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Review Article

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New Insights on the Relationship between Arginase-1, Tumor Associated Neutrophils, Perineural Invasion, Neoadjuvant therapy and Prognosis in Rectal Cancer Patients

Nicola Sarandria

Scientific Director – Planet Healthcare Srl, Healthcare Director Olimpia Medical Center, Italy

Corresponding author
Nicola Sarandria MD, MBA, PhD, Scientific Director – Planet Healthcare Srl, Healthcare Director Olimpia Medical Center, Italy.

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ABSTRACT

Introduction: Rectal cancer is one of the most common cancers with a high epidemiologic burden. Need for new prognostic and predictive indicators and therapies is urgent in the field to improve clinical outcomes and gather a better understanding of the disease. Indicators such as Arginase-1 (produced also by neutrophils) that has been found to be a potent immuno-depressor (eg. T cell immunodepression), neural-tumor relation, eg. the schwann cells-tumor interaction (including the recalling of MDSC), that can act as immunosuppressors for instance through the increase in production of TGF beta. This paper aims to shed new light on this complex set of interactions and their possible roles in prognosis, predictivity and basis for new drugs discoveries.

Methodology: As part of the project a quantitative analysis of tumor-associated neutrophils (TANs) on formalin embedded sections of rectal cancer using CD66b and ARG1 immunohistochemical staining was conducted. A total of 65 patients with histologically confirmed rectal adenocarcinoma were retrospectively included and stratified into four groups according to neoadjuvant treatment modality. Clinical and pathological data were collected from medical records, including: perineural invasion, neoadjuvant treatment received and progression-free survival (DFS).

Conclusion and Discussion: Patients who did not undergo neoadjuvant therapy and had no perineural invasion from the tumor, had a statistically significant higher level of Arginase-1 and CD66b (neutrophils) and had a significantly worse prognosis (in terms of Disease-Free Survival) compared to patients who underwent neoadjuvanct therapy and had no perineural invasion (especially compared to those who underwent exclusively radiotherapy). When considering the whole group of patients, including perineural invasion, patients who underwent no-neoadj therapy had a significantly higher amount of Arginase-1 and tumor associated neutrophils (TANs) compared to the first group.

Introduction

Rectal cancer is epidemiologically speaking underneath the umbrella term CRC, Colorectal Cancer, being the third most common type of cancer and high cause of morbidity and mortality in the population.

Colorectal cancer (CRC) is the one of the most common types of cancer, having an incidence rising by 1 and 2 % yearly in people under the age of 55, with an increased rate of mortality in these young patients rising by 1 % yearly too. In the duration of a man's life the probability of colorectal cancer is 1 in 23 (women 1 in 25), this excluding particular risk factors, both in terms of lifestyle and/or genetics, such as AFP. Even though better screening, treatments and decrease in smoking has led to better prognosis, there is an urgent need for better diagnostic tools for better predictivity and prognosis management to better aid the patients. Diet and environmental changes have led to a noticeable increase in incidence at younger age groups compared with the past [1].

The most common histology of colorectal cancer, which is implicitly takes as point of focus when discussing CRC, is the adenocarcinoma. A malignant neoplasia arising from glandular epithelium lining the intestinal and rectal tract [2].

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The main stay therapy for these pathologies is surgery. Other than surgery the patient may receive adjuvant and neoadjuvant therapy (before the surgery), which could include radiotherapy and/or chemotherapy.

As knowledge in the field of oncology and specifically colorectal cancer advanced through the years, a rising need for a more updated terminology of the pathology arose, namely the need to leave the umbrella term Colorectal cancer behind and go into more clinically and pathologically precise and relevant terms. The difference in terms of pathology, risk factors, treatment and prognosis of rectal cancer compared to right colon cancer and left colon cancer is such that there is a need to leave "CRC" term behind and specify the pathology precisely.

Rectal cancer has many differences compared to colon (right and left sided) cancer. Protecting factors including ASA intake which have been shown to reduce risk of colon cancer have been shown not to be applicable in rectal cancer. Also, in terms of prevalence and prognosis.

In terms of tumour micro environment and immune infiltrate, which is directly or indirectly linked also to genetic background and possibly to flora of the gut, it has been shown that the more proximal colon cancers, the higher the immunogenicity, higher MSI mutations and higher response to immunotherapy [3].

Several co-factors have been shown in the literature to be of impact in onco-progression. Perineural invasion has been shown to have significantly worse prognosis impact, partly possibly due to the immuno-suppressive capabilities of the neuro-tumor relationship leading to a buildup in TGF-beta. This due to Schwann cells and SC-tumor interaction recalling myeloid derived suppressor cells [4].

Furthermore, Arginase-1 has been shown in literature to also be a strong immuno-suppressor, leading a suppression in activity of T cells. In fact, Arginase inhibitors have been shown to be potent T cells stimulators and good candidates for novel drug development in the field of immuno-oncology, also revealing inhibition of tumor metastasis [5].

The aims of this analytical study are to understand the relationship of Arginase-1, TANs and prognosis, in relations to perineural invasion and neoadjuvanct treatment modalities.

Methods

Patient Selection and Clinical Data Collection

Samples were provided by the regional tumor bank of Franche-Comté (University Hospital of Besançon, France; registration number BB-0033-00024). The project was approved by the scientific board of the biobank (#2508).

Samples are from Stage III rectal cancer patients. A total of 65 patients with histologically confirmed rectal adenocarcinoma were retrospectively included and stratified into four groups according to neoadjuvant treatment modality: 20 patients received no neoadjuvant therapy, 20 were treated with combined radiochemotherapy (RTCT), 20 with radiotherapy (RT) alone, and 5 with chemotherapy (CT) alone. The latter group was included despite its limited size, as neoadjuvant chemotherapy without radiotherapy is not a current standard of care and is typically restricted to clinical trial settings (e.g., NORAD), making such cases relatively rare.

Clinical and pathological data were collected from medical records. When available, the following variables were recorded: date of biopsy or surgical sampling (date_prlv), age, sex, histological subtype and grade, Dworak tumor regression grade, pathological TNM stage, vascular and perineural invasion, resection margin status (R), neoadjuvant treatment received (neoadj), date of progression (date_prog), progression-free survival (DFS), date of death (date_death), overall survival (OS), and molecular profiling data including KRAS, NRAS, BRAF mutation status, and microsatellite instability (MSI). Death was considered as the first progression event in the absence of documented disease progression.

Sample Selection

For each surgical specimen, the most representative FFPE tissue block was selected by a pathologist, based on morphological assessment. This approach ensured optimal preservation of tumor architecture and included the relevant tumor regions (invasive margin, tumor center, and luminal surface) required for downstream analysis.

Immunohistochemistry

Serial 3-4 µm sections were cut from each FFPE block and mounted on positively charged slides. Immunohistochemical staining was performed on a BenchMark ULTRA automated stainer (Roche Diagnostics) following the manufacturer's protocol. Pre-treatment involved heat-induced epitope retrieval (HIER) under optimized buffer conditions, followed by incubation with primary antibodies and detection using the OptiView DAB IHC Detection Kit.

Tonsil tissue (for CD66b) and liver tissue (for ARG1) served as positive controls. Negative controls were processed identically but omitting the primary antibody.

Procedure Summary

- **Deparaffinization and Rehydration:** Slides were baked at 72°C for 12 minutes, followed by solvent-based deparaffinization and rehydration.
- **Epitope Retrieval:** HIER was carried out using Ventana CC1 buffer (pH 8.5) at 95–100°C for 64 minutes (CD66b) or 32 minutes (ARG1).
- **Primary Antibody Incubation:** Antibodies were applied automatically at optimized dilutions and incubation times.
- **Detection:** Staining was visualized using a multimer-based DAB chromogen system, with hematoxylin counterstaining.

Table 1:

Antibody	CD66b	Arginase-1 (ARG1)
Clone/Reference	G10F5 (BD Pharmingen)	AB96183 (Abcam)
Type	Mouse monoclonal	Rabbit polyclonal
Dilution	1:50	1:2000
Pre-treatment	CC1 standard (pH 8.5, 64 min)	CC1 short (pH 8.5, 32 min)
Incubation	32 min at 37°C	32 min at 37°C
Detection	OptiView DAB	OptiView DAB

Detection of CD66b and Arginase-1 Staining

CD66b and Arginase-1 immunostainings were quantified on whole slides using QuPath v0.5. For each slide, representative regions were manually selected to reflect spatial heterogeneity of the immune infiltrate. These included the invasive front, the deep core of the tumor, and the luminal interface (figure 1). Region selection was performed by one observer and independently reviewed by a board-certified pathologist.

Due to the predominantly membranous and/or cytoplasmic localization of these markers in small cells, nuclear detection was sometimes hindered, particularly within dense immune infiltrates. To overcome this limitation, two nucleus detection strategies were applied: optical density (OD) sum (figure 2) and hematoxylin (H) based detection. The former offered higher sensitivity, capturing nuclei partially obscured by cytoplasmic staining, while the latter showed improved specificity by reducing false positives from stromal background or artifacts. Both approaches yielded comparable results overall, with minor variations depending on tissue context and marker expression. Therefore, both datasets were retained to enable downstream statistical comparison and determine the most robust quantification method. Positive cell detection was performed using optimized parameters, with thresholds validated by a pathologist. Results were expressed as the density of positive cells per µm [2].

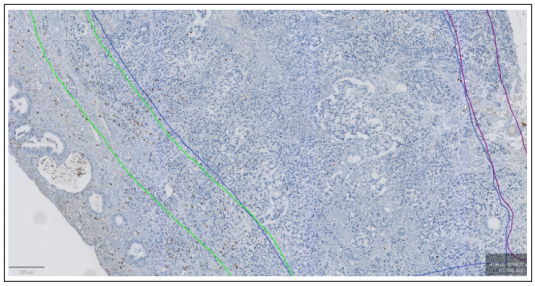

Figure 1: Representative annotation of selected tumor regions used for immunohistochemical quantification. The luminal interface is shown in green, the deep tumor core in blue, and the invasive front in purple. These regions were manually delineated on each slide

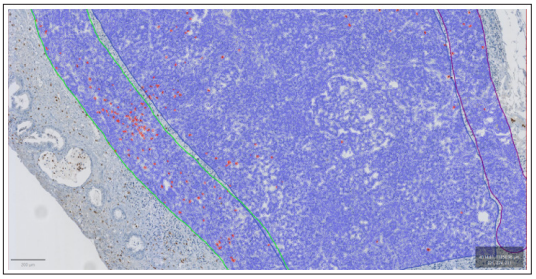

Figure 2: Representative annotation of selected tumor regions after detection with the optical density sum algorithm

Statistical Analysis Softwares

For the statistical analysis of the raw data, the following softwares were used:

- Microsoft Excel: For the division in groups and basic statistics such as median, mean.
- Graphpad Prism: For Survival Curves and their statistical analysis, Wilcoxon Test.

Measuring unit « Number of positive cells per square micrometer (cells/µm²) ».

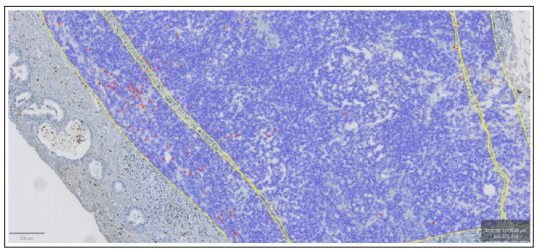

Figure 3: Representative annotation of selected tumor regions after detection with the hematoxylin algorithm

Results

Arginase-1 and TANs (Through CD 66b staining) concentration were analyzed.

From the 65 patients, those with perineural invasion were removed, for a first analysis without this factor which from literature was known to be an independent factor (and possible confounder in the analysis) of negative impact on prognosis and immune landscape of the tumor microenvironment.

A first analysis was performed using the patients who had no perineural invasion and dividing them into two groups. A group who received neoadjuvanct (neoadj) treatment and a second group who had no neoadjuvanct therapy.

In these 2 groups an analysis was performed on the possible differences in concentration in Arginase-1 and TANs in the Invasive Margine (IM) and in the Deep Core (DC).

As seen in Figure 4 below, a statistically significant difference in amount of Arginase-1 was recorded (P-value: <0.0001). Namely, that patient who had didn't underwent any neo-adjuvanct therapy, had a much higher amount of Arginase-1 in the IM zone compared to patients who underwent neo-adjuvanct therapy.

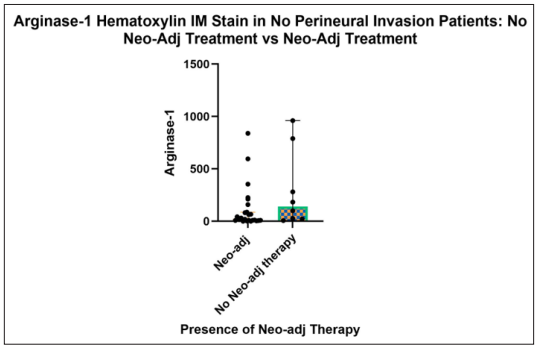

Figure 4: Scatter dot plot graph, 24 patients underwent neo-adjuvanct therapy and 8 had no neo-adjuvanct therapy. Staining performed on the Invasive Margin part of the section. The bar line shows median with 95 % CI. One sample Wilcoxon Test was perfromed (P-value: 0.007).

As seen in Figure 5 above, a statistically significant difference in number of TANs (CD66b Stained) was recorded (P-value: 0.015). Namely, that patient who had didn't underwent any neo-adjuvanct therapy, had a much higher number of TANs in the IM zone compared to patients who underwent neo-adjuvanct

therapy. This following the same trend as the amount of Arginase-1 present in the IM zone.

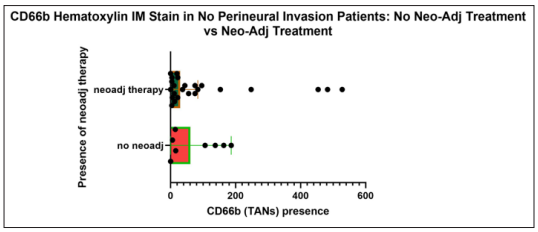

Figure 5: Scatter dot plot graph, 24 patients underwent neo-adjuvanct therapy and 8 had no neo-adjuvanct therapy. Staining performed on the Invasive Margin part of the section. The bar line shows median with 95 % CI. One sample Wilcoxon Test was performed (P-value: 0.015).

In Figure 6 below, a statistically significant difference in number of TANs (CD66b Stained) was recorded this time in the deep core (DC) (P-value: 0.015). Namely, that patient who had didn't underwent any neo-adjuvanct therapy, had a much higher number of TANs in the DC zone compared to patients who underwent neo-adjuvanct therapy.

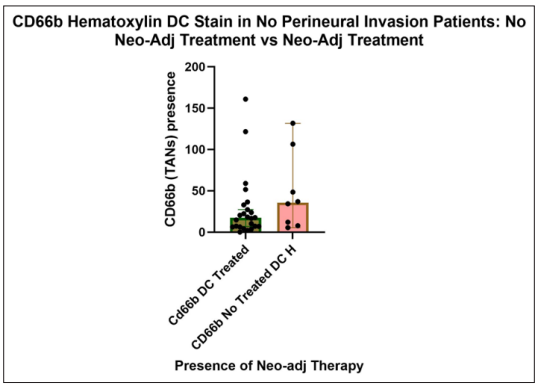

Figure 6: Scatter dot plot graph, 24 patients underwent neo-adjuvanct therapy and 8 had no neo-adjuvanct therapy. Staining performed on the Invasive Margin part of the section. The bar line shows median with 95 % CI. One sample Wilcoxon Test was performed (P-value: 0.007).

In Figures 7 and 8 below, a statistically significant difference in amount of Arginase-1 presence was recorded this time in the deep core (DC). Fig 7 using Mean as statistical parameter while Figure 8 using median as statistical parameter. Namely, that patient who had didn't underwent any neo-adjuvanct therapy, had a much higher number of TANs in the DC zone compared to patients who underwent neo-adjuvanct therapy. This following the same trend as the number of TANs present in the DC zone.

In figure 9 below, a statistically significant difference in amount of Arginase-1 presence was recorded this time in the IM between patients who didn't undergo any neo-adjuvant therapy comparing patients who had rectal cancer with perineural invasion vs no perineural invasion.

Same comparison between perineural and no perineural invasion was done amongst patients treated with neoadjuvanct therapy. Statistically significant difference in amount of Arginase-1 presence was recorded this time in the IM between the two groups, both using T-test with the mean (P-value: 0.01) and Wilcoxon Test with the Median (P-value: <0.0001).

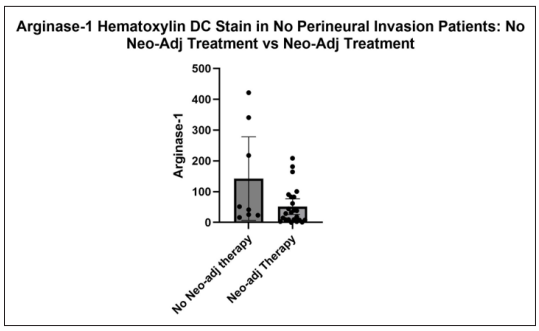

Figure 7: Scatter dot plot graph, 24 patients underwent neo-adjuvanct therapy and 8 had no neo-adjuvanct therapy. Staining performed on the Invasive Margin part of the section. The bar line shows mean with 95 % CI. One sample t-Test was performed (P-value: 0.04).

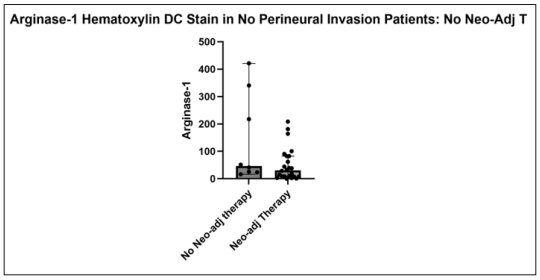

Figure 8: Scatter dot plot graph, 24 patients underwent neo-adjuvanct therapy and 8 had no neo-adjuvanct therapy. Staining performed on the Invasive Margin part of the section. The bar line shows median with 95 % CI. One sample Wilcoxon Test was performed (P-value: 0.007).

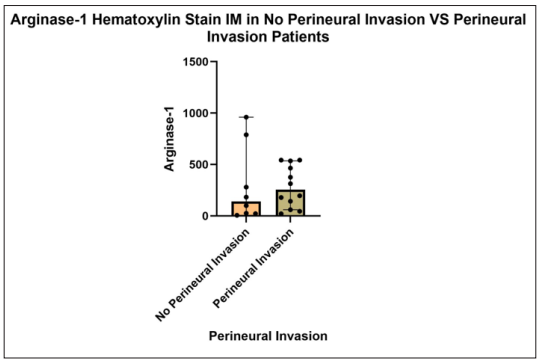

Figure 9: Scatter dot plot graph,in no-neo-adj group. Staining performed on the Invasive Margin part of the section. The bar line shows median with 95 % CI. One sample Wilcoxon Test was performed (P-value: 0.007).

In Figures 11 and 12 below, a statistically significant difference in amount of Arginase-1 presence was recorded in the IM and the DC of non-treated patients compared to the other groups (divided by modality of neoadjuvant treatment).

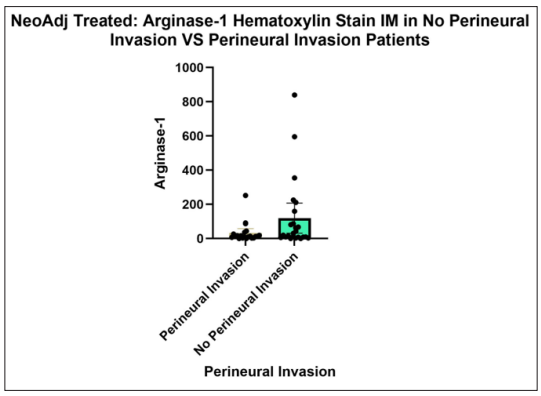

Figure 10: Scatter dot plot graph. Staining performed on the Invasive Margin part of the section. The bar line shows mean with 95 % CI. One sample t-Test was performed (P-value: 0.01)

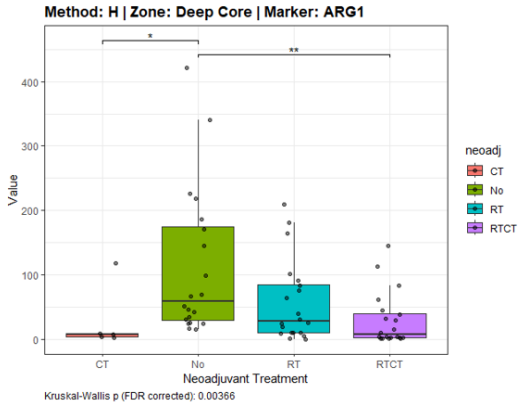

Figure 11:

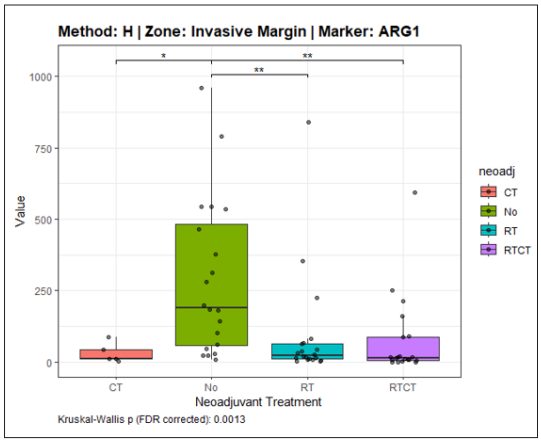

Figure 12:

A survival curve as seen in Figure 13 arose from the comparison of DFS between patients whose cancer had no perineural invasion that were not treated with neo-adjuvant therapy vs patients treated with neo-adjuvant therapy. P-Value: 0.003.

A survival curve as seen in Figure 14 arose from the comparison of DFS between patients whose cancer had no perineural invasion according to the different neoadjuvant treatment modalities (when including perineural invasion group too, no statistically different curves were recorded). Statistically significant curves were crecorded (to note the big difference between no neoadj group DFS curve and RT neoadj curve).

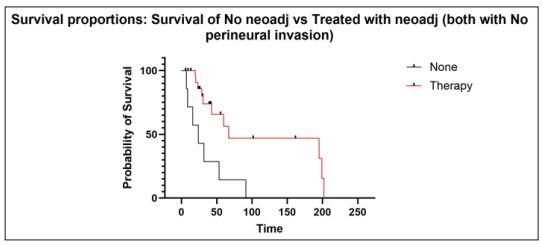

Figure 13: Survival curve. P-value: 0.003. DFS (disease free progression).

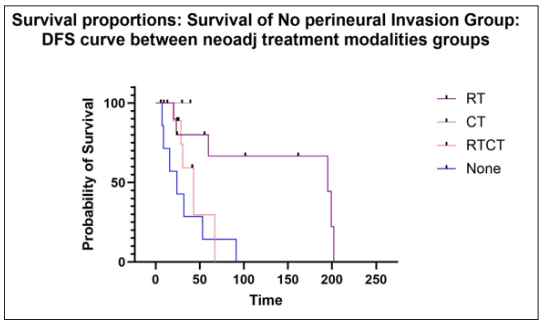

Figure 14: Survival curve. P-value: 0.003. DFS (disease free progression). Logrank (Mantel-Cox) test (P-value: 0.01).

Conclusion and Discussions

The results of this study reveal novel knowledge on arginase-1 and neutrophil in rectal cancer. No neoadjuvanct therapy patients have a statistically significant higher concentration of Arginase-1 in respect to patients treated with neoadjuvant therapy. The feature of perineural invasion in rectal cancer especially seems to be a very strong factor correlated with this difference, which is directly proportional with the concentration of neutrohils in the tumor (both in the marginal zone and in the deep core of the tumor tissue sample).

Furthermore, DFS was statistically significantly worse in the group of patients who were not treated with neoadjuvant therapy (when all these patients' tumors had no perineural invasion as a common denominator). When dividing these same patients according to treatment modality, only patients who received only radiotherapy had a statistically significant better prognosis in terms of DFS compared to patients who had no neoadj treatment (patients who also or only receive chemotherapy had a similar prognosis to those without treatment neoadj).

Extrapolating from the data of this study, arginase-1 seems to be a worsening factor to prognosis. This molecule has been found in literature to be a potent immuno-depressor (eg. T cell immunodepression), and arginase inhibitors are currently being thought as a potential new category of drugs potent against cancer and in cancer immunotherapies. In fact inhibiting arginase shows a strong boosting of immune reactions against cancer.

Perineural invasion was taken as an independent factor of negative prognosis. From this data, presence of neoadjuvanct therapy changes also the impact of perineural invasion presence on the concentration of Arg-1 within the tumour, inverting the relationship seen in the group as a whole (perineural invasion leading to higher Arg-1).

To keep in mind is the impact of RT and CT (such as 5-FU in the protocol of FOLFIRI or FOLFOX) in the neutrophil polarisation and tumour microenvironment.

Without taking in consideration the presence of neoadj therapy, the fact that perineural invasion seems to be correlated with this high level of arginase could be that the schwann cells tumor interaction (including the recalling of MDSC) act a immunosuppressors for instance through the increase in production of TGF beta.

This study sheds new light and need of further research on the relationship between Arginase-1, Tumor Associated Neutrophils, Perineural Invasion, Neoadjuvant therapy and Prognosis in Rectal Cancer Patients revealing potential prognostic and predictive factors and the importance of the possibility of seeing arginase-1 as a possible target molecule in the development of new drugs in the field of cancer immunotherapy. Patients who did not undergo neoadjuvant therapy and had no perineural invasion from the tumor, had a statistically significant higher level of Arginase-1 and CD66b (neutrophils) and had a significantly worse prognosis (in terms of Disease-Free Survival) compared to patients who underwent neoadjuvanct therapy and had no perineural invasion (especially compared to those who underwent exclusively radiotherapy). When considering the whole group of patients, including perineural invasion, patients who underwent no-neoadj therapy had a significantly higher amount of Arginase-1 and tumor associated neutrophils (TANs) compared to the first group.

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References

1. American Cancer Society. "Colorectal Cancer Statistics | How Common Is Colorectal Cancer?" American Cancer Society. 2023.
2. Peng Y, Tang H, Meng X, Shen Y, Hu D, et al. Histological grades of rectal cancer: whole-volume histogram analysis of apparent diffusion coefficient based on reduced field-of-view diffusion-weighted imaging. Quant Imaging Med Surg. 2020. 10: 243-256.
3. Boland CR, Goel A. Microsatellite instability in colorectal cancer. Gastroenterology. 2010. 138: 2073-2087.
4. Dudas J, Glueckert R, Greier MdC, Hofauer BG. The Relationships Among Perineural Invasion, Tumor–Nerve Interaction and Immunosuppression in Cancer. Onco. 2025. 5: 25.
5. Wang X, Xiang H, Toyoshima Y, Shen W, Shichi S, et al. Arginase-1 inhibition reduces migration ability and metastatic colonization of colon cancer cells. Cancer Metab. 2023. 11: 1.