Epithelial Human Leukocyte Antigen-DR Expression Predicts Reduced Recurrence Rates and Prolonged Survival in Rectal Cancer Patients

Elza C. de Bruin,1,3 Cornelis J.H. van de Velde,2 J. Han J.M. van Krieken,5 Corrie A.M. Marijnen,1,4 and Jan Paul Medema3

Abstract

Purpose: The development of local and distant recurrences is a major problem in the treatment of rectal cancer patients. In this study, we investigated whether epithelial human leukocyte antigen–DR (HLA-DR) expression allowed discrimination between high and low tumor recurrence rates, and analyzed the mechanism behind its expression.

Experimental Design: The role of IFNγ in HLA-DR expression was studied in rectal cancer cell lines and tumors by promoter-specific analyses of class II transactivator (CIITA). The predictive value of epithelial HLA-DR expression was investigated by immunohistochemical evaluation of 1,016 rectal tumors, obtained from a large prospective trial. Associations with recurrences and survival were determined by univariate and multivariate log-rank testing.

Results: HLA-DR was induced by IFNγ in rectal cancer cell lines. Activity of the IFNγ-inducible pIV-CIITA promoter correlated with epithelial HLA-DR expression in rectal tumors. Patients with HLA-DR–positive tumors developed less frequent local and distant recurrences [1.6% versus 9.1% (P = 0.0015) and 15.3% versus 29.9% (P < 0.0001), respectively, after 5 years of follow-up] and had better survival (78.6% versus 61.3%; P < 0.0001) than patients with HLA-DR–negative tumors. Epithelial HLA-DR was more often found in lower tumor-node-metastasis (TNM) stages. Next to TNM and circumferential resection margin, HLA-DR expression was independently associated with lower distant recurrence rates and prolonged survival.

Conclusions: Epithelial HLA-DR expression can be used as a marker to discriminate patients with high or low risk of developing recurrences. The possible involvement of IFNγ, the relationship with lower TNM stages, and the independent effect on recurrence development together suggest that the host immune response plays an important role in controlling tumor cells.

In the treatment of rectal cancer patients, local and distant recurrences are a major problem because these are associated with both high mortality and morbidity. The introduction of total mesorectal excision (TME) surgery in combination with preoperative radiotherapy has been shown to be useful in reducing local recurrences. However, more than 25% of the patients develop metastases within 5 years after surgical treatment with curative intent (1, 2).

We and others have shown that (colo)rectal cancer patients develop less frequent recurrences when high numbers of infiltrating immune cells are present in the tumor (3–6). An optimal antitumor immune response usually requires activation of CD4+ and CD8+ T lymphocytes with a tumor-associated antigen. CD8+ T cells are activated by antigen presented by human leukocyte antigen (HLA) class I molecules whereas CD4+ T cells are activated by antigen presented by HLA class II molecules, normally expressed on professional antigen-presenting cells such as dendritic cells and macrophages. We have recently shown that a subgroup of tumors expressed high levels of HLA class I and II and several other immune-related genes. Importantly, most of these tumors expressed the HLA class II protein HLA-DR on epithelial cells (7). These findings suggest an important role for the host immune response in targeting tumor cells, implying a better prognosis for patients with tumors expressing epithelial HLA-DR. Indeed, most studies on colorectal cancer patients have shown that epithelial expression of HLA-DR relates to a better prognosis (8–11). Because of the limited amount of data for rectal cancer patients, it is unclear whether the prognostic value of HLA-DR observed in colorectal cancer patients (with 33–44% rectal cancer patients) can also be applied specifically to rectal cancer patients. We therefore analyzed the expression of epithelial HLA-DR in a large number of rectal tumors, obtained from a prospective trial randomizing
surgery alone or short-term preoperative radiotherapy followed by surgery (1). The standardized high-quality surgery technique done in this trial decreased the number of recurrences due to inadequate surgery (12), and therefore allowed a reliable investigation of the influence of intrinsic tumor characteristics, such as HLA-DR expression, on recurrences and survival. In addition, we analyzed the involvement of IFNγ in epithelial HLA-DR expression in vivo.

**Materials and Methods**

**Cell lines.** Colon cancer cell line HT29, rectal cancer cell lines SW837 and SW1463, and Ramos B-cell line were all cultured in Iscove’s modified Dulbecco’s medium (Cambrex) supplemented with glutamine, penicillin/streptomycin, and 8% heat-inactivated FCS, at 37°C. Where indicated, exponentially growing cells were treated with 100 units/mL human recombinant IFN

**Immunohistochemistry.** Stainings were done as previously described (7). Briefly, 4-μm sections were deparaffinized and endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol for 30 min. Antigen retrieval was done by boiling for 10 min in 0.01 mol/L citrate buffer (pH 6.0). Preincubation with 20% normal goat serum in PBS/1% bovine serum albumin for 30 min was followed by overnight incubation with the prediluted monoclonal HLA-DR antibody (LN3; Signet Laboratories). Sections were incubated for 1 h with biotinylated goat anti-mouse (1:200; DakoCytomation) followed by incubation with streptavidin complexed with biotinylated peroxidase (DakoCytomation) and developed with 3,3-diaminobenzidine (DakoCytomation). Finally, sections were counterstained with Mayer’s hematoxylin. As a negative control, PBS alone was used in place of the primary antibody.

**Scoring.** For each tumor, one 2 mm-diameter punch was evaluated. Epithelial HLA-DR stainings were scored by determining the percentage of positively stained tumor epithelium. Most tumors (n = 674; 66%) were completely negative for epithelial HLA-DR. 126 (12%) tumors showed ≥20% positive epithelial, 52 (5%) tumors showed 20% to 50%, and 164 (16%) tumors showed ≥50% positive epithelial. To analyze the correlation between different variables, tumors were regarded positive if at least 20% of the epithelial cells stained positive for HLA-DR. This retrospectively selected cutoff value was chosen to ensure an adequate sample size in the HLA-DR-positive group. Analyses were repeated with ≥5% and ≥50% as cutoff values to show that results were independent of cutoff value.

**Statistical analysis.** All analyses were done with SPSS statistical software (version 12.0 for Windows, SPSS, Inc.). Data were analyzed with Mann-Whitney or Kruskal-Wallis tests to compare quantitative and ordered variables and with Student’s t tests to compare normally distributed data between two groups. χ² tests were used to compare proportions. A two-sided P value of ≤0.05 was considered statistically significant.

**Results**

**Role of IFNγ in epithelial HLA-DR expression in rectal cancer cell lines.** We have recently shown, following gene expression analysis on 47 nonirradiated rectal tumors, that a subgroup of tumors expressed HLA-DR, a member of the HLA class II
proteins, in epithelial cells (7). To obtain insight into the mechanism of epithelial HLA-DR expression, we first tested whether IFN-γ is able to induce HLA-DR expression in vitro in rectal cancer cells, as has been described for colon cancer cells (17). Indeed, fluorescence-activated cell sorting analysis showed up-regulated surface expression of HLA-DR following treatment with IFN-γ. Specifically, IFN-γ induced HLA-DR expression in SW1463 cells, which do not normally express HLA-DR, and markedly enhanced the HLA-DR expression in SW837 cells above the endogenous expression (Fig. 1A).

IFN-γ induces HLA-DR through the STAT1 signaling pathway, which promotes transcription of IRF1, leading to transcription of CIITA (18). Western blot analyses of rectal cancer cells treated with IFN-γ indeed showed rapid phosphorylation of STAT1 and up-regulation of IRF1 after 6 h on treatment (Fig. 1B). Together, the data show that IFN-γ is able to induce epithelial HLA-DR expression in these rectal cancer cells, and therefore suggest that epithelial HLA-DR expression in tumors might be driven by IFN-γ.

**Role of IFN-γ in epithelial HLA-DR expression in rectal tumors.** To investigate the role of IFN-γ in epithelial HLA-DR expression in vivo, we examined the promoter activity of CIITA in rectal tumors. CIITA is controlled by four independent promoters, of which promoter pIV has been described to regulate IFN-γ-induced CIITA expression, and pIII to regulate constitutive CIITA expression such as in B cells and some melanomas (19–21). PCR analyses, with promoter-specific primers, enabled us to determine the promoter activity in rectal tumors and showed both active pIII and pIV in HLA-DR–positive tumors. Whereas pIII-CIITA was detected in some HLA-DR–negative tumors, activity of pIV-CIITA could not be found in HLA-DR–negative tumors (Fig. 2, left). In addition, analysis of IFN-γ-treated cells showed that both promoters, pIII and pIV, were activated on IFN-γ treatment. Although SW837 displayed pIV-derived CIITA in the absence of IFN-γ, its activity was markedly enhanced on IFN-γ treatment (Fig. 2, right). Together, these observations suggest that IFN-γ is likely involved in epithelial HLA-DR expression in vivo.

**Correlation between epithelial HLA-DR expression and patients’ characteristics.** We previously analyzed 47 rectal cancer patients for epithelial HLA-DR expression and showed epithelial HLA-DR expression in 9 tumors (7). Interestingly, none of the patients with HLA-DR–positive tumors developed recurrences after 3 years of follow-up, whereas 19% of the patients with HLA-DR–negative tumors developed recurrences within this period of time. The number of patients is obviously too small to provide reliable data. To further investigate this, we evaluated HLA-DR expression in 1,016 paraffin-embedded rectal tumors obtained from a prospective trial randomizing for preoperative radiotherapy, followed by surgery. Most tumors (66%) did not show any HLA-DR expression in the epithelial compartment. These were grouped together with the 12% of tumors that showed <20% positivity and considered HLA-DR negative. The 216 (21%) tumors that expressed
tumors were classified as being HLA-DR positive if at least 20% of
the epithelial tumor cells stained positive. To evaluate the
consistency of the data, the analyses have been repeated with
the cutoff values ≥5% and ≥50%. These analyses showed
similar results (i.e. significantly lower recurrence rates and
better survival for patients with HLA-DR–positive tumors).

The curves in Fig. 3A showed that epithelial HLA-DR can
discriminate patients with high or low recurrence risks.
Nevertheless, several important variables favor the HLA-DR–
positive group (Table 1). Curves that are corrected for the
effects of TNM, CRM, and treatment are displayed in Fig. 3B.
These curves also showed better prognosis for patients with
HLA-DR–positive tumors.

Stratified analysis based on the treatment regimen revealed
that both nonirradiated and irradiated patients had lower
distant recurrence rates and better survival when the tumor
stained positive for HLA-DR (Table 2). We also observed better
local control for HLA-DR–positive tumors in irradiated patients
(P = 0.011), and a similar trend in nonirradiated patients
(P = 0.064). Interestingly, none of the irradiated patients with
HLA-DR–positive tumors developed local recurrences within
5 years of surgery. Stratification based on TNM showed a
comparable trend for TNM stage I/II and TNM stage III/IV:
lower recurrence rates and better survival for the HLA-
DR–positive group. Although the predictive value of epithelial
HLA-DR on recurrence rates was not statistically significant for
the CRM-positive tumors (local recurrences, P = 0.11; distant
recurrences, P = 0.10), it was associated with a better survival
(P = 0.01) for this group of patients. For the CRM-negative
tumors, positive HLA-DR was significantly associated with lower
recurrence rates and prolonged survival.

The independent predictive value for HLA-DR was tested in
multivariate Cox regression analysis with backward stepwise
elimination in the context of the following variables: CRM,
TNM stage, differentiation, distance to anal verge, and treat-
ment. No significant interactions were observed between the
variables. Although statistically not significant, epithelial HLA-
DR expression tended to be predictive for local recurrences
(hazard ratio, 2.4; P = 0.07) in addition to CRM, TNM, and
treatment. Importantly, HLA-DR expression on epithelial tumor
cells had independent prognostic value for distant recurrence
rate (hazard ratio, 1.6; P = 0.01) and survival (hazard ratio, 1.4;
P = 0.01) in additional to TNM and CRM (Table 3).

| Table 1. Clinical and pathologic characteristics of
patients with HLA-DR–positive and HLA-DR–
negative tumors |
<table>
<thead>
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<tbody>
<tr>
<td>HLA-DR</td>
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<tr>
<td>n (%)</td>
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<tr>
<td>---</td>
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<tr>
<td>Age (median)</td>
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<tr>
<td>Sex</td>
</tr>
<tr>
<td>Female</td>
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<tr>
<td>Male</td>
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<tr>
<td>Treatment</td>
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<tr>
<td>TME</td>
</tr>
<tr>
<td>RT + TME</td>
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<tr>
<td>TNM stage</td>
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<tr>
<td>I</td>
</tr>
<tr>
<td>II</td>
</tr>
<tr>
<td>III</td>
</tr>
<tr>
<td>IV</td>
</tr>
<tr>
<td>Differentiation</td>
</tr>
<tr>
<td>Well</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td>Poor/undif</td>
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<tr>
<td>Distance to anal verge, cm</td>
</tr>
<tr>
<td>≥5</td>
</tr>
<tr>
<td>5-10</td>
</tr>
<tr>
<td>&lt;5</td>
</tr>
<tr>
<td>Operation type</td>
</tr>
<tr>
<td>LAR</td>
</tr>
<tr>
<td>APR</td>
</tr>
<tr>
<td>Hartmann</td>
</tr>
<tr>
<td>CRM</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
</tr>
</tbody>
</table>

Abbreviations: RT, radiotherapy; undif, undifferentiated; LAR, low
anterior resection; APR, abdominoperineal resection.

HLA-DR in at least 20% of the epithelial cells were considered
HLA-DR positive.

HLA-DR expression in relation to clinicopathologic variables
is shown in Table 1. Epithelial HLA-DR expression was
associated with lower TNM stages, and HLA-DR–positive
tumors had significantly more negative CRMs. HLA-DR–
positive tumors were more often found in irradiated patients,
but analysis with the percentage of HLA-DR–positive cells as a
continuous variable revealed no significant difference between
irradiated and nonirradiated patients (P = 0.137). The
association with TNM stages and CRM remained statistically
significant (P < 0.001 and P = 0.015, respectively).

Epithelial HLA-DR expression is related to a better prognosis for
rectal cancer patients. The association between epithelial HLA-
DR expression in tumors and the development of recurrences in
the cancer patients is displayed in Fig. 3. Local recurrence rates
were significantly lower for patients with HLA-DR–positive
tumors compared with patients with HLA-DR–negative
tumors: 1.6% versus 9.1% at 5 years of follow-up (P =
0.0015; Fig. 3A, left). A similar result was found for distant
recurrence rates: 15.3% versus 29.9% (P < 0.0001; Fig. 3A,
middle). The lower number of recurrences resulted in prolonged
overall survival for patients with epithelial HLA-DR–positive
tumors when compared with patients with HLA-DR–negative
tumors: 78.6% versus 61.3% (P < 0.0001) of the patients were
still alive at 5 years of surgery (Fig. 3A, right). In this study,
tumors were classified as being HLA-DR positive if at least 20% of

In this study, we found that HLA-DR was expressed on
epithelial cells in ~20% of rectal tumors, and showed that
rectal cancer patients with HLA-DR–positive tumors had better
survival than patients with HLA-DR–negative tumors. In
addition, epithelial HLA-DR expression correlated with a
reduction in both local and distant recurrence rates.

The expression of HLA-DR was inducible by IFNγ in rectal
cancer cell lines, which is consistent with published data for
colon cancer cell lines (17). The influence of IFNγ on HLA-
DR expression in vivo was previously suggested following detection
of IFNγ mRNA in HLA-DR–positive tumors (11). Nevertheless,
the presence of IFNγ can also be a consequence of epithelial
HLA-DR because HLA-DR–expressing tumor cells recruit and
activate CD4+ T helper 1 and natural killer cells, resulting in
local production of IFNγ (22, 23). To obtain more insight into
HLA-DR expression in vivo, we analyzed CIITA promoter

Discussion
activity and showed that activity of pIV correlated with HLA-DR expression, in contrast to that of pIII, which is involved in constitutive expression (20, 21). This suggests that epithelial HLA-DR expression in vivo is regulated by CIITA via the IFN-γ-inducible pIV promoter. It therefore follows that HLA-DR expression could result from the presence of IFN-γ.

Epithelial HLA-DR expression as prognostic factor has previously been described for colorectal cancer patients (8–11).

Table 2. Prognostic values of epithelial HLA-DR expression for local and distant recurrences and survival on stratification for treatment, TNM, or CRM

<table>
<thead>
<tr>
<th>HLA-DR</th>
<th>Local recurrences</th>
<th></th>
<th></th>
<th>Distant recurrences</th>
<th></th>
<th></th>
<th>Overall survival</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pos</td>
<td>Neg</td>
<td>P</td>
<td>HR (95% CI)</td>
<td>Pos</td>
<td>Neg</td>
<td>P</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Overall</td>
<td>1.6</td>
<td>9.5</td>
<td>0.002</td>
<td>3.9 (1.6-9.7)</td>
<td>15.3</td>
<td>29.9</td>
<td>&lt;0.0001</td>
<td>2.1 (1.5-3.1)</td>
</tr>
<tr>
<td>TME</td>
<td>3.5</td>
<td>10.6</td>
<td>0.06</td>
<td>2.5 (0.9-7.1)</td>
<td>14.6</td>
<td>30.2</td>
<td>0.005</td>
<td>2.1 (1.2-3.8)</td>
</tr>
<tr>
<td>RT + TME</td>
<td>0.0</td>
<td>7.2</td>
<td>0.01</td>
<td>8.7 (1.1-64.5)</td>
<td>16.0</td>
<td>29.5</td>
<td>0.002</td>
<td>2.1 (1.3-3.5)</td>
</tr>
<tr>
<td>TNM I/II</td>
<td>0.0</td>
<td>4.1</td>
<td>0.08</td>
<td>3.4 (0.8-14.6)</td>
<td>10.7</td>
<td>15.9</td>
<td>0.15</td>
<td>1.5 (0.9-2.5)</td>
</tr>
<tr>
<td>TNM III/IV</td>
<td>4.8</td>
<td>16.1</td>
<td>0.07</td>
<td>2.8 (0.9-9.0)</td>
<td>30.4</td>
<td>49.4</td>
<td>0.01</td>
<td>1.9 (1.1-3.3)</td>
</tr>
<tr>
<td>CRM</td>
<td>1.2</td>
<td>7.3</td>
<td>0.01</td>
<td>3.5 (1.2-9.7)</td>
<td>12.1</td>
<td>25.1</td>
<td>0.0005</td>
<td>2.2 (1.4-3.3)</td>
</tr>
<tr>
<td>CRM+</td>
<td>4.3</td>
<td>18.6</td>
<td>0.11</td>
<td>4.5 (0.6-33.6)</td>
<td>38.9</td>
<td>53.2</td>
<td>0.10</td>
<td>1.8 (0.9-3.6)</td>
</tr>
</tbody>
</table>

NOTE: Five-year recurrence rates and survival for patients with HLA-DR–positive tumors (pos) and HLA-DR–negative tumors (neg) and P values were determined by univariate log-rank analyses. Hazard ratios (HR) with 95% confidence intervals (95% CI) were determined by Cox regression analysis with HLA-DR as covariate.
However, stratified analyses according to tumor location revealed better prognosis for HLA-DR–positive tumors in colon cancer patients but not in rectal cancer patients (9). The prognostic value of HLA-DR obtained in colorectal patients might be skewed by its association with microsatellite instability (9), which is a rare event in rectal tumors (24, 25).

To our knowledge, this is the first comprehensive study investigating a correlation between epithelial HLA-DR expression and survival of rectal cancer patients. The high number of patients studied and the standardized high-quality TME surgery enabled us to show remarkably lower recurrence rates and better survival for rectal cancer patients with HLA-DR–positive tumors. Interestingly, the results did not depend on the cutoff value because similar results were obtained with the cutoff values ≥5%, ≥20%, and ≥50% HLA-DR–positive epithelium.

Several mechanisms have been proposed to explain the good prognosis for colorectal cancer patients with HLA-DR–positive tumors. A role for IFNγ has been described, as it affects multiple genes involved in apoptosis, cell growth, and genetic instability (10, 26–28). Other studies focused on immunologic tumor control. Animal experiments showed that CIITA-transfected tumor cells were rejected via activation of the host immune response (22, 23). In vitro experiments with human tumor cells also showed enhanced immunogenicity on (IFNγ-induced) expression of CIITA (29, 30). In agreement with this, we and others have shown that high amounts of tumor infiltrating lymphocytes correlate with a better prognosis in (colo)rectal cancer patients (3, 4, 6). In our study, HLA-DR–positive tumors were more often found in low TNM stages. This might be explained by reduced proliferation of HLA-DR–positive cells (10), as well as by immunologic control of the primary tumor.

The less frequent distant recurrence development supports the hypothesis that HLA-DR–positive tumor cells exert an immunologic control. Whereas HLA-DR expression independently predicted lower distant recurrence rates and prolonged survival, its predictive value for local recurrences was not statistically significant in the context of TNM, CRM, and treatment. This loss of significance indicates different regulation of local versus distant recurrences. Whereas the immune response plays an important role in preventing HLA-DR–positive cells from metastasizing to lymph nodes or other organs, local control also depends on other mechanisms, such as the effect of radiotherapy.

TNM stage, CRM, and radiotherapy were independently associated with local control, whereas HLA-DR only showed a trend toward better local control. These data indicate that radiation and a negative CRM could be more important factors than epithelial HLA-DR expression in preventing local recurrences. The role of HLA-DR expression may, however, be underestimated due to the small number of events.

In conclusion, epithelial HLA-DR expression is associated with a reduced recurrence rates and better survival. Because this expression is an intrinsic tumor characteristic, evaluation of HLA-DR expression can be used to identify patients with high risk for disease recurrences who need adjuvant treatment (31). In addition, we showed that epithelial HLA-DR expression in rectal tumors is likely to result from the presence of IFNγ. It is also known that HLA-DR expression enhances the immune system and results in local IFNγ production. This implies a self-amplifying system between HLA-DR and IFNγ. If this is the case, then the induction of HLA-DR could be a promising therapeutic strategy. A recently published phase II trial in metastatic melanoma patients showed a long-lasting expression of HLA-DR on IFNγ treatment (32), indicating that up-regulation of HLA-DR can indeed be achieved by administration of IFNγ in vivo. In these melanoma patients, HLA-DR induction did not correlate with tumor regression. However, melanomas are not directly comparable to rectal tumors because they are known to be highly immunogenic and have several mechanisms to escape immune destruction (26, 33–38). It remains to be elucidated whether treatment-induced HLA-DR expression will improve prognosis to comparable levels as endogenous epithelial HLA-DR expression in rectal cancer patients. In light of our study, future investigation into the therapeutic benefit of HLA-DR induction for the treatment of rectal cancer patients is warranted.

**Acknowledgments**

We thank Peter van den Elsen (Leiden University Medical Center, Leiden, the Netherlands) for kindly providing the CIITA promoter-specific primers, and Fiona Kimberley (LEXOR, Academic Medical Center, the Netherlands) for critical reading of the manuscript.

**Table 3. Prognostic values of epithelial HLA-DR expression for distant and local recurrences and survival in multivariate analysis**

<table>
<thead>
<tr>
<th></th>
<th>Local recurrence</th>
<th>Distant recurrence</th>
<th>Survival</th>
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<tbody>
<tr>
<td><strong>HR (95% CI)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HLA-DR</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Negative</td>
<td>2.4 (0.9-6.0)</td>
<td>1.6 (1.1-2.4)</td>
<td>1</td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
<td>1</td>
<td>1.4 (1.1-1.9)</td>
</tr>
<tr>
<td>CRM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>1</td>
<td>1.7 (1.3-2.1)</td>
</tr>
<tr>
<td>Positive</td>
<td>1.9 (1.1-3.3)</td>
<td>1.8 (1.3-2.4)</td>
<td>1</td>
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<tr>
<td>TNM stage</td>
<td></td>
<td></td>
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<tr>
<td>I</td>
<td>3.9 (1.3-11.7)</td>
<td>2.5 (1.6-4.1)</td>
<td>1.3 (1.1-1.7)</td>
</tr>
<tr>
<td>II</td>
<td>8.2 (2.9-23.4)</td>
<td>6.4 (4.1-10.0)</td>
<td>1</td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
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<tr>
<td>Distance to anal verge, cm</td>
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<tr>
<td>≥10</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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<tr>
<td>5-10</td>
<td>1.4 (1.0-2.0)</td>
<td>ns</td>
<td>ns</td>
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<tr>
<td>&lt;5</td>
<td>1.8 (1.3-2.5)</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
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<tr>
<td>TME</td>
<td>1.8 (1.0-3.0)</td>
<td>ns</td>
<td>ns</td>
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<tr>
<td>RT + TME</td>
<td>1</td>
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**NOTE:** P values and hazard ratios with 95% confidence intervals were determined by Cox regression analysis. "ns" indicates that this variable was not statistically significant in the backward stepwise logistic regression analysis and was therefore eliminated.
are predicted by the nonspecific immune response; specific immune response has only a systemic effect—a histopathological and immunohistochemical study. BMC Cancer 2001;1:7.


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