Ankersmit Laboratory
for Diagnosis & Regeneration in Thoracic Diseases & Applied Immunology

ORIGINAL RESEARCH

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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

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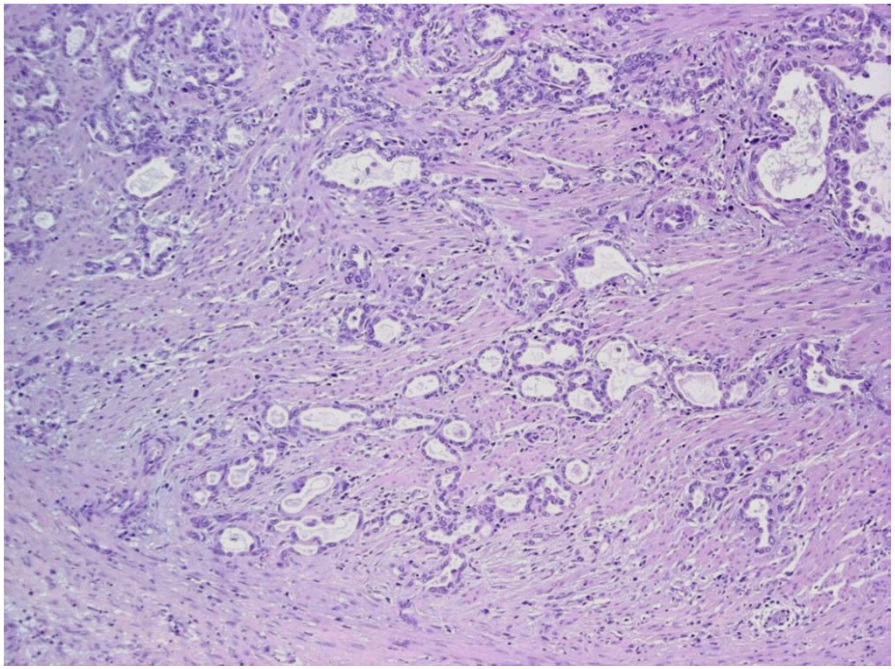
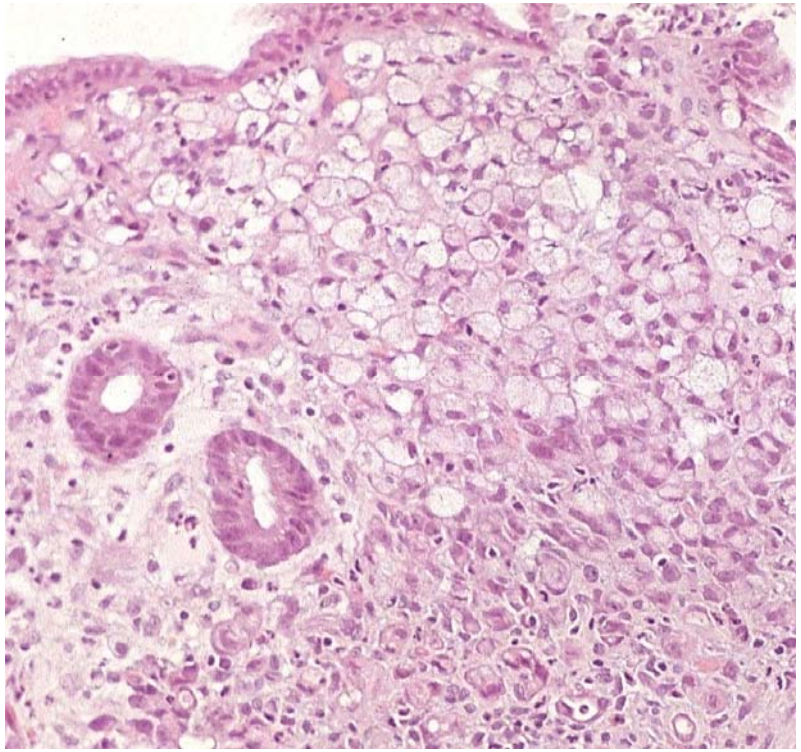
Vast majority are adenocarcinomas

- Intestinal type



- Diffuse type



Introduction – gastric cancer

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- 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

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

Patients

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- 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

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- 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

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- 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

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- 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

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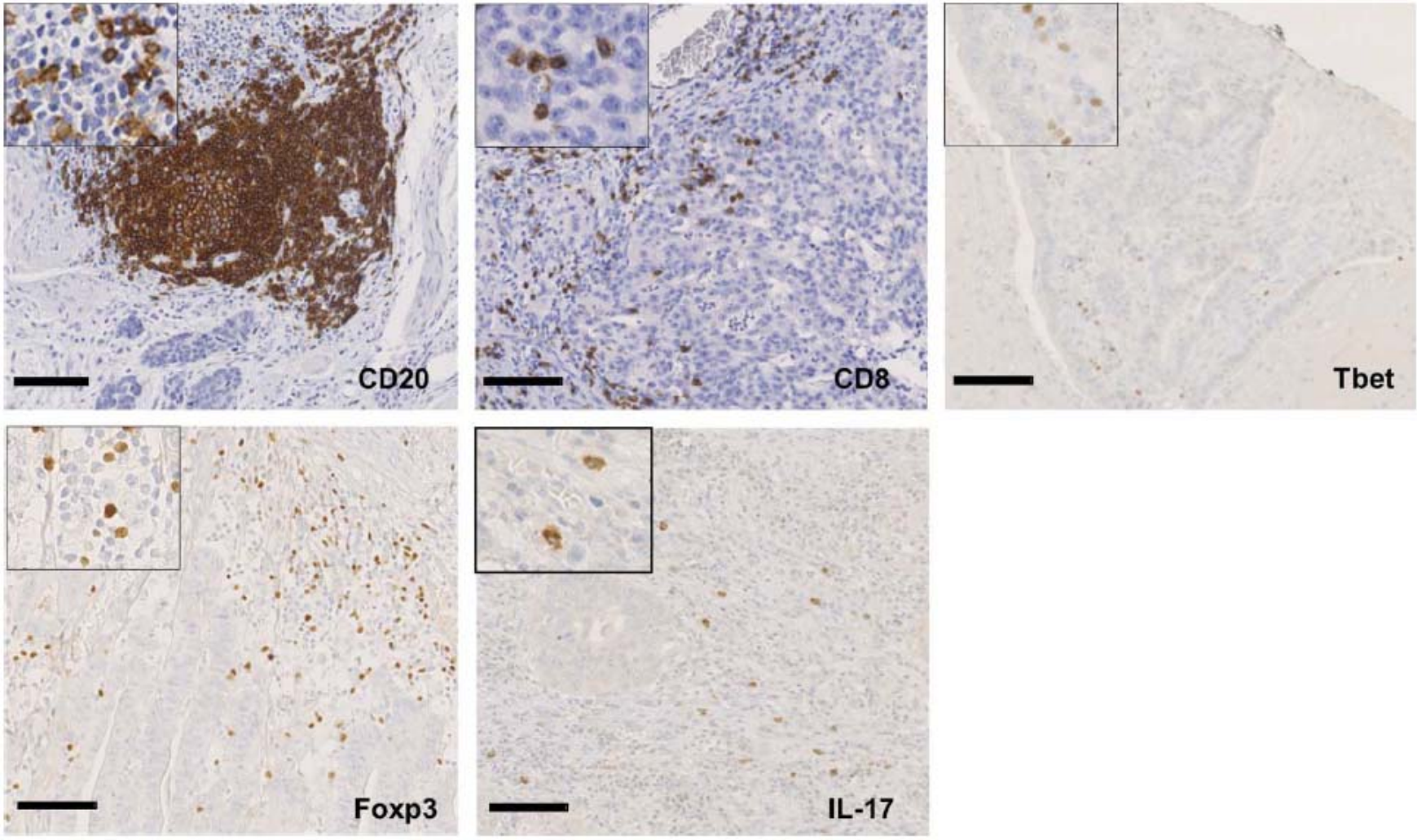
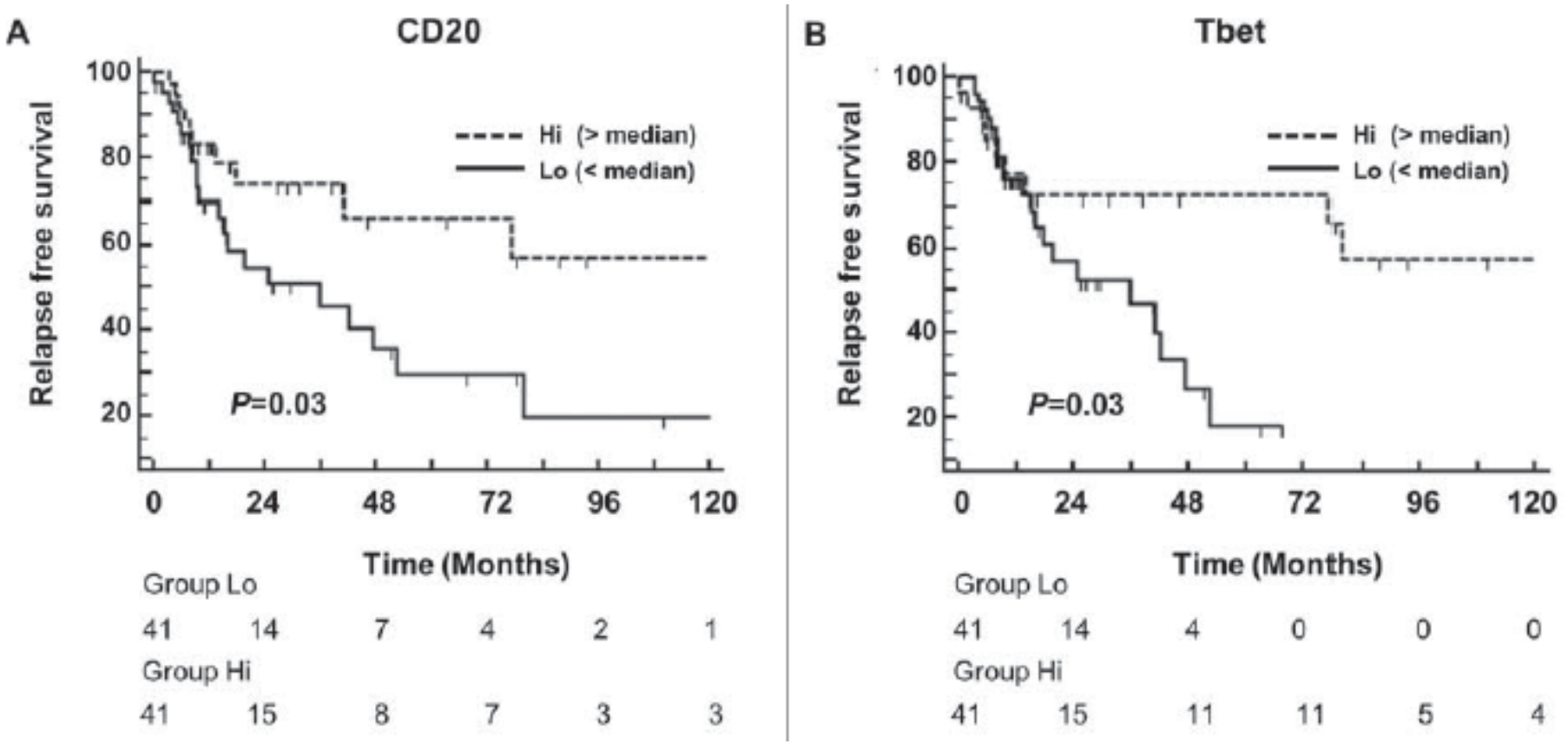


Results

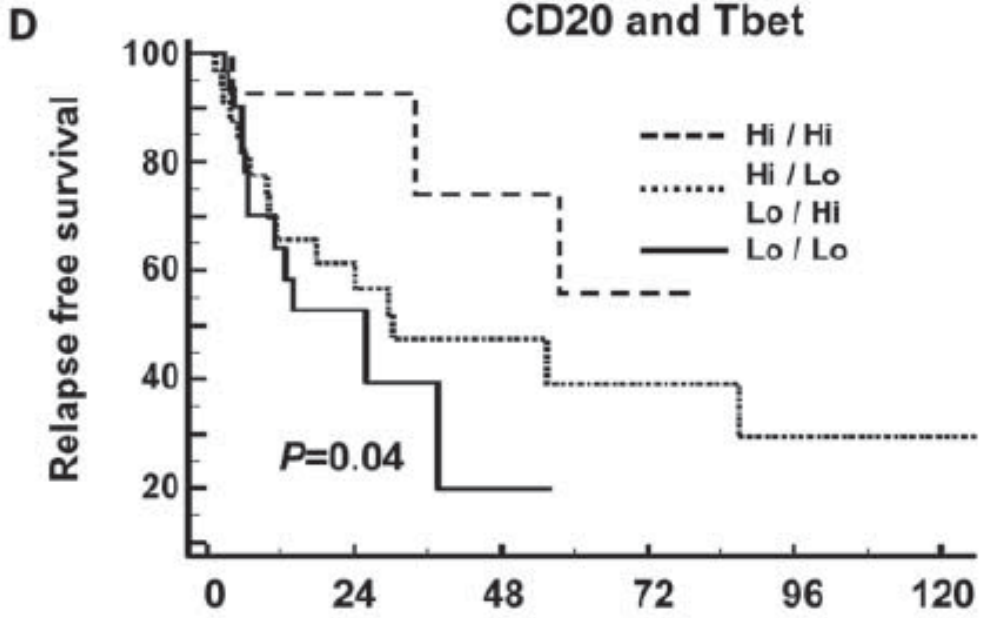
Table 1. Univariate and multivariate analyses of relapse-free survival according to clinical prognostic factors and immune infiltrates

	Univariate			Multivariate		
	HR	95% CI	P	HR	95% CI	P
T stage		0.8–3.5	0.15		0.7–4.8	0.22
1–2	1			1		
3–4	1.7			1.8		
N stage		1.1–4.5	0.02		0.4–3	0.7
N0	1			1		
N+	2.25			1.2		
Histological type		0.5–2	0.9			
Intestinal	1					
Diffuse	1.15					
Neoadjuvant therapy		0.8–3.6	0.08		0.3–1.1	0.06
Yes	1			1		
No	1.8			0.5		
CD20		0.24–0.9	0.03		0.2–1	0.04
<median	1			1		
>median	0.48			0.4		
TUMOR STROMA:						
CD8		0.5–1.3	0.25			
<median	1					
>median	0.65					
Foxp3		1–4	0.04		0.7–3.5	0.1
<median	1			1		
>median	2			1.6		
IL-17		0.5–2.3	0.7			
<median	1					
>median	1.15					
Tbet		0.2–0.96	0.03		0.2–1.2	0.1
<median	1			1		
>median	0.48			0.5		

Results

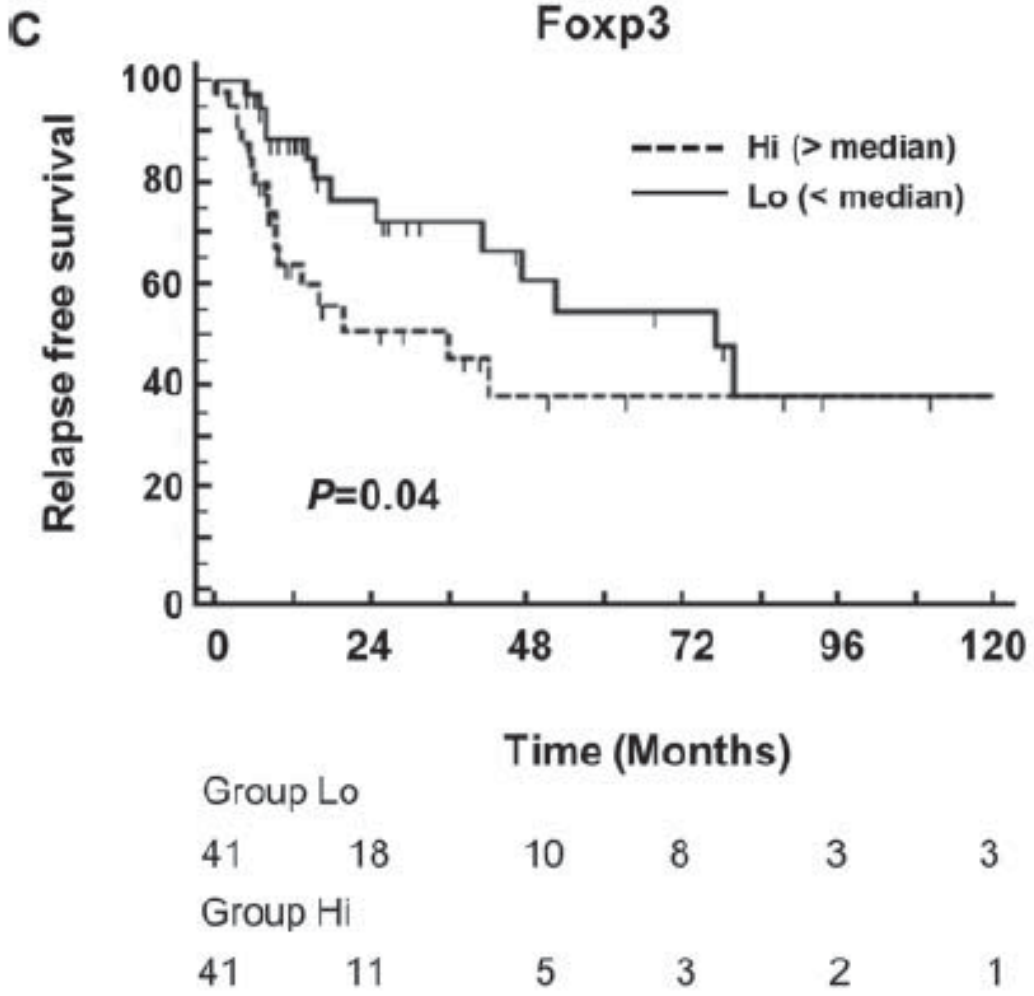

A
CD20
Relapse free survival
100
80
60
40
20
Hi (> median)
Lo (< median)
P=0.03
0
24
48
72
96
120
Time (Months)
Group Lo
41 14 7 4 2 1
Group Hi
41 15 8 7 3 3
B
Tbet
Relapse free survival
100
80
60
40
20
Hi (> median)
Lo (< median)
P=0.03
0
24
48
72
96
120
Time (Months)
Group Lo
41 14 4 0 0 0
Group Hi
41 15 11 11 5 4

Results



Group Lo/Lo					
15	6	4	4	1	0
Group Hi/Lo or Lo/Hi					
35	15	9	6	4	4
Group Hi/Hi					
32	9	2	1	0	0

Results


C
Foxp3
Relapse free survival
100
80
60
40
20
0
Hi (> median)
Lo (< median)
P=0.04
0
24
48
72
96
120
Time (Months)
Group Lo
41
18
10
8
3
3
Group Hi
41
11
5
3
2
1

Results

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- 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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	Univariate			Multivariate		
	HR	95% CI	P	HR	95% CI	P
INVASIVE MARGIN:						
CD8		0.5–2	0.95			
<median	1					
>median	0.98					
Foxp3		0.6–2.5	0.5			
<median	1					
>median	1.23					
IL-17		0.45–1.8	0.7			
<median	1					
>median	0.88					
Tbet		0.45–1.8	0.8			
<median	1					
>median	0.9					

Results

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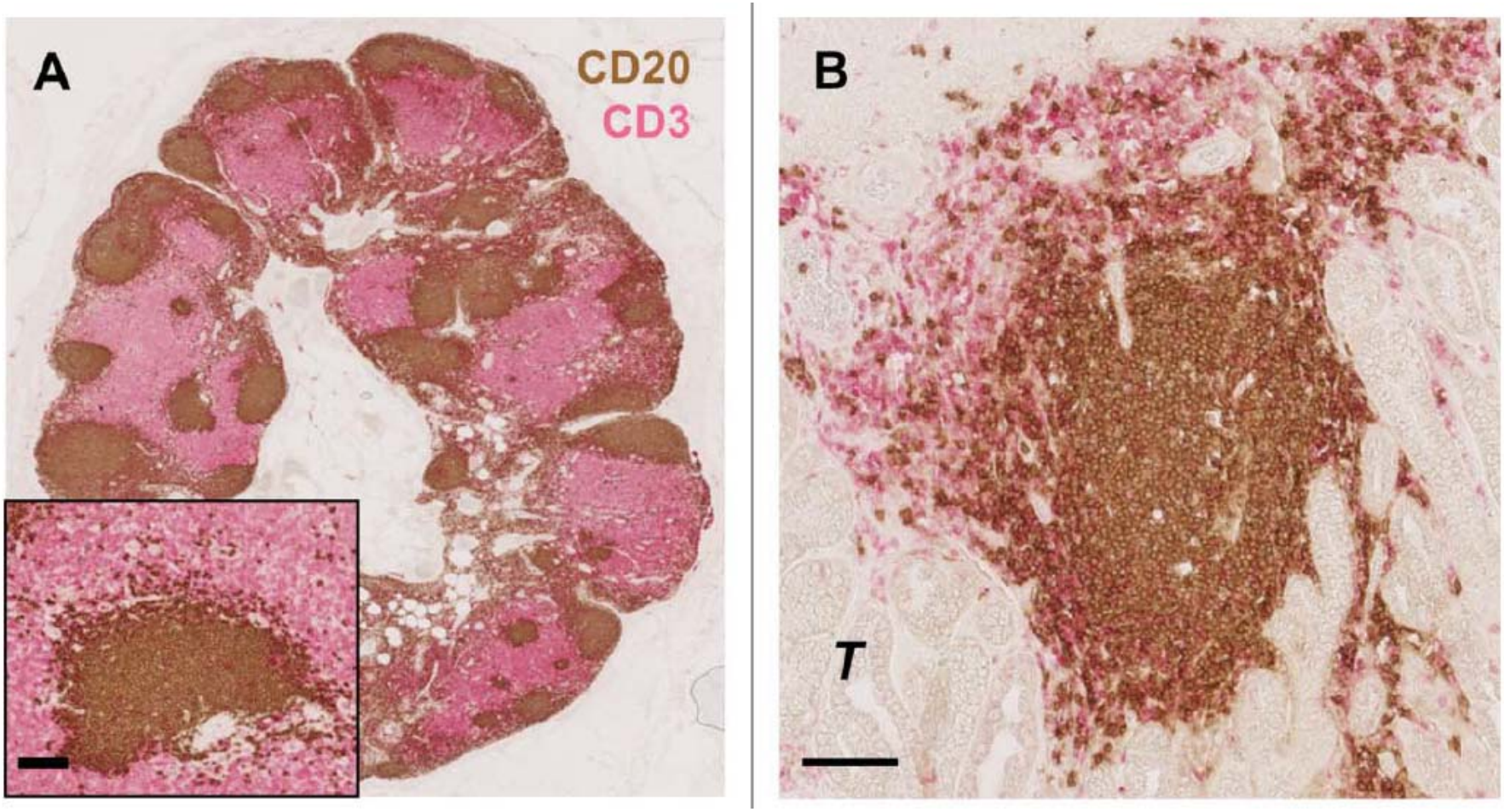

Results

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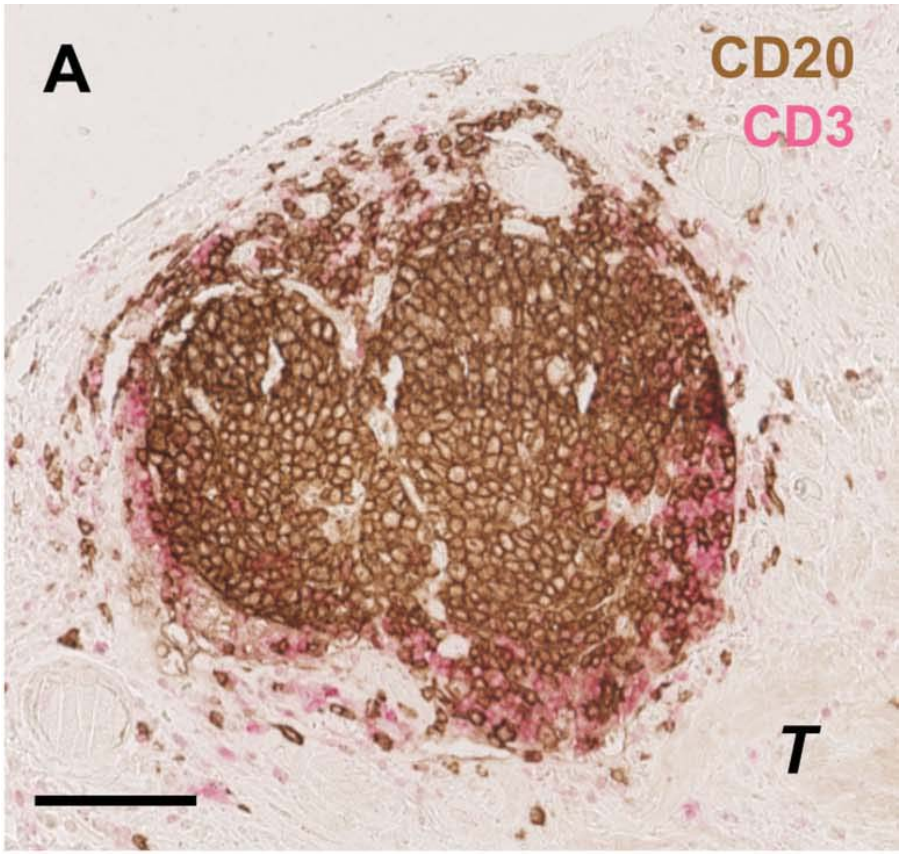
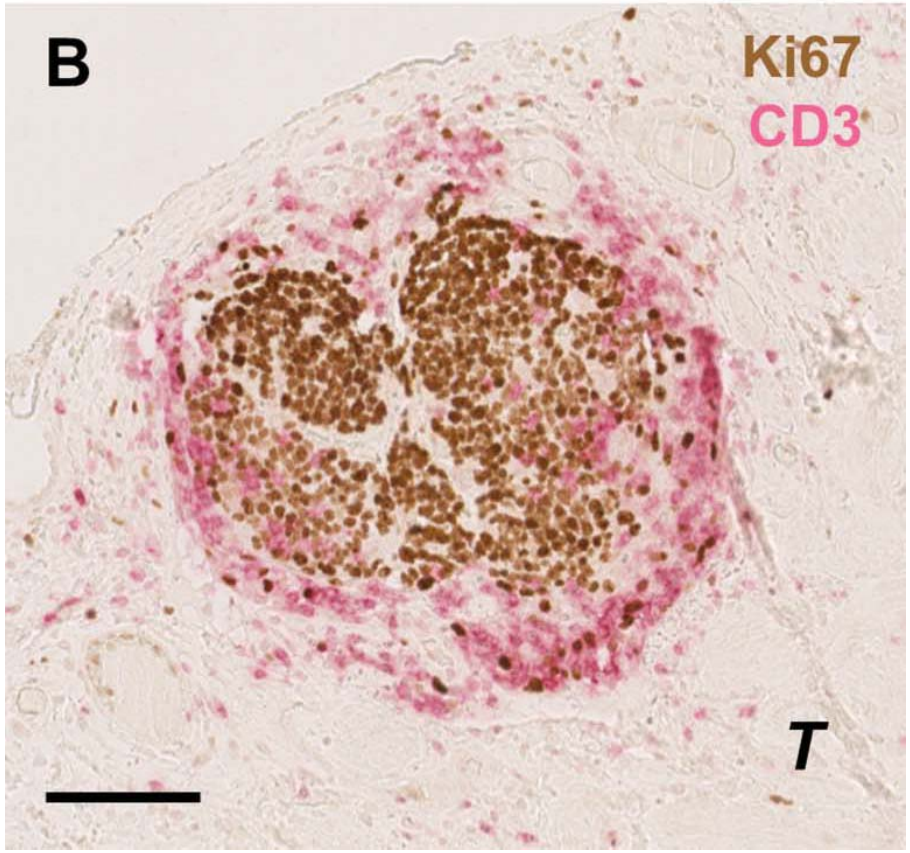
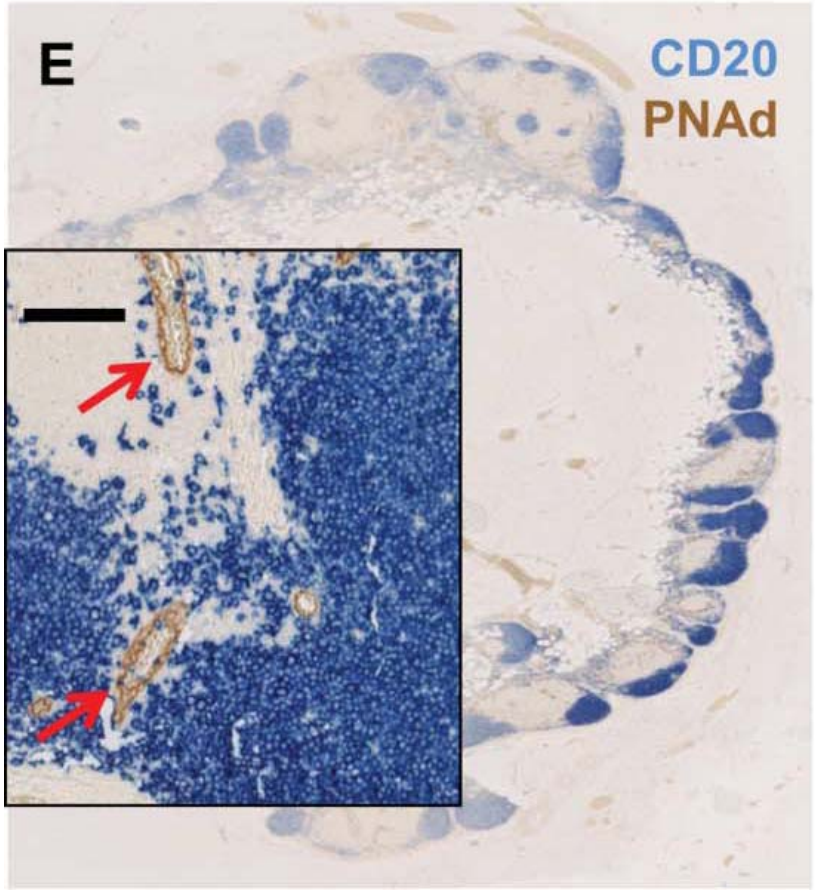
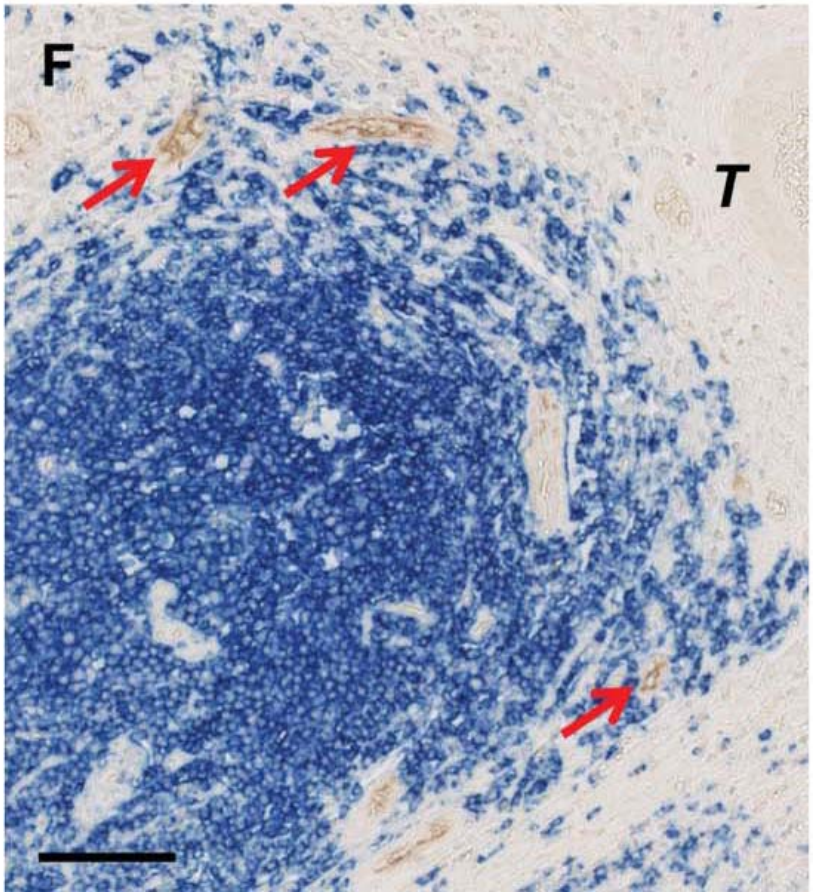




Results





PNAd = peripheral node addressin;
red arrows: HEVs = PNAd+ high endothelial venules

External validation using transcriptomic data

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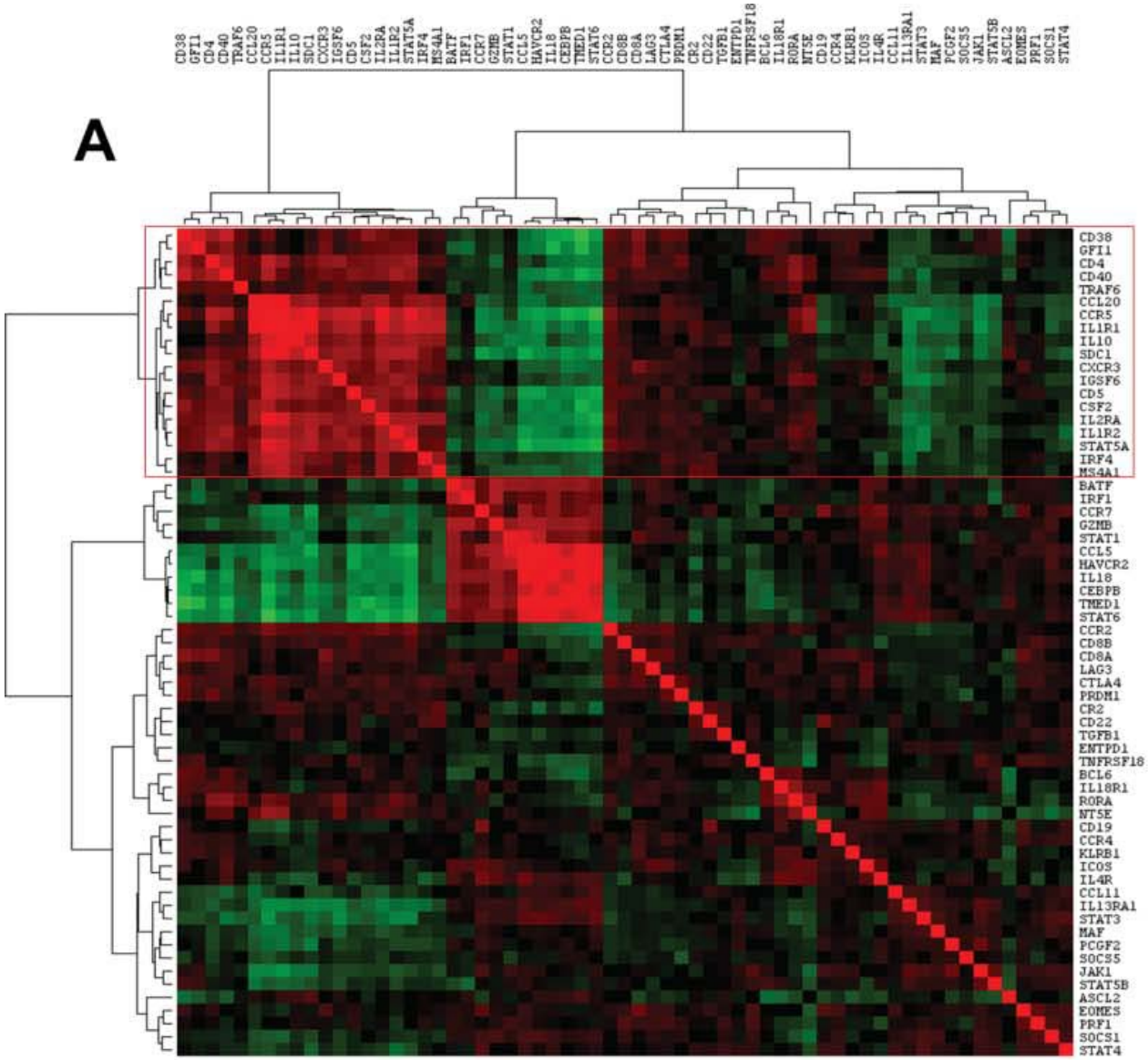
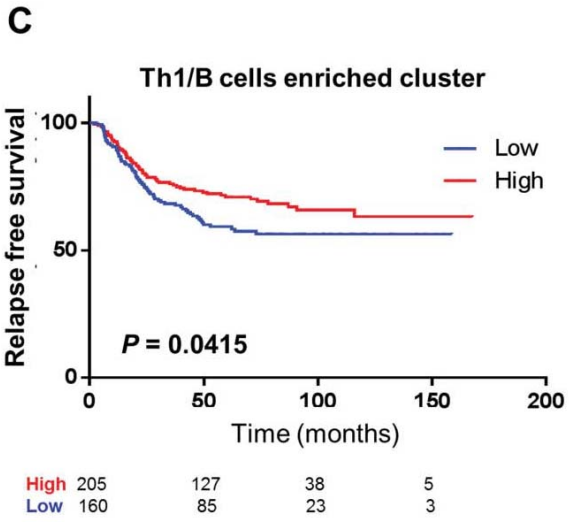
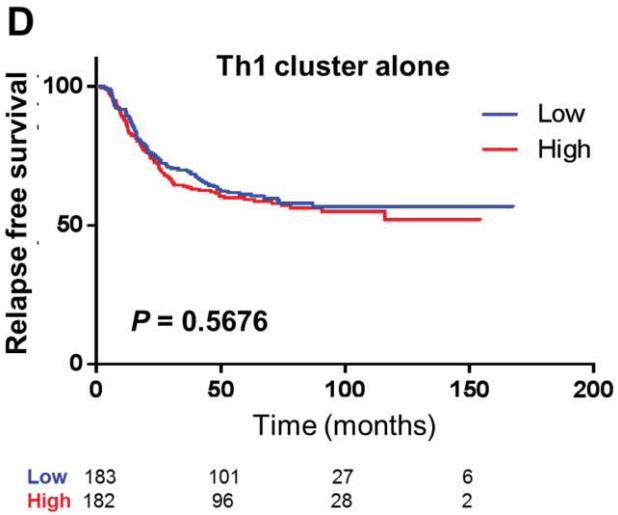
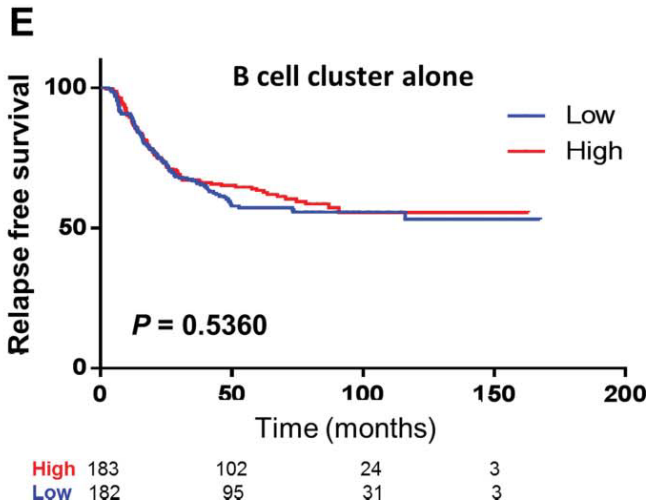
- Independent public large scale transcriptomic data set
  - 365 patients with stage I-III gastric cancers
  - Selection of 63 genes in accordance with their expression in immune cells of interest


A
CD38
GFI1
CD4
CD40
TRAF6
CCL20
CCR5
IL1R1
IL10
SDC1
CXCR3
IGSF6
CD5
CSF2
IL2RA
IL1R2
STAT5A
IRF4
MS4A1
BATF
IRF1
CCR7
GZMB
STAT1
CCL5
HAVCR2
IL18
CEBPB
TMED1
STAT6
CCR2
CD8B
CD8A
LAG3
CTLA4
PRDM1
CR2
CD22
TGFB1
ENTPD1
TNFRSF18
BCL6
IL18R1
RORA
NT5E
CD19
CCR4
KLRB1
ICOS
IL4R
CCL11
IL13RA1
STAT3
MAF
PCGF2
SOCS5
JAK1
STAT5B
ASCL2
EOMES
PRF1
SOCS1
STAT4


C
Th1/B cells enriched cluster
Relapse free survival
100
50
0
Low
High
P = 0.0415
0
50
100
150
200
Time (months)
High 205 127 38 5
Low 160 85 23 3


D
Th1 cluster alone
Relapse free survival
100
50
0
Low
High
P = 0.5676
0
50
100
150
200
Time (months)
Low 183 101 27 6
High 182 96 28 2


E
B cell cluster alone
Relapse free survival
100
50
0
Low
High
P = 0.5360
0
50
100
150
200
Time (months)
High 183 102 24 3
Low 182 95 31 3

Conclusion

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- 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

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- 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

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+

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?