Prognostic utility of immune markers and validation of Immunoscore® in stage III colon carcinoma (CC) patients (pts) treated with adjuvant FOLFOX in a phase III trial [NCCTG N0147 (Alliance)]

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Background

Immunoscore was developed based on CD3+ and CD8+ T-cell density and location in primary CC-pts with paired stages and varying treatment and follow-up. We determined if individual immune markers and/or Immunoscore can predict disease-free survival (DFS) in resected stage III CC pts from FOLFOX arm of a phase III trial.

Objectives

To determine if individual immune markers and/or the Immunoscore are prognostic in resected stage III CC pts from FOLFOX arm of a phase III trial.

Methods

PATIENTS:

Stage III colon cancer patients (N=650) randomly selected from the FOLFOX arm enrolled in NCCTG N0147, a phase III randomized controlled trial of FOLFOX +/- cetuximab (NCT00786274)

IMMUNE MARKERS:

- Density of CD3+ and CD8+ T-cells, or CD20+ B-lymphocytes, in central tumor (CT) and invasive margin (IM) was evaluated by IHC and quantified using image analysis software.

- Immunoscore® was calculated using densities of CD3+ and CD8+ in both CT and IM by an algorithm implemented on a cohort including stage II and III pts (Galon J, J Clin Invest 2013;123:3450-60)

- High risk: Immunoscore of 0 or 1
- Low risk: Immunoscores of 2, 3, and 4

STATISTICAL ANALYSIS:

- Primary outcome: Disease-free survival (DFS). Associations evaluated by Kaplan-Meier curves and multivariable Cox model adjusting for age, gender, race, T/N stage, and dMMR for CD8 and/or CD3.

- Model selection procedure (backwards selection) was used to determine the most important immune markers in predicting DFS.

- Cox and O’Quigley method, highest hazard ratio (HR) and lowest p-value, and False Discovery Rate were used to identify the most important immune markers in predicting DFS.

- Pre-defined cut-offs were used to validate the standardized Immunoscore®.

- Immunoscore® and DFS

Results

Table 2. CD3IM risk group and patient characteristics

<table>
<thead>
<tr>
<th>Immunoscore*</th>
<th>HR (95% CI)</th>
<th>p-adj</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3IM 0-1</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>CD3IM 3-4</td>
<td>1.87 (0.87, 4.01)</td>
<td>0.133</td>
</tr>
<tr>
<td>CD3IM 0-1</td>
<td>1.20 (0.53, 2.72)</td>
<td>0.64</td>
</tr>
<tr>
<td>CD3IM 2-3</td>
<td>1.0 (0.43, 2.29)</td>
<td>1.0</td>
</tr>
<tr>
<td>CD3IM 3-4</td>
<td>0.76 (0.36, 1.61)</td>
<td>0.51</td>
</tr>
<tr>
<td>CD3IM 2-3</td>
<td>0.99 (0.43, 2.31)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Model selection and risk group distribution

Model Selection:

- When adjusted for known factors and after backwards selection, only CD3+ IMF remained in the model, i.e., was most important Immunoscore predictor in predicting DFS.

Risk Group Determination:

- Density of 690 for CD3+ IM was identified as optimal cutoff to define low vs. high risk groups:

  - High risk: CD3+ IM ≥ 690
  - Low risk: CD3+ IM < 690

Conclusions

- Densities of CD3+ and CD8+ especially at IM, were individually prognostic in FOLFOX-treated stage III CC-pts. In IM, high CD8+ association with outcome.

- Immunoscore® (using pre-defined cut-offs) was strongly prognostic, and this result provides validation in a phase III clinical trial.

- In resected stage III CCs, risk classification based on immune marker(s) or Immunoscore provides clinically useful prognostic stratification.