Improvement of the multimodality treatment of oesophageal cancer
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Mutational analysis of EGFR, KRAS and BRAF in oesophageal cancer: a surgical patient series and review of the literature

Submitted

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Abstract

Introduction
Although neoadjuvant chemoradiation has lowered the risk for local recurrence after surgery in patients with oesophageal cancer, novel therapies are being explored to further improve outcome results. The epidermal growth factor receptor (EGFR) and its downstream factors KRAS and BRAF are frequently mutated oncogenes in various human malignancies. Given recent reports of responsive cases of oesophageal cancer to targeted therapy, we aimed to determine the mutation frequencies of KRAS, EGFR and BRAF in pretreatment oesophageal tumour biopsies.

Methods
Archival biopsy specimens were obtained from 41 patients who had been treated for oesophageal cancer with chemoradiotherapy followed by surgical resection. In 30 patients, biopsies contained more than 50% tumour cells. DNA mutations in KRAS, EGFR and BRAF could be examined in 21 patients. The literature on these mutations is reviewed.

Results
KRAS, EGFR and BRAF mutations were not detected in any of the investigated cancer tissue samples. That is, one possible deletion of EGFR in exon 19 that was identified in a patient with a pathological complete response (pCR) could not be confirmed by fragmentation analysis. Reported frequencies of KRAS, EGFR and BRAF mutations in oesophageal cancer vary largely.

Conclusion
In this series of oesophageal cancer patients with potentially curable disease, KRAS, EGFR and BRAF mutations were absent. On the basis of these results, caution is warranted when introducing targeted therapies in the treatment of non-metastatic oesophageal cancer.
Introduction

Oesophageal cancer currently represents the sixth most frequent cause of death from cancer worldwide. It is an aggressive disease with early lymphatic and haematogenous dissemination. Five-year survival rates rarely exceed 30% after intentionally curative oesophagectomy. Improvement in oesophageal cancer treatment includes better selection of patients with an unfavourable prognosis from the outset in whom intensive (local) therapy will only do harm. These patients should be discriminated from those patients who can be cured. Neoadjuvant chemoradiation shows promising results, with pathological complete response rates ranging from 13 to 49%, but the need for novel strategies to improve treatment outcome is still warranted.1

It is widely accepted that self-sufficiency in growth signals and insensitivity to growth-inhibitory signals play an important role in the development of cancer.2 Increasing evidence exists that carcinogenesis must be understood in terms of accumulation of mutations in regulatory genes, including activation of oncogenes and inactivation or loss of tumour-suppressor genes.3 With the recent advances in drug development, there are emerging possibilities to utilize antagonists of growth factor signal transduction – and activated kinases-pathways in targeted therapy. Possibly, knowledge of the mutational status of oesophageal tumours may open doors to targeted therapies.

The Ras-Raf-MEK-ERK kinase-pathway is an important mediator of a number of cellular fates including cellular responses to growth signals, proliferation and survival.4

KRAS genes are localized on the human chromosome 12. KRAS mutations have been detected in lung, pancreatic, colon, small intestinal, and stomach cancer.5-7 In oesophageal cancer, previous reports of KRAS mutations have resulted in widely varying results.8-11

In colorectal and lung cancer, the mutational status of KRAS dictates responsiveness to therapies against the epidermal growth factor receptor (EGFR).12,13 On the other hand, an EGFR mutation is closely associated with a favourable response to treatment with tyrosine kinase (TK) inhibitors.14,15 In vitro studies have indicated that inhibition of EGFR function may be a worthwhile approach for treating oesophageal cancers.16

BRAF is part of the raf family genes. These genes encode highly conserved serine/threonine cytoplasmic kinases and play important roles in proliferation, differentiation and programmed cell death. Recent data have shown that BRAF is mutated in about 7% of cancers.17

The aim of this retrospective study was to determine the incidence of mutations in KRAS, EGFR and BRAF genes in oesophageal cancer biopsies that were taken prior to chemoradiotherapy followed by surgical resection.
Material and methods

Patients
Between 1997 and 2007, 81 patients with oesophageal cancer were treated with concurrent chemoradiation at our institute. Only surgically resected cases, for whom we could standardize evaluation of histopathological response, were enrolled in the present study (Figure 1). Follow-up data were collected until death or March 31, 2010.

Figure 1: Flow chart of patient selection for the present study

Chemoradiotherapy and surgical treatment
At our institute, neoadjuvant chemoradiation for patients with oesophageal cancer has been given since January 1997. The preferred regimen consisted of two cycles of cisplatin 75 mg/m² and intravenous 5-fluorouracil (FU) 800 mg/m², in combination with 50 Gray in 25 fractions. For patients with hearing loss or diminished renal function, cisplatin was considered to be contraindicated. These patients received a regimen that consisted of carboplatin targeted at an “area under the curve” of 2, and paclitaxel 50 mg/m², combined with 50.4 in 28 fractions.
Exclusion criteria for oesophageal resection were progressive disease during chemoradiation, tumour invasion into neighbouring organs, distant metastasis and insufficient cardiopulmonary reserve. If possible, surgery was planned 5-6 weeks after the completion of the administered regimen. Both transhiatal and transthoracic oesophageal resections were performed.

**Tissue collection**
Archival specimens of oesophageal cancer biopsies obtained at the time of primary diagnosis were collected to analyse the gene status of EGFR, KRAS and BRAF. An experienced pathologist (MLvV) examined all biopsies and marked the areas consisting of at least 50% tumour tissue.

**DNA Isolation**
Paraffin-embedded tissue sections were macrodissected, and serial 10-μm paraffin sections were deparaffinized by standard procedures and incubated for 16 hours in 1 M sodium thiocyanate at 37°C, followed by 2 × 5 min wash in phosphate-buffered saline (Dulbecco). Subsequently, the region containing the highest percentage of tumour cells (at least 50%) was microdissected. The tissue was then transferred to a tube containing digestion buffer: 2 mg/ml proteinase-K (Roche Diagnostics, Pleasanton, California, USA) in 10 mM Tris–HCl (pH 8.0), 50 mM KCl, 2.5 mM MgCl₂, 0.5% Tween-80 and 0.1 mg/ml gelatin. Tubes were incubated for 24 hours at 55°C, proteinase-K was then heat inactivated at 80°C for 10 minutes, and after centrifugation the supernatant was transferred to a clean tube and stored at 4°C until use.

**KRAS mutational analysis**
Mutational analysis of KRAS was performed by polymerase chain reaction (PCR) amplification and high-resolution melting (HRM) using a Light Cycler 480-II system (Roche Diagnostics, Penzberg, Germany) and primers flanking the KRAS hotspot region of codon 12 and 13. \(^{19,20}\)

The reaction mixtures with a final volume of 10 μl contained: 500 nM forward primer, 100 nM reverse primer, 500 nM unlabelled probe with a 3-conjugated C₃ spacer, and 10 ng genomic DNA in 1× LightScanner Master mix (Idaho Technologies, Salt Lake City, UT, USA). The cycling and melting conditions were as follows: one cycle of 30 sec 95°C; 50 cycles of 5 sec 95°C; 30 sec 64°C and one cycle of 60 sec 64°C, 20 sec 95°C, 20 sec 55°C; with a final melting in two steps: Melt 1 (55–78°C at 0.06°C/sec) followed by Melt 2 (78–95°C, 0.06°C/sec) and continuously recording of the fluorescent level. The change of fluorescence was converted to a melting peak by plotting the negative derivative of the fluorescent signal corresponding to the temperature (−dF/dT) on the LightCycler software.
The analysis of Real-Time PCR and HRM data was performed using the LightCycler® 480SW1.5 (Roche) software.

For sample analysis, amplification and cycle threshold (CT) values were checked (sigmoid curve, CT<40). Probe- and amplification melt were analysed (negative derivative of fluorescence against the temperature). Data were normalized, temperature-adjusted and a difference plot was generated. A reference control (i.e. pooled DNA isolated from blood of 7 women, fragmented into 600 bp fragments) was used to normalize melting profiles of the other samples against this predefined horizontal baseline. Products (in duplo) with different probe melting curves against the wild-type were considered mutated and were sequenced (in duplo) with the M13 Forward primer (TGT AAA ACG ACG GCC AGT).20

**EGFR mutational analysis**

For EGFR mutational analysis, exons 18–21 were PCR amplified using exon-specific primers: exon 18 forward primer (18-F): 5′-GCTGAGGTGACCCCTTGCTCTC-3'; exon 18 reverse primer (18-R): 5′-CTCCCCACCAGACCATGA-3'; 19-F: 5′-CATGCGTCTTACCTGGAA-3'; 20-F: 5′-CATGCGTCTTACCTGGAA-3'; 20-R: 5′-AGCAGGTACTGGGAGCCAAT-3'; 21-F: 5′-CTTCAGCACAGGGCTTCTC-3' and 21-R: 5′-TGCTCTCCTTCTGCATGGTA-3'. After an initial round of PCR amplification, PCR products were visualized by agarose gel electrophoresis to check the quality of the PCR products. Subsequently, PCR fragments were purified and subjected to cycle sequence reactions using BigDye Terminators (DNA sequencing kit, Applied Biosystems, Foster City, California, USA). The sequence fragments were precipitated and analysed using an automated sequencer (ABI3700).

In addition, we have carried out PCR fragment analysis using a fluorescently labelled (FAM) primer on the automated sequencer for sensitive detection of the exon 19 deletion (19-F primer: 5′-FAM-CATGCGTCTTACCTGGAA-3').

**BRAF mutational analysis**

For BRAF codon V600E mutation analysis, a similar procedure as for EGFR mutation analysis was followed. After amplification by PCR and both forward and reverse sequence, reactions were repeated for confirmation. The following primers were used: forward 5′-TGTAASACGACCGAGCTGATGTCATATTTTCTCAGATGGAC-3', and reverse 5′-CAACTGTTCAAACTGTAGGGAC-3'.

**Histopathological evaluation of surgical resection specimens**

Biopsies and corresponding surgically resected primary tumours were paraffin-embedded after fixation with formaldehyde. Serial sections of each block were cut and stained with
haematoxylin and eosin (H&E). Conventional histopathological examination was performed on the H&E stained slides. The histopathological response to chemoradiation was evaluated according to the classification as described by Mandard et al\textsuperscript{21}: Tumour Regression Grade (TRG) 1: no residual cancer (complete regression); TRG 2: rare residual cancer cells; TRG 3: fibrosis outgrowing residual cancer; TRG 4: residual cancer outgrowing fibrosis; TRG 5: absence of regressive change.

**Results**

**Characteristics**
In this retrospective series, 41 patients underwent oesophageal resection after neoadjuvant chemoradiation (Figure 1). In 30 patients, more than 50% tumour cells was present in the biopsy obtained before the start of treatment. Their median age was 59 (range 41-76) years, and 22 patients were male (73%). Twenty-four (80%) patients had an adenocarcinoma and 6 (20%) had a squamous cell carcinoma. Stage distribution was as follows: stage II 8 (27%) patients, stage III 17 (57%) patients and stage IVa 5 (17%) patients. The majority (27 patients; 90%) was treated with the 5FU/cisplatin chemoradiation scheme.

**KRAS**
KRAS mutation status could be determined in 23 out of 30 patients, because in the remaining cases, the amount of DNA quality was too limited. No mutations were detected. Positive controls (DNA embedded lung tumours) were positive.

**EGFR**
As most reported mutations in EGFR are in exon 19 and 21, we first determined these mutations. In samples with sufficient DNA, we also performed sequence analysis for exon 18 and 20.

For exon 19, 21 samples were evaluable. In none of them, a deletion was detected. One possible deletion was not reproduced after repeated fragment analysis on new isolated DNA with sufficient tumour. Exon 21 could be analysed in 14 samples, but no mutations were detected.

EGFR exon 18 could be analysed in only 9 samples. No mutations were detected. For EGFR exon 20 analysis, 12 samples could be analysed. In none of them, a mutation was detected.

Positive controls (formalin-fixed paraffin-embedded lung tissue) for exon 21 and exon 19 deletions were all positive.
**BRAF**

BRAF mutation analysis could be performed in 21 cases. In none of them, a BRAF mutation was demonstrated.

**Treatment outcome**

Pathological complete response (pCR) was observed in 10 out of 30 (33%) patients. These pCR patients had a better survival than non-pCR patients (3-year survival rate of 69% versus 46%, respectively; \( p = 0.04 \)). Since we did not detect any mutations in KRAS, EGFR or BRAF, no correlations between histopathological features and mutational status could be made.

**Review of the literature**

In Table 1, an overview is given of published studies on KRAS, EGFR or BRAF mutations in oesophageal cancer.\(^{4,8-11,22-29}\) In Table 2, a summary of studies on targeted agents in patients with locally advanced oesophageal cancer is presented.\(^{30-42}\)

### Table 1: Summary of published studies on KRAS, EGFR and BRAF mutations in oesophageal cancer

<table>
<thead>
<tr>
<th>Authors</th>
<th>Number of patients</th>
<th>Histology</th>
<th>Tumour percentage</th>
<th>Method</th>
<th>Number of mutations</th>
<th>Type of mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyronis et al(^4)</td>
<td>30</td>
<td>SCC</td>
<td>NM</td>
<td>RFLP</td>
<td>5 (16%)</td>
<td>Point mutation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>codon 12</td>
</tr>
<tr>
<td>Sommerer et al(^9)</td>
<td>19</td>
<td>AC</td>
<td>NM</td>
<td>DNA sequencing</td>
<td>4 (21%)</td>
<td>Point mutation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>codon 12</td>
</tr>
<tr>
<td>Janmaat et al(^10)</td>
<td>23</td>
<td>AC/SCC</td>
<td>NM</td>
<td>DNA sequencing</td>
<td>2 (9%)</td>
<td>Point mutation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>codon 12</td>
</tr>
<tr>
<td>Meltzer et al(^11)</td>
<td>5</td>
<td>AC</td>
<td>NM</td>
<td>DNA sequencing</td>
<td>0 (0%)</td>
<td>NA</td>
</tr>
<tr>
<td>Lord et al(^12)</td>
<td>23</td>
<td>AC</td>
<td>NM</td>
<td>REMS-PCR</td>
<td>7 (30%)</td>
<td>Point mutation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>codon 12</td>
</tr>
<tr>
<td>Casson et al(^13)</td>
<td>19</td>
<td>AC</td>
<td>&gt;80%</td>
<td>DNA hybridization and sequencing</td>
<td>1 (5%)</td>
<td>Point mutation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>codon 12</td>
</tr>
<tr>
<td>Trautmann et al(^14)</td>
<td>11</td>
<td>AC</td>
<td>NM</td>
<td>RFLP</td>
<td>4 (36%)</td>
<td>Point mutation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>codon 12</td>
</tr>
<tr>
<td>Lorenzen et al(^15)</td>
<td>37</td>
<td>SCC</td>
<td>≥60%</td>
<td>DNA sequencing</td>
<td>0 (0%)</td>
<td>NA</td>
</tr>
<tr>
<td>Present study</td>
<td>23</td>
<td>AC/SCC</td>
<td>≥50%</td>
<td>HRM</td>
<td>0 (0%)</td>
<td>NA</td>
</tr>
</tbody>
</table>
In this study, including a limited group of oesophageal cancer patients, who underwent neoadjuvant chemoradiation followed by surgery, no mutations in KRAS, EGFR and BRAF genes were found.

Our institution has a broad experience in performing diagnostic mutational analyses for different types of tumours on a routine basis. There is a continuous strive to refine existing techniques, to explore new techniques, and there are strict rules on the validation.

**Discussion**

<table>
<thead>
<tr>
<th>EGFR</th>
<th>Number of patients</th>
<th>Histology</th>
<th>Tumour percentage</th>
<th>Method</th>
<th>Number of mutations</th>
<th>Type of mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Janmaat et al⁴⁴</td>
<td>26</td>
<td>AC/SCC</td>
<td>NM</td>
<td>DNA sequencing</td>
<td>0 (0%)</td>
<td>NA</td>
</tr>
<tr>
<td>Sudo et al²³</td>
<td>50</td>
<td>AC/SCC</td>
<td>NM</td>
<td>DNA sequencing</td>
<td>1 (2%)</td>
<td>Codon 719 exon 18</td>
</tr>
<tr>
<td>Puhringer-Op-perman et al⁶⁶</td>
<td>105</td>
<td>AC</td>
<td>≥ 70%</td>
<td>SSCP</td>
<td>1 (1%)</td>
<td>Silent mutation codon</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>In-frame deletion delE746-A750 exon 19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>False start codon 1719 exon 18</td>
</tr>
<tr>
<td>Kwak et al⁷⁷</td>
<td>17</td>
<td>AC</td>
<td>≥ 50%</td>
<td>DNA sequencing</td>
<td>2 (12%)</td>
<td>Missense L858R exon 21</td>
</tr>
<tr>
<td>Hanawa et al⁸⁴</td>
<td>40</td>
<td>SCC</td>
<td>NM</td>
<td>DNA sequencing</td>
<td>0 (0%)</td>
<td>NA</td>
</tr>
<tr>
<td>Sunpaweravong et al⁴⁹</td>
<td>48</td>
<td>SCC</td>
<td>NM</td>
<td>DNA sequencing</td>
<td>0 (0%)</td>
<td>NA</td>
</tr>
<tr>
<td>Present study</td>
<td>21</td>
<td>AC/SCC</td>
<td>≥ 50%</td>
<td>DNA sequencing + fragment analysis</td>
<td>0 (0%)</td>
<td>NA</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BRAF</th>
<th>Number of patients</th>
<th>Histology</th>
<th>Tumour percentage</th>
<th>Method</th>
<th>Number of mutations</th>
<th>Type of mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyronis et al⁶</td>
<td>30</td>
<td>SCC</td>
<td>NM</td>
<td>RFLP</td>
<td>0 (0%)</td>
<td>Mutation T1796A (p.VAL599GLU)</td>
</tr>
<tr>
<td>Sommerer et al⁹</td>
<td>19</td>
<td>AC</td>
<td>NM</td>
<td>DNA sequencing</td>
<td>2 (11%)</td>
<td>NA</td>
</tr>
<tr>
<td>Present study</td>
<td>21</td>
<td>AC/SCC</td>
<td>≥ 50%</td>
<td>DNA sequencing</td>
<td>0 (0%)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: SCC: squamous cell carcinoma; AC: adenocarcinoma; NM: not mentioned; RFLP: restriction fragment length polymorphism; REMS-PCR: restriction endonuclease-mediated selective-polymerase chain reaction; DNA: Deoxyribonucleic acid; HRM: High resolution melting analysis; SSCP: single-strand conformation polymorphism; NA: not applicable.
Chapter 6

of positive results. For example, in the present series, one possible deletion was not reproduced after repeated fragmentation analysis on new isolated DNA. Furthermore, positive control samples were included and revealed the expected mutations. Regarding our tissue selection, an important inclusion criterion was that the biopsy had to consist of at least 50% tumour cells. This threshold should have precluded the possibility of too limited sensitivity. Still, a significant proportion of the samples had to be rejected because of poor DNA quality. Most biopsies (mostly one per patient) had been taken many years

<table>
<thead>
<tr>
<th>Author</th>
<th>Agents</th>
<th>Number of patients</th>
<th>Histology</th>
<th>ORR or pCR rate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anti-epidermal growth factor receptor antibodies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enzinger et al&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cetuximab + Cis/CPT/RT</td>
<td>17</td>
<td>17 AC</td>
<td>13% (2/15)</td>
</tr>
<tr>
<td>Safran et al&lt;sup&gt;g&lt;/sup&gt;</td>
<td>Cetuximab + Carbo/paclitaxel/RT</td>
<td>57</td>
<td>45 AC &amp; 12 SCC</td>
<td>28% (13/46)</td>
</tr>
<tr>
<td>De Vita et al&lt;sup&gt;h&lt;/sup&gt;</td>
<td>Cetuximab + FOLFOX/RT</td>
<td>27</td>
<td>9 AC &amp; 18 SCC</td>
<td>40% (4/10)</td>
</tr>
<tr>
<td>Ruhstaller et al&lt;sup&gt;i&lt;/sup&gt;</td>
<td>Cetuximab + Cis/docetaxel/RT</td>
<td>28</td>
<td>15 AC &amp; 13 SCC</td>
<td>32% (9/28)</td>
</tr>
<tr>
<td>Agarwala et al&lt;sup&gt;a4&lt;/sup&gt;</td>
<td>Cetuximab + RT</td>
<td>40</td>
<td>31 AC &amp; 9 SCC</td>
<td>36% (13/36)</td>
</tr>
<tr>
<td><strong>Anti-epidermal growth factor tyrosinase kinase inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sunpaweravong et al&lt;sup&gt;a5&lt;/sup&gt;</td>
<td>Gefitinib + 5FU/Cis +/- RT</td>
<td>27</td>
<td>NS</td>
<td>25% (1/4)</td>
</tr>
<tr>
<td>Javle et al&lt;sup&gt;a6&lt;/sup&gt;</td>
<td>Gefitinib/oxaliplatin/RT</td>
<td>6</td>
<td>6 AC</td>
<td>33% (2/6)</td>
</tr>
<tr>
<td>Rodriguez et al&lt;sup&gt;a7&lt;/sup&gt;</td>
<td>Gefitinib/5FU/Cis/RT pre- and post-operatively</td>
<td>80</td>
<td>75 AC &amp; 5 SCC</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Anti-Her-2/neu monoclonal antibodies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Safran et al&lt;sup&gt;a8&lt;/sup&gt;</td>
<td>Trastuzumab + Cis/paclitaxel/RT</td>
<td>19</td>
<td>19 AC</td>
<td>50% (3/6)</td>
</tr>
<tr>
<td><strong>Anti-vascular endothelial growth factor monoclonal antibodies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ilson et al&lt;sup&gt;a9&lt;/sup&gt;</td>
<td>Bevacizumab + Cis/CPT/RT</td>
<td>18</td>
<td>18 AC</td>
<td>10% (1/10)</td>
</tr>
<tr>
<td><strong>Other targeted therapies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Govindan et al&lt;sup&gt;a10&lt;/sup&gt;</td>
<td>Celecoxib + 5FU/Cis/RT</td>
<td>31</td>
<td>NS</td>
<td>23% (5/22)</td>
</tr>
<tr>
<td>Enzinger et al&lt;sup&gt;a11&lt;/sup&gt;</td>
<td>Celecoxib + Cis/CPT/RT</td>
<td>36</td>
<td>30 AC &amp; 6 SCC</td>
<td>44% (11/25)</td>
</tr>
<tr>
<td>Dawson et al&lt;sup&gt;a12&lt;/sup&gt;</td>
<td>Celecoxib + 5FU/Cis/RT</td>
<td>13</td>
<td>10 AC &amp; 3 SCC</td>
<td>17% (1/6)</td>
</tr>
</tbody>
</table>

Abbreviations: Cis: cisplatin; CPT: irinotecan; RT: radiation therapy; Carbo: carboplatin; FOLFOX: leucovorin/5-fluorouracil/oxaliplatin; 5FU: 5-fluorouracil; Her: Human epidermal growth factor receptor; AC: adenocarcinoma; SCC: squamous cell carcinoma; ORR: objective response rate; pCR: pathologic complete response; NS: not stated
ago, which could explain this result. We chose to perform these analyses on pretreatment biopsies. In surgical resection specimens, the mutational status may have been influenced by the effects of chemoradiation.

In four out of eight studies reporting on KRAS mutation status in oesophageal cancer (Table 1), less than 10% KRAS mutated cases were identified. On the other hand, in two other studies a KRAS mutation was identified in more than 30% of patients. Due to the small number of patients in each study it is hard to draw conclusions, but there does not seem to be a correlation between histological type or method of mutational analysis and KRAS gene status.

While EGFR is commonly overexpressed, mutations in EGFR are not frequently found in oesophageal cancers. For example, in a large series of 105 samples of oesophageal adenocarcinoma, exons 19 and 21 were analysed and only one silent mutation in exon 19 was found. Kwak et al analysed 17 oesophageal adenocarcinomas and in two (12%) cases, an EGFR mutation was documented. In a phase II study on gefitinib, all of the 26 evaluated oesophageal tumours were wild type for exons 18 to 21 of the EGFR gene. This study did show that clinical outcome was significantly better in patients demonstrating high EGFR expression.

In accordance with our results, no BRAF mutation could be detected in a study on 30 patients with oesophageal squamous cell carcinoma. In an other study, 2 (11%) out of 19 Barrett’s cancers had a BRAF mutation.

It is unclear why the findings in all these studies vary to such an extent. As can be seen in Table 1, there are differences in study numbers, histology and methodology. Furthermore, there are also geographical differences. An explanation for the variety in results can not be detected. Unfortunately, due to the lack of mutations in KRAS, EGFR and BRAF in the present study, no correlation with clinicopathological features could be made.

Although the frequency of mutational abnormalities in oesophageal cancer has been investigated in relatively small studies, many studies have already been undertaken to test new targeted agents in oesophageal cancer patients. It should be noted that eight out of 13 studies were published as abstracts only. A major challenge in the evaluation of targeted therapies is to identify a valid target. It is clear that the molecular pathways that drive oncogenesis involve complex hierarchical interactions. Discarding a single empirically-chosen signal may therefore lead to no or limited clinical effect unless this signal is critically required for maintenance of the malignant phenotype.

With evolving knowledge of molecular and genetic defects in the various subtypes of oesophageal cancer, rationally designed targeted therapies can be tested in the clinical setting. At the moment, there are several ongoing phase III evaluations of anti-EGFR and
anti-VEGF-agents in patients with oesophageal cancer.\textsuperscript{45} These trials are expected to elucidate the role of these targeted agents as monotherapy or in combination with chemoradiotherapy.

As for now, targeted therapy in the treatment of oesophageal cancer is still in development and underlying mechanisms are to be explored. On the basis of our results, caution is warranted when introducing targeted therapies in the treatment of non-metastatic oesophageal cancer. Clinical studies have to be combined with mutational testing. The crux will be to synchronize our laboratory techniques, validation protocols, and data analysis.
References

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