10 Current Diagnosis and Future Impact of Micrometastases for Therapeutic Strategies in Adenocarcinoma of the Esophagus, Gastric Cardia, and Upper Gastric Third

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Abstract Esophageal and gastric cancers are aggressive neoplasms with a poor prognosis. Although postoperative mortality has declined and rates of complete resection have improved considerably, 5 year survival rates are still very low. Early metastatic relapse after complete resection of an apparently localized primary lesion indicates that disseminated tumor cells, undetectable by current methods, may already have been present at the time of surgery, even in patients with seemingly early tumor stages. Occult residual tumor disease is suggested when either bone marrow or lymph nodes from which tumor relapse may originate are affected by micrometastatic lesions undetectable by conventional histopathology. The presence of single tumor cells detected by immunohistological methods is increasingly regarded as a clinically relevant prognostic factor. The use of antibodies against tumor-associated targets enables detection of individual epithelial tumor cells in lymph nodes and in bone marrow in various tumor entities. The potential role and benefit of an antibody-based treatment as a therapeutic target would be of particular interest in tumors with a notoriously poor prognosis such as esophageal cancer and cardia cancer.

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10.1 Introduction

In recent decades, the incidence of esophageal adenocarcinoma in the United States and Western Europe has risen at a more rapid rate than any other malignant neoplasm (Blot et al. 1991; Devesa et al. 1998; Bytzer et al. 1999; Vizcaino et al. 2002; Botterweck et al. 2000; Blot and McLaughlin 1999; Trivers et al. 2008).

Gastric cancer is the second most common malignancy worldwide (Parkin et al. 2001; Parkin 2004), and surgical treatment remains the only curative management option (Sano et al. 2004).

The incidence of gastric cardia cancer has increased recently in the West, and this trend is in contrast with a decrease in more distal cancer (Jeon et al. 2006; Orengo et al. 2006; Walther et al. 2001).

According to the prevailing classification, three types of esophagogastric cancer are differentiated: type I is defined as adenocarcinoma in Barrett's esophagus as long as it develops

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within 1–5 cm proximal to the Z-Line. Type II is the "true" carcinoma of the cardia originating from the cardial mucosa. Type III is the subcardial or fundic carcinoma of the stomach infiltrating into the mucosa or submucosa of the distal esophagus (Siewert et al. 1987).

Barrett's dysplasia of the distal esophagus may be causative particularly in type I but may also exist concomitantly in the other types. The term "wanderer between two worlds" reflects the topographic pattern of cardia cancer between the thoracic and abdominal cavity.

Surgical treatment is controversial and varies widely as to the extent of esophageal and gastric resection. Treatment options therefore encompass esophagectomy, limited resection of the esophagogastric junction, esophagogastrectomy, and extended gastrectomy, hereby resulting in different levels of lymphatic clearance.

The importance of lymph node yield and ratio of afflicted lymph nodes with its prognostic relevance and stage migration of the tumors influenced by the surgical approach (transhiatal vs. thoracoabdominal) has been previously described by our own group (Bogoevski et al. 2008). The operative procedure depends on stage, exact localization of the primary tumor, and the patient's general condition.

Although surgical techniques have improved, the overall prognosis for patients remains poor primarily due to local recurrence and the development of distant metastases.

Stage, grade, and status of resection margins are currently accepted as the most accurate pathologic variables predicting survival.

However, even in patients with seemingly early tumors (T1, N0), tumor relapse may occur. This reflects the shortcomings of the current pathologic staging system to sufficiently discriminate patients with a high risk to develop tumor recurrence from those who carry a lower risk. Thus, effort continues to identify new prognosticators of tumor relapse that indicate the need for adjuvant therapy.

Occult residual tumor disease is suggested when either bone marrow or lymph nodes from which tumor relapse may originate are affected by micrometastatic lesions undetectable by conventional histopathology (Pantel and Brakenhoff 2004).

The use of antibodies against tumor-associated targets enables detection of individual epithelial tumor cells both in lymph nodes (Byrne et al. 1987; Passlick et al. 1994; Raymond and Leong 1989) and in bone marrow in various tumor entities (pancreas, breast etc) (Latza et al. 1990).

These immunohistochemical analyses have been accepted as an addendum in the last UICC classification for pancreatic, nonsmall lung, and esophageal cancer (Hermanek et al. 1999).

Nonetheless, the clinical significance of immunohistochemical assessment of nodal micrometastases (Izbicki et al. 1997; Hosch et al. 2001; Komukai et al. 2002; Waterman et al. 2004) is still controversial (Momburg et al. 1987; Pantel et al. 1994; Z'Graggen et al. 2001; Kasper et al. 1987; Bogoevski et al. 2004), e.g., due to putative sampling errors (Hermanek et al. 1999).

10.2 Incidence of Nodal Micrometastases

Results of current studies have shown that in patients with adenocarcinoma of the esophagus and esophagogastric junction, tumor cells can be detected by immunohistochemistry at a relatively high frequency in regional lymph nodes that have been judged to be "tumor-free" by routine histopathological methods.

Mueller et al. (2000) have shown that 42% of the patients with pN0 staged type II/III-tumors had detectable tumor cells in the regional lymph nodes by immunohistochemistry. Similar studies have been done in patients with gastric carcinoma, with rates from 23.5% in early gastric carcinoma to over 90% in more advanced stages (Maehara et al. 1996; Siewert et al. 1996).

Our group showed a 49% incidence of nodal microinvolvement in lymph nodes classified to

be "tumor-free" in conventional histopathology in patients with an adenocarcinoma of the esophagogastric junction (Schurr et al. 2006).

According to histopathology, lower mediastinal lymph node metastases were found in 24% of type I tumors and 10% of type II tumors. When positive disseminated tumor cells were additionally considered, mediastinal lymph node involvement increased to 40% in type I patients and 33% in type II patients. Similarly, in the paracardial and upper abdominal lymph node compartment a higher frequency of lymph node involvement was found by immunohistochemical staining.

The prevalence of nodal microinvolvement in esophageal cancer was first evaluated in both squamous cell carcinoma and adenocarcinoma by our group (Izbicki et al. 1997).

A total of 399 lymph nodes obtained from 68 patients were found to be free of tumor by routine histopathological analysis and were studied further for isolated tumor cells by immunohistochemical analysis with the monoclonal antiepithelial-cell antibody Ber-EP4. Of the 399 "tumor free" lymph nodes, 67 (17%), obtained from 42 of the 68 patients, contained Ber-EP4–positive tumor cells.

The incidence of nodal microinvolvement in patients with adenocarcinoma of the esophagus was higher in later (pT2/3 = 36%) than in earlier tumor stages (pT1 = 11%) (Koenig et al. 2009).

An important counter-argument challenging the reliability of immunohistochemical assays is that of sampling error.

Factors which might influence this are the number of lymph nodes dissected during the course of resection, the number of lymph nodes assessed by immunohistochemistry, the number of lymph node sections, and the level of these sections within the lymph nodes. Previously, several authors have suggested that the ratio of positive lymph nodes detected by conventional histopathology should be used for a refined pN staging in esophageal and gastric cancer (Koenig et al. 2009).

Apart from staging accuracy, the surgical impetus of radical lymphadenectomy is to remove the surrounding loco-regional soft and lymphatic tissue in the vicinity of the tumor. The importance of lymph node yield and ratio and its influence on stage migration, and therefore as a strong independent prognostic factor on survival, was previously described by our group (Bogoevski et al. 2008). It is not only that the global presence or absence of nodal involvement may serve as a tool for the differentiation of "high-risk" from "low-risk" patients, but also the ratio of immunohistochemically affected lymph nodes to the total number of lymph nodes seems to enable improved risk stratification of cancer patients.

However, extensive removal of the lymphatic tissue carries the ability to uncover the correct pN-status, and immunohistochemistry is a helpful tool to refine the risk stratification in these solid pathologies.

Neoadjuvant treatment modalities were developed to improve local tumor control as well as to reduce lymph node metastases and distant metastases. Prenzel et al. (2007) previously evaluated the influence of neoadjuvant chemoradiation on nodal microinvolvement. A total of 1,186 lymph nodes of 52 patients of both adenocarcinoma and squamous cell carcinoma were diagnosed as negative for metastases in routine histopathology. A major histopathologic response (<10% vital residual tumor cells) was shown by 42.3%, whereas in 30 tumors, only a minor response (>10% vital residual cells) was present. Major response was shown by 19 of 32 patients (59.4%) with pN0-status. Of these, only four patients had a nodal microinvolvement which was significantly reduced compared to those with minor response (9 of 13 patients). Due to the small number of patients in this setting, future studies will show whether this can be further confirmed.

10.3 Mode of Spread

Schurr et al. investigated the role of the mediastinal lymphadenectomy in carcinomas of the

Table 10.1 Positive Lymph Nodes in Histopathology (Hematoxylin and Eosin staining) and immunohistochemistry (Ber-Ep4þ cells) in the Mediastinal, Paracardial, and Upper Abdominal Lymph Nodes

pN0/pN1	No. of patients (%) (n=45 for Type I and n=40 for Type II)	
	Type I	Type II
	Mediastinal	
Histopathology	11 (24%)	4 (10%)
Histopathology and Ber-Ep4þ cells	18 (40%)	13 (33%)
	Paracardial	
Histopathology	15 (33%)	21 (53%)
Histopathology and Ber-Ep4þ cells	17 (38%)	35 (88%)
	Upper abdominal	
Histopathology	8 (17%)	16 (40%)
Histopathology and Ber-Ep4þ cells	13 (29%)	17 (43%)

esophagogastric junction. Frequency, location, and prognostic significance of lymph node metastases detected both histopathologically and immunohistochemically were analyzed in patients with an adenocarcinoma of the esophagogastric junction. The differences of histopathological lymph node involvement between type I and II cancer are shown in Table 10.1.

Immunostaining showed that in type I carcinoma, nodal microinvolvement occurred to mediastinal in 40%, to paracardial in 38%, and to upper abdominal nodes in 29%, whereas in type II carcinoma, nodal microinvolvement corresponded to 33% to the mediastinal, 88% to the paracardial, and 43% to the upper abdominal compartment. Combined assessment of lymph nodes by histopathology and immunohistochemistry raised the numbers of positive patients in the three compartments. Remarkably, in type II carcinoma, an overlap of nodal involvement was detected by conventional histopathology and immunostaining in the mediastinal lymph node compartment. This resulted in nodal involvement detected either by conventional histopathology or immunohistochemistry in a total of 33% of patients with type II carcinoma.

Potential metastatic spread to the lymph nodes of the abdominal and mediastinal compartments indicate that cardia carcinoma behaves like a “wanderer between two worlds” (Schurr et al. 2006).

In esophageal carcinoma, the frequency of metastasis in certain lymph node groups is influenced by the location of the primary tumor. Akiyama published data on 236 patients about this aspect of metastatic spread (Akiyama et al. 1994).

Patients with carcinoma of the upper esophagus had metastases in the neck lymph nodes in 44.1%, upper mediastinum in 50.0%, middle mediastinum in 20.6%, and lower mediastinum in 5.9%. Remarkably, 14.7% of the cases presented with metastases in the upper gastric area.

Carcinoma of the mid-esophagus was associated with metastasis in the neck in 32.9%, the upper mediastinum in 38.1%, the mid-mediastinum 41.0%, the lower mediastinum 20.2%, and in the upper gastric region in 42.5%. Carcinoma of the lower esophagus was associated with 29.4% positive lymph nodes in the neck and 30.9% in the upper mediastinum, 48.5% had metastases in the middle mediastinum, 35.3% in the lower mediastinum, and 69.1% in the upper gastric area.

In summary, the incidence of metastases in the superior mediastinum is high, even in patients with primary tumors located in the lower esophagus. These data underline the importance of extensive lymph node sampling for correct staging.

10.4 Effect of Nodal Microinvolvement on Survival

Immunohistochemistry neither is a clinical routine nor is its use universal because the results of previous studies have been inconclusive. In esophageal carcinoma and cardia carcinoma, previous studies that evaluated the value of nodal microinvolvement with respect to improved risk stratification provide inconsistent data (Siewert and Stein 1998; Casson et al. 1994).

Mueller et al. showed that micrometastases in "tumor-free" lymph nodes have a prognostic impact. In this study, the presence of micrometastases in the lymph nodes of the tumors of the esophagogastric junction (type I, II, III) has shown different rates of tumor cell detection by immunohistochemistry according to the location of the tumor. In comparison, the rate of micrometastases was significantly higher in type II/III tumors compared with type I tumors. Patients with pN0 status and no micrometastases had a mean survival time of 85.8 months, whereas pN0-patients with immunohistochemically detected micrometastases 45.5 months, which was similar to patients with a pN1-status (45.2 months).

Schurr et al. described that after a median observation time of 27 months, the presence of nodal microinvolvement was associated with significantly reduced disease-specific survival. The Kaplan-Meyer-Analysis showed a significant survival benefit for patients negative in immunohistochemistry (Fig. 10.1).

The median disease-specific survival was 87 months for patients without nodal microinvolvement, and 16.8 months for patients with microinvolvement. The estimated 2 and 5-year survival rates were 77 and 39% for patients without and 62 and 21% for those with nodal microinvolvement.

Additionally, micrometastases to mediastinal lymph nodes for type II carcinoma and abdominal micrometastases for type I carcinoma strongly predicted the outcome, thus elucidating

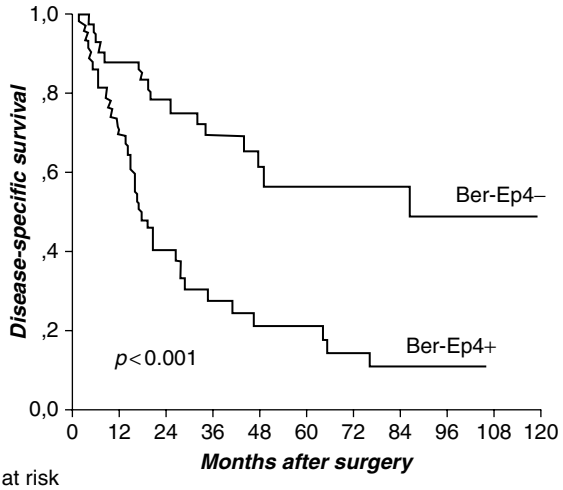

Fig. 10.1 Effect of nodal microinvolvement on disease-specific survival (all patients)

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the role of micrometastases "crossing" the diaphragmatic border. These results show that "proximal" cardia carcinoma located mainly in the distal esophagus may spread "downwards" to the upper abdominal lymph node compartment as may "true" junctional cardia carcinoma, located mainly in the Z-line, metastasize "upwards" to the lower mediastinal lymph nodes. The presence of nodal microinvolvement both in type I and type II carcinoma had a highly significant, independent impact on survival regardless of pT-stage and grading. Patients who had, apart from overt lymph node metastases, additional, occult tumor cells in lymph nodes classified to be "tumor-free" by conventional histopathology, showed significantly shorter disease-specific survival as compared with pN1-patients without such cells. On the other hand, pN0 patients who had Ber-Ep4+ cells in their lymph nodes showed impaired survival (Fig. 10.2).

The median survival was 65 months (95%-CI: 2–113) vs. a median not reached for the presence/absence of the Ber-Ep4+ cells. The prognostic effect of Ber-Ep4+ cells was confirmed by the finding that disease-specific survival of pN0 Ber-Ep4+ patients was similar to pN1-patients (Fig. 10.3).

Disease-specific survival revealed a 2.77 higher independent risk for patients who had nodal microinvolvement (Schurr et al. 2006).

The conclusion of these results is that it is highly suggestive that even single, occult tumor cells in lymph nodes of patients with cardia carcinoma have a strong malignant potential and may contribute to metastatic relapse whether or not overt lymph nodes metastases are assessed by conventional histopathology.

Therefore, transdiaphragmatic removal of both lymph node compartments seems to be mandatory with respect to oncological requirements. Moreover, these results indicate that complementary immunohistochemical analysis of lymph nodes in addition to conventional histopathology yields a distinct increase of staging accuracy, thereby providing a potential tool to identify "at risk" patients who will not be cured by surgery alone.

In patients with esophageal carcinoma, isolated tumor cells in lymph nodes by immunohistochemical analysis are strong prognostic-ators. Izbicki et al. had shown that Ber-EP4–positive cells found in "tumor free" nodes were independently predictive of significantly reduced relapse-free survival and overall survival. They

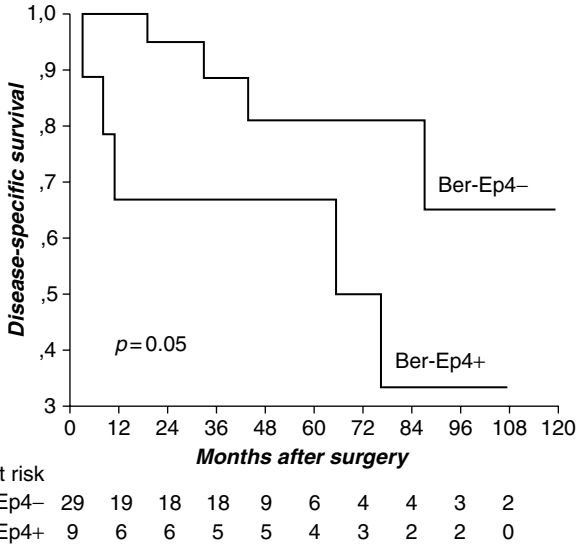

Fig. 10.2 Effect of nodal microinvolvement on disease-specific survival (type I and II carcinomas)

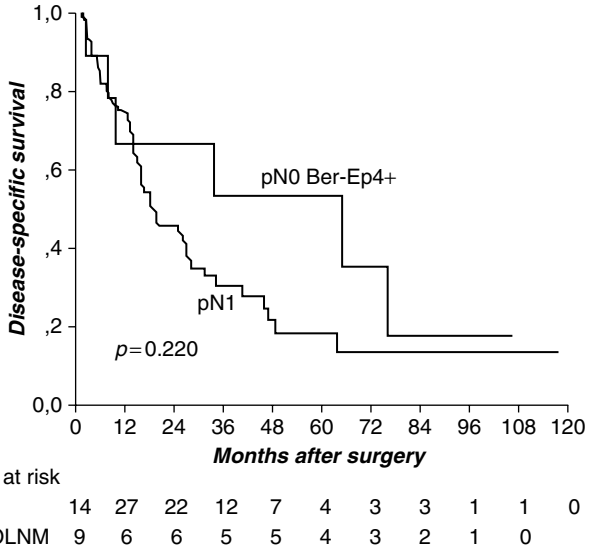

Fig. 10.3 Effect of nodal microinvolvement on disease-specific survival (pN0 with nodal microinvolvement vs. pN1-patients)

predicted relapse both in patients without nodal metastases and in those with regional lymphnode involvement (Izbicki et al. 1997).

Koenig et al. (2009) described that in patients with adenocarcinoma survival was associated with a worse median overall survival (20 months vs. 28 months; $p=0.029$). The 5 year survival probability accounted for 65% in patients without nodal microinvolvement, whereas that in patients with immunohistochemically detectable tumor cells was 0%.

Furthermore, in this study, multivariate analysis showed that micrometastatic lymph node ratio was the most powerful predictive variable for overall survival in patients with esophageal carcinoma irrespective of histopathological tumor type, followed by pT-stage and substratification of patients according to conventional nodal staging (pN0 vs. pN1).

10.5 Current and Future Perspectives

The potential role of an antibody-based treatment as a therapeutic target has been intensely evaluated in numerous types of human cancer. HER-2 gene amplification and protein overexpression occurs in about 20% of breast cancers (Zhang et al. 2003) and is routinely used as the target of an antibody-based therapy (trastuzumab) in metastatic HER-2-positive breast cancer (Baselga et al. 1999; Leyland-Jones 2002; Tripathy et al. 2004).

More recently, adjuvant trastuzumab application was also shown to be dramatically effective in *HER-2*-positive breast cancer patients (Tuma 2005).

The potential benefit of trastuzumab in other tumor entities is largely unknown. HER-2 positivity has been described in most human tumor types but with a highly variable frequency (Ross and McKenna 2001; Allgayer et al. 2000; Safran et al. 2001).

This especially applies for immunohistochemical studies where different reagents and definitions of positivity resulted in an extremely wide range of HER-2 positivity in almost all tumor types.

Despite this, there is evidence for a possible response of HER-2-positive nonbreast cancers to trastuzumab (Langer et al. 2004; Kollmannsberger et al. 1999; Locati et al. 2005).

Applying trastuzumab as additional treatment option would be of particular interest in

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tumors with a notoriously poor prognosis such as esophageal cancer and cardia cancer.

Several studies indeed suggested that HER-2 amplification/overexpression may be relevant for these tumor entities. HER-2 overexpression was reported in 0–83% of esophageal cancer, with a tendency towards higher rates of positivity in adenocarcinoma (10–83%) (al-Kasspooles et al. 1993; Geddert et al. 2002; Walch et al. 2001; Jankowski et al. 1992; Flejou et al. 1994; Nakamura et al. 1994; Hardwick et al. 1995, 1997; Kim et al. 1997; Polkowski et al. 1999; Sauter et al. 1993; Duhaylongsod et al. 1995; Friess et al. 1999; Trudgill et al. 2003; Safran et al. 2004) compared to squamous cell carcinomas (0–56%) (Friess et al. 1999; Hardwick et al. 1997; Mimura et al. 2005; Akamatsu et al. 2003; Lam et al. 1998; Suo et al. 1992, 1995; Suwanagool et al. 1993). A similar variability was observed in amplification analyses. Different methods for analysis (Southern blot or FISH) and definitions of amplification have resulted in amplification frequencies ranging from 15 to 100% in adenocarcinomas (al-Kasspooles et al. 1993; Geddert et al. 2002; Walch et al. 2000a, b, 2001; Jankowski et al. 1992; Persons et al. 1998; Brien et al. 2000) and from 0 to 25% in squamous cell carcinomas of the esophagus (Friess et al. 1999; Mimura et al. 2005; Suo et al. 1995; Ikeda et al. 1996; Tanaka et al. 1997). In a phase I/II study by Safran et al. (2007), trastuzumab was weekly used in combination with paclitaxel, cisplatin, and radiation for advanced esophagogastric junction adenocarcinoma. This combination was well tolerated without an increased incidence of cardiotoxicity or esophagitis when the full dose of trastuzumab was used. HER-2 was overexpressed in 33% (similar to the rates in breast cancer) of the patients. But only those patients with advanced loco-regional and distant adenopathy were included, and therefore, these cases did not receive surgical resection after chemoradiotherapy. Hence, endoscopic responses were used to assess. Five of ten patients in the trastuzumab arm and 10 of 13 patients in the control arm had no tumor on postchemoradiation endoscopic biopsy. However, it should be noted that the negative predictive value of a postchemoradiotherapy endoscopic biopsy is low. A randomized study of patients receiving chemoradiation with and without trastuzumab is needed.

Considering the encouraging results of clinical trials in breast cancer, it could be speculated that trastuzumab might also represent a possible option for HER-2 amplified esophageal adenocarcinomas or cardiacacinoma of the esophagogastric junction after resection of the primary tumor.

Clinical trials investigating the response of HER-2 amplified in these cancer types to trastuzumab are needed.

References

Akamatsu M, Matsumoto T, Oka K et al (2003) C-erbB-2 oncoprotein expression related to chemoradioresistance in esophageal squamous cell carcinoma. Int J Radiat Oncol Biol Phys 57:1323–1327

Akiyama H, Tsurumaru M, Udagawa H et al (1994) Radical lymph node dissection for cancer of the thoracic esophagus. Ann Surg 220(3):364–372

al-Kasspooles M, Moore JH, Orringer MB et al (1993) Amplification and over-expression of the EGFR and erbB-2 genes in human esophageal adenocarcinomas. Int J Cancer 54:213–219

Allgayer H, Babic R, Gruetzner KU et al (2000) c-erbB-2 is of independent prognostic relevance in gastric cancer and is associated with the expression of tumor-associated protease systems. J Clin Oncol 18:2201–2209

Baselga J, Tripathy D, Mendelsohn J et al (1999) Phase II study of weekly intravenous trastuzumab (Herceptin) in patients with HER2/neu-overexpressing metastatic breast cancer. Semin Oncol 26:78–83

Blot WJ, McLaughlin JK (1999) The changing epidemiology of esophageal cancer. Semin Oncol 26(suppl 15):2–8

Blot WJ, Devesa SS, Kneller RW et al (1991) Rising incidence of adenocarcinoma of the esophagus and gastric cardia. JAMA 265:1287–1289

Bogoevski D, Yekebas EF, Schurr P et al (2004) Mode of spread in the early phase of lymphatic metastasis in pancreatic ductal adenocarcinoma: prognostic significance of nodal microinvolvement. Ann Surg 240(6):993–1000

Bogoevski D, Onken F, Koenig A et al (2008) Is it time for a new TNM classification in esophageal carcinoma? Ann Surg Apr 247(4):633–641

Botterweck AA, Schouten LJ, Volovics A et al (2000) Trends in incidence of adenocarcinoma of the oesophagus and gastric cardia in ten European countries. Int J Epidemiol 29:645–654

Brien TP, Odze RD, Sheehan CE et al (2000) HER-2/neu gene amplification by FISH predicts poor survival in Barrett's esophagus-associated adenocarcinoma. Hum Pathol 31:35–39

Byrne J, Waldron R, McAvinchey D et al (1987) The use of monoclonal antibodies for the histopathological detection of mammary axillary micrometastases. Eur J Surg Oncol 13(5):409–411

Bytzer P, Christensen PB, Damkier P et al (1999) Adenocarcinoma of the esophagus and Barrett's esophagus: a population-based study. Am J Gastroenterol 94:86–91

Casson AG, Rusch VW, Ginsberg RJ et al (1994) Lymph node mapping of esophageal cancer. Ann Thorac Surg 58:1569–1570

Devesa SS, Blot WJ, Fraumeni JF Jr (1998) Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. Cancer 83:2049–2053

Duhaylongsod FG, Gottfried MR, Iglehart JD et al (1995) The significance of c-erb B-2 and p53 immunoreactivity in patients with adenocarcinoma of the esophagus. Ann Surg 221:677–683

Flejou JF, Paraf F, Muzeau F et al (1994) Expression of c-erbB-2 oncogene product in Barrett's adenocarcinoma: pathological and prognostic correlations. J Clin Pathol 47:23–26

Friess H, Fukuda A, Tang WH et al (1999) Concomitant analysis of the epidermal growth factor receptor family in esophageal cancer: overexpression of epidermal growth factor receptor mRNA but not of c-erbB-2 and c-erbB-3. World J Surg 23:1010–1018

Geddert H, Zeriouh M, Wolter M et al (2002) Gene amplification and protein overexpression of c-erb-b2 in Barrett's carcinoma and its precursor lesions. Am J Clin Pathol 118:60–66

Hardwick RH, Shepherd NA, Moorghen M et al (1995) c-erbB-2 overexpression in the dysplasia/carcinoma sequence of Barrett's oesophagus. J Clin Pathol 48:129–132

Hardwick RH, Barham CP, Ozua P et al (1997) Immunohistochemical detection of p53 and c-erbB-2 in oesophageal carcinoma; no correlation with prognosis. Eur J Surg Oncol 23: 30–35

Hermanek P, Hutter RV, Sobin LH et al (1999) International Union Against Cancer. Classification of isolated tumor cells and micrometastasis. Cancer 86(12):2668–2673

Hosch SB, Stoecklein NH, Pichlmeier U et al (2001) Esophageal cancer: the mode of lymphatic tumor cell spread and its prognostic significance. J Clin Oncol 19(7):1970–1975

Ikeda Y, Ozawa S, Ando N et al (1996) Meanings of c-erbB and int-2 amplification in superficial esophageal squamous cell carcinomas. Ann Thorac Surg 62:835–838

Izbicki JR, Hosch SB, Pichlmeier U et al (1997) Prognostic value of immunohistochemically identifiable tumor cells in lymph nodes of patients with completely resected esophageal cancer. N Engl J Med 337(17):1188–1194

Jankowski J, Coghill G, Hopwood D et al (1992) Oncogenes and onco-suppressor gene in adenocarcinoma of the oesophagus. Gut 33:1033–1038

Jeon J, Luebeck EG, Moolgavkar SH (2006) Age effects and temporal trends in adenocarcinoma of the esophagus and gastric cardia (United States). Cancer Causes Control 17:971–981

Kasper M, Stosiek P, Typlt H et al (1987) Histological evaluation of three new monoclonal anti-cytokeratin antibodies. 1. Normal tissues. Eur J Cancer Clin Oncol 23(2):137–147

Kim R, Clarke MR, Melhem MF et al (1997) Expression of p53, PCNA, and C-erbB-2 in Barrett's metaplasia and adenocarcinoma. Dig Dis Sci 42:2453–2462

Koenig A, Prenzel KL, Bogoevski D et al (2009) Strong impact of micrometastatic tumor cell load in patients with esophageal carcinoma. Ann Surg Oncol 16:454–462

Kollmannsberger C, Pressler H, Mayer F et al (1999) Cisplatin-refractory, HER2/neu-expressing germ-cell cancer: induction of remission by the monoclonal antibody trastuzumab. Ann Oncol 10:1393–1394

Komukai S, Nishimaki T, Suzuki T et al (2002) Significance of immunohistochemical nodal micrometastasis as a prognostic indicator in

potentially curable oesophageal carcinoma. Br J Surg 89(2):213–219

Lam KY, Tin L, Ma L (1998) C-erbB-2 protein expression in oesophageal squamous epithelium from oesophageal squamous cell carcinomas, with special reference to histological grade of carcinoma and pre-invasive lesions. Eur J Surg Oncol 24:431–435

Langer CJ, Stephenson P, Thor A et al (2004) Trastuzumab in the treatment of advanced non-small-cell lung cancer: is there a role? Focus on Eastern Cooperative Oncology Group Study 2598. J Clin Oncol 22:1180–1187

Latza U, Niedobitek G, Schwarting R et al (1990) Ber-EP4: new monoclonal antibody which distinguishes epithelia from mesothelial. J Clin Pathol 43(3):213–219

Leyland-Jones B (2002) Trastuzumab: hopes and realities. Lancet Oncol 3:137–144

Locati LD, Rinaldi G, Bossi P et al (2005) Herceptin plus chemotherapy in relapsed and/or metastatic salivary gland cancer. Oral Oncol 41:97–98

Maehara Y, Oshiro T, Endo K et al (1996) Clinical significance of occult micrometastasis lymph nodes from patients with early gastric cancer who died of recurrence. Surgery 119(4):397–402

Mimura K, Kono K, Hanawa M et al (2005) Frequencies of HER-2/neu expression and gene amplification in patients with oesophageal squamous cell carcinoma. Br J Cancer 92: 1253–1260

Momburg F, Moldenhauer G, Hammerling GJ et al (1987) Immunohistochemical study of the expression of a Mr 34, 000 human epithelium-specific surface glycoprotein in normal and malignant tissues. Cancer Res 47(11):2883–2891

Mueller JD, Stein HJ, Oyang T et al (2000) Frequency and clinical impact of lymph node micrometastasis and tumor cell microinvolvement in patients with adenocarcinoma of the esophagogastric junction. Cancer 89(9):1874–1882

Nakamura T, Nekarda H, Hoelscher AH et al (1994) Prognostic value of DNA ploidy and c-erbB-2 oncoprotein overexpression in adenocarcinoma of Barrett's esophagus. Cancer 73:1785–1794

Orengo MA, Casella C, Fontana V et al (2006) AIRT Working Group. Trends in incidence rates of oesophagus and gastric cancer in Italy by subsite and histology, 1986-1997. Eur J Gastroenterol Hepatol 18:739–746

Pantel K, Brakenhoff RH (2004) Dissecting the metastatic cascade. Nat Rev Cancer 4(6):448–456

Pantel K, Schlimok G, Angstwurm M et al (1994) Methodological analysis of immunocytochemical screening for disseminated epithelial tumor cells in bone marrow. J Hematother 3(3): 165–173

Parkin DM (2004) International variation. Oncogene 23:6329–6340

Parkin DM, Bray FI, Devesa SS (2001) Cancer burden in the year 2000. The global picture. Eur J Cancer 37(suppl 8):S4–S66

Passlick B, Izbicki JR, Kubuschok B et al (1994) Immunohistochemical assessment of individual tumor cells in lymph nodes of patients with non-small-cell lung cancer. J Clin Oncol 12(9): 1827–1832

Persons DL, Croughan WS, Borelli KA et al (1998) Interphase cytogenetics of esophageal adenocarcinoma and precursor lesions. Cancer Genet Cytogenet 106:11–17

Polkowski W, van Sandick JW, Offerhaus GJ et al (1999) Prognostic value of Lauren classification and c-erbB-2 oncogene overexpression in adenocarcinoma of the esophagus and gastroesophageal junction. Ann Surg Oncol 6:290–297

Prenzel KL, Koenig A, Schneider PM et al (2007) Reduced incidence of nodal micrometastasis after major response to neoadjuvant chemoradiation in locally advanced esophageal cancer. Ann Surg Oncol 14(2):954–959

Raymond WA, Leong AS (1989) Immunoperoxidase staining in the detection of lymph node metastases in stage I breast cancer. Pathology; 21(1): 11–5

Ross JS, McKenna BJ (2001) The HER-2/neu oncogene in tumors of the gastrointestinal tract. Cancer Invest 19:554–568

Safran H, Steinhoff M, Mangray S et al (2001) Overexpression of the HER-2/neu oncogene in pancreatic adenocarcinoma. Am J Clin Oncol 24:496–499

Safran H, DiPetrillo T, Nadeem A et al (2004) Trastuzumab, paclitaxel, cisplatin, and radiation for adenocarcinoma of the esophagus: a phase I study. Cancer Invest 22:670–677

Safran H, DiPetrillo T, Akerman P et al (2007) Phase I/II study of trastuzumab, paclitaxel, cisplatin and radiation for locally advanced, HER2 overexpressing, esophageal adenocarcinoma. Int J Radiat Oncol Biol Phys 67(2):405–409

Sano T, Sasako M, Yamamoto S et al (2004) Gastric cancer surgery: morbidity and mortality results from a prospective randomized controlled trial comparing D2 and extended para-aortic lymphadenectomy-Japan Clinical Oncology Group study 9501. J Clin Oncol 22:2767–2773

Sauter ER, Keller SM, Erner S et al (1993) HER-2/neu: a differentiation marker in adenocarcinoma of the esophagus. Cancer Lett 75:41–44

Schurr PG, Yekebas EF, Kaifi JT et al (2006) Lymphatic spread and microinvolvement in adenocarcinoma of the esophago-gastric junction. J Surg Oncol 94(4):307–315

Siewert JR, Stein HJ (1998) Classification of adenocarcinoma of the oesophagogastric junction. Br J Surg 85:1457–1459

Siewert JR, Holscher AH, Becker K et al (1987) Cardia cancer: attempt at a therapeutically relevant classification. Chirurg 58:25–32

Siewert JR, Kestlmeier R, Busch R et al (1996) Benefits of D2 lymph node dissection for patients with gastric cancer and pN0 and pN1 lymph node metastases. Br J Surg 83(8):1144–1147

Suo Z, Holm R, Nesland JM (1992) Squamous cell carcinomas, an immunohistochemical and ultrastructural study. Anticancer Res 12:2025–2031

Suo Z, Su W, Holm R et al (1995) Lack of expression of c-erbB-2 oncoprotein in human esophageal squamous cell carcinomas. Anticancer Res 15:2797–2798

Suwanagool P, Parichatikanond P, Maeda S (1993) Expression of c-erbB-2 oncoprotein in primary human tumors: an immunohistochemistry study. Asian Pac J Allergy Immunol 11:119–122

Tanaka S, Mori M, Akiyoshi T et al (1997) Coexpression of Grb7 with epidermal growth factor receptor or Her2/erbB2 in human advanced esophageal carcinoma. Cancer Res 57:28–31

Tripathy D, Slamon DJ, Cobleigh M et al (2004) Safety of treatment of metastatic breast cancer with trastuzumab beyond disease progression. J Clin Oncol 22:1063–1070

Trivers KF, Sabatino SA, Stewart SL (2008) Trends in esophageal cancer incidence by histology, United States, 1998-2003. Int J Cancer 123(6): 1422–1428

Trudgill NJ, Suvarna SK, Royds JA et al (2003) Cell cycle regulation in patients with intestinal metaplasia at the gastro-oesophageal junction. Mol Pathol 56:313–317

Tuma RS (2005) Trastuzumab trials steal show at ASCO meeting. J Natl Cancer Inst 97:870–871

Vizcaino AP, Moreno V, Lambert R et al (2002) Time trends incidence of both major histologic types of esophageal carcinomas in selected countries, 1973–1995. Int J Cancer 99:860–868

Walch A, Bink K, Gais P et al (2000a) Evaluation of c-erbB-2 overexpression and Her-2/neu gene copy number heterogeneity in Barrett's adenocarcinoma. Anal Cell Pathol 20:25–32

Walch AK, Zitzelsberger HF, Bink K et al (2000b) Molecular genetic changes in metastatic primary Barrett's adenocarcinoma and related lymph node metastases: comparison with nonmetastatic Barrett's adenocarcinoma. Mod Pathol 13:814–824

Walch A, Specht K, Bink K et al (2001) Her-2/neu gene amplification, elevated mRNA expression, and protein overexpression in the metaplasia-dysplasia-adenocarcinoma sequence of Barrett's esophagus. Lab Invest 81:791–801

Walther C, Zilling T, Perfekt R et al (2001) Increasing prevalence of adenocarcinoma of the oesophagus and gastro-oesophageal junction: a study of the Swedish population between 1970 and 1997. Eur J Surg 167:748–757

Waterman TA, Hagen JA, Peters JH et al (2004) The prognostic importance of immunohistochemically detected node metastases in resected esophageal adenocarcinoma. Ann Thorac Surg 78(4):1161–1169

Z'Graggen K, Centeno BA, Fernandez-del Castillo C et al (2001) Biological implications of tumor cells in blood and bone marrow of pancreatic cancer patients. Surgery 129(5):537–546

Zhang D, Salto-Tellez M, Do E et al (2003) Evaluation of HER-2/neu oncogene status in breast tumors on tissue microarrays. Hum Pathol 34:362–368