p53 expression in human rectal tissue after radiotherapy: upregulation in normal mucosa versus functional loss in rectal carcinomas

C.A.M. Marijnen¹², E. Kapiteijn², I.D. Nagtegaal²³, A.A. Mulder-Stamp³, C.J.H. van de Velde², P.I. Schrier¹, L.T.C. Peltenburg¹, J.H.J.M. van Krieken⁴

Departments of Clinical Oncology¹, Surgery² and Pathology³, Leiden University Medical Centre, Leiden; Department of Pathology⁵, University Medical Centre St. Radboud, Nijmegen, The Netherlands

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INTRODUCTION

The high local recurrence rate is a major problem in rectal cancer. Preoperative radiotherapy (RT) has been shown to be useful in reducing the number of local recurrences. However, a major disadvantage of preoperative RT is the over-treatment of a subset of patients. Therefore prognostic markers for the tumour response to RT are needed. The tumour suppressor gene p53 has been extensively studied for its prognostic value. In several studies, overexpression of the p53 protein has been shown to correlate with patient survival, a finding that has not been confirmed in other studies.

One of the functions of p53 in normal cells is to respond to DNA damage by causing either cell cycle arrest or by forcing damaged cells to go into apoptosis. Mutations in the p53 gene lead to a functionally inactive protein. The p53 gene is one of the most commonly inactivated genes in cancer and plays an important role in the multistage development of colorectal cancer. The stability of the p53 protein is regulated by binding to MDM2, a protein that degrades p53 and consequently inactivates the transcriptional function of p53. Because of this regulation, wild type (wt) p53 is highly unstable, with a half-life of minutes, and therefore hard to detect by immunohistochemistry (IHC). Mutations in p53 prevent degradation by MDM2, allowing stabilisation and detection of the protein by IHC. Interpretation of IHC is complicated because other genetic alterations like frameshift mutations or deletions can lead to truncation or complete loss of p53, which precludes detection with IHC. Furthermore, MDM2 overexpression can prevent detection of wild type p53. Therefore, negative staining for p53 indicates either wild type p53 or a non-functional gene.

After ionising irradiation the half-life of wt p53 increases significantly because of phosphorylation of the protein, which inhibits degradation of the protein by MDM2 and thus allows its detection by IHC. Accumulation of wt p53 normally leads to transcription of several downstream target genes, such as p21 and GADD45. The induction of the CDK inhibitor p21 after ionising radiation leads to a G1 growth arrest, thus allowing the cell to repair the damage. Apart from induction by wt p53, activation of the p21 gene can also occur through mechanisms independent of p53. TGF-β, the BRCA1 gene products and Nerve Growth Factor are examples of factors that promote p21 transcription by p53-independent mechanisms. In addition to a role in the repair process, p21 has an important function during differentiation of cells.

In cell lines, the effects of ionising radiation on the expression of p53 and p21 have extensively been studied. After ionising radiation a rapid increase of wt p53 is observed, normalising within 48-72 hours. A subsequent increase of p21 expression is found in cells with wt p53, however, this is not observed in cells with inactive p53. In normal intestinal tissue of irradiated mice, a rapid increase of p53 as well as of p21 positive cells is reported. In p53- mice no increase in p21 expression was observed after irradiation, indicating that wt p53 is mandatory for upregulation of p21.

Presence of wt p53, however, does not guarantee a functional intact pathway. Induction of p53 after irradiation without upregulation of p21 has been reported, suggesting disruption of the pathway downstream of p53. Expression of p21 after irradiation can thus be used as an indicator of defects in the pathway downstream of p53.

The relationship between p53 and p21 after irradiation has been investigated in the normal intestinal mucosa of mice, but little is known about tumours in vivo. We therefore evaluated the direct effect of ionising radiation on the expression of p53 and p21 in...
normal mucosa and rectal carcinoma in vivo, by analysing a large number of tumours of rectal cancer patients participating in a randomised trial. One half of the patients received short-term preoperative RT within one week followed by surgery, and the other half underwent surgery only. This trial disclosed a unique series of samples serving as an in vivo model for the functional activity of the p53 protein.

By careful evaluation of expression patterns of both p53 and p21 we suggest new criteria for determination of p53 mutations on IHC. Furthermore, we show that in tumours with p53 wild type the downstream pathway is often disrupted.

METHODS
Patients and treatment
All tumours used for analysis were derived from rectal cancer patients, randomised in a large multicenter trial in which the effect of short-term, preoperative RT (5x5 Gy) in combination with total mesorectal excision (TME) surgery was investigated. They were randomised to either RT followed by surgery or surgery alone. The patients assigned to preoperative RT received a total dose of 25 Gy in 5 fractions during 5-7 days. Irradiated patients in whom the interval between RT and operation exceeded 8 days were excluded from analysis. Standardised routine pathologic examination was performed in the laboratories of the referring hospitals as described by Quirke et al. Tumour staging was performed using the Tumour-Node-Metastasis (TNM) classification.

Tumours
The expression of p53 and p21 were evaluated in tumour samples from the first 103 patients entered in the trial from the 12 hospitals contributing the most patients. Of these patients, 51 received preoperative RT. To compare the expression of p53 before and after RT in individual tumours, 32 pretreatment biopsies of irradiated patients were collected, analysed for p53 expression and compared with the corresponding irradiated tumour specimen. The other 19 biopsies were either not available or too small to analyse.

To evaluate the kinetics of p53 degradation after ionising radiation, we analysed p53 expression in tumour and normal tissue with varying intervals between the last fraction of RT and surgery of 1, 3, 5 or 7 days. Because most patients underwent surgery after 3 days, we additionally stained 53 samples to extend the different groups to 20 samples. Only 15 patients had an interval of 7 days in the trial, leading to 75 tumours in total. Both tumour and normal tissue were stained for all samples.

Colorectal tumours are considered mucinous when mucin covers more than 50% of the microscopically observed areas. From the literature, it is known that mucinous tumours are more often wild type p53. To evaluate the effect of RT on the expression of p53 in wt tumours, we additionally analysed all tumours with 90-100% mucinous areas from patients randomised in the trial.

Immunohistochemistry
Tissue samples of the primary tumours were fixed in 4% phosphate-buffered formalin, dehydrated and embedded in paraffin. Tissue sections of 4 µm were cut and mounted onto 3-aminopropyltriethoxysilane (APES) pre-coated slides. Serial sections were stained with hematoxylin and eosin or processed for immunohistochemistry.
p53 and p21\textsuperscript{waf1} expression were assessed by immunohistochemical investigation with the following antibodies: anti-p53 (mAb NCL-p53-DO-7, Novocastra Laboratories Ltd., Newcastle, United Kingdom) and anti-p21 (WAF 1 (Ab-1), Oncogene Research Products, Cambridge, Massachusetts). In brief, sections were deparaffinised in xylene and rehydrated. Endogenous peroxidase activity was blocked by 1% hydrogen peroxide for 20 minutes. For non-enzymatic epitope retrieval, 0.01 M citrate buffer (pH 6.0) was used. After overnight incubation with the primary antibody (dilutions: p53 1/2000, p21\textsuperscript{waf1} 1/250) in 1% phosphate-buffered saline/bovine serum albumin (1% PBS-BSA), the secondary biotin-conjugated antibody and a tertiary complex of streptavidin-avidin-biotin conjugated to 3-amino-9-ethylcarbazole (AEC) or 3',3'-diaminobenzidine (DAB) were applied. Finally, the sections were counterstained with haematoxylin. Incubation with PBS instead of the primary antibody served as a negative control.

**Scoring**
All slides were evaluated semi-quantitatively and independently by two investigators (CAMM and EK). Sections that were categorised discrepantly were discussed together with an independent investigator (JHJMvK). Nuclear p53 and p21\textsuperscript{waf1} staining were scored in tumour tissue in the following categories: 0%, 1-5%, 5-15%, 16-25%, 26-75% and >75%. Normal mucosal tissue was scored when present in the same block. p53 expression in normal mucosa was scored in the same categories as the tumour tissue. For p21\textsuperscript{waf1}, normal mucosal tissue was scored as totally positive, apical cells positive or totally negative. Since some mucinous tumours contain relatively few tumour cells, p53 was only scored in three categories in these tumours: 0%, 1-25% and 26-100% positive cells.

In order to analyse the correlation between different variables, p21\textsuperscript{waf1} was regarded positive if more than 5% of the tumour cells stained positive. p53 in tumours was divided in three categories: 0% (negative), 1-25% (low) and >25% (positive) to evaluate the influence of RT on the expression of p53.

**Data collection and statistics**
All data were entered in a database and analysed with Mann-Whitney tests to compare quantitative and ordered variables and with Student’s t-tests to analyse differences in normally distributed data between the two groups. Chi-square tests were used to compare proportions. A two-sided P-value of 0.05 or less was considered statistically significant.

**RESULTS**

**Patient characteristics**
The mean age was 62 years in the irradiated group and 63 years in the unirradiated group. Thirty-one percent of the irradiated patients had a TNM stage III tumour, vs. 40% of the unirradiated patients. There was no difference in the distribution of gender, type of operation or tumour type in both treatment arms.

**p53**
To assess the influence of RT on p53 expression \textit{in vivo}, we examined 103 rectal tumours and normal mucosa. Nuclear expression of p53 was observed in both irradiated and non-irradiated tumours (Figure 1A). A complete absence of p53 after RT was observed in 8
tumours (Figure 1B), whereas irradiated normal mucosa as well as stromal tissue showed widely distributed p53 staining (Figure 1C). p53 expression in tumour tissue in samples from both treatment arms is displayed in Figure 2. In the non-irradiated group, slightly more tumours were found with 1-5% or 6-15% of the cells expressing p53, while in the irradiated group more tumours expressed p53 in 76-100% of the cells. These findings suggest that tumours with low p53 expression (1-25%) might contain wt p53 that can be upregulated by irradiation. When the whole group was evaluated this difference could no longer be observed (P=0.39), because of the small numbers of tumours in these categories.

p53 expression in normal mucosa was determined when present, which was in 38 samples of the irradiated group and in 28 of the non-irradiated group. Only one non-irradiated normal mucosa sample showed p53 expression in >5% of the cells, whereas this was present in 36/38 (95%) of the irradiated normal mucosa samples (P<0.001, Table 1). This clearly demonstrates upregulation of p53 in normal tissue after irradiation.

To evaluate the kinetics of p53 upregulation after RT in vivo, we selected tumours and normal mucosa of 75 patients with an interval between RT and surgery of 1, 3, 5 or 7 days. The percentage of p53-positive tumours ranged between 55% and 80% and did not vary significantly between the different intervals.

In all normal mucosal tissue samples p53 expression was still observed up to 7 days after the last fraction of RT. There was, however, a decrease in the percentage of positive cells over time.

### Table 1. Distribution of p53 expressing cells in normal mucosa of irradiated and non-irradiated patients.

<table>
<thead>
<tr>
<th></th>
<th>RT+TME n=38</th>
<th>TME n=28</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0%</td>
<td>0</td>
<td>12</td>
<td>43</td>
</tr>
<tr>
<td>1-5%</td>
<td>2</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>6-15%</td>
<td>7</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>16-25%</td>
<td>2</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>26-75%</td>
<td>26</td>
<td>68</td>
<td>1</td>
</tr>
<tr>
<td>76-100%</td>
<td>1</td>
<td>3</td>
<td>-</td>
</tr>
</tbody>
</table>

### p53 in biopsies

Since the percentage of p53-positive tumour cells over the various categories in irradiated tumours was not different from that for unirradiated tumour samples, we compared p53 expression before and after irradiation in individual tumours by evaluating the 32 available pre operative biopsies. The distribution of the p53 expression in the biopsies as well as in the corresponding irradiated tumours is given in Table 2. Five negative biopsies had corresponding irradiated p53-negative tumours, indicating that these tumours represent non-functional p53. Seven biopsies with negative or low p53 expression, showed upregulation of p53 in the corresponding tumours after irradiation, suggesting the presence of wt p53. 19 biopsies showed p53 expression in more than 25% of the cells. Since the patients had not been irradiated at the time of biopsy, this is most likely due to mutant p53.
Table 2. Relation between $p53$ expression in non-irradiated biopsies and corresponding irradiated tumours.*

<table>
<thead>
<tr>
<th>Tumour Biopsy</th>
<th>0%</th>
<th>1-25%</th>
<th>26-100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>5</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>1-25%</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>26-100%</td>
<td>0</td>
<td>2</td>
<td>17</td>
</tr>
</tbody>
</table>

* Numbers in the cells represent numbers of tumours.

$p53$ in mucinous tumours

To evaluate the effect of RT on $p53$ expression in a group of tumours that most probably contained wild-type $p53$, we analysed $p53$ expression in all 100% mucinous tumours in the trial. Results are depicted in Figure 3. In the non-irradiated group, 11 of 18 mucinous tumours showed low $p53$ expression (1-25%) vs. 12 of 52 non-mucinous tumours (61% vs. 23%), suggesting that $wt\,p53$ is frequently present in mucinous tumours. Eighteen of the 24 irradiated mucinous tumours were more $p53$-positive compared to only 4 of 18 of the non-irradiated mucinous tumours, suggesting upregulation of $wt\,p53$ after RT in this group.

$p21\,waf1$

To evaluate the effect of radiotherapy on the expression of $p21\,waf1$ in rectal cancer in vivo we compared irradiated and non-irradiated tumours and normal mucosa. Nuclear expression of $p21\,waf1$ was observed in irradiated tumours and in non-irradiated tumours. $p21\,waf1$ expression in tumours in both treatment arms is displayed in Figure 4, demonstrating similar $p21\,waf1$ expression in both treatment arms. Normal mucosa was present in 29 samples of each treatment arm and showed widely distributed $p21\,waf1$ staining in 97% (28 of 29) of the irradiated cases and was negative in 76% (22 of 29) of the unirradiated cases ($P<0.001$). Occasionally, the unirradiated mucosa showed some $p21\,waf1$ positive cells in the upper part of the crypts. This suggests that radiotherapy induces $p21\,waf1$ expression in normal cells, but has no influence on the $p21\,waf1$ expression in rectal tumour cells.

Relationship between $p53$ and $p21\,waf1$

To investigate whether $p21\,waf1$ expression in vivo is dependent on the $p53$ status, we analysed the relationship between $p53$ and $p21\,waf1$. This relationship for irradiated and non-irradiated tumour tissue is represented in Table 3. In the irradiated group, none of the eight $p53$-negative tumours showed $p21\,waf1$ expression, in line with the functional absence of $p53$. Of the 36 irradiated tumours positive for $p53$, 9 (25%) were also positive for $p21\,waf1$. In the unirradiated group 33 tumours were positive for $p53$, of which 6 (18%) were also positive for $p21\,waf1$. These percentages for $p21\,waf1$ positivity in irradiated and unirradiated tumours show that upregulation of $p21\,waf1$ by $p53$ after radiotherapy is not very common.

In normal mucosa, 28 samples in each group could be analysed for both $p21\,waf1$ and $p53$ expression. Of the unirradiated samples, 20 of 28 were negative for $p21\,waf1$ and $p53$ expression, however, of the irradiated samples 26 of 28 were positive for both $p53$ and $p21\,waf1$ expression. This indicates that in normal tissue expression of $p53$ and $p21\,waf1$ is clearly increased after irradiation.
The status of p53 expression in untreated biopsies in relation to the corresponding irradiated tumours is shown in Figure 5. In this figure, we included expression of p21\textsuperscript{waf1} as a marker for the functionality of p53. Of the 7 tumours in Table 2 with probably wild-type p53, only two tumours showed upregulation of p21\textsuperscript{waf1}, and 5 were negative for p21\textsuperscript{waf1} (Figure 1D-1F).

Of the 19 tumours with a p53-positive biopsy, 17 showed p53 positivity in the tumour, indicating the presence of mutant p53. Three of these tumours showed p21\textsuperscript{waf1} expression (Figure 1G-1I). Although the numbers were small, these results indicate that the presence of wt p53 does not necessarily lead to upregulation of p21\textsuperscript{waf1}, while the presence of mutant p53 does not exclude p21\textsuperscript{waf1} overexpression.

**DISCUSSION**

This study was undertaken to evaluate the \textit{in vivo} effect of radiotherapy on the expression of p53 and p21\textsuperscript{waf1} in normal rectal mucosa and rectal carcinoma.

For the first time, we demonstrate that in normal cells p53 as well as p21\textsuperscript{waf1} are upregulated in humans \textit{in vivo} after short-term preoperative radiotherapy. In tumour cells however, no difference in the expression of p53 or p21\textsuperscript{waf1} in rectal tumours could be observed between the irradiated and non-irradiated groups. These results indicate that p53 protein in rectal tumours does not respond to irradiation, suggesting a very high frequency of p53 abnormalities. We conclude that the p53-p21\textsuperscript{waf1} pathway is disrupted in nearly all tumours, but that there are different underlying mechanisms.

**p53 in normal mucosa**

In normal cells, p53 can generally not be detected by immunohistochemistry, whereas stabilised p53 can be detected. Stabilisation may occur either through mutation (in cancer) or through phosphorylation of the protein (e.g. after radiotherapy). All irradiated normal mucosa samples showed overexpression of p53, confirming that \textit{in vivo} wt p53 is upregulated after irradiation. We detected p53 expression 7 days after the last fraction of radiotherapy, which is even later than the reported p53 expression found in the large intestine of mice (3 days after irradiation).23

### Table 3. Relationship between p53 and p21 expression in irradiated and non-irradiated tumours.*

<table>
<thead>
<tr>
<th>Irradiated tumours</th>
<th>p21 negative</th>
<th>p21 positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53 negative</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>p53 low</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>p53 positive</td>
<td>27</td>
<td>9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-irradiated tumours</th>
<th>p21 negative</th>
<th>p21 positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53 negative</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>p53 low</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>p53 positive</td>
<td>27</td>
<td>6</td>
</tr>
</tbody>
</table>

* Numbers represent numbers of tumours.
Figure 1. Expression of p53 and p21 in tumour biopsies, tumours and normal mucosa.
A: Non-irradiated sample, showing p53-positive tumour cells (→), whereas normal mucosal cells (←) are p53-negative. B: Irradiated sample, showing a tumour completely negative for p53 (→), with p53-positive normal mucosal cells (←) and stromal cells. C: p53-positive normal mucosa after irradiation.
D, E, F: Samples from the same patient. D: Non-irradiated tumour biopsy with 1-25% of the cells p53 positive. E: Corresponding irradiated tumour, showing >25% of the cells p53 positive, indicative for wild type p53. F: Same tumour as E, showing no p21wat staining in tumour cells, indicative for a disrupted pathway. Stromal cells are clearly positive.
G, H, I: Samples from the same patient. G: Non-irradiated tumour biopsy with >25% of cells p53 positive, indicative for mutant p53. H: Corresponding irradiated tumour, showing >25% of the cells p53 positive. I: Same tumour as H, showing p21wat-positive cells throughout the tumour, indicating a p53 independent upregulation of p21wat. RT: irradiated, no RT: non-irradiated. (for full-colour figure, see page 181)
p53 expression in rectal tissue after radiotherapy

Figure 2. Distribution of percentage of p53 expressing cells over the treatment arms. No difference is observed (P=0.39). RT+TME: radiotherapy followed by surgery, TME: surgery only.

Figure 3. Distribution of p53 expression in non-mucinous, mucinous and irradiated mucinous tumours. A shift from low p53 expression in the mucinous tumours towards p53 positivity in the irradiated tumours is observed, indicative for the upregulation of wild type p53 after irradiation.

Figure 4. Distribution of p21 expression over the treatment arms. No difference is observed (P=0.36). RT+TME: radiotherapy followed by surgery, TME: surgery only.

Figure 5. Flow chart demonstrating the relationship between the p53 expression in untreated biopsies and the irradiated tumours. p21 expression in the corresponding tumours is displayed.
p21\textsuperscript{waft} in normal mucosa
In unirradiated mucosa, we observed expression of p21\textsuperscript{waft} in cells located in the upper part of the crypts. This has been described before and this apical expression is thought to be limited to differentiated and non-proliferating cells.\textsuperscript{21} After radiotherapy expression of p21\textsuperscript{waft} in normal mucosa was upregulated throughout the crypt and demonstrates upregulation of p21\textsuperscript{waft} by wt p53 after ionising irradiation. These data confirm that \textit{in vivo} a functional p53-p21\textsuperscript{waft} pathway can be demonstrated using IHC.

p53 in tumours
The clear upregulation of wt p53 in normal mucosa suggests that tumours in the irradiated group completely negative for p53 do not represent tumours with functional p53. Because no significant difference was found in the number of p53-negative tumours in both treatment arms, we propose that all tumours completely devoid of p53-positive cells contain either a frameshift or truncating p53 mutation or have MDM2 overexpression.

Previously we have shown that p53 positivity by immunohistochemistry in nonirradiated carcinomas almost always represents mutated p53.\textsuperscript{30} In the irradiated group however, p53-positive tumours can either have mutated or upregulated p53. Because the number of p53-positive tumours was not significantly different between both treatment arms, it is likely that in the irradiated group high p53 expression was in the great majority of cases caused by mutation and not by radiation-induced upregulation of wt p53. The slight difference between both treatment arms in the distribution of tumours with low p53 expression, suggests that tumours expressing p53 in 1-25% of the cells might contain wt p53. In conclusion, we propose that tumours completely negative for p53 or showing p53 overexpression represent tumours with non-functional p53, and p53 expression in 1-25% of the cells is indicative for wt p53. This means that in our study, in 84% of the tumours p53 was non-functional, a higher percentage than usually reported for colorectal cancer (40-80%).\textsuperscript{4,31} The results of p53 expression in mucinous tumours are in agreement with this hypothesis. The higher number of unirradiated mucinous tumours with low p53 expression vs. the relatively low frequency in the irradiated group, confirms the assumption that tumours with low p53 expression (1-25% of the cells positive) represent tumours with wild type protein, that becomes upregulated after radiotherapy.

The observed upregulation after irradiation in p53 negative biopsies, seems in contrast with the conclusion that p53-negative samples contain mutated p53. This might be explained by the small size of these samples, preventing the detection of p53-positive cells in biopsies. In a study with 5x5 Gy preoperative radiotherapy, the irradiated as well as the unirradiated group showed p53 positivity in the tumours, while the biopsies were negative.\textsuperscript{4} This observed increase in the non-irradiated tumours confirms that biopsies can be too small to reliably indicate p53 status. Another study comparing nonirradiated biopsies with irradiated surgical samples, showed no increase in the expression of p53 after radiotherapy.\textsuperscript{5} Apart from the size of the biopsies, this might be explained by the fact that in this study the median interval between radiotherapy and surgery was 14 days, allowing the degradation of radiation-induced stabilised wt p53.
p21<sup>waf1</sup> in tumours

Our results indicate that the expression of p21<sup>waf1</sup> in tumour tissue does not change after radiotherapy. In contrast, loss of p21<sup>waf1</sup> expression after radiotherapy in initially positive colorectal tumours has been described. In addition, the average interval between radiotherapy and surgery in that study was 12 weeks, which might allow for outgrowth of a subset of p21<sup>waf1</sup> negative tumour cells.

The expression of p21<sup>waf1</sup> observed in tumours completely negative for p53 indicates that p21<sup>waf1</sup> transcription in tumours is not always dependent on p53, as has been described before. In addition to this, we observed that upregulation of wt p53 by irradiation does not necessarily lead to increased expression of p21<sup>waf1</sup>. On basis of these arguments it must be concluded that a high percentage of rectal cancer tumours contain p53 mutations or show a failure in the signaling downstream of p53. Consequently, the number of rectal carcinomas with functionally active p53 is very limited.

In the literature, the overexpression of p53 in colorectal carcinomas varies between 40% and 80%. The variation may be explained by patient selection and by the various cut-off points used for p53 positivity. Furthermore, none of the studies differentiate between tumours absolutely negative for p53 and tumours with a low number of positive cells, thus obscuring p53-mutated tumours in a group that is usually considered wild type. This might explain the contradictory results concerning the prognostic value of p53 overexpression. We suggest that the downstream pathway of p53 is often disrupted in p53 wild type tumours, complicating interpretation even further. Therefore, we believe that the value of p53 as prognostic marker requires reconsideration. Using this new information we will develop an assay for p53 assessment that we will use to study the prognostic value of p53 in rectal cancer.

REFERENCES
Chapter 9