PRESENCE OF A HIGH AMOUNT OF STROMA AND DOWNREGULATION OF SMAD4 PREDICT FOR WORSE SURVIVAL FOR STAGE I-II COLON CANCER PATIENTS.

Wilma E. Mesker¹, Gerrit-Jan Liefers², Elaine Johnstone³, Hans Morreau³, Hans J. Tanke², David Kerr⁴, Rob A.E.M. Tollenaar¹.  
1. Department of Surgery, 2. Department of Molecular Cell Biology, 3. Department of Pathology Leiden University Medical Center (LUMC), Leiden, The Netherlands 4. Department of Clinical Pharmacology, University of Oxford, UK.

Introduction.  
For stage I-II colon cancer a significant number (5%-25%) of patients have recurrent disease within 5 years. There is need to identify these high-risk patients as they might benefit from additional treatment. Stromato-tissue surrounding the cancer cells plays an important role in the tumor behavior. The proportion of intra-tumor stroma was evaluated for the identification of high-risk patients. In addition, protein expression of the markers SMAD4 involved in pathways related to stroma production and epithelial-to-mesenchymal transition (EMT) was analyzed.

Method.  
In a retrospective study of 135 patients with stage I-II colon cancer, the amount of stroma was estimated on routine haematoxylin-eosin stained histological sections. Sections were also immunohistochemically stained for β-catenin, TGF-β, p21 and SMAD4.

Results.  
Of 135 analyzed patients 34 (25.2%) showed a high proportion of stroma (stroma-high) and 101 (74.8%) a low proportion (stroma-low). Significant differences in overall survival and disease-free-survival were observed between the two groups, with stroma-high patients showing poor survival (OS p<0.001, HZ 2.73, CI 1.73-4.30; DFS p<0.001, HZ 2.43, CI 1.55-3.82). A high-risk group was identified with stroma-high and SMAD4 loss (OS p=0.008, HZ 7.98, CI 4.12-15.44, DFS p=0.005, HZ 6.57, CI 3.43-12.96). 12 of 14 (85.7%) patients died within 3 years. For the stroma-low group this difference was not significant (OS p=0.937, DFS p=0.685). Percentages of 5 year follow up were 7.1% for stroma-high/SMAD4-negative patients and 80.3% for stroma-high/SMAD4-positive patients (Tables 3 and 4). A group of “high risk” patients with low survival time showing a high amount of intra-tumor stroma and negative SMAD4 staining could be distinguished with additional independent prognostic value.

In a logistic regression analysis the interaction between the variables high intra-tumor stroma and loss of SMAD4 were found to be strongly related (HZ 5.42, CI 2.13-13.82, p<0.001) indicating that SMAD4 staining can be a specific marker to select “high risk” patients.

Conclusions.  
Conventional haematoxylin-eosin stained tumor slides contain percentages of stroma and SMAD4 expression that can be used to select patients for adjuvant therapy or to follow patients that might benefit from adjuvant treatment.

Immunostaining for SMAD4.  
From 118 patients stained for SMAD4, positive nuclear staining was seen in 28 (23.5%). There was a significant difference in survival time between the SMAD4 positive and the SMAD4 negative patients (OS p=0.006, DFS p=0.022). Twelve of the 14 (85.7%) stroma-high/SMAD4-negative patients died within 3 years. For the stroma-low group this difference was not significant (OS p=0.937, DFS p=0.685). Percentages of 5 year follow up were 7.1% for stroma-high/SMAD4-negative patients and 80.3% for stroma-high/SMAD4-positive patients (Tables 3 and 4). A group of “high risk” patients with low survival time showing a high amount of intra-tumor stroma and negative SMAD4 staining could be distinguished with additional independent prognostic value.

Kaplan-Meier survival curves for stroma-high patients and stroma-low patients with positive and negative SMAD4 staining: (a) OS, (b) DFS. A. Stroma-low / SMAD4-positive. B. Stroma-high / SMAD4-positive. C. Stroma-high / SMAD4-negative. D. Stroma-high / SMAD4-positive.

Results of SMAD4 staining relative to the amount of stroma in the primary tumor.

<table>
<thead>
<tr>
<th>Stroma high</th>
<th>Stroma low</th>
<th>SMAD4 negative</th>
<th>Perc. of patients</th>
<th>Perc. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SMAD4 positive</td>
<td>11.9% (7.1%)</td>
<td>11.9% (85.7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11.9% (57.1%)</td>
<td>64.4% (80.3%)</td>
</tr>
</tbody>
</table>

Carcinoma percentage estimated as more than 80% in a patient with a long OS/DFS. a. H&E staining, b. cytokeratin staining for carcinoma cells, c. vimentin staining of stromal compartments. Carcinoma percentage estimated as 30% in a patient with a short OS/DFS. d. H&E staining, e. cytokeratin staining, vimentin staining.

Kaplan-Meier survival curves for stroma-high patients and stroma-low patients with positive and negative SMAD4 staining: (a) OS, (b) DFS. A. Stroma-low / SMAD4-positive. B. Stroma-high / SMAD4-positive. C. Stroma-high / SMAD4-negative. D. Stroma-high / SMAD4-positive.

Results.  
We analyzed the standard arm of patients as part of this Victor trial that were additionally treated with IFU (IV of bolus). A significant decreased overall and disease free survival was found for patients with a high amount of stroma.

Conclusions.  
These results show that tumor-stroma ratio as a single parameter or in combination with SMAD4 immunohistochemistry can further select for a patient population with specific bad prognosis. When confirmed in series from other institutions our approach might contribute to a better selection of high risk stage I and II patients that might benefit from adjuvant treatment.

Consequently, prospective studies to select patients for a randomized clinical study in which adjuvant therapy is selectively applied in stage I and II colorectal cancer should follow. For stage III disease this parameter selects for patients with worse outcome for which current standard therapy is not effective.