Early response evaluation and prediction in neoadjuvant-treated patients with esophageal cancer

Joerg Theisen, Bernd Krause, Christian Peschel, Roland Schmid, Hans Geinitz, Helmut Friess

Abstract

Since the introduction of multimodal therapy regimens, the prognosis of esophageal cancer has improved. There is undoubtedly true for patients with surgically resected tumors in the case of a response to neoadjuvant chemotherapy or chemoradiation. Important conclusions can be drawn from this regarding the indication for perioperative therapies, the radicality of surgery, or the surgical indications. Thus, most of the current research in this field is aimed at the early identification of this subset of patients, at the beginning of, or even before, neoadjuvant treatment. Conventional staging tools have failed to predict responses to neoadjuvant therapy. However, molecular imaging methods, e.g. positron emission tomography (PET)-scans, have shown promising results in the early selection of responders and non-responders during the course of neoadjuvant therapy, allowing physicians to alter the treatment plan accordingly. Even more desirable is the identification of potential responders before the start of neoadjuvant therapy. Preliminary molecular data on biopsy specimens demonstrate the possibility of early response prediction in these patients. We present the current knowledge on response evaluation and prediction in esophageal cancer and draw conclusions for future clinical practice and studies in this review.

Key words: Esophageal cancer; Response prediction; Individualized therapy

INTRODUCTION

The last decade has seen a change in the therapy of patients with esophageal cancer. For many years, the standard therapy for locally advanced lesions has been surgical resection. However, overall survival for patients with locally advanced tumors after resection remains poor, with a five-year survival rate between 10% and 30%[1,2]. Most patients still present with an advanced tumor stage; therefore, multimodal therapy regimens have been introduced, some using neoadjuvant chemotherapy or radiochemotherapy followed by radical resection, whereas others use adjuvant protocols[3]. Furthermore, trials have been presented in which surgery was omitted completely. Currently,
there is no evidence-based international agreement on one or the other multimodal approach. The results have been conflicting. Some have demonstrated a benefit for neoadjuvant radiochemotherapy, others for neoadjuvant chemotherapy, and some favour an adjuvant approach. Other published studies did not find any difference in survival for a multimodal concept. There are multiple reasons for these marginal differences and for the controversies in the preferred regimens, such as the heterogeneity of the groups analyzed, mixing squamous cell cancer of the esophagus with esophageal adenocarcinoma or analyzing true esophageal adenocarcinoma (Barrett’s cancer) together with carcinomas of the esophago-gastric junction and proximal gastric carcinomas. It is very important to stress that esophageal adenocarcinoma and squamous cell cancer of the esophagus have to be considered as completely different entities and therefore separate analysis is mandatory. Additionally several protocols for chemotherapy and radiochemotherapy have been used. However, careful analysis of the published results indicates that there is always a subset of patients benefiting from the multimodal approach. These are patients who show a response to the respective neoadjuvant or adjuvant protocol. This is the case for all the different regimens applied. Unfortunately, in most studies the treatment response is not clearly defined. The current gold standard for response assessment is the pathohistological statement of the amount of viable tumor cells within the resected specimen. This gold standard might be an adequate tool for all adjuvant protocols because of the opportunity to tailor postresection therapy accordingly. However, the current trend favors neoadjuvant studies. The Patterns of Care studies in the US showed that neoadjuvant chemoradiation therapy increased from approximately 10% during 1992-1994 to approximately 26% in 1996-1999 for locally advanced esophageal carcinomas. A meta-analysis of the survival benefit in the neoadjuvant setting found an increase in survival for neoadjuvant radiochemotherapy in squamous cell cancer of the esophagus and to a lesser extent for adenocarcinoma using chemotherapy alone. Protocols using radiochemotherapy in the neoadjuvant setting in patients with esophageal adenocarcinoma did show a benefit compared to chemotherapy alone. For this preoperative approach, the pathohistological assessment of response is available too late to modify any preoperative protocols. Therefore, current research activities aim to identify predictors of response early or even before the neoadjuvant concepts are applied to individualize therapies according to the respective tumour behaviour.

There are several theoretical components available for the prediction of pretherapeutic response. Demographic data, initial staging imaging tools, biopsies, and serum. Pure clinical response evaluation after neoadjuvant therapy for esophageal cancer is highly inaccurate. Despite numerous studies in this field, no clear reliable candidate marker predicting response was identified. However, recent studies using new technologies such as gene chips or molecular imaging have shown promising early results towards a response prediction or response evaluation.

| Grade | Description | Response |
|-------|-------------|----------|
| 1a    | No residual tumor/tumor bed | Responder |
| 1b    | < 10% residual tumor/tumor bed | Responder |
| 2     | 10%-50% residual tumor/tumor bed | Partial responder |
| 3     | > 50% residual tumor/tumor bed | Non-responder |

### HISTOPATHOLOGICAL RESPONSE

The current gold standard for response prediction is the histopathological assessment of the tumor regression grade as described by Mandard et al. and slightly modified by Becker et al. for esophageal and gastric tumors. This regression grading system stratifies response based on the biological effect of radiation or chemotherapy on the amount of remaining viable tumor cells. There are three to five grades based on the ratio of fibrosis to remaining viable tumor content. Complete pathohistological response means that there are no viable residual tumor cells present. Partial response is defined as the presence of tumor cells scattered through the fibrosis, and minimal regression showing residual cancer outgrowing fibrosis. Absence of any regressive changes is considered a no-change situation. For esophageal and gastric tumors, Becker et al. described a clinically useful four-grade classification based on an estimation of the percentage of vital tumor tissue in relation to the tumor bed (Table 1).

Patients with no or < 10% residual tumor cells (tumor regression score 1) are classified as responders. All other tumors (tumor regression score 2: 10%-50% residual tumor cells and tumor regression score 3: > 50% residual tumor cells) were classified as non responders. This tumor response grading system is clearly associated with survival and currently serves as the gold standard for response assessment. However, this prognostic system is not available prior to neoadjuvant therapy and therefore is more useful in the potential adjuvant setting.

To date, a predictor of response based on demographics or conventional imaging information has not emerged. Performance status, primary location or age has not been shown to be associated with pathological response.

### APPROACH TO RESPONSE ASSESSMENT

In addition to the availability of components such as serum or biopsies, several methods have been used to predict as early as possible the response to neoadjuvant therapy. In the past, most studies used immunohistochemical methods to assess the response. The approach to the analyzed proteins has been either chemotherapy drugs driven or proteins have been chosen that have been shown to play a role in the behaviour of certain malignancies. However, single potential biomarkers have failed to sufficiently predict response to neoadjuvant therapy. This is not surprising considering the complex interactions between all the gene products expressed by the cells, and the many proteins.
involved in numerous cellular functions, such as apoptosis, DNA repair, and the metabolism and detoxification of drugs; all of which contribute to the individual response of a given tumor. Therefore, it is likely that multiple markers are needed to define the sensitivity or resistance of tumors to specific drugs. In recent years, new technologies using microarray gene chips or proteomics have been used for response prediction studies. These technologies enable the analysis of thousands of genes at the same time with a single biopsy specimen at the RNA/DNA or protein level.

Molecular imaging has emerged in recent years as an important technique. Many studies have been published showing a significant correlation of tumor metabolism and pathohistological response. Based on these results, therapy regimens have been successfully changed according to the response behaviour. Unfortunately, this information is not available prior to the therapy. At least two weeks of the neoadjuvant chemotherapy or radiochemotherapy has to be given before the molecular imaging is able to distinguish between responders and non-responders.

**MOLECULAR RESPONSE PREDICTION IN ESOPHAGEAL SQUAMOUS CELL CANCER**

Patients with locally advanced squamous cell cancer of the esophagus are currently treated with either neoadjuvant radiochemotherapy or definitive radiotherapy. These regimens differ in the radiation dosages used. In the neoadjuvant setting, approximately 45 Gray are administered followed by esophagectomy with lymphadenectomy. Radiochemotherapy without resection applies approximately 50 to 60 Gray to the tumor region and the corresponding lymph nodes areas.

In a retrospective study of 68 patients with locally advanced esophageal squamous cell cancer who received a multimodal treatment with 5-Fluorouracil (5-FU) based radiochemotherapy, Sarbia and colleagues examined the correlation of the presence of genetic polymorphisms in genes involved in folate metabolism with the response behaviour and outcome\[^{[11]}\]. The DNA of pretherapeutic biopsies was genotyped for common genetic polymorphisms of MTHFR (5,10-methylenetetrahydrofolate reductase), MTR (Methionine synthase), and TS (thymidylate synthase) tandem repeat polymorphisms. Tumors with an MTR wild-type genotype showed a shorter survival in contrast to tumors with an MTR variant genotype. This correlated with the response behaviour, where tumors with an MTR variant genotype responded more frequently to the neoadjuvant radiochemotherapy. In a subsequent study by Sarbia and colleagues, the expression of proteins involved in DNA repair and/or cell-cycle regulation, including p53 (phosphorylated at Ser15), EGFR (Epidermal Growth Factor Receptor), ATM (ataxia-telangiectasia mutated) protein kinase (phosphorylated at Ser1981), and checkpoint kinase 2 (CHK2) (phosphorylated at Thr68), was correlated with the response to RCTx and with overall survival\[^{[23]}\]. Tumors that were positive for CHK2 expression more frequently showed clinically determined regression after RCTx than tumors that were negative for CHK2 expression, whereas other parameters did not correlate with tumour regression. Expression of ATM correlated with expression of CHK2 and p53-phospho. In contrast to histopathological response evaluation, none of the molecular parameters under investigation correlated with overall survival.

Other studies aimed to identify genes or proteins involved in resistance to 5-FU or cisplatin, found that 5-FU metabolism pathway genes, such as TS (thymidylate synthase), TP (thymidine phosphorylase), and DPD (dihydropyrimidine dehydrogenase), or genes involved in DNA-repair mechanisms, such as ERCC-1 (excision repair cross complementing), GSTP-1 (Glutathione S-transferase), or nm23-H1, were somewhat predictive for the response behaviour in patients with neoadjuvant radiochemotherapy. These studies were done either by immunohistochemistry or quantitative RT-PCR technologies\[^{[13,14]}\].

Metallothionein (MT) is a small protein involved in many patho-physiological processes such as detoxification, cell proliferation, apoptosis, and therapy resistance. Kishi et al\[^{[15]}\] demonstrated that high expression of this protein is associated with a poor prognosis due to non-response in patients with localized squamous cell cancer of the esophagus who received neoadjuvant radiochemotherapy. However, no such association could be found in another study by Harpole et al\[^{[16]}\].

To date, however, none of these studies has shown one independent predictive factor on multivariate analysis. With regard to molecular markers, the p53 gene is one of the most widely investigated genes in human cancer. It has been found as a prognostic indicator in many different carcinomas\[^{[17,18]}\]. It plays a crucial role in repairing DNA of damaged cells, is involved in triggering apoptosis, and might be intrinsically involved in the response to radiochemotherapy. Several studies have examined p53 expression and the response to radiochemotherapy in esophageal cancer\[^{[19,20]}\]. In patients with squamous cell cancer of the esophagus, Seitz et al\[^{[21]}\] used immunohistochemistry to demonstrate a significant association of p53 overexpression and decreased response rates. Other groups have not found this association\[^{[22]}\]. It is postulated that this difference might be due to gene deletion, failure of transcription, or a non-stabilizing mutation, all of which might lead to loss of p53 function\[^{[23]}\].

The protein p21 is transcriptionally regulated by p53 by ionising radiation. This can cause cell cycle arrest and apoptosis. p21 is involved in disruption of regulatory networks and might be a good candidate gene for radioresistance prediction. However, as with many other single gene studies, the published results have been controversial. Some studies describe a positive correlation of p21 positivity and response or survival, while others were not able to demonstrate a correlation of p21 expression and response to radiochemotherapy\[^{[24-26]}\].

Much research has been done in the past in studying COX-2 (Cyclooxygenase-2) expression and response in esophageal cancer. COX-2 plays an important role in
prostaglandin synthesis and is involved in angiogenesis and tumor growth. Results from cervical cancer demonstrating a predictive potential of COX-2 mRNA expression have been adapted to squamous cell cancer of the esophagus[27]. A study by Takatori et al[29] of 29 patients with esophageal squamous cell cancer who received neoadjuvant radiochemotherapy, found that high COX-2 mRNA expression in tumor biopsies was significantly associated with a poor response to radiochemotherapy and ultimately with a poor survival in these patients.

Another important aspect associated with molecular response prediction is growth regulation. Several proteins have been studied, such as epidermal growth factor receptor (EGFR), HER-2 (human epidermal growth factor receptor), and different cyclins. The results have been conflicting; for example HER-2 and cyclin D1 expressions have been found to be correlated with response to neoadjuvant radiochemotherapy in patients with squamous cell carcinoma of the esophagus, but failed to be predictive for the overall survival[29]. By studying EGFR and proliferating cell nuclear antigen, Hickey et al[30] reported an inverse relationship of response and expression in immunostaining of biopsies of patients with esophageal squamous cell cancer.

More convincing evidence was demonstrated by analysing VEGF (vascular endothelial growth factor) in the context of response prediction. VEGF is the major angiogenic factor in pathological angiogenesis. Angiogenesis plays a very important role in the promotion of tumor growth and formation of metastases. In a study by Shimada et al[31], co-expression of p53, TP, and VEGF (analysed by immunohistochemistry) was correlated to the response behaviour and survival in patients with squamous cell cancer of the esophagus. In a multivariate analysis, only VEGF emerged as a predictor of response to the neoadjuvant radiochemotherapy. Its expression was associated with a high incidence on non-responders and significantly worse survival. These results were supported by a study from Gorski and colleagues, who demonstrated that blocking the activity of VEGF enhances the effects of radiochemotherapy on the tumor[32]. The mechanisms behind this effect are not fully understood, but angiogenic factors seem to be a valuable clinical target for influencing the response behaviour in neoadjuvant treated squamous cell cancer of the esophagus.

One of the reasons for the conflicting results is the fact that most studies focused on single or few gene expression analyses. Tumor tissue has a very heterogeneous gene profile; therefore, the likelihood of finding a single gene responsible for the regulation of tumor resistance or sensitivity is very low. Recently, the introduction of RNA/DNA or protein microarrays opened the door for a variety of studies of molecular tumor profiling. Duong et al[33] were able to demonstrate a positive predictive value of 100% and a negative predictive value of 79% in regards to response prediction in 21 patients with squamous cell carcinoma of the esophagus receiving neoadjuvant radiochemotherapy using SVM (support vector machine) modelling and LOOCV (Leave-one-out cross-validation) analyses as a multigene classifier. A 32-gene classifier was used to predict response to neoadjuvant radiochemotherapy. By further analyzing the specific genes involved in response prediction, most of the sequences were found to be involved in the apoptosis and angiogenesis pathways.

Serum markers
Several serum markers have been tested to predict response to neoadjuvant radiochemotherapy in patients with esophageal squamous cell cancer, including CEA (carcino-embryonic antigen), VEGF, and CYFRA (cytokeratin fragment) 21-1[34,35]. CYFRA was the only marker showing a close correlation between its serum level and response, but it has not been studied in a large prospective clinical trial.

Molecular imaging
The introduction of molecular imaging, such as FDG-PET (fluoro-deoxy-glucose-positron emission tomography) has changed the field with respect to the previous disappointing results of the conventional image methods in predicting response to neoadjuvant therapies. CT-scan, MRI, or EUS have failed to accurately predict tumor resistance or sensitivity. A recent meta-analysis found FDG-PET to be more accurate than a CT-scan for the measurement of treatment response[36]. There was no differentiation possible between inflammation, scars, and remnant carcinoma. FDG-PET is based on the high glucose metabolism of a tumor compared to normal tissue, enabling a good differentiation of tumor areas from non-tumor tissue. Recently, PET-CT-scans have combined the metabolism with anatomic location[37]. The relative changes in FDG uptake have been used for early response evaluation in a variety of tumor entities, such as breast[38], lung[39], and colon[40] cancer, as well as in Hodgkin’s[41] and non-Hodgkin’s lymphoma[42]. There have been encouraging results in predicting response in patients with esophageal squamous cell cancer. Most studies performed the PET examination before the neoadjuvant radiochemotherapy and at the end of the preoperative protocol[43-48]. The relative decrease in standardized uptake value (SUV) between these two examinations served as a discriminator for response or non-response. The results were then correlated to the histopathological response assessment. By applying this approach, Brücher et al[46] were able to demonstrate a correlation between SUV decrease and histopathological response in 24 patients with esophageal squamous cell cancer. This was confirmed by other authors. Some even suggested that the absolute SUV of the initial PET was sufficient to predict the response behaviour[49]. Recently, studies have been published with PET scans performed initially and two weeks into the neoadjuvant radiochemotherapy[49]. With the cut-off point at 30% reduction in SUV, a separation of responders from non-responders was possible after only two weeks with a sensitivity of 93%, a specificity of 88%, and an accuracy of 79%. Notably, non-responding patients who stopped the neoadjuvant chemotherapy after two weeks did not show a difference in survival compared to the patients who previously received the entire three months of chemo-
therapy. Without any decrease in survival, 2.5 months of chemotherapy could be avoided in these patients. At this time point, therapeutic regimens could be individualized based on the PET results. Performing the 2nd PET-scan after one week of radiochemotherapy failed to predict the pathological response.\(^{[29]}\) Table 2 shows a summary of the current available results of PET guided response evaluation on esophageal squamous cell cancer.

### Table 2: Studies that have assessed the role of a PET scan in the response prediction of patients with neoadjuvant treated squamous cell cancer of the esophagus

| Author            | \(n\) | RCTx         | 2nd PET       | Correlation of PET with histopathological response yes/no |
|-------------------|------|--------------|---------------|---------------------------------------------------------|
| Song et al\(^{[44]}\), 2005 | 32   | Cis/Cap/46 Gy | 4 wk          | Yes                                                     |
| Wieder et al\(^{[46]}\), 2004 | 38   | 5 FU/40 Gy   | During RCTx   | Yes                                                     |
| Flamen et al\(^{[9]}\), 2004 | 27   | Cis/5-FU/40 Gy | After RCTx    | Yes (tumor/liver ratio)                                 |
| Kato et al\(^{[45]}\), 2002 | 10   | 5-FU/30 Gy   | After RCTx    | No                                                      |
| Brücher et al\(^{[48]}\), 2001 | 24   | 5-FU/30 Gy   | After RCTx    | Yes                                                     |

PET: Positron emission tomography; Cis: Cisplatin; Cap: Capectabine; 5-FU: 5-Flourouracil; Gy: Gray; RCTx: Radiochemotherapy.

In patients with adenocarcinoma of the esophagus, the multimodal concepts differ considerably compared to the treatment regimens in squamous cell cancer. Some groups apply chemotherapy alone; others prefer a combination of radiation and chemotherapy in the multimodal setting. Therefore, it is difficult to compare the results of different studies. Additionally, adenocarcinoma of the esophagus is a much more heterogeneous tissue, with areas of invasive cancer, high-grade intraepithelial neoplasia, and the precursor lesion specialized intestinal metaplasia (Barrett’s metaplasia) adjacent to each other. By taking single biopsies there is always a high risk of sampling error.

With regard to molecular response prediction, more work has been published for adenocarcinomas compared to squamous cell cancer of the esophagus. The introduction of quantitative high-throughput RT-PCR technologies, such as TaqMan, greatly increased the likelihood of identifying potential genes, or groups of genes, involved in response prediction. Most of the published studies focused on the analyses of drug targets involved in the metabolism of the most commonly used chemotherapy agents, such as 5-FU and platinum compounds. In a study by Lange et al\(^{[54]}\) the quantitative RNA expression in biopsies of Barrett’s carcinomas prior to the neoadjuvant chemotherapy of genes involved in the 5-FU metabolism (TS, TP, DPD, MTHFR, MAP7 (Mitogen-activated protein), and ELF3 (eukaryotic initiation factor) and platinum related genes, caldesmon, ERCC1, ERCC4, HER-2/neu, GADD45, and MRP1) were determined and compared to the histopathological response assessment of the post-operative specimen. There was a significant correlation between the pretherapeutic expression levels of MTHFR, caldesmon, and MRP1 with the histopathological response. Other groups found an association between TS, ERRC1, DPD, and GADD45 (growth arrest and DNA damage-inducible gene) and response.\(^{[51,52]}\)

In the past, p53 has been one of the most studied genes with regard to response prediction in esophageal adenocarcinoma, as well as in squamous cell cancer of the esophagus. Several trials found an inverse correlation between p53 positive tumors identified immunohistochemically and response. The entire apoptosis pathway seems to play a crucial role in the chemosensitivity of neoadjuvant treated Barrett carcinomas. Genes such as c-erbB-2, p53, p21, ki76, and bcl-2 (B-cell lymphoma) have been shown to be involved in response prediction.\(^{[53]}\) These results were obtained either by immunohistochemistry or by quantitative RT-PCR methods.

The introduction of microarray technologies opened an entirely new area of response prediction on biopsy material. This has led to a large increase in published studies that have enhanced our understanding of the biology of esophageal adenocarcinoma. Problems still arise from the difficulties in analysing the enormous amounts of data generated by these arrays. Different analytical approaches are currently available, such as unsupervised hierarchical cluster analysis of LOOCV analysis. The first published studies demonstrated promising results in identifying a cluster of 30-100 genes closely related to the response behaviour of these tumors. Furthermore, these gene expression studies identified some very interesting new genes that might serve as targets for new therapeutic approaches.\(^{[54,55]}\)

More recently, proteomic profiling has become feasible. First studies on cell lines assessing the chemosensitivity of a variety of cell lines, including esophageal cancer cell lines, provided the basis for the prediction of drug response based on protein markers.\(^{[56]}\) A combination of these technologies might hold great promise for the future with regard to response prediction.

Currently, there is no reliable molecular marker for tumor response to neoadjuvant therapy. Early results are promising. Luthra et al\(^{[57]}\) and Ashida et al\(^{[58]}\) reported hierarchical clustering of gene expression profiles of esophageal carcinoma segregated samples into two major groups that correlated with response and identified genes differentially expressed between long- and short-term survival after neoadjuvant therapy for esophageal cancer. Additionally, Duong and colleagues\(^{[59]}\) used a 52-gene classifier to predict response. These are all preliminary results, and to date, no clinical recommendations can be drawn from this.
Molecular imaging
In Barrett’s cancer, the use of PET-scan for the evaluation of response has been widely studied. These studies suggested that changes in FDG uptake in response to therapy correlates with the pathological response and predicts the risk of local recurrence and survival. Unfortunately, there have been no data published exclusively investigating the role of FDG-PET in adenocarcinoma of the esophagus. Therefore, it is difficult to assess the usefulness of PET imaging in predicting response to neoadjuvant chemotherapy in adenocarcinomas of the esophagus. The only good prospective trials were published in 2006 by Ott et al[37] followed by Lordick et al[40] in 2007. In the initial study on 65 patients with esophageal adenocarcinoma and some carcinoma at the cardia, the authors demonstrated that by taking a SUV decrease greater than 35% as the precondition of response, a prediction was possible during the neoadjuvant therapy after only two weeks. These results were correlated to the pathohistological response assessment on the operative specimen. This study was followed by a prospective trial where, for the first time, the therapy was tailored according to the changes in SUV uptake after two weeks. The responding group continued on chemotherapy followed by resection, whereas the group of patients who did not respond to the neoadjuvant chemotherapy discontinued chemotherapy after two weeks and proceeded directly to surgery. Complete histopathological response was noted in almost 60% of the PET responders in contrast to the PET non-responders, in which no histological response was noted. This first prospective trial confirmed the feasibility of a PET guided treatment plan after only two weeks of neoadjuvant chemotherapy. Other groups have also shown the value of PET scan in response prediction[38-40]. Based on these results, two major questions arose: (1) What treatment changes have to be made to the non-responders to increase the number of patients responding to the therapy? (2) Is it really necessary in the responder group to complete the entire cycle of neoadjuvant chemotherapy or is the biological selection after two weeks already sufficient for a prolongation in survival?

Table 3 lists the currently available data for PET guided response prediction in Barrett’s cancer.

| Author            | n  | CTx                  | 2nd PET                  | Correlation of PET with histopathological response yes/no |
|-------------------|----|----------------------|--------------------------|--------------------------------------------------------|
| Lordick et al[37], 2007 | 110 | Cis/5-FU             | 2 wk during CTx          | Yes                                                   |
| Ott et al[37], 2006   | 65  | Cis/5-FU             | 2 wk during CTx          | Yes                                                   |
| Gillham et al[37], 2006 | 29  | Cis/5-FU             | After CTx                | No                                                    |
| Levine et al[37], 2006 | 52  | Cis/5-FU             | After CTx                | Yes                                                   |
| Swisher et al[37], 2004 | 73  | Cis/5-FU/50 Gy       | After RCTx               | Yes                                                   |
| Brink et al[37], 2004  | 13  | Cis/5-FU             | After CTx                | No                                                    |
| Arslan et al[37], 2002 | 22  | Cis/Taxol/50 Gy      | After RCTx               | Yes                                                   |
| Weber et al[37], 2001  | 40  | Cis/5-FU             | 2 wk during CTx          | Yes                                                   |

Cis: Cisplatin; Irin: Irinotecan; Carbo: Carboplatin; 5-FU: 5 Flurouracil; Gy: Gray; RCTx: Radiochemotherapy; CTx: Chemotherapy.

REFERENCES
1. Altorki N, Kent M, Ferrara C, Port J. Three-field lymph node dissection for squamous cell and adenocarcinoma of the esophagus. Ann Surg 2002; 236: 177-183
2. Lerut T, Coosemans W, De Leyn P, Deneffe G, Topal B, Van de Ven C, Van Raemdonck D. Reflections on three field lymphadenectomy in carcinoma of the esophagus and gastro-esophageal junction. Hepatogastroenterology 1999; 46: 717-725
3. Geh JJ, Crelin AM, Glynne-Jones R. Preoperative (neoadjuvant) chemoradiotherapy in oesophageal cancer. Br J Surg 2001; 88: 338-356
4. Siewert JR, Ott K. Are squamous and adenocarcinomas of the esophagus the same disease? Semin Radiat Oncol 2007; 17: 38-44
5. Suntharalingam M, Moughan J, Coia LR, Krasna MJ, Kachnic L, Haller DG, Willett CG, John MJ, Minsky BD, Owen JB. The national practice for patients receiving radiation therapy...
for carcinoma of the esophagus: results of the 1996-1999 Patterns of Care Study. Int J Radiat Oncol Biol Phys 2003; 56: 981-987
23 Catalano V, Baldelli AM, Giordani P, Cascinu S. Molecular markers predictive of response to chemoradiotherapy in gastrointestinal tumors. Crit Rev Oncol Hematol 2001; 38: 93-104
24 Nakamura T, Hayashi K, Ota M, Ide H, Takasaki K, Mitsuhashi M. Expression of p21(Waf1/Cip1) predicts response and survival of esophageal cancer patients treated by chemoradiotherapy. Dis Esophagus 2004; 17: 315-321
25 Nakashima S, Natsugoe S, Matsumoto M, Kijima F, Takebayashi Y, Okumura H, Shimada M, Nakano S, Kusano C, Baba M, Takao S, Aikou T. Expression of p53 and p21 is useful for the prediction of preoperative chemotherapeutic effects in esophageal carcinoma. Anticancer Res 2000; 20: 1953-1957
26 Okumura H, Natsugoe S, Matsumoto M, Matakai Y, Takahori H, Ishigami S, Takao S, Aikou T. The predictive value of p53, p53R2, and p21 for the effect of chemoradiotherapy on esophageal squamous cell carcinoma. Br J Cancer 2005; 92: 284-289
27 Kim YB, Kim GE, Pyo HR, Cho NH, Keum KC, Lee CG, Seong J, Suh CO, Park TK. Differential cyclooxygenase-2 expression in squamous cell carcinoma and adenocarcinoma of the uterine cervix. Int J Radiat Oncol Biol Phys 2004; 60: 822-829
28 Takatori H, Natsugoe S, Okumura H, Matsumoto M, Uchikado Y, Sotoyama T, Sasaki K, Tamotsu K, Owaki T, Ishigami S, Aikou T. Cyclooxygenase-2 expression is related to prognosis in patients with esophageal squamous cell carcinoma. Br J Surg 2008; 94: 397-402
29 Akamatsu M, Matsumoto T, Oka K, Yamashita S, Sonoue H, Kijayama Y, Tsurumaru M, Sasai K. c-erbB-2 oncoprotein expression related to chemoradioresistance in esophageal squamous cell carcinoma. Int J Radiat Oncol Biol Phys 2003; 57: 1323-1327
30 Hickey K, Grehan D, Reid IM, O’Brien S, Walsh TN, Hennessy TP. Expression of epidermal growth factor receptor and proliferating cell nuclear antigen predicts response of esophageal squamous cell carcinoma to chemoradiotherapy. Cancer 1994; 74: 1693-1698
31 Shimada H, Hoshino T, Okazumi S, Matsubara H, Funami Y, Nabeya Y, Hayashi H, Takeda A, Shiratori T, Uno T, Ito H, Ouchi T. Expression of angiogenic factors predicts response to chemoradiotherapy and prognosis of esophageal squamous cell carcinoma. Br J Cancer 2002, 86: 552-557
32 Gorski DH, Beckett MA, Jaskowiak NT, Calvin DP, Maurer HJ, Salloum RM, Seetharam S, Koons A, Hari DM, Kufe DW, Weichselbaum RR. Blockage of the vascular endothelial growth factor stress response increases the antitumor effects of ionizing radiation. Cancer Res 1999; 59: 3374-3378
33 Schmidt U, Begley CG. Cancer diagnosis and microarrays. Int J Biochem Cell Biol 2003; 35: 119-124
34 Duong C, Greenawalt DM, Kowalczyk A, Ciavarella ML, Raskutti G, Murray WK, Phillips WA, Thomas RJ. Pre-treatment gene expression profiles can be used to predict response to neoadjuvant chemoradiotherapy in esophageal cancer. Ann Surg Oncol 2007; 14: 3602-3609
35 Quillien V, Raoul JL, Laurent JF, Meunier B, Le Prise E. Comparison of Cyfria 21-L, TPA and SCC tumor markers in esophageal squamous cell carcinoma. Oncol Rep 1998; 5: 1561-1565
36 Nakamura T, Ide H, Eguchi R, Hayashi K, Takasaki K, Watanabe S. CYFRA 21-1 as a tumor marker for squamous cell carcinoma of the esophagus. Dis Esophagus 1998; 11: 35-39
37 Westerterp M, van Westreenen HL, Reitsma JB, Hoekstra OS, Stoker J, Fockens P, Jager PL, van Eck-Smit BL, Plukker JT, van Lanschot JJ, Sloof GW. Esophageal cancer: CT, endoscopic US, and FDG PET for assessment of response to neoadjuvant therapy—systematic review. Radiology 2005; 236: 841-851
38 Bar-Shalom R, Guralnik L, Tsalik M, Leidemann M, Frenkel A, Gaitini D, Ben-Nun A, Keidar Z, Israel O. The additional chemotherapy for squamous cell carcinoma of the esophagus: do histological assessment and p53 overexpression predict chemo-responsiveness? Eur J Cancer 1997; 33: 1221-1225

Becker K, Mueller JD, Schultmacher C, Ott K, Funk U, Busch R, Bottcher K, Sievert JR, Höfler H. Histomorphology and grading of regression in gastric carcinoma treated with neo-adjuvant chemotherapy. Cancer 2003; 98: 1521-1530
10 Chirieac LR, Swisher SG, Amani RA, Komaki RR, Correa AM, Morris JS, Roth JA, Rashid A, Hamilton SR, Wu TT. Post-therapy pathologic stage predicts survival in patients with esophageal carcinoma receiving preoperative chemoradiation. Cancer 2005; 103: 1347-1353
11 Barcia M, Stahl M, von Weyher C, Weirich G, Pühringer-Oppermann F. The prognostic significance of genetic polymorphisms (Methylenetetrahydrofolate Reductase C677T, Methionine Synthase A2756G, Thymidilate Synthase tandem repeat polymorphism) in multimodally treated oesophageal squamous cell carcinoma. Br J Cancer 2006; 94: 203-207
12 Barcia M, Ott N, Pühringer-Oppermann F, Brücher BL. The predictive value of molecular markers (p53, EGFR, ATM, CHK2) in multimodally treated squamous cell carcinoma of the oesophagus. Br J Cancer 2007; 97: 1404-1408
13 Joshi MB, Shirota Y, Danenberg KD, Conlon DH, Salonga DS, Herndon JE 2nd, Danenberg PV, Harpole DH Jr. High gene expression of TSI, GSTP1, and ERCC1 are risk factors for survival in patients treated with trimodality therapy for esophageal cancer. Clin Cancer Res 2005; 11: 2215-2221
14 Gillham CM, Reynolds J, Hollywood D. Predicting the response of localised oesophageal cancer to neo-adjuvant chemoradiation. World J Surg Oncol 2007; 5: 97
15 Kishi K, Dokic Y, Miyata H, Yano M, Tanaka M, Monden M. Prediction of the response to chemoradiation and prognosis in oesophageal squamous cancer. Br J Surg 2002; 89: 597-603
16 Harpole DH Jr, Moore MB, Herndon JE 2nd, Aloia T, D’Amico TA, Sparr T, Parr A, Linolla I, Allegra C. The prognostic value of molecular marker analysis in patients treated with trimodality therapy for esophageal cancer. Clin Cancer Res 2001; 7: 562-569
17 Quinlan DC, Davidson AG, Summers CL, Warden HE, Doshi HM. Accumulation of p53 protein correlates with a poor prognosis in human lung cancer. Cancer Res 1992; 52: 4828-4831
18 Thomas DJ, Robinson M, King P, Hasan T, Charlton R, Martin J, Carr TW, Neal DE. p53 expression and clinical outcome in prostate cancer. Br J Urol 1993; 72: 778-781
19 Ribeiro U Jr, Finkelstein SD, Safalet-Ribeiro AV, Landreneau RJ, Clarke MR, Bakker A, Swalsky PA, Gooding WE, Posner MC. p53 sequence analysis predicts treatment response and outcome of patients with esophageal carcinoma. Cancer 1998; 83: 7-18
20 Lowe SW, Ruley HE, Jacks T, Housman DE. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. Cell 1993; 74: 957-967
21 Seitz JF, Perrier H, Monges G, Giovannini M, Gourmelen J. [Multivariate analysis of the prognostic and predictive factors of response to concomitant radiochemotherapy in epithelioderms of the esophagus. Value of immunodetection of protein p53] Gastroenterol Clin Biol 1995; 19: 465-474
22 Lam KY, Law S, Ma LT, Ong SK, Wong J. Pre-operative
value of PET/CT over PET in FDG imaging of oesophageal cancer. Eur J Nucl Med Mol Imaging 2005; 32: 918-924
39 Schelling M, Avril N, Nährig J, Kuhn W, Römer W, Sattler D, Werner M, Dose J, Janicke F, Graeff H, Schweiger M. Positron emission tomography using [18F]Fluorodeoxyglucose for monitoring primary chemotherapy in breast cancer. J Clin Oncol 2000; 18: 1689-1695
40 Thomas DM, Mitchell PL, Berlangieri SU, Tochon-Danguy H, Knight S, Clarke CP, Scott AM. Positron emission tomography in assessing response to neoadjuvant chemotherapy for non-small-cell lung cancer. Med J Aust 1998; 169: 227
41 Yoshikawa T, Fukuda H, Fujitani T, Iwata R, Ido T, Murakawa Y, Gamo M, Ishioka C, Kanamaru R. FDG PET evaluation of residual masses and regrowth of abdominal lymph node metastases from colon cancer treated with CT during chemotherapy. Clin Nucl Med 1999; 24: 261-263
42 Huelenscheidt B, Sautter-Bihl ML, Lang O, Maul FD, Fischer J, Margenthaler HG, Bihl H. Whole body positron emission tomography in the treatment of Hodgkin disease. Cancer 2001; 91: 302-310
43 Okada J, Yoshikawa K, Inazeki K, Minoshima S, Uno K, Itami J, Kuyama J, Maruno H, Arimizu N. The use of FDG-PET in the detection and management of malignant lymphoma: correlation with pathologic uptake of prognosis. J Nucl Med 1991; 32: 686-691
44 Song SY, Kim JH, Ryu JS, Lee GH, Kim SB, Park SL, Song HY, Cho KJ, Ahn SD, Lee SW, Shin SN, Choi EK. FDG-PET in the prediction of pathologic response after neoadjuvant chemoradiotherapy in locally advanced, resectable esophagogastric cancer. Int J Radiat Oncol Biol Phys 2005; 63: 1053-1059
45 Wieder HA, Brücher BL, Zsarnemann F, Becker K, Lordick F, Beer A, Schweiger M, Fink U, Siewert JR, Stein HJ, Weber WA. Time course of tumor metabolic activity during chemoradiotherapy of esophageal squamous cell carcinoma and response to treatment. J Clin Oncol 2004; 22: 900-908
46 Flamenc P, Van Cutsen E, Lerut A, Cambier JP, Haustermans K, Bormans G, De Leyn P, Van Raemdonck D, De Wever W, Eckers N, Maes A, Mortelmans L. Positron emission tomography for assessment of the response of chemoradiotherapy in locally advanced oesophageal cancer. Ann Oncol 2002; 13: 361-368
47 Kato H, Kuwano H, Nakajima M, Miyazaki T, Yoshikawa M, Masuda N, Fukuchi M, Manda R, Tsukada K, Orikuchi N, Endo K. Usefulness of positron emission tomography for assessment of the response of neoadjuvant chemoradiotherapy in patients with esophageal cancer. Am J Surg 2002; 184: 279-283
48 Brücher BL, Weber W, Bauer M, Fink U, Avril N, Stein HJ, Werner M, Zimmermann F, Siewert JR, Schweiger M. Neoadjuvant therapy of esophageal squamous cell carcinoma: response evaluation by positron emission tomography. Ann Surg 2001; 233: 300-309
49 Gillham CM, Lucey JA, Keoghan M, Duffy GJ, Malik V, Raouf AA, O’Byrne K, Hollywood D, Muldoon C, Reynolds JV. (18)FDG uptake during induction chemoradiation for oesophageal cancer fails to predict histomorphological tumour response. Br J Cancer 2006; 95: 1174-1179
50 Langer R, Specht K, Becker K, Ewald P, Bekesch M, Sarbia M, Busch R, Feitlach M, Stein HJ, Siewert JR, Höfler H. Association of pretherapeutic expression of chemotheraphy-related genes with response to neoadjuvant chemotherapy in Barrett carcinoma. Clin Cancer Res 2005; 11: 7462-7469
51 Theisen J, Danenberg K, Ott K, Becker K, Danenberg P, Stein HJ, Siewert JR. Predictors of response and survival for neoadjuvant treated patients with esophageal adenocarcinoma. Dis Esophagus 2008; 21: 601-606
52 Warnecke-Eberz U, Metzger R, Miyazono F, Baldus SE, Neiss S, Brabender J, Schaefer H, Doerfler W, Bollschweiler E, Dienes HP, Mueller RP, Danenberg PV, Hoelscher AH, Schneider PM. High specificity of quantitative excision repair cross-complementing 1 messenger RNA expression for prediction of minor histopathological response to neoadjuvant radiochemotherapy in esophageal cancer. Clin Cancer Res 2004; 10: 3794-3799
53 Duahayongsod FG, Gottfried MR, Iglehart JD, Vaughan AL, Wolfe WG. The significance of c-erb B-2 and p53 immunoreactivity in patients with adenocarcinoma of the esophagus. Ann Surg 1995; 221: 677-683; discussion 683-684
54 Luthra R, Luthra MG, Izzo J, Wu TT, Lopez-Alvarez E, Malhotra U, Choi IS, Zhang L, Ajani JA. Biomarkers of response to preoperative chemoradiation in esophageal cancers. Semin Oncol 2006; 33: S2-S5
55 Schauer M, Janssen KP, Rimmkus C, Raggi M, Feith M, Friess H, Theisen J. Microarray based response prediction in esophageal adenocarcinomas. Clin Cancer Res 2009; In press
56 Ma Y, Ding Z, Qian Y, Shi X, Castranova V, Harner EJ, Guo L. Predicting cancer drug response by proteomic profiling. Clin Cancer Res 2006; 12: 4583-4589
57 Luthra R, Wu TT, Luthra MG, Izzo J, Lopez-Alvarez E, Zhang L, Bailey J, Lee JH, Bressler R, Rashid A, Swisher SG, Ajani JA. Gene expression profiling of localized esophageal carcinomas: correlation with pathologic response to preoperative chemoradiation. J Clin Oncol 2006; 24: 259-267
58 Ashida A, Boku N, Aoyagi K, Sato H, Tsubosa Y, Minashi K, Muto M, Ohtsu A, Ochiai A, Yoshida T, Yoshida S, Sasaki H. Expression profiling of esophageal squamous cell carcinoma patients treated with definitive chemoradiotherapy: clinical implications. Int J Oncol 2008; 28: 1345-1352
59 Ott K, Weber WA, Lordick F, Becker K, Busch R, Herrmann K, Wieder H, Fink U, Schweiger M, Siewert JR. Metabolic imaging predicts response, survival, and recurrence in adenocarcinomas of the esophagogastric junction. J Clin Oncol 2006; 24: 4692-4698
60 Lordick F, Ott K, Krause BJ, Weber WA, Becker K, Stein HJ, Lorentzen S, Schuster T, Wieder H, Herrmann K, Bredenkamp R, Höfler H, Fink U, Poschel C, Schweiger M, Siewert JR. PET to assess early metabolic response and to guide treatment of adenocarcinoma of the esophagogastric junction: the MUCONII phase II trial. Lancet Oncol 2007; 8: 797-805
61 Levine EA, Farmer MR, Clark P, Mishra G, Ho C, Geisinger KR, Melin SA, Lovato J, Oaks T, Blackstock AW. Predictive value of 18-fluoro-deoxy-glucose-positron emission tomography (18F-FDG-PET) in the identification of responders to chemoradiation therapy for the treatment of locally advanced esophageal cancer. Ann Surg 2006; 243: 472-478
62 Swisher SG, Erasmus J, Maish M, Correa AM, Macapinlac H, Ajani JA, Cox JD, Komaki RR, Hong D, Lee HK, Putnam JB Jr, Rice DC, Smythe WR, Thai L, Vaporiyan AA, Walsh GL, Wu TT, Roth JA. 2-Fluoro-2-deoxy-D-glucose positron emission tomography imaging is predictive of pathologic response and survival after preoperative chemoradiation in patients with esophageal carcinoma. Cancer 2004; 101: 1776-1785
63 Brink I, Hentschel M, Bley TA, Walch A, Mix M, Kleimaier M, Moser E, Imdahl A. Effects of neoadjuvant radio-chemotherapy on 18F-FDG-PET in esophageal carcinoma. Eur J Surg Oncol 2004; 30: 544-550
64 Arslan N, Miller TR, Dehdashfi S, Battafarano RJ, Siegel BA. Evaluation of response to neoadjuvant therapy by quantitave 2-deoxy-2-[18F]fluoro-D-glucose with positron emission tomography in patients with esophageal cancer. Mol Imaging Biol 2002; 4: 301-310
65 Weber WA, Ott K, Becker K, Dittler HJ, Helmberger H, Avril NE, Meisseltschläger G, Busch R, Siewert JR, Schweiger M, Fink U. Prediction of response to preoperative chemotheraphy in adenocarcinomas of the esophagogastric junction by metabolic imaging. J Clin Oncol 2001; 19: 3058-3065

S-Editor Li LF L-Editor Stewart GJ E-Editor Lin YP