHC, immunohistochemistry; IM, intestinal metaplasia.

pointed out that 20% of gallbladder cancers have equivocal expression; these cases could be analyzed for amplification of the *HER2/neu* gene.[48]

The advent of next-generation sequencing technologies has identified ErbB2 mutations in some solid tumors. These activating mutations also appear to respond to targeted therapies and deserve further exploration in gallbladder cancer. There are regional differences in HER2 amplification status between Asian and Western populations with gastric cancer. Whether these differences also apply to gallbladder cancer should be investigated further. Finally, we conclude that more than 10% of patients with advanced gallbladder cancers in Chile could benefit from treatment of HER2/neu, and clinical trials of a targeted therapy should be encouraged.

REFERENCES

1. Eslick GD: Epidemiology of gallbladder cancer. *Gastroenterol Clin North Am* 39:307–330, 2010
2. Andia ME, Hsing AW, Andreotti G, et al: Geographic variation of gallbladder cancer mortality and risk factors in Chile: a population-based ecologic study. *Int J Cancer* 123:1411–1416, 2008
3. Andia KM, Gederlini GA, Ferreccio RC: [Gallbladder cancer: trend and risk distribution in Chile]. *Rev Med Chil* 134:565–574, 2006
4. Begnami MD, Fukuda E, Fregnani JH, et al: Prognostic implications of altered human epidermal growth factor receptors (HERs) in gastric carcinomas: HER2 and HER3 are predictors of poor outcome. *J Clin Oncol* 29:3030–3036, 2011
5. Tafe LJ, Tsongalis GJ: The human epidermal growth factor receptor 2 (HER2). *Clin Chem Lab Med* 50:23–30, 2012
6. Menard S, Tagliabue E, Campiglio M, et al: Role of HER2 gene overexpression in breast carcinoma. *J Cell Physiol* 182:150–162, 2000
7. Mitri Z, Constantine T, O'Regan R: The HER2 receptor in breast cancer: pathophysiology: clinical use, and new advances in therapy. *Chemother Res Pract* 2012:743193, 2012
8. Gutierrez C, Schiff R: HER2: biology, detection, and clinical implications. *Arch Pathol Lab Med* 135:55–62, 2011
9. Wang Y, Liu Y, Du Y, et al: The predictive role of phosphatase and tensin homolog (PTEN) loss, phosphoinositol-3 (PI3) kinase (PIK3CA) mutation, and PI3K pathway activation in sensitivity to trastuzumab in HER2-positive breast cancer: a meta-analysis. *Curr Med Res Opin* 29:633–642, 2013
10. Segatto O, Pelicci G, Giuli S, et al: Shc products are substrates of erbB-2 kinase. *Oncogene* 8:2105–2112, 1993
11. Yang XR, Chang-Claude J, Goode EL, et al: Associations of breast cancer risk factors with tumor subtypes: a pooled analysis from the

Breast Cancer Association Consortium studies. *J Natl Cancer Inst* 103:250–263, 2011

12. Moasser MM: The oncogene HER2: its signaling and transforming functions and its role in human cancer pathogenesis. *Oncogene* 26:6469–6487, 2007
13. Shadeo A, Lam WL: Comprehensive copy number profiles of breast cancer cell model genomes. *Breast Cancer Res* 8:R9, 2006
14. Nishimura R, Osako T, Okumura Y, et al: Changes in the ER, PgR, HER2, p53 and Ki-67 biological markers between primary and recurrent breast cancer: discordance rates and prognosis. *World Surg Oncol* 9:131, 2011
15. Turner NH, Di Leo A: HER2 discordance between primary and metastatic breast cancer: assessing the clinical impact. *Cancer Treat Rev* 39:947–957, 2013
16. Chua T, Merrett ND: Clinicopathologic factors associated with HER2-positive gastric cancer and its impact on survival outcomes: a systematic review. *Int J Cancer* 130:2845–2856, 2012
17. English DP, Roque DM, Santin AD: HER2 expression beyond breast cancer: therapeutic implications for gynecologic malignancies. *Mol Diagn Ther* 17:85–99, 2013
18. Penault-Llorca F, Chenard MP, Bouche O, et al: HER2 and gastric cancer: recommendations for clinical practice in 2011 (in French). *Ann Pathol* 31:78–87, 2011
19. Boku N: HER2-positive gastric cancer. *Gastric Cancer* (in press)
20. Wong H, Yau T: Targeted therapy in the management of advanced gastric cancer: are we making progress in the era of personalized medicine? *Oncologist* 17:346–358, 2012
21. Terashima M, Kitada K, Ochiai A, et al: Impact of expression of human epidermal growth factor receptors EGFR and ERBB2 on survival in stage II/III gastric cancer. *Clin Cancer Res* 18:5992–6000, 2012
22. Thibault C, Khodari W, Lequoy M, et al: HER2 status for prognosis and prediction of treatment efficacy in adenocarcinomas: a review. *Crit Rev Oncol Hematol* 88:123–133, 2013
23. Wang S, Zheng G, Chen L, et al: Effect of HER-2/neu over-expression on prognosis in gastric cancer: a meta-analysis. *Asian Pacific J Cancer Prevent* 12:1417–1423, 2011
24. Stern HM: Improving treatment of HER2-positive cancers: opportunities and challenges. *Sci Transl Med* 4:127rv2, 2012
25. Jelovac D, Emens LA: HER2-directed therapy for metastatic breast cancer. *Oncology* 27:166–175, 2013
26. Harder J, Waiz O, Otto F, et al: EGFR and HER2 expression in advanced biliary tract cancer. *World J Gastroenterol* 15:4511–4517, 2009
27. Kaufman M, Mehrotra B, Limaye S, et al: EGFR expression in gallbladder carcinoma in North America. *Int J Med Sci* 5:285–291, 2008
28. Pignochino Y, Sarotto I, Peraldo-Neia C, et al: Targeting EGFR/HER2 pathways enhances the antiproliferative effect of gemcitabine in biliary tract and gallbladder carcinomas. *BMC Cancer* 10:631, 2010
29. Puhalla H, Wrba F, Kandioler D, et al: Expression of p21(Wafl/Cip1), p57(Kip2) and HER2/neu in patients with gallbladder cancer. *Anticancer Res* 27:1679–1684, 2007
30. Shafizadeh N, Grenert JP, Sahai V, et al: Epidermal growth factor receptor and HER-2/neu status by immunohistochemistry and fluorescence in situ hybridization in adenocarcinomas of the biliary tree and gallbladder. *Hum Pathol* 41:485–492, 2010
31. Su WC, Shiesh SC, Liu HS, et al: Expression of oncogene products HER2/Neu and Ras and fibrosis-related growth factors bFGF, TGF-beta, and PDGF in bile from biliary malignancies and inflammatory disorders. *Digest Dis Sci* 46:1387–1392, 2001
32. Toledo C, Matus CE, Barraza X, et al: Expression of HER2 and bradykinin B receptors in precursor lesions of gallbladder carcinoma. *World J Gastroenterol* 18:1208–1215, 2012
33. Kim YW, Huh SH, Park YK, et al: Expression of the c-erb-B2 and p53 protein in gallbladder carcinomas. *Oncol Rep* 8:1127–1132, 2001
34. Matsuyama S, Kitajima Y, Sumi K, et al: Gallbladder cancers rarely overexpress HER-2/neu, demonstrated by Hercep test. *Oncol Rep* 11:815–819, 2004
35. Nakazawa K, Dobashi Y, Suzuki S, et al: Amplification and overexpression of c-erbB-2, epidermal growth factor receptor, and c-met in biliary tract cancers. *J Pathol* 206:356–365, 2005
36. Chaube A, Tewari M, Garbyal RS, et al: Preliminary study of p53 and c-erbB-2 expression in gallbladder cancer in Indian patients. *BMC Cancer* 6:126, 2006
37. Kumari N, Kapoor VK, Krishnani N, et al: Role of C-erbB2 expression in gallbladder cancer. *Indian J Pathol Microbiol* 55:75–79, 2012
38. Kawamoto T, Krishnamurthy S, Tarco E, et al: HER receptor family: novel candidate for targeted therapy for gallbladder and extrahepatic bile duct cancer. *Gastrointest Cancer Res* 1:221–227, 2007
39. Summary of ASCO/CAP HER2 Guideline Recommendations. 2012. http://www.cap.org/apps/docs/committees/immunohistochemistry/summary_of_recommendations.pdf
40. Hofmann M, Stoss O, Shi D, et al: Assessment of a HER2 scoring system for gastric cancer: results from a validation study. *Histopathology* 52:797–805, 2008
41. Stenzinger A, von Winterfeld M, Aulmann S, et al: Quantitative analysis of diagnostic guidelines for HER2-status assessment. *J Mol Diagn* 14:199–205, 2012
42. Ruschoff J, Dietel M, Baretton G, et al: HER2 diagnostics in gastric cancer-guideline validation and development of standardized immunohistochemical testing. *Virchows Arch* 457:299–307, 2010
43. Schrohl AS, Pedersen HC, Jensen SS, et al: Human epidermal growth factor receptor 2 (HER2) immunoreactivity: specificity of three pharmacodiagnostic antibodies. *Histopathology* 59:975–983, 2011
44. Ananiev J, Gulubova M, Manolova I, et al: Prognostic significance of HER2/neu expression in gastric cancer. *Wien Klin Wochenschr* 123:450–454, 2011
45. Yoon HH, Shi Q, Sukov WR, et al: Adverse prognostic impact of intratumor heterogeneous HER2 gene amplification in patients with esophageal adenocarcinoma. *J Clin Oncol* 30:3932–3938, 2012
46. Tafe LJ, Janjigian YY, Zaidinski M, et al: Human epidermal growth factor receptor 2 testing in gastroesophageal cancer: correlation between immunohistochemistry and fluorescence in situ hybridization. *Arch Pathol Lab Med* 135:1460–1465, 2011
47. Martin V, Camponovo A, Ghisletta M, et al: Internal quality assurance program for ERBB2 (HER2) testing improves the selection of breast cancer patients for treatment with trastuzumab. *Pathol Res Int* 2012:261857, 2012
48. Allison KH, Dintzis SM, Schmidt RA: Frequency of HER2 heterogeneity by fluorescence in situ hybridization according to CAP expert panel recommendations: time for a new look at how to report heterogeneity. *Am J Clin Pathol* 136:864–871, 2011

Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.