SUMMARY OF THE DOCTORAL THESIS

THE IMPACT OF THE IMMUNOSUPPRESSIVE MOLECULES PD-L1 AND SIGLEC-15 ON TILs ASSOCIATED WITH GASTRIC ONCOGENESIS

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Introduction

Despite important advances in diagnostic techniques and therapeutic tools, the prognosis of advanced gastric cancer (GC) remains unfavorable due to late detection, tumor heterogeneity, and limited efficacy of systemic therapies. This clinical reality highlights the need to identify new biomarkers involved in the molecular mechanisms of GC, as well as new therapeutic targets to improve patient stratification and therapeutic management.

Overall objectives

This doctoral thesis aims to contribute to a deeper understanding of the immuno-oncological architecture of GC by characterizing the immunohistochemical expression patterns of tumor lymphocytic infiltrates (TILs), programmed death ligand-1 (PD-L1), and sialic acid-binding immunoglobulin-like lectin 15 (Siglec-15), followed by their correlation with clinicopathological data and aggressiveness parameters in gastric adenocarcinoma (GAC). Thus, by identifying alternative immune escape pathways, such as Siglec-15, we can contribute to the development of new combination therapies or patient selection strategies, moving towards more personalized and effective approaches in CG immunotherapy.

General methodology

The four studies from the doctoral thesis included 133 patients diagnosed with GAC between 2020 and 2021 within the Department of Pathological Anatomy of the County Clinical Hospital of Târgu Mureș, Romania. Tissue fragments obtained from gastric resection specimens were stained with hematoxylin-eosin, followed by immunohistochemical (IHC) staining using BechMark GX, Ventana Medical Systems Inc., (Tucson, AZ, USA), and Dako Autostainer Link 48 (Agilent Technologies, Glostrup, Denmark) automated immunostainers. All IHC staining was performed according to the manufacturer's protocols. For digital morphometry, the analyzed slides were scanned using the Leica Aperio AT2 scanner (Leica Biosystems, Wetzlar, Germany). Subsequently, the QuPath digital morphometry program (version 0.5.0) developed by the Institute of Cancer Research, Northern Ireland, was used to assess the number/density of immune cells. Statistical analysis was performed with GraphPad Prism, version 10.4.1 (GraphPad Software, Boston, USA) and Microsoft Excel (Microsoft Office 365, version 2408, Washington, USA).

Study no. 1. Prognostic value and histopathological implications of the Klintrup–Mäkinen (KM) score in gastric adenocarcinomas

Aim: The study analyzed the feasibility of using the KM score as a semiquantitative method for assessing peritumoral immune infiltrate in the routine histopathological practice of GAC.

In the second phase, the prognostic significance of the KM score was evaluated through correlation with clinicopathological parameters: pTNM classification, histological differentiation degree, lymph node status, and presence of lymphovascular and perineural invasion.

Through this approach, the research aimed to contribute to increasing prognostic accuracy and to identifying subgroups of patients who could benefit from personalized therapeutic protocols, depending on the peritumoral immune response.

Conclusions: A low KM score has been associated with a more aggressive biological behavior, being more frequently reported in poorly differentiated and advanced stages of GAC, with lymphovascular and perineural invasion. Although it does not replace IHC or molecular tests, it may improve the accuracy of the prognosis of patients with GAC. Integrating the KM score into the histopathological evaluation could facilitate the identification of patients eligible for immunotherapy and contribute to a personalized management of GAC.

Study no. 2. Evaluation of the expression and prognostic impact of the biomarkers Siglec-15 and PD-L1 in gastric adenocarcinomas

Aim: This study evaluated the tumor expression of SIGLEC-15 in GAC and analyzed its correlations with clinicopathological parameters and its influence on patient survival. In parallel, PD-L1 expression was investigated with the aim of exploring a possible association between the two immunological biomarkers in GAC. Therefore, a deeper understanding of their interaction could contribute to a more accurate prognostic stratification and form the basis for new immunomodulatory therapeutic approaches.

Conclusions: The present study highlighted the clinical significance of SIGLEC-15 expression in GAC. Increased SIGLEC-15 expression was associated with lymphatic, vascular, and perineural invasion, as well as significantly reduced overall survival. Moreover, patients with overexpression of both markers (SIGLEC-15 and PD-L1-1) had the worst survival rate. These elements suggest that SIGLEC-15 not only reflects the aggressive biological behavior of GAC but also adds prognostic value when evaluated together with PD-L1. This dual approach may improve prognostic stratification of patients and support the development of combined immune checkpoint inhibition strategies in GAC.

Study no. 3. Digital quantification of $CD4^+$ and $CD8^+$ lymphocytes and correlation with PD-L1 expression in gastric adenocarcinomas

Aim: The main aim of the study was to quantify by digital morphometry the density of $CD8^+$ and $CD4^+$ T lymphocytes, with determination of the $CD8^+/CD4^+$ ratio, in association with histopathological aggressiveness parameters and overall survival in. In the secondary stage, the relationship between $CD8^+$ T lymphocytes and PD-L1 was analyzed, in an attempt to respond to the uncertainty of the prognostic value of PD-L1 as a single biomarker in resectable GAC.

Conclusions: In the present study, the increased density of $CD8^+$ lymphocyte infiltrate quantified by digital morphometry was not associated with histopathological parameters of tumor aggressiveness. On the other hand, patients with a rich CD8+ lymphocyte infiltrate showed extended overall survival. All these aspects emphasize the potential role of CD8 as a prognostic marker in GAC. The $CD8^+/CD4^+$ ratio followed the same statistical pattern, but with less significant results. In contrast, the density of $CD8^+$ lymphocyte infiltrate did not correlate with the CPS score.

In conclusion, the results of the study supported the integration of TILs in future prognostic models and provided a biological basis for research that will investigate immunomodulatory strategies in patients with resectable GAC.

Study no. 4. Digital assessment of macrophages and the relationship with SIGLEC-15 expression in the tumor microenvironment of gastric adenocarcinomas

Aim: This study investigated the relationship between $CD68^+$ macrophages quantified by digital morphometry, histopathological aggressiveness factors (tumor size, pT3–4/pN1–3, lymphatic/vascular/perineural invasions) and overall survival in resected GAC. In a secondary phase, the correlation between $CD68^+$ macrophages and SIGLEC-15 expression in the tumor microenvironment of GAC was analyzed.

Conclusions: The results of the study demonstrated that macrophage-rich GACs were more aggressive and had significantly reduced overall survival. The strong correlation observed between macrophage density and SIGLEC-15 expression highlighted a distinct GAC phenotype, potentially independent of the classical PD-L1-induced immune profile. Together, these results underscored the prognostic relevance of macrophages and suggested that the use of combination immunotherapies could improve the stratification and prognosis of patients with operable GCA.

General conclusions

Increased SIGLEC-15 expression and coexpression of SIGLEC-15 and PD-L1 were associated with advanced histopathological stages of GAC and reduced overall survival. Macrophage density showed a strong correlation with SIGLEC-15 expression. Patients with GAC and a tumor microenvironment rich in macrophages showed a more aggressive biological behavior, with a significantly reduced overall survival. The presence of $CD8^+$ lymphocytes in TILs from the intra- and peritumoral stroma was associated with a less aggressive tumor behavior. $CD8^+$ lymphocyte expression did not significantly correlate with PD-L1. The $CD8^+/CD4^+$ ratio showed an inferior association with aggressiveness parameters, and the independent assessment of $CD8^+$ was sufficient to estimate the prognosis in GAC. A low Klintrup–Mäkinen (KM) score was associated with poorly differentiated types, pT-3 stages, and the presence of lymphovascular and perineural invasion.