Long-Term Survival after Radical Resection for Pancreatic Head and Ampullary Cancer: A Potential Role for the EGF-R

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Key Words

Pancreatic head cancer · Ampullary cancer · Survival, ampullary/pancreatic head cancers · Prognostic factors · Epithelial growth factor receptor · C-myc · Tissue microarray

Abstract

Background/Aim: Pancreatic cancer has a dismal prognosis. Ampullary cancer (defined as cancer of the ampulla of Vater or the distal common bile duct) has a better prognosis and is thought to be a biologically different tumor. The aim of this study was to find factors that could predict survival after radical (R-0) resection for pancreatic head and ampullary cancers. **Methods:** We analyzed clinical and pathological data from 93 patients who underwent a true R-0 resection for pancreatic head or ampullary cancer. Furthermore, we performed a tissue microarray protein expression analysis for several growth factor receptors and oncogenes: HER-2, EGF-R, ER, PR, C-myc, p53, p16, RB-1, and chromogranin A as a neuroendocrine differentiation marker. **Results:** Median survival (14 vs. 42 months) and time to recurrence (16 vs. 42 months) were significantly longer for ampullary than for pancreatic head cancers. Preoperative pain, perineural invasion, lymph node metastasis, and tumor differentiation grade are indicators of a poor survival. No differences in protein expression were found between groups, except for

EGF-R which was expressed more in pancreatic head cancers ($p = 0.026$). **Conclusions:** Outcomes for ampullary cancers are better than for pancreatic head cancers. This different biological behavior can possibly be explained by differences in EGF-R expression.

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Introduction

Of all gastrointestinal malignancies, pancreatic cancer has the poorest prognosis, with a 5-year survival of less than 5% [1, 2]. Men are more frequently affected by this disease than women (relative risk 1.5) [2]. Ampullary cancers, however, carry a better prognosis [3]. This evident difference may be due to earlier clinical presentation or different biological behavior. Curative resection offers the only chance of cure, but is possible in only 10% of the patients [4]. Adjuvant chemotherapy and chemoradiotherapy after curative resection are of limited value [5]. Even after a macroscopically radical resection (R-0), distant micrometastases probably already exist [6], and tumor cells are often observed at one or more edges of the resected specimen (R-1) [7, 8]. Reported relevant prognostic variables for survival after resection are: tumor size, lymph node metastasis, histological differentiation, and resection status [9–12]. Most studies include R-0 as

well as nonradical (R-1/R-2) resections. To eliminate the apparent effect of R-1 status on (local) recurrence and survival, it would be interesting to evaluate true R-0 resections only.

The aim of this study was to determine clinical, histological, and molecular factors that could predict recurrence and survival after R-0 resection. Special focus is on the differences between ampullary (defined as cancer of the ampulla of Vater or distal common bile duct) and pancreatic head cancers. Additionally a tissue microarray (TMA) analysis of resected tumors (R-0) was added in order to search for relevant molecular factors [13, 14]. For immunohistochemical staining, we selected common antibodies raised against proteins that are frequently expressed in (pancreatic and ampullary) cancers such as: retinoblastoma (RB-1) [15, 16], p16 [17], C-myc [18], and p53 [17]. Potential targets for therapy: HER-2 [19] and epithelial growth factor receptor (EGF-R) [20]. Potential markers for a male/female difference: estrogen receptor (ER) and progesterone receptor (PR) [21]. Chromogranin A was used as a neuroendocrine differentiation marker.

Materials and Methods

From a consecutive series of 176 resections for pancreatic head and ampullary adenocarcinomas, specimens were revisited. For the purpose of this study we defined ampullary cancer as cancer of ampulla of Vater and distal common bile duct. Duodenal tumors were not included. Pancreatic cancers adjacent or close to the ampulla were classified as pancreatic head cancer. R-0 was defined as absence of macroscopic and microscopic residual tumor >1 mm from the margin of the resection specimen. Even if there were lymph node metastases restricted to the resected area, the resection was considered R-0. Seventy patients had an R-1 and 6 patients an R-2 resection. In 7 cases the pathologist was not able to define whether a true R-0 resection was performed. Therefore, 93 patients were evaluated in this study.

One pathologist reviewed all specimens of R-0 resected tumors. Histological differentiation, tumor size (T), location and extent, as well as vasoinvasive growth (small blood vessels), perineural invasive growth, and lymph node metastasis (N) were assessed. Staging was performed according to the UICC classification, 2002 [22].

Preoperative data, including age, gender, weight loss, pain (back pain and pain in the epigastric region), jaundice, and diabetes mellitus, were obtained from the clinical records.

Preoperative Staging and Surgical Techniques

All patients underwent conventional ultrasonography and/or computed tomographic scanning. Most patients (76%) underwent endoscopic retrograde cholangiopancreatography and subsequent preoperative biliary drainage using endoprotheses. After excluding extraregional and distant metastases, an estimation of the resectability was made by judging the involvement of com-

mon hepatic artery and superior mesenteric vein and portal vein. Dissection of the pancreas was to the left of superior mesenteric vein/portal vein. Histological examination of the frozen section of the remaining pancreatic surface was performed. In case of a standard Whipple (SW) resection, one third of the stomach was also resected. Only tumors without macroscopic infiltration of the postpyloric duodenum and in absence of positive lymph nodes along the pylorus were treated by a pylorus-preserving pancreaticoduodenectomy (PPPD). Lymph nodes were dissected on the right-hand side of superior mesenteric vein/superior mesenteric artery up to the celiac trunk and in the hepatoduodenal ligament along the common hepatic artery. Perioperative data included type of operation performed (PPPD or SW), blood loss (ml), and transfusions (units). Postoperative data included complications, hospital stay, and adjuvant therapy (5-FU and radiotherapy).

Tissue Microarray

We identified 75 patients for whom paraffin blocks were available. In all other cases paraffin blocks were either lost or insufficient. The TMA was constructed as described by Kononen et al. [13]. For each carcinoma, we prepared three tissue cores of 0.6 mm in diameter from the paraffin tissue block to ensure adequate representation of the neoplastic cells. The tissue cores from each carcinoma were then mounted in linear arrays in a paraffin TMA block. Tissue cores from various organs (pancreas, duodenum, and gallbladder) were used as controls, for orientation purposes, and to estimate background labeling for each of the immunohistochemical markers. Immunohistochemistry labeling was performed according to standard protocols. In brief, 4- μ m sections for each TMA were transferred to StarFrost® slides (Starfrost, Berlin, Germany), and immunostaining was performed using the UltraVision Large Volume Detection System Anti-Polyvalent, HRP (Lab Vision, Fremont, Calif., USA) after deparaffinization. Microwave (700 W) pretreatment was performed for 15 min using citrate buffer (100 mM citric monohydrate, pH 6.0). Antibodies (clone) used for immunostaining were ER- α (1D5), PR (PgR636), p16 (E6H4), p53 (DO-7), RB-1 (RB-1), EGF-R (H11), and HER2 (C-erbB-2) (DAKO, Glostrup, Denmark); chromogranin A (LK2H10) (Biogenex, San Ramon, Calif., USA), and C-myc (9E10) (Santa Cruz Biotechnology, Santa Cruz, Calif., USA).

TMA Scoring Strategy

A pathologist and an experienced analyst examined the TMA slides for each immunohistochemical marker with a multiobserver microscope. Both observers were blinded to the type of cancer and its location on the slide. Only moderate or strong labeling was scored as positive. Weak or 'blush' labeling was ignored. A two-tiered scheme (positive or negative) was used for scoring the TMA. A proportion exceeding 1% of positive cells was regarded as protein overexpression. A negative or positive score for each carcinoma was determined after examining the three tissue cores. Focal and diffuse positive scores were combined for the sake of statistical analysis.

Statistics

Survival analysis consisted of overall survival and time to recurrence. Curves were calculated by the Kaplan-Meier method, followed by log-rank tests. Factors, which showed to be significant in the univariate analysis, were entered into a multivariate Cox proportional hazards model to evaluate their independent prog-

Table 1. Group comparisons

	Factor positive (%)			p (Fisher's exact t test)
	head (n = 56)	ampullary (n = 37)	total (n = 93)	
<i>Preoperative factors</i>				
Male sex	37 (66)	19 (51)	37 (39)	0.191
Age, years	63	63	63	0.993
Pain	31 (55)	20 (56)	51 (55)	1
Jaundice	49 (88)	30 (83)	79 (86)	0.76
Pruritus	13 (23)	15 (44)	28 (31)	0.059
Weight loss	45 (80)	28 (78)	73 (79)	0.796
>5 kg	23 (51)	18 (64)	41 (56)	0.335
Obstruction	5 (9)	5 (14)	10 (11)	0.505
Diabetes	10 (18)	8 (22)	18 (20)	0.603
Endoprosthesis	44 (79)	27 (71)	71 (78)	1
<i>Perioperative factors</i>				
PPPD	28 (50)	18 (49)	46 (50)	1
PV resection	1 (2)	0	1 (1)	1
SMA resection	0	1 (3)	1 (1)	0.398
Blood loss, ml	2,700	1,950	2,400	0.028 ^a
>1 liter	5 (9)	6 (18)	11 (13)	0.324
Transfusions, U	2.4	1.5	1.2	0.038 ^a
Transfused patients	22 (41)	7 (21)	29 (33)	0.064
Complications	24 (44)	20 (54)	44 (48)	0.396
Leakage pancreaticojejunostomy			5 (5)	
Pancreatic fistula			6 (6)	
Leakage biliary anastomosis			1 (1)	
Abdominal abscess			11 (12)	
Minor			17 (18)	
Mortality			2 (2)	
Hospitalization, months	28	21		0.048 ^a
Radiation/5-Fu	19 (34)	9 (24)	28 (30)	0.363
<i>Pathological factors</i>				
T1	7 (12)	8 (22)		not tested
T2	15 (27)	16 (43)		not tested
T3	34 (61)	13 (35)		not tested
Diameter <2 cm	23 (41)	28 (76)	51 (55)	0.001
N	29 (52)	26 (70)	55 (59.1)	0.088
M	0	0	0	
G1	6 (11)	7 (19)		0.509 ^b
G2	43 (79)	25 (68)		
G3	7 (12)	5 (13)		
R-0 resection	56 (100)	37 (100)		
Perineural invasion	31 (55)	7 (19)	38 (41)	0.001
Vasoinvasive	11 (20)	5 (14)	16 (17)	0.579
<i>Survival</i>				
Recurrences	41 (73)	26 (70)	67 (72)	0.816
Local	7 (18)	6 (26)		0.497 ^b
Distant	18 (46)	8 (35)		
Both	12 (31)	9 (39)		
Deaths	51 (91)	28 (76)	79 (85)	0.073
Median survival, months	142	42		0.001 ^c
Median time to recurrence, months	16	34		0.024 ^c

	Factor positive (%)			p (Fisher's exact t test)
	head (n = 56)	ampullary (n = 37)	total (n = 93)	
<i>Tissue microarray</i>				
ER-α	0 (0)	1 (3.7)	1 (1)	0.365
PR	5 (11)	1 (3.7)	6 (80)	0.406
p16	10 (21)	9 (33)	19 (25)	0.28
p53	9 (20)	5 (19)	14 (19)	1
RB-1	28 (62)	20 (77)	48 (64)	0.293
C-myc	19 (41)	16 (59)	35 (47)	0.154
Chromogranin A	10 (22)	6 (22)	16 (21)	1
EGF-R	11 (24)	1 (3.7)	12 (16)	0.026
HER-2	4 (9)	3 (11)	7 (9)	1

PPPD = Pylorus-preserving pancreaticoduodenectomy; PV = portal vein; SMA = superior mesenteric artery; T = primary tumor; N = positive lymph nodes; M = metastasis; G = differentiation grade.

^a t test; ^b chi-square for 3 × 2 tables; ^c log-rank test.

nostic value by backward elimination. To determine whether factors differed between patients with ampullary versus pancreatic head carcinomas, appropriate interaction terms were used. p (two-sided) < 0.05 was considered statistically significant. All calculations were performed using SPSS version 13.0 (SPSS, Chicago, Ill., USA).

Results

Survival

The follow-up period was more than 10 (median 12) years. Median survival and time to recurrence were significantly longer for ampullary than for pancreatic head cancers (14 and 42 months, p = 0.001, and 16 and 34 months, p = 0.024, respectively; table 1). Survival curves are shown in figure 1. There were no differences between groups with regard to site of recurrence (p = 0.497).

Preoperative Factors

No differences in preoperative factors were observed between both groups. Survival was better for women than for men in the pancreatic head cancer group, but not in the ampullary cancer group. Preoperative abdominal and back pain significantly influenced survival and time to recurrence for both groups. Jaundice was a predictor of survival and time to recurrence in ampullary cancers. Weight loss and diabetes mellitus did not influence survival (tables 1, 2).

Table 2. Univariate analysis

Factor positive	Direction of effect	Relation to survival			Time to recurrence		
		head (n = 56)	ampullary (n = 37)	pooled ^a (n = 93)	head (n = 56)	ampullary (n = 37)	pooled ^a (n = 93)
Male sex	–	0.028	0.688	0.046	0.072	0.614	0.086
Pain	–	0.018	0.001	0.000	0.006	0.000	0.000
Jaundice	+	0.364	0.029	0.044	0.773	0.017	0.097
N	–	0.064	0.013	0.004	0.016	0.006	0.000
G	–	0.034	0.624	0.012	0.046	0.384	0.012
Perineural invasion	–	0.273	0.001	0.025	0.053	0.000	0.001
C-myc	+	0.034	0.968	0.078	0.015	0.858	0.038
EGF-R	–	0.084	not tested	0.080	0.037	not tested	0.042

Pain = Preoperative back pain and abdominal pain; N = positive lymph nodes; G = differentiation grade.
^aTest for trend.

Fig. 1. Kaplan-Meier survival curves for ampullary and pancreatic head cancers ($p = 0.001$, log-rank test).

At risk	Time (months)							
	12	24	36	48	60	72	84	120
Pancreatic head (n = 56)	31	22	15	11	10	10	5	3
Ampullary (n = 37)	32	27	19	16	14	13	13	10

Perioperative Findings

The surgical technique used (PPPD vs. SW) did not correlate with survival ($p = 1.000$). Blood losses were higher and blood transfusions (units) more often required in the pancreatic head cancer group ($p = 0.028$ and

0.038). Hospitalization was longer for pancreatic head cancers ($p = 0.048$). However, these factors did not influence survival (tables 1, 2).

Mortality and Morbidity

Two patients (2%) died postoperatively because of aspiration pneumonia and sepsis. These patients remained in our analysis because of the intent-to-treat principle. Postoperative complications occurred in 44 cases (48%). In 5 patients (5%), leakage of the pancreaticojejunostomy was seen, defined as the presence of amylase in drainage or abdominal fluid ($>3 \times$ serum concentration). Six patients developed a pancreaticocutaneous fistula. Leakage of the biliary anastomosis occurred in 1 patient. Eleven patients (12%) had an intra-abdominal abscess. Minor complications were found in 17 patients (18%). Complications did not influence survival or time to recurrence (tables 1, 2).

Adjuvant Therapy

Adjuvant therapy (5-FU and radiotherapy) was given to 28 patients, distributed evenly between both groups ($p = 0.363$; table 1). Survival data are omitted because of bias. These patients participated in a trial, in which we compared the effects of chemoradiation with those of surgery alone [3].

Pathological Factors

Pancreatic head tumors were larger ($p = 0.001$) than ampullary tumors. Perineural invasion was also significantly more often observed in this group ($p = 0.001$).

The strongest negative histological factor for survival and time to recurrence was lymph node involvement in

Fig. 2. Kaplan-Meier curves for EGF-R expression in pancreatic head cancers, time to recurrence ($p = 0.037$, log-rank test).

At risk		Time (months)							
		12	24	36	48	60	72	84	120
Positive	(n = 11)	5	2	1	0				
Negative	(n = 35)	19	16	9	8	8	6	4	2

the resected specimen. Tumor diameter as well as extent of the tumor did not influence survival. Histological grading was correlated with survival and time to recurrence for pancreatic head cancers. Invasive growth into intrapancreatic perineural tissue significantly influenced survival ($p = 0.001$) and time to recurrence ($p = 0.000$) in ampullary cancers. Tumor invasion of small surrounding blood vessels was not correlated with survival (tables 1, 2).

Tissue Microarray

The overall failure rate secondary to lack of interpretable neoplastic tissue was 3%. In all other cases in whom cores were without malignant glands, interpretation of the labeling pattern was possible with the remaining tissue cores from the same carcinoma. Although histologically very similar, adenocarcinomas of the ampullary region are thought to be biologically different from pancreatic ductal adenocarcinomas. However, for most tested proteins, the labeling was not significantly different between both groups. Interestingly, EGF-R is expressed more frequently in pancreatic head than in ampullary cancers ($p = 0.026$). For pancreatic head cancers, EGF-R

Fig. 3. Kaplan-Meier survival curves for C-myc expression in pancreatic head cancers ($p = 0.034$, log-rank test).

At risk		Time (months)							
		12	24	36	48	60	72	84	120
Positive	(n = 19)	14	10	7	5	5	4	2	2
Negative	(n = 27)	15	9	5	3	2	2	2	0

overexpression is an indicator for a shorter time to recurrence ($p = 0.037$; fig. 2). A trend was observed towards a poor survival (median 14 vs. 25 months; $p = 0.084$). Furthermore, no long-term survivors were observed in the EGF-R-positive group. C-myc overexpression is correlated with improved survival ($p = 0.034$; fig. 3) and longer time to recurrence ($p = 0.015$). It is clear that these last two factors are of significance for pancreatic head cancers, but not for ampullary cancers (tables 1, 2).

Multivariate Analysis

All factors that were significant in the univariate analysis were entered into a Cox regression model. Independent prognostic factors for survival for the pancreatic head group were: preoperative back pain and abdominal pain (B_{exp} : 0.548/ $p = 0.05$) and differentiation grade (2.004/0.016). No independent prognostic factors were found for ampullary cancers.

For time to recurrence independent prognostic factors for the pancreatic head group were: preoperative back pain and abdominal pain (0.446/0.015), positive lymph nodes (0.491/0.033), and EGF-R overexpression (0.448/0.034). Independent prognostic factors for

ampullary cancers were preoperative back pain and abdominal pain (0.224/0.007) and perineural invasion (0.199/0.031).

Discussion

Median survival and time to recurrence after R-0 resection were significantly longer for ampullary than for pancreatic head cancers. This evident difference may be due to earlier clinical presentation or different biological behavior. We found no differences in preoperative factors between both groups.

Preoperative back pain and abdominal pain was found in 51 patients (55%). It was also an independent negative prognostic factor for time to recurrence for both groups and for survival in univariate analysis. We further found that intrapancreatic perineural invasion was a negative prognostic factor for survival (not for pancreatic head cancers, $p = 0.273$) and time to recurrence in the univariate analysis (both groups). Preoperative pain as a negative prognostic factor has been reported earlier by Ridder and Klempnauer [23] and Okusaka et al. [24]. Forty-one percent of the patients with preoperative pain had intrapancreatic perineural growth, and 55% of the patients with perineural growth had preoperative pain. These findings suggest that pain is not always caused by intrapancreatic perineural growth and vice versa. Indeed, pain is usually interpreted as resulting from tumor infiltration into extrapancreatic (retropancreatic) splanchnic nerves and thus may indicate advanced tumor growth beyond the borders of the pancreas.

In the present study, 72% of all patients had recurrence of their cancer, and almost half of all recurrences were local (table 1). This is similar to previously reported rates in two large trials on adjuvant therapy done by Klinkenbijl et al. [3] and Neoptolemos et al. [25]. Although both studies included only 18–22% R-1 resections, the reported rate of recurrence was approximately 70%, and 37–52% of these recurrences were local.

Possibly our definition of R-0 resection may have included some ‘irradical’ resections. Verbeke et al. [26] suggested that R-1 resection can be underestimated, when the pathological examination is not completely standardized. These authors compared pathological examinations for two consecutive periods with and without a highly standardized protocol. The number of R-0 resections for pancreatic cancer during the standardized period was lower than during the nonstandardized period ($p = 0.009$). Interestingly, long-term survival for pancreatic

cancer was not predicted by resection status in either cohort. A similar observation was made by Neoptolemos et al. [12, 25] in a subanalysis of their trial on adjuvant therapy. They found that the resection status was not an independent prognostic factor for survival. Only after omission of nodal status and differentiation grade, the resection status became significant [12, 25].

It is intuitive that R-1 resection is a predictor of a poor survival, and indeed long-term survivors are sparse after R-1 resection. Therefore, R-0 resection is often considered to be the most important factor predicting a favorable outcome. Consequently, extended resections, including extensive clearing of retroperitoneal soft tissue and lymphatic tissue, have been advocated. Several randomized controlled trials [27–29] were performed, but failed to show a survival benefit for extended surgery. In a recent study performed by Hishinuma et al. [30], 27 patients who had undergone extended resection were studied at autopsy. Most resections were R-0 (25/27), 3 patients died postoperatively, the majority died of metastatic disease, and only 4 patients died due to local recurrence. Evidence of local recurrence was found in 18 of 24 patients (75%) [30]. This study clearly shows that even after extended surgery, local recurrences are still frequent. Possibly R-0 and R-1 resections are both accompanied by (occult) metastatic disease. Indeed, in an overview of advanced molecular detection techniques [6], it was shown that tumor cells can be found pre- and perioperatively in peritoneal lavage fluids, liver, blood, ‘tumor-negative’ lymph nodes, and bone marrow of patients without ‘conventional’ evidence of metastatic disease. As a result, R-0 resection offers an opportunity for long-term survival for a limited number of patients. However, in the majority of the cases, resection cannot provide cure, and recurrence remains imminent. This further emphasizes the importance of additional prognostic parameters to predict outcomes after ‘R-0’ (‘R < 1’) resection and to select patients in need of (aggressive) systemic adjuvant therapy.

The TMA technique proved to be a simple, efficient, and relatively inexpensive method to explore the protein expression in large groups of patients. Our failure rate of 3% is acceptable and did not cause statistical problems. The expression of p53 and p16 proteins is well known in pancreatic cancer [17]. Nevertheless, their prognostic value remains unclear. We found a similar expression of p16 (21–33%) (table 1) to that reported in previous studies (13–59%) [17]. The expression of p53 in our study was lower (19–20%) than previously reported (35–69%) [17]. This may be due to our techniques or a selection bias in our

group (R-0). RB-1 protein expression has been reported previously in tissue and cell lines, the exact prevalence, however, was unclear [31, 32]. We found RB-1 to be positive in 62–77% of the cases. C-myc, a proto-oncogene, expression has been previously reported [18]. We found improved survival for patients with overexpression of this protein. This is counterintuitive, since, as a known proto-oncogene, it is thought to promote oncogenesis and tumor growth. Alternatively, C-myc expression is thought to be an early step [18] in the rapid oncogenesis of pancreatic cancer [33] and may be an indicator of a relatively early point in tumor progression. Hypothetically, this implicates an early-stage disease and thus a less aggressive nature. Nevertheless, the exact role of C-myc remains unclear. We found 22% of the cancers to be positive for chromogranin A, a neuroendocrine differentiation marker. This is similar to the previous report on neuroendocrine differentiation by Tezel et al. [34]. However, these authors did not find any chromogranin A-positive tumors in their specimens, in contrast to 18–36% expression of other neuroendocrine markers (NCAM, NSE, synaptophysin, CD57). This may be due to differences in antigen retrieval, immunohistochemical techniques, and/or antibody clones used. HER-2 was positive in only 9–11% of the cases and did not show a correlation with survival. This is comparable to previously published results (8–58%) [17].

EGF-R, a well-known growth factor receptor, was positive in ampullary cancers (4%), but significantly more often in pancreatic head cancers (24%; $p = 0.026$). This is in accordance with expression rates reported previously (28–68%) [17]. The difference in EGF-R expression between pancreatic head and ampullary cancers was previously suggested by Friess et al. [35]. Although their study did not provide matched clinical and survival data, they suggested a role for the EGF-R in the less favorable outcomes of pancreatic cancers. In our study, EGF-R overexpression was a negative prognostic factor for time to recurrence ($p = 0.037$) and possibly survival ($p = 0.084$). This effect on survival has been described previously in several studies, while others found no effect [17, 36]. For head and neck cancer, the EGF-R expression was shown to be correlated with survival and relapse [37]. In breast and colorectal cancers, however, no correlation was evident [38, 39].

In conclusion, the differences in biological behavior between pancreatic head and ampullary cancers could be confirmed in this study. The poor prognosis of patients with pancreatic head cancer can possibly be related to the increased EGF-R expression of these tumors as compared with ampullary cancers. Our data support the rationale to use drugs that have recently been designed to target the EGF-R selectively (Tarceva, Iressa, Erbitux) [40] in adjuvant therapy regimens.

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