Tumor infiltration by Tbet⁺ effector T cells and CD20⁺ B cells is associated with survival in gastric cancer patients

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Introduction – gastric cancer

Vast majority are adenocarcinomas

- Intestinal type
- Diffuse type
Introduction – gastric cancer

• World‘s third leading cause of cancer mortality
• Annually 723 000 deaths worldwide
• >70% occur in developing countries
• Poor prognosis
• Helicobacter pylori-induced chronic gastritis is a major risk factor
Aim

• Prognostic significance of tumor infiltration by CD8 and CD4 T-cells, and B lymphocytes in patients with localized gastric cancer?
Patients

- Retrospective cohort
- 82 patients with localized gastric cancer, treated by surgery
- January 1993 - December 2013
- Median follow-up: 27 months

- 42 received neoadjuvant 5-FU and cisplatin-based chemotherapy
- Exclusion criteria: distant metastatic lesions
Methods

• Immunohistochemistry on FFPE surgical specimen
  • Tumor core
  • Invasive margin
    • T cells:
      • IL-17+
      • CD8+
      • Foxp3+
      • Tbet+
    • B cells:
      • CD20+

• Presence of H. pylori was assessed by May-Grundwald Giemsa staining
Methods

- Immunohistochemistry
  - CD8, T-bet, Foxp3 and IL-17:
    - Number of positively stained cells was counted in 3 consecutive high power fields (x40)
    - Mean count of 3 fields was used for statistical analysis
  - CD20:
    - Counted the number of CD20+ lymphoid aggregates in the whole tumor area
Methods

• Statistical analyses
  • Relapse-free survival (RFS): from date of diagnosis until the date of metastatic relapse (local or metastatic) or death, or the last follow-up
  • Alive or dead patients without relapse were censored at the last follow-up
  • Statistical significance level: p<0.05
Results

CD20  CD8  Tbet

Foxp3  IL-17
# Results

Table 1. Univariate and multivariate analyses of relapse-free survival according to clinical prognostic factors and immune infiltrates

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th>Multivariate</th>
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<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
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<tr>
<td><strong>T stage</strong></td>
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<tr>
<td>1–2</td>
<td>1</td>
<td>0.8–3.5</td>
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<td>3–4</td>
<td>1.7</td>
<td>1</td>
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<tr>
<td><strong>N stage</strong></td>
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<tr>
<td>N0</td>
<td>1</td>
<td>1.1–4.5</td>
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<tr>
<td>N+</td>
<td>2.25</td>
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<tr>
<td><strong>Histological type</strong></td>
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<tr>
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<td>1</td>
<td>0.5–2</td>
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<tr>
<td>Diffuse</td>
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<tr>
<td><strong>Neoadjuvant therapy</strong></td>
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<tr>
<td>Yes</td>
<td>1</td>
<td>0.8–3.6</td>
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<tr>
<td>No</td>
<td>1.8</td>
<td>1</td>
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<tr>
<td><strong>CD20</strong></td>
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<tr>
<td>&lt; median</td>
<td>1</td>
<td>0.24–0.9</td>
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<tr>
<td>&gt; median</td>
<td>0.48</td>
<td></td>
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<tr>
<td><strong>TUMOR STROMA</strong></td>
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<tr>
<td>CD8</td>
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<td>0.5–1.3</td>
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<tr>
<td>&gt; median</td>
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<td>1</td>
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<tr>
<td>&gt; median</td>
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<td>IL-17</td>
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<td>0.5–2.3</td>
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<tr>
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<tr>
<td>Tbet</td>
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<td>0.2–0.96</td>
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<tr>
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<td>0.48</td>
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</tbody>
</table>
Results

![Graphs showing relapse-free survival for CD20 and Tbet with statistical significance](image)

- **CD20**
  - Group Lo: 41, 14
  - Group Hi: 41, 15
  - Time (Months): 7, 4, 2, 1
  - Hazard ratio: \( P = 0.03 \)

- **Tbet**
  - Group Lo: 41, 14
  - Group Hi: 41, 15
  - Time (Months): 4, 0, 0, 0
  - Hazard ratio: \( P = 0.03 \)
Results

![Graph showing CD20 and Tbet](image)

- **Group Lo/Lo**
  - 15 cases:
    - 6 cases at 4 months
    - 4 cases at 4 months
    - 1 case at 1 month
    - 0 cases at 0 months

- **Group Hi/Lo or Lo/Hi**
  - 35 cases:
    - 15 cases at 9 months
    - 6 cases at 6 months
    - 4 cases at 4 months
    - 4 cases at 4 months

- **Group Hi/Hi**
  - 32 cases:
    - 9 cases at 2 months
    - 1 case at 1 month
    - 0 cases at 0 months
    - 0 cases at 0 months
Results

![Graph showing relapse-free survival over time for two groups: Group Lo and Group Hi.](image)

- **Group Lo**: 41 patients, with relapse-free survival rates as follows:
  - Time: 10, 8, 3, 3

- **Group Hi**: 41 patients, with relapse-free survival rates as follows:
  - Time: 5, 3, 2, 1

- **P-value**: 0.04
Results

• NO significant association with gastric cancer prognosis:
  • CD8+ T cell density
  • IL-17+ T-cell density

• Association with better relapse-free survival:
  • High infiltration of Tbet+ T cells
  • High numbers of CD20+ B-cell follicles
  • Low infiltration of Foxp3+ T cells

• NO influence:
  • Treatment with neoadjuvant chemotherapy
  • Histological tumor type (diffuse versus intestinal)
  • Presence/absence of H.pylori infection
## Results

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</tbody>
</table>
Results
Results

A

CD20
CD3

B

Ki67
CD3

Andrea Beer
Results

PNAd = peripheral node addressin;
red arrows: HEVs = PNAd+ high endothelial venules
External validation using transcriptomic data

- Independent public large scale transcriptomic data set
  - 365 patients with stage I-III gastric cancers
  - Selection of 63 genes in accordance with their expression in immune cells of interest
Conclusion

• Tumor infiltration by B and Th1 T cells
  • could affect gastric cancer prognosis
  • may be used to better define the outcome of patients with localized gastric cancer
Discussion

• Increasing evidence that development of Th1 adaptive immunity is associated with improved outcome in patients afflicted with a variety of cancer types

• Patients with colorectal carcinoma: presence of mRNA encoding molecules expressed by Th1 cells (such as T-bet) has been shown to correlate with reduced metastatic invasion and increased survival

• Gastric cancer patients: more favorable outcome for patients with gastric tumors highly infiltrated with Tbet+ cells

• Lung cancer: tertiary lymphoid structures were associated with prognosis

• Cutaneous melanoma, breast cancer, ovarian cancer: favorable effect of tumor-infiltrating B cells on patient prognosis

• Colorectal cancer: paradoxical association of improved clinical prognosis and a high density of FoxP3+ tumor-infiltrating Tregs
Own opinion

+ used entire slide instead of tumor microarrays
  Long-time follow-up

- „Low“/“high“ density
  According to which criterion is the selection of the counted HPFs made?
  “core”/”margin”
  Applicability in daily practice?