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Gastric carcinoma with lymphoid stroma

in the era of the immune context and immunotherapies

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Background and aims

Novel cancer immunotherapies can selectively block the cancer evasion of immune surveillance. Phase II/III clinical trials with antibodies directed against CTLA-4 and PD-1/PD-L1 immune inhibitory checkpoints are currently ongoing in GC and are new attractive therapeutic strategies for GC patients. Recently, The Cancer Genome Atlas (TCGA) network proposed a four-tiered molecular classification of GC [1], that could help to guide optimal selection of therapy. EBV+ and MSI- high GC are two molecular subtypes in which the PD-1/PD-L1 blockade may be particularly beneficial [1,2]. Moreover, a morphological subtype of GC has been associated to MSI-high status and EBV infection, that is Gastric Carcinoma with Lymphoid Stroma (GCLS) [3,4,5]. Hence, the abundant immune infiltrate and the molecular features of GCLS offer an attractive landscape to study tumour immune micro-environment, immune inhibitory checkpoints and their relationship with GC cells. The **aim** of this study was to analyse the clinico-pathological features, EBV infection, MSI, PD-L1 status and tumour immune microenvironment in GCLS.

Material and methods

Twenty-four GCLSs, selected from a series of 1088 surgically resected GC patients, were analysed by: RNA *in situ* hybridisation (EBER) for EBV, PCR/fragment analysis for MSI, and immunohistochemistry (IHC) for cytokeratin (CK) AE1/AE3, CD3, CD8 and PD-L1. PD-L1 immunoreactivity was evaluated separately for tumour immune and epithelial cells. The Immunoreactivity Scoring System (IRS) recently described by Boger C *et al* was applied [6]. Double immunofluorescence for CK/PD-L1 and CD68/PD-L1 was performed in selected cases. CD3+, CD8+ T cell densities and CD8/CD3 *ratio* (CD8/CD3R) were calculated both in the tumour centre (TC) and at the invasive front (IF) by digital analysis (Definines®).

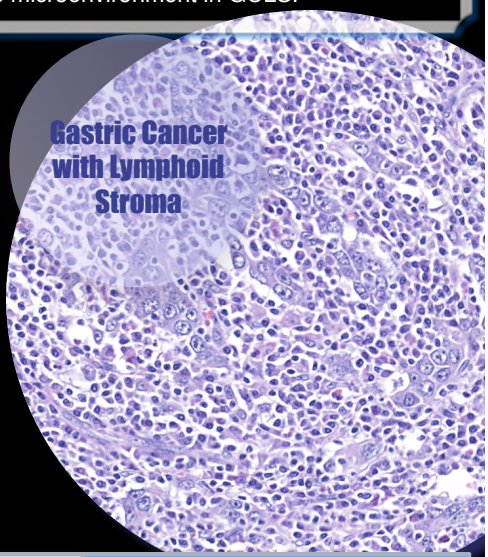
A tissue microarray (TMA) was constructed from a control group of 54 non-GCLSs and analysed by IHC for PD-L1 and by EBER.

Results

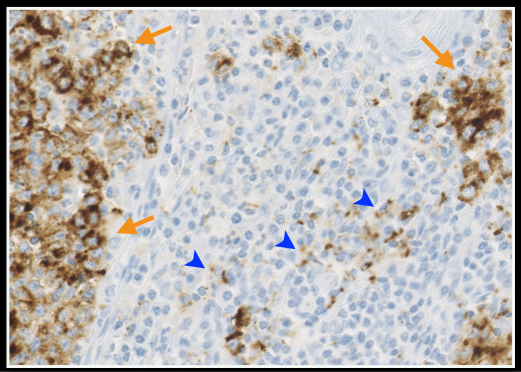
Regarding EBV and MSI *status*, 3 groups were identified: EBV+/microsatellite stable (MSS) (n=16), EBV-/MSI-high (n=4), and EBV-/MSS (n=4). IRS>2 was restricted to EBV+ (6/16; 37.5%) and MSI-high (2/4; 50%) GCLSs.

Overall, CD8/CD3R at the IF exceeded CD8/CD3R in the TC ($p<0.001$). CD8/CD3R was significantly higher (p=0.008) in EBV+ (n=16) than in EBV- (n=8) GCLSs.

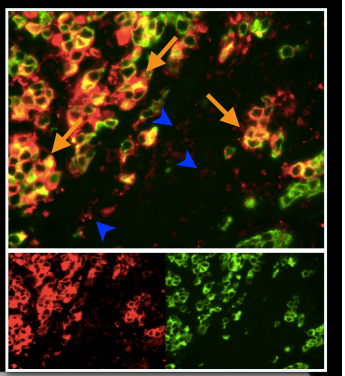
By comparison with non-GCLSs, GCLSs were significantly associated with EBV infection (66.7% *versus* 5.56%, $p<0.001$) and PD-L1 protein expression (33.3% *versus* 13.0%, $p=0.04$). Moreover, GCLSs harbour distinctive clinico- pathological features: younger age ($p=0.03$), proximal location ($p=0.04$), indeterminate group of Lauren's classification ($p<0.001$), lower lymphatic invasion ($p=0.02$), lower pTNM stage ($p=0.002$) and better overall survival ($p=0.01$).



Tumour epithelial cells: **negative** (IRS≤2) Tumour immune cells: **negative** (<1%)	Tumour epithelial cells: **positive** (IRS>2) Tumour immune cells: **positive** (≥1%)	Tumour epithelial cells: **negative** (IRS≤2) Tumour immune cells: **positive** (≥1%)
n=2 (8.3%)	n=8 (33.3%)	n=14 (58.3%)



PD-L1 expression. Membranous, linear and strong PD-L1 expression was observed in tumour epithelial cells (arrows), whereas cytoplasmic dotted/granular pattern was observed in stromal immune cells (arrowheads). This GCLS case harboured PD-L1 amplification.



Conclusions

GCLSs are characterised by distinctive clinico-pathological features, EBV infection (66.7%), PD-L1 expression (33.3%) and high CD8/CD3R (at the IF and in EBV+ cases). In keeping with the recent molecular data, PD-L1 expression (IRS>2) was restricted to EBV+ and MSI-high cases, reinforcing the potential implications of immunotherapy in these molecular subtypes of GCLS.

[1] TCGA. Comprehensive molecular characterization of gastric adenocarcinoma. Nature 2014; 513(7517), 202-9; [2] Le DT *et al*. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med 2015; 372(26), 2509-20; [3] Chiaravalli AM *et al*. The role of histological investigation in prognostic evaluation of advanced gastric cancer. Analysis of histological structure and molecular changes compared with invasive pattern and stage. Virchows Arch 2001; 439(2), 158-69; [4] Grogg KL, Lohse CM, Pankratz VS et al. Lymphocyte-rich gastric cancer: associations with Epstein-Barr virus, microsatellite instability, histology, and survival. Mod Pathol 2003; 16(7), 641-51; [5] Lü BJ *et al*. Gastric medullary carcinoma, a distinct entity associated with microsatellite instability-H, prominent intraepithelial lymphocytes and improved prognosis. Histopathology 2004; 45(5), 485-92; [6] Böger C *et al*. Oncotarget. PD-L1 is an independent prognostic predictor in gastric cancer of Western patients. Oncotarget 2016; 7(17), 24269-83.