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COMMUNICATION

# Prognostic Value of Increased her2 Expression in Cancers of Pancreas and Biliary Tree

**Alper Ata · Ayşe Polat · Ebru Serinsöz · Mehmet Ali Sungur · Ali Arican**

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**Abstract** Increased HER2 expression has a prognostic, and predictive value in many solid cancer types, predominantly in breast cancer. However the effects of HER2 on survival from cancers of pancreas, gall bladder, cholangiocellular, and ampullary region are not known. In this study, the effects of increased HER2 expression on these types of cancer have been analyzed. Immunohistochemical HER2 staining was performed in 31 (44.9 %) female, and 38 (55.1 %) male patients with a mean age of 65±10 years, and various parameters, mostly survival rates of patients with pancreas ($n$=30; 43.5 %), gall bladder ($n$=17; 24.6 %), cholangiocellular ($n$=12; 17.4 %), and ampullary region ($n$=10; 14.5 %) carcinomas were evaluated. Strong (3 +) membranous staining for HER2 was observed in 2 patients with gall bladder cancers (11.76 % of all gall bladder cancers). In 2.90 % of all cases strong membranous staining (2+ or 3+) was observed. Weak (1+) membranous staining was noted in one (3.33 %) pancreatic, and one cholangiocellular (8.33 %) cancer patient, and in none of the ampullary region patient membranous staining for HER2 was observed. Since only scarce number of patients demonstrated membranous staining for HER2, survival analysis was not performed on these patients. Based on cytoplasmic HER2 staining scores, the patients were divided into weakly (0–3 pts; $n$=17 patients; 24.66 %), moderate (4–5 pts; $n$=22; 31.88 %), and strongly (6–7 pts; $n$=30; 43.46 %) stained groups. Patients whose specimens demonstrated borderline statistical significant ($p$=0.052) low staining for HER2 had higher survival rates when compared with other cases. Increased HER2 expression has no prognostic, and predictive value in cancers of pancreas, biliary tract, and ampulla vateri. If HER2 will be evaluated in these types of cancer, membranous, as well as cytoplasmic staining properties should be taken into account.

**Keywords** HER2 · Cancers of pancreas, and biliary tract · Survival

A. Ata (✉)
Department of Medical Oncology, Mersin State Hospital, Mersin, Turkey
e-mail: dralperata@yahoo.com

A. Polat · E. Serinsöz
Department of Pathology, Mersin University School of Medicine, Mersin, Turkey

A. Polat
e-mail: aysep@yahoo.com

E. Serinsöz
e-mail: ebruserinsoz@yahoo.co.uk

M. A. Sungur
Department of Biostatistics, Mersin University School of Medicine, Mersin, Turkey
e-mail: malisungur@yahoo.com

A. Arican
Department of Medical Oncology, Mersin University School of Medicine, Mersin, Turkey
e-mail: dr.arican@hotmail.com

## Introduction and Objective

In some cancers very important developments have been made, and mortality rates of these diseases decreased markedly [1, 2]. However, unfortunately, in cancers of pancreas, gall bladder, cholangiocellular, and ampullary region, therapeutical advances could not be achieved. The most important reason is that biological characteristics of these diseases have not been fully elucidated.

Human epidermal growth factor receptor 2 (HER2) oncogen, is a protooncogen located on the long arm of the 17. chromosome [3]. It secretes a protein with intracellular thyrosine kinase activity. Increased HER2 expression plays a role in epithelial cell growth, differentiation, and angiogenesis

[4]. Increased HER2 expression is associated with a worse prognosis especially in breast, and gastric cancers. Drugs inducing HER2 signal inhibition like trastuzumab, and lapatinib have demonstrated marked improvements especially in the treatment of breast cancer [5–7]. HER2 oncogen activity can be demonstrated using immunohistochemical methods. The basic principle of these methods is the affinity of monoclonal –polyclonal antibodies to HER2 protein which demonstrated an increased expression.

Increased HER2 expression is seen in 17–45 % of the pancreatic cancer patients [8]. Prognostic value of increasing rates of HER2 expression is still debatable. Some studies have associated this increase with lower survival rates, while others have advocated the opposite thesis [9–11].

Increased HER2 expression has been observed in a wide range (5–76 %) of the biliary tract cancer patients [12, 13]. In these patients any correlation between rate of HER2 expression, and prognosis can not be revealed so far [14, 15].

Increased HER2 expression has been observed in 6.1 % of the ampullary cancer patients [16]. In these patients any correlation between rate of HER2 expression, and prognosis can not be revealed.

The aim of this study is to demonstrate HER2 expression rate in cancers of pancreas, gall bladder, and biliary tract using immunohistochemical assays, and also assess its prognostic value. In this study we will try to enlighten different biological behaviours of these diseases with relatively worse survival data.

## Material and Method

Between May 2002–July 2011, a total of 69 patients with pancreas, gall bladder, cholangiocellular, and ampullary cancer were included in the study, in Mersin University Faculty of Medicine. Patients who lost to follow-up of our hospital for any reason, those with unavailable survival data, and inconclusive histopathological data, cases without any adenocarcinoma of pancreas, and biliary tract were excluded from the study.

Demographic data (age, gender), information about current, and past medical history, location, stage (based on AJCC criteria), and grade of the disease, treatment received, follow-up periods, progression, and survival of the disease were recorded. Using immunohistochemical assays performed with the aid of the department of pathology. HER2 expression rates were assessed in postoperative tissue samples or diagnostic biopsy specimens of patients with unresectable tumors.

All histopathological preparations of archival material stained with hematoxylin-eosin which belonged to biopsied or operated patients were re-evaluated. At least one block of each case containing especially both tumoral, and intact tissue was selected. Sections prepared from these blocks were stained immunohistochemically with HER2 antibody. These slide preparations were examined under microscope (Nikon Eclipse 80i) using similar magnifications by the same experienced pathologist. Microfilms were obtained using Nikon digital camera, DS-L1.

Five-micron-cuts obtained from paraffin blocks were subjected to deparaffinization, and fixation with alcohol for further examination. Then, Polyclonal Rabbit, HER2 antibody (Neomarkers, 1:40 dilution) was applied using Avidin Biotin complex immune peroxidase method. In immunohistochemical staining method, a biochemical analysis kit (Lab-Vision, Ultravision Large Volume Detection System Anti-Polyvalent, HRP, Ready to Use), and for background staining Meier hematoxylin dye were used. As a positive control, breast tumor whose positivity was previously established was utilized.

All surfaces of the cut sections were evaluated under microscope firstly at 40 X, and then 200 X, and 400 X magnifications. For scoring, scoring system used in gastric cancers was used as a basis [17]. This scoring system demonstrates some differences from that used in breast cancer [18]. In both scoring systems, membranous staining is evaluated in 4 categories as negative, weak, moderate, and strong.

In our study cytoplasmic staining were evaluated, too. In each case cytoplasmic staining, its extend, and intensity were estimated. As for extend of staining the cases were categorized as grade 0 (no tumoral staining), 1 (< 25 %), 2 (25–50 %), 3 (50–75 %), and 4 (>75 %) according to the percentage of the stained tumoral cells. Intensity of staining were graded as 0 (no staining), 1 (weak) 2 (moderate), and 3 (diffuse) staining intensity. For each case, total staining score was estimated by summing intensity, and density of staining. Total staining score was classified as weak (0–3 pts), moderate (4–5 pts), and diffuse (6–7 pts) staining.

### Statistical Analysis

Distribution of variables was analyzed using Shapiro-Wilks test, and for intergroup comparisons dependent on the distribution pattern one-way ANOVA or Kruskal-Wallis tests was used. To analyze the relationship among categorical variables, chi-square test was utilized. In the analysis of survival times Kaplan-Meier method was employed, and for their comparisons log-rank test was utilized. For the analysis of the relationship between continuous variables, and survival times a Cox-regression model was formulated. Statistical analyses were realized using SPSS v.11.5 package program, and results with a $p$ value of 0.05 was considered statistically significant.

## Results

The patients were analyzed according to their diagnoses in 4 groups as pancreatic, gall bladder, cholangiocellular, and

ampullary region cancers Among a total of 69 patients, pancreatic ($n$=30, 43.5 %), gall bladder ($n$=17; 24.6 %), cholangiocellular ($n$=12; 17.40 %), and ampullary region cancers ($n$=10; 14.5 %) were detected. Mean age of all patients was 65.02±10.37 years. Study population consisted of 31 (44.9 %) female, and 38 (55.1 %) male patients. Distribution of age, and gender of the patients according to the diseases is seen in Tables 1 and 2.

Diagnoses of the diseases were made in biopsied ($n$=26 patients; 37.7 %) or surgically excised ($n$=43; 62.3 %) specimens.

Median follow-up periods of the patients were also estimated as for pancreatic (5.5 months), gall bladder (10 months), cholangiocellular (5.5 months), and ampullary region (6 months) cancers. A statistically significant difference was not found as for the follow-up period ($p$=0.320).

Survival data of all patients were recorded. Ten (33.3 %) pancreatic, 3 (17.6 %) gall bladder, 5 (41.7 %) cholangiocellular, and 5 (50.0 %) ampullary region cancer patients were still alive at the termination of the study. Changes in survival times of the patients were evaluated in consideration of their disease states.

Median survival curve was analyzed, and median survival times for cancers of pancreas, gall bladder, cholangiocellular, and ampullary region were calculated as 10, 11, 8, and 33 months, respectively. Differences among survival times for the diseases were not statistically significant ($p$=0.980). However, though not statistically significant, ampullary region cancer patients had longer survival times. As a remarkable finding, a patient who had an unexpectedly longer survival time of 66 months influenced survival curve of our patients.

In all patients both membranous, and cytoplasmic staining patterns for HER2 were evaluated. In only 4 (5.7 %) patients positive membranous staining pattern was observed. Two (2.8 %) of these patients demonstrated 1 +, while the other 2 (2.8 %) patients 3+ staining intensity. Cancer cells demonstrated membranous staining pattern for HER2 oncoprotein in 3.38 % of pancreatic, 11.76 % of gall bladder, and 8.33 % of cholangiocellular cancer patients. In none of the ampullary region cancer patients, membranous staining pattern was observed. However because of scarcity of the patients with membranous staining patterns, healthy interpretations could not be made between these cases, and the patients in whom membranous staining pattern could not be displayed.

**Table 1** Demographic features of the patients (Ages)

| | n (%) | Mean±SD | Min-Max |
|---|---|---|---|
| Pancreatic Cancer | 30 (43,5) | 64,77±8,63 | 47-80 |
| Gallbladder Cancer | 17 (24,6) | 64,29±10,89 | 46-87 |
| Cholangiocellular Cancer | 12 (17,4) | 64,42±13,42 | 38-83 |
| Periampullary Cancer | 10 (14,5) | 67,80±11,54 | 44-81 |
| Total | 69 (100,0) | 65,03±10,38 | 38-87 |

Immunohistochemical cytoplasmic staining patterns were also evaluated for HER2 oncoprotein. Based on these fundamental principles, distribution of the patients according to cytoplasmic staining scores is seen in Table 3.

A statistically significant difference was not seen between cytoplasmic staining groups as for median survival rates ($p$>0.05). However patients in whom weak staining patterns were demonstrated lived longer than those with moderate, and strong staining characteristics with a statistically borderline significant ($p$=0.052) difference between these groups.

In weakly cytoplasmic stained group only lymphovascular invasion was less frequently observed in moderate and strongly stained groups ($p$=0.024). Apart from this parametre, any difference was not seen between groups as for other parametres ($p$>0,05). Besides, any association between tumor markers, and cytoplasmic staining characteristics could not be demonstrated ($p$>0,05).

## Discussion

After acknowledgement of clinical importance of increases in HER2 oncogen expression in breast cancer patients, similar investigations have been conducted in many other cancer types. However nowadays, only results obtained in beast and gastric cancer patients have been reflected on daily practice [19].

Immunohistochemically, HER2 oncoprotein can demonstrate two different staining patterns as membranous, and cytoplasmic. Demonstration of both higher importance of membranous staining rather than cytoplasmic staining in breast cancers, and lack of specificity of cytoplasmic staining has led to the establishment of immunohistochemical assessment standards for membranous staining. However clinical importance of cytoplasmic staining both in breast, and other types of cancer has been also claimed [20–24].

In some studies which also included pancreatic cancer patients, an association between malign behaviour of the disease, and HER2 expression rate has been demonstrated [25, 26]. However Bergman et al. demonstrated lack of increased HER2 expression in patients with anaplastic pancreatic cancer with an agressive course, and worse prognosis, and contrary to other investigators claimed lack of any correlation between malign behavioral pattern, and HER2 expression [27]. In another study, authors asserted that rate of MUC4 expression in pancreatic cancer increases, and it also plays a role in the pathogenesis of this cancer type. As has been demonstrated in various studies, MUC4 occupies the same location on the cell surface as HER2, and it is influenced by HER2 activity [28].

**Table 2** Demographic features of the patients (Sex)

| | Pancreatic Cancer | Gallbladder Cancer | Cholangiocellular Cancer | Periampullary Cancer | Total |
|---|---|---|---|---|---|
| Female | | | | | |
| n | 8, | 13, | 6, | 4, | 31, |
| Sex % | 25,8 %, | 41,9 %, | 19,4 %, | 12,9 %, | 100,0 %, |
| Disease % | 26,7 % | 76,5 % | 50,0 % | 40,0 % | 44,9 % |
| Male | | | | | |
| n | | 4, | 6, | 6, | 38, |
| Sex % | 22, | 10,5 %, | 15,8 %, | 15,8 %, | 100,0 %, |
| Disease % | 57,9 %, 73,3 % | 23,5 % | 50,0 % | 60,0 % | 55,1 % |
| Total | | | | | |
| n | 30, | 17, | 12, | 10, | 69, |
| Sex % | 43,5 %, | 24,6 %, | 17,4 %, | 14,5 %, | 100,0 %, |
| Disease % | 100,0 % | 100,0 % | 100,0 % | 100,0 % | 100,0 % |

Firstly in 1995, an association between increased rate of HER2 expression, and worse prognosis was claimed to exist in pancreatic cancer patients. Cases with ampulla vateri tumors were also included in this investigation [9]. Similarly, Komoto et al. accepted increased HER2 expression as an independent worse prognostic parameter for pancreatic cancers [12].

However some studies have suggested lack of any correlation between increase in HER2 expression, and prognosis in pancreatic cancer patients. In a separate study which detected a negative linear correlation between increase in HER2 oncoprotein expression, and grade of the tumor, could not reveal any association with the stage of the cancer [29]. Another study also found similar results, and could not detect a relationship between increased HER2 amplification, and stage of the disease or survival rates [30].

In patients with gall bladder cancers, HER2 expression was investigated using immunohistochemical methods, and increased HER2 expression was correlated with increased tumor size (T), but its relationship with survival rates could not be demonstrated [13, 31].

Despite contrary opinions increases in HER2 expression have been claimed to play a role in the pathogenesis of cholangiocellular cancer starting from the first stages of the disease [16, 32]. Prognostic role of increased HER2 expression in cholangiocellular carcinomas is still debatable [14, 33, 34]. Therefore, many authors advocated that routine HER2 testing in biliary tract cancers is not a must, and suggested that HER2 status does not alter treatment modalities. However, as demonstrated in biliary tract cancer cell cultures, favourable outcomes have been obtained by EGFR/HER2 receptor blocker monotherapy or its combination with gemcitabine. Especially in cholangiocellular cancer cell cultures, their beneficial effects were more prominent [35].

A summary of the studies published so far which have investigated the correlation between characteristic features of pancreatic, cholecystic, cholangiocellular, and ampullary region cancers, and HER2 oncoprotein is presented in Table 4.

As seen in our review of the literature, HER2 status varies greatly in pancreatic, and biliary tract cancers. Evaluation of our study results has displayed that incidence of cancers with HER2 positive membranous staining patterns are lower than those published for pancreatic cancer, but in accordance with literature as for gall bladder, and cholgiocellular cancers. In none of the ampullary region cancer patients included in our study increased HER2 expression could be demonstrated. In the largest series in the literature, Baumhoer et al. had shown amplification of FISH, and HER2 genes in 5 of 82 patients with ampulla vateri cancers [16].

**Table 3** Distribution of the patients with cytoplasmic staining with HER. (There is no statistical significant difference between areas of the cancer, $p$=0,963.)

| Disease | Cytoplasmic Staining | | | |
|---|---|---|---|---|
| | Weak | Moderate | Diffuse | Total |
| Pancreatic Cancer | 9 (30,0 %) | 10 (33,3 %) | 11 (36,7 %) | 30 (100,0 %) |
| Gallbladder Cancer | 4 (23,5 %) | 5 (29,4 %) | 8 (47,1 %) | 17 (100,0 %) |
| Cholangiocellular Cancer | 3 (16,7 %) | 4 (33,3 % | 6 (50,0 %) | 12 (100,0 %) |
| Periampullary Cancer | 1 (20,0 %) | 3 (30,0 %) | 5 (50,0 %) | 10 (100,0 %) |
| Total | 17 (24,6 %) | 22 (31,9 %) | 30 (43,5 %) | 69 (100,0 %) |

**Table 4** Studies published which have investigated the correlation between characteristic features of pancreatic, gallbladder, cholangiocellular, and periampullary cancers, and HER2 oncoprotein (IHC; immunohistochemistry, FISH; fluorescence in situ hybridization, CISH; chromogenic in situ hybridization)

| Study, year | Area of cancer | Number of patients (n) | Staining pattern | HER2 staining (%) | Method | Result/Conclusion |
|---|---|---|---|---|---|---|
| Lei, 1995 [9] | Pancreatic, Periampullary | 21 | Membranous | 47,6, 33,3 | IHC | İncreased HER2 expression is correlated with poor prognosis |
| Tsiambas, 2006 [29] | Pancreatic | 50 | Membranous | 16,0 | IHC CISH | İncreased HER2 expression is correlated with poor tumoral differantiation |
| Sharif, 2008 [30] | Pancreatic | 63 | | 26,0 | FISH | İncreased HER2 expression is NOT correlated with poor prognosis |
| Komoto, 2009 [11] | Pancreatic | 129 | Membranous | 61,7 | IHC | İncreased HER2 expression is correlated with poor prognosis |
| Puhalla, 2007 [13] | Gallbladder | 55 | Membranous | 13,0 | IHC | İncreased HER2 expression is correlated with T score but NOT poor prognosis |
| Baumhoer, 2008 [16] | Periampullary | 82 | | 6,0 | FISH | İncreased HER2 expression is NOT different between benign and malignant lesions |
| Kaufman, 2008 [31] | Gallbladder | 16 | Membranous | 6,2 | IHC | İncreased HER2 expression is correlated with higher survival rates |
| Yoshikawa, 2008 [14] | Cholangiocellular (intrahepatic and extrahepatic) | 236 | Membranous | 0,9 and 8,5 | IHC | İncreased HER2 expression is correlated with poor tumoral differantiation |
| Endo, 2002 [33] | Cholangiocellular | 71 | Membranous | 30,0 | IHC | İncreased HER2 expression is correlated with stage of the disease |
| Nakazawa, 2005 [34] | Gallbladder, Cholangiocellular, Periampullary | 221 | Membranous | 15.7, 5.1 and 11.5 | IHC FISH | HER2 positivity is a target of therapy of these disease |
| Harder, 2009 [15] | Gallbladder and Cholangiocellular (intrahepatic and extrahepatic) | 124 | Membranous | 20,2 (all) | IHC FISH | İncreased HER2 expression is NOT correlated with differantiation, stage and response to chemotherapy |
| Pignochino, 2010 [35] | Gallbladder and Cholangiocellular, (extrahepatic) | 49 | Membranous | 10, 0 and 26,5 | IHC FISH | HER2 positivity is a target of therapy of these disease |
| Shafizadeh, 2010 [38] | Gallbladder and Cholangiocellular | 51 | Membranous | 4,0 (all) | IHC FISH | İncreased HER2 expression is correlated with poor tumoral differantiation |

İmmunohistochemical methods are mostly used in the evaluation of increased HER2 expression. However, immunohistochemical assessments can be influenced from sensitivity, and specificity of antibodies used, tissue types (frozen or fixed by formalin) and scoring methods which show individual differences [36]. If considered carefully, different antibodies have been used in various studies which is the most important reason for detection of such a wide spectrum of increases reported in the literature for HER2 expression. As a solution for these discrepancies, we can advise compliance with the recommendations proposed by ASCO for breast cancer [18]. One of the difficulties encountered in immunohistochemical studies is generally very small sizes of tumoral tissue specimens examined for HER2 expression. This issue is more prominent especially in patients diagnosed based on biopsized specimens. In fact, it is noteworthy that almost all of our patients who demonstrated membranous staining patterns were diagnosed based on examination of surgical specimens, rather than biopsized material

Strong membranous staining was observed in patients with gall bladder cancers. These patients had also demonstrated strong cytoplasmic staining intensity which is a recognized characteristics especially for breast cancers [37]. It is not possible to arrive at statistical conclusions because of scarcity of our patients demonstrating membranous staining. Therefore healthy interpretations could not be made predominantly about survival rates, and also other parametres as TNM state, vascular-neural invasion, and tumor markers. However we want to stress that none of our patients with membranous staining patterns had any metastatic disease

In recent years, prognostic value of staining of intracellular part of HER2 oncoprotein (ie. cytoplasmic staining) has been demonstrated in bladder, colon, pancreas, thyroid, and nasopharynx carcinomas [20–24]. As seen in Fig. 1, though with statistically borderline significance ($p$=0.052), in patients who demonstrated weak cytoplasmic staining had longer survival times relative to those with moderate and strong staining intensity. We couldn't find any association between cytoplasmic staining characteristics, age, gender, TNM state, and tumor markers. We only observed that lymphovascular invasion was more rarely seen in weakly stained group when compared with those moderately, and strongly stained groups ($p$=0,024). As far as we know, any literature study has not investigated cytoplasmic staining pattern of HER2 in cancers of pancreas, biliary tract, and ampullary region so far.

In this study we investigated the incidence, and the impact of increased HER2 expression in cancers of pancreas, biliary tract, and ampullary region on survival. In a very small proportion of our study population, strong membranous staining was observed. Due to scarcity of patients, we couldn't demonstrate any association of the characteristic features of HER2 staining with the survival rates. When we investigated cytoplasmic staining feature for HER2, although with borderline statistical significance, we have seen that patients with lower scores had longer survival times relative to those with moderate, and strong staining intensities.

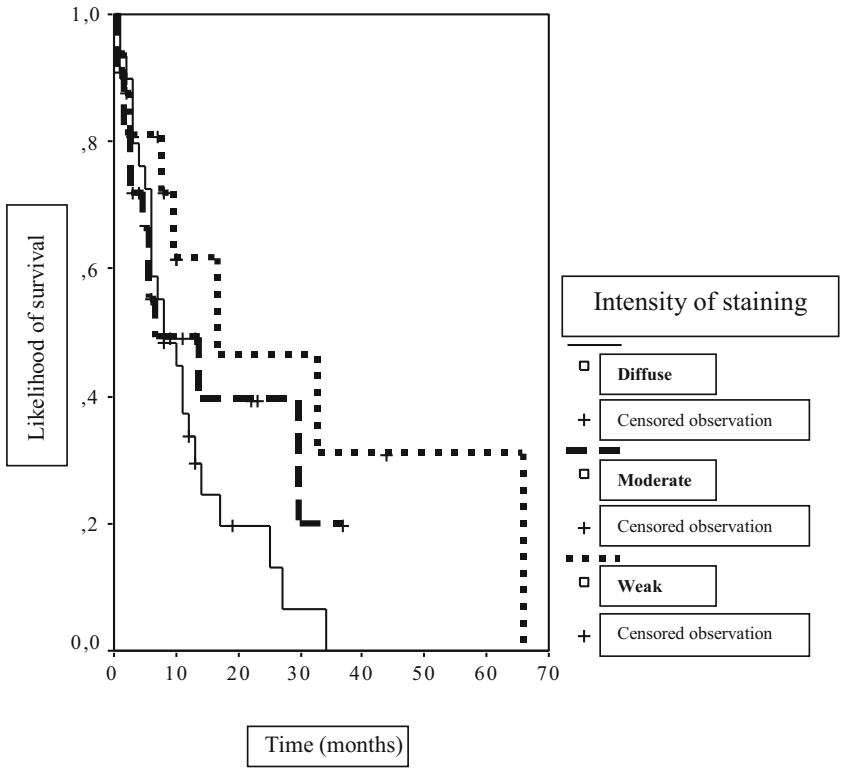


**Fig. 1** Survival curve of all patients based on their cytoplasmic staining characteristics

In conclusion, before initiation of pharmaceutical investigations, further studies performed with innovative laboratory methods analyzing gene expressions in larger patient series are required. However under the light of our study, and literature findings, we think that differences in biological behaviours of these cancers with relatively shorter survival times have not been elucidated with current level of knowledge.

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