Abstract. Members of the epidermal growth factor receptor, EGFR, family are interesting as targets for radionuclide therapy using targeting agents labeled with α- or β-emitting radionuclides, especially when EGFR-positive colorectal carcinomas, CRC, are resistant to EGFR inhibiting agents like cetuximab and various tyrosine kinase inhibitors. The expression of EGFR, HER2 and HER3 was therefore analyzed in CRC samples from primary tumors, corresponding lymph node metastases and, in a few cases, liver metastases. The expression of HER2 and EGFR was scored from immunohistochemical preparations using the HercepTest criteria 0, 1+, 2+ or 3+ for cellular membrane staining while HER3 expression was scored as no, weak or strong cytoplasm staining. Material from 60 patients was analyzed. The number of EGFR 2+ or 3+ positive primary tumors was 16 out of 56 (29%) and for lymph node metastases 8 out of 56 (14%) whereas only one out of nine (11%) liver metastases were positive. Thus, there was lower EGFR positivity in the metastases. Only one among 53 patients was strongly HER2 positive and this in both the primary tumor and the metastasis. Eight out of 49 primary tumors (16%) were strongly HER3 positive and the corresponding numbers for lymph node metastases were 9 out of 49 (18%) and for liver metastases 2 out of 9 (22%). The observed number of strongly EGFR positive cases was somewhat low but EGFR might be, for the cases with high EGFR expression in metastases, a target for radionuclide therapy. HER2 seems not to be of such interest due to rare expression, neither HER3 due to mainly expression in the cytoplasm. The requirements for successful EGFR targeted radionuclide therapy are discussed, as well as patient inclusion criteria related to radionuclide therapy.

Introduction

There is, so far, no successful curative treatment for the majority of patients with disseminated colorectal carcinoma (CRC). The disease represents the third most frequent malignancy worldwide, with about one million new cases diagnosed yearly (1). The incidence and mortality rates of CRC are higher in western than in eastern countries. However, the incidence rates have increased rapidly in some previously low incidence areas, such as Shanghai, China (2). The majority of CRC patients are diagnosed with resectable localized disease. However, even after potentially curative surgical excisions, about half of the patients will develop local recurrences or distant metastases, which in most cases will be fatal. Combinations of irinotecan, oxaliplatin, and 5-fluorouracil with leucovorin have some efficacy, but the prognosis remains poor with median survival of ~18-20 months (3,4). This has led to the hope that receptor-mediated tumor targeted therapy with radionuclides could improve the response and survival rates.

For receptor targeted radionuclide therapy to be an effective complement or alternative to chemotherapy, it is necessary that the disseminated tumor cells and metastases express the target structure to a similar extent as the corresponding primary tumors. When the target for radionuclide therapy is a growth factor receptor in the epidermal...
growth factor receptor (EGFR) family, there are several indications that when the expression is high in the primary tumor, it is often also high in the metastases (5). The reason for this is probably that the receptor expressing tumor cells are dependent on the growth stimulation from the growth factor-receptor interactions. If tumor cells, e.g. due to genomic instability, lose the receptor expression they might also lose their growth advantage and be overgrown by tumor cells with high receptor expression (5).

The EGFR family members are EGFR (ErbB-1/HER1), HER2 (ErbB-2), HER3 (ErbB-3), and HER4 (ErbB-4). They have an extracellular ligand binding domain, a hydrophobic transmembrane domain and an intracellular domain with protein-tyrosine kinase activity. However, HER3 has no intrinsic tyrosine kinase activity but is instead signaling via dimerisation with any of the other receptors. EGFR and five other ligands bind to EGFR and neuregulins (NRG) are the ligands for HER3 and HER4. HER2 has, so far, no known ligand but is activated via dimerisation with any of the other receptors (6,7). Overexpression of EGFR and HER2 has often been associated with malignant transformation. It has been stated that EGFR is often overexpressed in primary CRC and associated with poor survival (8,9). EGFR positive cells have also been detected in peripheral blood from colon cancer patients (10,11). No expression of the mutated EGFRvIII receptor has, to our knowledge, so far been found in CRC (5,12).

Targeted therapy using non-radioactive agents is a clinical reality for tumors expressing high levels of EGFR or HER2 although resistance has been reported (6,7,13-15). Both EGFR and HER2 seem to be good targets also for radionuclide based tumor therapy and whether this is the case for colorectal carcinomas is the subject of this study. It remains to be determined whether also HER3 and HER4 receptors are suitable for radionuclide-targeted therapy. One problem seems to be that HER3 and HER4, in immunohistochemical staining, often is in the cytoplasm. This staining pattern is not understood and it cannot be excluded that there is also a fraction of the receptors in the cellular membrane since both HER3 and HER4 contain transmembrane regions. Cytoplasm staining of HER3 has been reported for several tumor types such as pharyngeal, esophageal and base of tongue tumors as well as for CRC (16-19). However, some membrane HER3 staining has also been reported for CRC (19,20). The role of HER4 for tumor growth is not clear and HER4 was not determined whether also HER3 and HER4 receptors are suitable for radionuclide-targeted therapy. One problem seems to be that HER3 and HER4, in immunohistochemical staining, often is in the cytoplasm. This staining pattern is not understood and it cannot be excluded that there is also a fraction of the receptors in the cellular membrane since both HER3 and HER4 contain transmembrane regions. Cytoplasm staining of HER3 has been reported for several tumor types such as pharyngeal, esophageal and base of tongue tumors as well as for CRC (16-19). However, some membrane HER3 staining has also been reported for CRC (19,20). The role of HER4 for tumor growth is not clear and HER4 was not analyzed in this study.

EGFR-family targeted radionuclide therapy is mainly planned to target the native receptors and the effect of radiation is probably not, when the dose is high, dependent on whether the targeting agent interferes with intracellular signaling cascades. The cell killing properties of ionizing radiation are well known and treatment induced resistance has, to our knowledge, not been reported. With this background we investigated the expression of EGFR, HER2 and HER3 in CRC with immunohistochemical methods.

Materials and methods

Patients and samples. Sixty patients with primary node positive CRC who were diagnosed and treated in the Second
mounted in organic mounting medium (Pertex ®, Histolab, were dehydrated through graded alcohol to xylene and stained in Harris haematoxylin (Sigma). Finally, the slides were counterstained with Mayer's haematoxylin and mounted with Aquamount (BDH Ltd.).

The scoring was based on a scale where 0 corresponded to completely negative staining, 1+ corresponded to faint perceptible staining of the tumor cell membranes, 2+ corresponded to moderate staining of the entire tumor cell membranes and 3+ was strong circumferential staining of the entire tumor cell membranes creating a fishnet pattern. The HER2 staining was scored using the HercepTest scoring criterion. The HER2-score was based on a scale where 0 corresponded to tumor cells that were completely negative, 1+ corresponded to faint perceptible staining of the tumor cell membranes, 2+ corresponded to moderate staining of the entire tumor cell membranes and 3+ was strong circumferential staining of the entire tumor cell membranes creating a fishnet pattern. The Canadian and the Dako HercepTest guidelines (21) that require >10% of the tumor cells to be stained were applied. Cytoplasmic staining was considered non-specific and was not included in the scoring. As positive controls we used in house positive control tissue sections as well as positive control sections supplied by Dako. As negative controls we used normal tissue, which are expected not to express HER2, e.g. connective tissue seen in the same sections as the tumor cells. In the metastases sections we used lymphocytes and the surrounding capsule of the lymph nodes as negative internal controls. The expression pattern of EGFR is similar to that of HER2 and EGFR expression was therefore evaluated using the same scoring criterion as for HER2. As EGFR positive controls we used in house positive control skin tissue sections. As negative controls we used connective tissue seen in the same sections as the tumor cells. In the metastases sections we used lymphocytes and the surrounding capsule of the lymph nodes as negative internal controls.

HER3 expression was evaluated using the Elite ABC kit (Vectastain, Vector Laboratories). Blocking serum was applied for 15 min and followed by incubation with rabbit anti-human c-erbB-2 oncoprotein (code no. A 0485, Dako) diluted 1:350. Sections were then incubated with the biotinylated secondary antibody and were visualized by using the peroxidase substrate 3-amino-9-ethyl-carbazole (AEC) (Sigma A-5754) as chromogen. Finally, the sections were counterstained with Mayer's haematoxylin and mounted with Aquamount (BDH Ltd., Poole, UK).

HER3 staining. The HER3 staining was performed as previously described (16,17). After deparaffinization, the sections were incubated in methanol and hydrogen peroxide for 30 min quenching endogenous peroxidase. Antigen retrieval was done in a water bath at 95-98°C, pH 6.0 for 40 min. Thereafter the slides were treated with 1% hydrogen peroxide for 15 min and then washed in distilled water. Immunohistochemical staining was performed using the Elite ABC kit (Vectastain, Vector Laboratories, Burlingame, CA, USA). Blocking serum was applied for 15 min and followed by incubation with rabbit anti-human c-erbB-2 oncoprotein (code no. A 0485, Dako) diluted 1:350. Sections were then incubated with the biotinylated secondary antibody and were visualized by using the peroxidase substrate 3-amino-9-ethyl-carbazole (AEC) (Sigma A-5754) as chromogen. Finally, the sections were counterstained with Mayer's haematoxylin and mounted with Aquamount (BDH Ltd., Poole, UK).

HER3 staining. The HER3 staining was performed as previously described (16,17). After deparaffinization, the sections were incubated in methanol and hydrogen peroxide for 30 min quenching endogenous peroxidase. Antigen retrieval was done in a water bath at 95-98°C, pH 6.0 for 40 min. Thereafter the slides were washed in distilled water. Immunohistochemical stainings were performed using the Elite ABC kit (Vectastain, Vector Laboratories). Blocking serum was applied for 15 min and followed by incubation with the monoclonal antibody MAB4021 (Chemicon, Temecula, CA) diluted 1:1000. Sections were then incubated with a biotinylated secondary antibody and were visualized by using the peroxidase substrate 3-amino-9-ethyl-carbazole (AEC) (Sigma A-5754) as chromogen. Finally, the sections were counterstained with Mayer's haematoxylin and mounted with Aquamount (BDH Ltd.).

EGFR and HER2 scores. The HER2 expression was scored using the HercepTest scoring criterion. The HER2-score was based on a scale where 0 corresponded to tumor cells that were completely negative, 1+ corresponded to faint perceptible staining of the tumor cell membranes, 2+ corresponded to moderate staining of the entire tumor cell membranes and 3+ was strong circumferential staining of the entire tumor cell membranes creating a fishnet pattern. The Canadian and the Dako HercepTest guidelines (21) that require >10% of the tumor cells to be stained were applied. Cytoplasmic staining was considered non-specific and was not included in the scoring. As positive controls we used in house positive control tissue sections as well as positive control sections supplied by Dako. As negative controls we used normal tissue, which are expected not to express HER2, e.g. connective tissue seen in the same sections as the tumor cells. In the metastases sections we used lymphocytes and the surrounding capsule of the lymph nodes as negative internal controls. The expression pattern of EGFR is similar to that of HER2 and EGFR expression was therefore evaluated using the same scoring criterion as for HER2. As EGFR positive controls we used in house positive control skin tissue sections. As negative controls we used connective tissue seen in the same sections as the tumor cells. In the metastases sections we used lymphocytes and the surrounding capsule of the lymph nodes as negative internal controls.

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Results

EGFR expression in primary tumor and lymph node metastasis. The fraction of EGFR expressing primary tumors was somewhat low, see Table II. Only 16 out of 56 (29%) primary
tumors had 2 or 3+ scores. The EGFR expression in the corresponding lymph node metastases was even lower with only 8 (14%) being positive. As can be seen in Table III, two cases increased from 0-1+ in the primary tumors to 2+ - 3+ in the lymph node metastases. The opposite change, down-regulation in metastases, was observed in 10 cases.

**Table III. Major results from the EGFR-score analyses.**

<table>
<thead>
<tr>
<th>EGFR-score characteristics</th>
<th>Fraction</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary tumors with 2+ or 3+</td>
<td>16/56</td>
<td>29%</td>
</tr>
<tr>
<td>Lymph node metastases with 2+ or 3+</td>
<td>8/56</td>
<td>14%</td>
</tr>
<tr>
<td>Patients who had 0 or 1+ in primary tumors and changed to 2+ or 3+ in lymph node metastases</td>
<td>2/56</td>
<td>4%</td>
</tr>
<tr>
<td>Patients who had 2+ or 3+ in primary tumors and changed to 0 or 1+ in lymph node metastases</td>
<td>10/56</td>
<td>18%</td>
</tr>
</tbody>
</table>

**Table IV. HER2-scores for the analyzed primary CRC and the corresponding lymph node metastases (n=55).**

<table>
<thead>
<tr>
<th>Primary tumors, HER2-scores</th>
<th>Lymph node metastases, HER2-scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2-scores</td>
<td>0 1+ 2+ 3+</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>0</td>
<td>51 0 0 0</td>
</tr>
<tr>
<td>1+</td>
<td>2 1 0 0</td>
</tr>
<tr>
<td>2+</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>3+</td>
<td>0 0 0 1</td>
</tr>
</tbody>
</table>

The scoring was based on a scale where 0 corresponded to completely negative staining, 1+ corresponded to faint perceptible staining of the tumor cell membranes, 2+ corresponded to moderate staining of the entire tumor cell membranes and 3+ was strong circumferential staining of the entire tumor cell membranes creating a fishnet pattern.

**Table V. HER3-scores for the analyzed primary CRC and the corresponding lymph node metastases (n=49).**

<table>
<thead>
<tr>
<th>Primary tumors, HER3-scores</th>
<th>Lymph node metastases, HER3-scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER3-scores</td>
<td>0 W S</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>0</td>
<td>29 4 1</td>
</tr>
<tr>
<td>W</td>
<td>3 4 0</td>
</tr>
<tr>
<td>S</td>
<td>0 0 8</td>
</tr>
</tbody>
</table>

0, no stained cells; W, weak cellular staining of cytoplasm with or without weak stained granules; S, strong granular cytoplasmatic staining.

**HER2 expression in primary tumor and lymph node metastasis.** HER2 was strongly expressed in only one primary tumor and in this case also in the corresponding metastasis (Table IV). No other strongly HER2 stained samples were found.

**HER3 expression in primary tumor and lymph node metastasis.** Of 49, 15 (31%) of the primary tumors expressed HER3 and strongly in 8 (16%). Lymph node expression was slightly higher, 17 out of 49 (35%) and strongly in 9 (18%). Overall there was fairly good correspondence in HER3 expression between primary tumors and lymph node metastases (Table V).

**EGFR, HER2 and HER3 expression in liver metastases.** The expression of EGFR, HER2 and HER3 was analyzed in liver metastases from 10 patients (Table VI) and these receptors were also analyzed in primary tumors and lymph node metastases from the same patients. Samples from three patients were strongly EGFR expressing (2+) in the primary tumors and in two of the corresponding lymph node metastases, but none of them strongly expressed EGFR in the liver metastases. Only one liver metastasis expressed EGFR strongly while the corresponding primary tumor and lymph node metastasis samples did not. None of these samples were HER2 positive. Two patients expressed HER3 strongly in the primary tumors and lymph node metastases while only one of them strongly expressed HER3 in the liver metastases.

**Morphological appearance.** Typical immunohistochemical stainings are shown in Fig. 1. The EGFR staining of CRC tissue (primary tumor, lymph node metastasis and liver metastasis; a, b and c, respectively) all showed mosaic or fishnet staining patterns, typical for cell membrane staining. The normal liver tissue, shown in d, had a weak EGFR cell membrane staining in the hepatocytes. Strong HER2 cell membrane stainings from the only HER2 positive case (primary tumor and lymph node metastasis) are seen in Fig. 1e and f. The HER3 stainings had a granular pattern in the cytoplasm and this was seen in primary tumors (g) as well as in the lymph node (h) and liver metastases (i). Thus, HER3 did not have a cell membrane staining pattern.

**Discussion**

**EGFR expression in CRC.** There is a surprisingly wide span between previously published levels on EGFR-expression in
Table VI. EGFR, HER2 and HER3 scores from primary tumors, lymph node metastases and liver metastases (n=10).

<table>
<thead>
<tr>
<th>Sample</th>
<th>PT</th>
<th>LNM</th>
<th>LM</th>
<th>PT</th>
<th>LNM</th>
<th>LM</th>
<th>PT</th>
<th>LNM</th>
<th>LM</th>
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</thead>
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<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1+</td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>1+</td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>7</td>
<td>2+</td>
<td>1+</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>S</td>
<td>S</td>
<td>W</td>
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<tr>
<td>10</td>
<td>2+</td>
<td>2+</td>
<td>1+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

PT, primary tumor; LNM, lymph node metastases; LM, liver metastases. The scoring was based on the same criteria as described in the Tables IV and V. -, not possible to evaluate due to lack of tumor cells in the section.

Figure 1. Typical brown IHC EGFR-staining of sections from a primary colorectal carcinoma cancer (a) and the corresponding lymph node (b) and liver (c) metastasis. Note the homogeneous membrane staining of virtually all tumor cells. EGFR staining of apparently normal liver tissue (taken some distance from a liver metastasis) is shown in (d). Typical brown IHC HER2-stainings of sections from the only HER2 positive primary colorectal carcinoma cancer (e) and the corresponding lymph node metastasis (f). Immunohistochemical HER3-stainings (brown) of sections from a primary colorectal carcinoma cancer (g) and the corresponding lymph node (h) and liver metastases (i).
primary tumors of CRC. The fraction of tumor cells being 
EGFR-positive varied in the range 20-95% (12,22-35). The 
variations in expression of EGFR in lymph node (28,30,33) 
and liver metastases (29-35) were similarly high. Our values 
for the Hangzhou patients are in the lower part of the ranges. 
Independent of low or high values there is, in the literature, 
often a rather good concordance on EGFR expression between 
primary tumors and corresponding metastases, whether lymph 
node or liver metastases are considered (5,28,30-32). However, 
our results indicate lower EGFR positivity in the metastases. 
Downregulation in metastases have recently been indicated 
also in other studies (20,33-35).

**HER2 expression in CRC.** HER2 has been reported to be 
expressed in primary colorectal carcinomas but the levels vary 
within an extremely wide range, 3-82% (22,23,25,36-42). In 
our study the HER2 expression was nearly zero (one positive 
case out of 55). In a report by Knoesel et al (39) summarizing 
10 previously published CRC studies during 1994-2001, 
including 1007 patient cases, more than half of the cases were 
HER2 positive. HER2 expression in metastases has been less 
studied but reported to be in the range 36-54% (28,38,39) with 
our present study as an exception. Thus, the lack of HER2 
expression in our material is striking, and is reasonably not due 
to errors in the analysis since we had well documented positive 
and negative controls and an extensive experience in HER2 
alyses. For example, the obtained HER2 results are low in 
comparison to the results from other tumor types studied at our 
laboratory (applying the same IHC techniques and scoring 
criteria), e.g. breast (43) and urinary bladder (44,45). The level 
is actually as low as in squamous carcinomas, e.g. laryngeal 
(16), esophageal (17) and oral cavity and base of tongue (18) 
cancers. The variations in CRC data are probably mainly due 
to variations in the scoring criteria (cut off levels and lack of 
discrimination between low expression and overexpression). 
Considering only our data, HER2 is not of interest for targeted 
radionuclide therapy.

**HER3 expression in CRC.** HER3 has previously been reported 
to be expressed in 36-89% of CRC (5,23,25,46-48). In our 
study, the fraction strongly HER3 expressing cases were only 
16 and 18% for primary tumors and lymph node metastases, 
respectively. If also weak staining is included, the fractions 
increased to ~31 and 35%. Cytoplasm staining was observed 
in our CRC samples and such HER3 staining has previously 
also been observed in other types of cancer (16-19). Membrane 
HER3 staining of CRC cells has, to our knowledge, only 
been reported in two studies (19,20). The human protein atlas 
shows mainly cytoplasm HER3 staining but also some cases 
with nuclear staining (http://www.proteinatlas.org). Clear cell 
membrane HER3 staining, as for EGFR and HER2, cannot 
be found in the atlas. HER3 has a transmembrane region and it 
is possible that HER3 most often is situated in intracellular 
membranes and only visiting the outer cell membrane 
ocasionally. Since outer cell membrane localization is 
necessary for targeted radionuclide therapy using macromolecules 
and peptides, HER3 seems not an interesting target for radionuclide therapy.

**EGFR expression in normal tissues.** We only discuss EGFR 
here since HER2 and HER3 seems not to be candidates for 
targeted radionuclide therapy of CRC. The expression of 
EGFR in normal tissues has been characterized many years 
ago (49,50). Distributions of EGFR in various tissues can 
also be found at the human protein atlas (http://www.
proteinatlas.org) showing that EGFR is expressed in skin, liver, 
digestive tract and reproductive organs. EGFR staining in 
colon is shown in Fig. 1d. EGFR is attractive as target mainly 
when the uptake of radioactivity is higher in the tumor, and 
studied metastases, than in most normal tissues. This can, in 
the patient setting, best be studied through nuclear medicine 
techniques such as PET (positron emission tomography) or 
SPECT (single photon emission tomography) applying tracer 
amounts of the targeting agent labeled with radionuclides 
suitable for imaging, e.g. ⁶⁷Ga (PET) and ¹¹¹In (SPECT). 
Such methods provide information on whether metastases 
can be targeted or not and give information about the uptake 
in normal tissues (51).

**Reasons for variations in EGFR expression.** The reported 
variations in EGFR expression, in tumors and metastases, 
between different studies must, to a large part, be due 
to variations in the applied cut off levels and lack of 
discrimination between low expression and overexpression. 
In some studies, EGFR positivity was scored positive if at least 
half of all cells in the tumor were stained while in other 
studies positivity was scored if only one or a few EGFR 
positive tumor cells were found. There are also differences in 
IHC retrieval techniques between the laboratories. Variations 
are probably also due to different patient inclusion criteria 
etiological differences.

**EGFR targeted radionuclide therapy.** In spite of the large 
variations in EGFR expression between different studies and 
a possible decreased expression in metastases, there is a 
reasonably good chance that EGFR targeted radionuclide 
therapy can be of value for selected patients, i.e. those with 
verified EGFR expression in metastases. EGFR targeted 
radionuclide therapy could actually be feasible for the subgroup 
of patients with EGFR expression and which are resistant 
therapy with substances that interfere with downstream 
EGFR signaling (e.g. cetuximab and various tyrosine kinase 
inhibitors) due to K-Ras (52-57) or other mutations (58). 
EGFR expression has been reported to be independent of 
K-Ras mutations (59). As long as the EGFR is expressed, 
toxic radionuclides coupled to an EGFR-binding substance 
could be of therapy value.

**Inclusion criteria.** We do not discuss ‘conventional’ patient 
inclusion criteria such as age and health status, only those 
aspects that are unique for targeted radionuclide therapy. It is 
important to decide how the immunohistochemical techniques 
and scoring systems (cut off level) should be designed to 
allow for relevant patient inclusion. It seems not possible to 
attribute which criteria that is most relevant for CRC 
since the EGFR positive tumor cells, whether few or many, 
might be the most malignant and those that form disseminated 
disease. For example, increased EGFR expression has been 
reported in the invasive margin of CRC (60), which indicates 
that a low cut-off could be reasonable. The HercepTest applied 
in a standardized way for analysis of HER2 in breast cancer
Design of targeting agents. The design of suitable radio-labeled EGFR-binding agents with high binding to EGFR-positive CRC cells and low uptake in critical normal tissues is a challenge. However, there is hope for a favorable development since new knowledge is continuously emerging about biodistribution, pharmacokinetics and cellular processing of different types of targeting agents and the research on molecular design of new agents is rapidly expanding. The development of peptides and small proteins, e.g. affibody molecules, is one strategy (61,62). The area of antibody engineering is also rapidly developing and various forms of antibody fragments are developed such as minimal recognizing units, single chain fragments, scFv, and dimeric scFv (63). Liposomes containing toxic radionuclides and conjugated units, single chain fragments, scFv, and dimeric scFv (63). Liposomes containing toxic radionuclides and conjugated with targeting agents might be of special interest for killing of disseminated tumor cells that remain in the systemic circulation (64). Suitable radionuclides for therapy, that are commercially available, are the ß-emitters 177Lu and 90Y while the c-emeitter 211At is a future candidate (51.65,66).

To conclude, EGFR might be a target for radionuclide therapy in CRC patients with EGFR positive metastases. HER2 and HER3 seem not to be of such interest, at least based on the present results.

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References


