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HISTOPATHOLOGICAL AND MOLECULAR CHANGES IN ADVANCED COLON TUMORS – A RETROSPECTIVE STUDY

PHD THESIS SUMMARY

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CUPRINS

List of abbreviations

HP – Histopathology

MSI – Microsatellite Instability

CMS – Consesus Molecular Subtypes

TME – Tumor Microenvironment

IHC – Immunohistochemistry

TNM – Tumor-Node-Metastasis

AJCC – American Joint Committee on Cancer

UICC – Union for International Cancer Control

ESMO – European Society of Medical Oncology

NCCN – National Comprehensive Cancer Network

ADK – Adenocarcinoma

NOS – Not Otherwise Specified

WHO – World Health Organization

CAP – College of American Pathologists

TIF – Tumor Invasion Front

PNI – Perineural Invasion

LVI – Lymphovascular Invasion

OS – Overall Survival Rate

EMVI – Extramural Venous Invasion

IMVI – Intramural Venous Invasion

TB – Tumor Budding

EMT – Epithelial-Mesenchymal Transition

ITB – Intratumoral Budding

TSCs – Tumor Stem Cells

NSTCs – Non-Stem Tumor Cells

PDCs – Poorly Differentiated Clusters

CAFs – Cancer Associated Fibroblasts

ECM – Extracellular Matrix

STR – Stroma/Tumor Ratio

TAMs – Tumor-Associated Macrophages

TANs – Tumor-Associated Neutrophils

TILs – Tumor-Infiltrating Lymphocytes

NK – Natural Killers

TIEs – Tumor-Infiltrating Eosinophils

PFS – Progression-Free Survival

MMR – Mismatch-Repair System

MSI-H – High Microsatellite Instability

dMMR – deficient MMR system

pMMR – proficient MMR system

MSS – Microsatellite Stability

GTP – Guanosine-5'-triphosphate

H&E – Hematoxylin and eosin

FFPE – Formalin-Fixed Paraffin-Embedded

Introduction

Colon cancer represents a major global health problem, being the fourth most common type of cancer and the fifth leading cause of cancer death according to GLOBOCAN 2022, with a significantly higher prevalence in Asia (46.6%) and Europe (30%) compared to Africa (< 5%), affecting men more than women, and with a considerably higher incidence in high-income countries compared to low-income countries [1–3]. According to GLOBOCAN 2022 data, in Europe, colon cancer is the second most common type of cancer, constituting 12% of all cases, with the highest incidence in Denmark and the highest mortality in Hungary; in Romania, it has a frequency of 12.9%, being more prevalent in men (14.1%) than in women (11.6%), and represents the third leading cause of death after lung cancer and breast cancer [4–7]. These data highlight significant variations in the incidence and mortality of colon cancer, influenced by geographical, gender, and socio-economic factors, showing that in high-income countries, increased incidence is associated with lifestyle and early diagnosis, while reduced mortality is due to effective treatments, whereas in low-income countries, incidence is lower but mortality is higher due to limited access to diagnosis and treatment; in Europe, differences between countries like Denmark and Hungary reflect variations in healthcare systems, screening programs, and medical practices, emphasizing the importance of screening programs and access to adequate treatments for reducing the global impact of colon cancer.

In the last 10 – 15 years, advances in understanding the histopathological (HP) and molecular changes of colon cancer have elucidated some of the genetic and epigenetic mechanisms of carcinogenesis, facilitating the development of personalized and precise treatments targeting molecular biomarkers such as BRAF, KRAS, and NRAS mutations, as well as microsatellite instability (MSI), through the use of innovative therapies (e.g., anti-VEGF antibodies, anti-EGFR antibodies, and immunotherapy) to halt tumor progression [8–13]. The integration of HP and molecular findings has significantly improved the understanding of colon cancer subtypes, exemplified by the Consensus Molecular Subtypes (CMS) classification, currently used in research, providing a comprehensive perspective on tumor heterogeneity and facilitating the identification of new prognostic and therapeutic prediction biomarkers [10,12,14,15]. Recent research has highlighted the prevalence of tumor heterogeneity and the concept of spatial heterogeneity as essential HP biomarkers to be evaluated in colon cancer, revealing the variability of genetic abnormalities and their uneven distribution reflected morphologically, with major clinical implications for disease progression and therapeutic response [14–17]. Additionally, recent studies have underscored the crucial role

of the tumor microenvironment (TME) in the progression of colon cancer, and understanding the complex interactions between tumor cells and its components (stroma and the immune cell network) has opened new perspectives for guiding therapeutic decisions (e.g., immunotherapy) and the development of targeted therapies [18,19]. In the context of scientific advancements regarding molecular biomarkers for colon cancer, the CDX2 protein has proven to be a promising prognostic factor, addressing the current deficit of specific biomarkers, given that the BRAF gene is the only validated independent prognostic molecular biomarker recommended by international guidelines [12,20]. Studies indicate the crucial role of the CDX2 gene in tumor suppression, and its deletion is associated with aggressive tumor behavior; furthermore, the variability of its intratumoral immunohistochemical (IHC) expression, due to interactions with the TME, underscores the importance of evaluating it in the context of tumor heterogeneity [12,20–23].

The motivation for colon cancer research stems from its significant impact on public health and the need for innovative approaches in molecular and HP evaluation. This requires the integration of fields such as Oncology, Surgery, Pathology, Genetics, and Molecular Biology to adequately address the complexity of this disease. In the Romanian healthcare system, identifying accessible and cost-effective HP and molecular biomarkers is essential for optimizing the oncological management of this type of cancer. The relevance of this research is highlighted by recent advances in understanding the genetic and molecular mechanisms of colon cancer, which facilitate the discovery of new biomarkers and the development of personalized therapeutic strategies. The novelty of this research topic lies in integrating recent findings on tumor heterogeneity in the context of genetic and epigenetic variability and identifying new biomarkers to advance precision oncology. This study aims to evaluate HP and molecular changes in advanced colon cancer, highlighting the insufficiency of current conventional biomarkers and investigating new HP and molecular biomarkers recently proposed and under validation to improve prognostic evaluation and guide therapeutic decisions. The study employed a retrospective and multifaceted approach, combining descriptive and inferential analysis of clinicopathological data with HP and IHC evaluation of tumor tissue samples from 97 cases of advanced colon cancer. The results revealed significant correlations between conventional and new biomarkers, as well as between the new biomarkers and oncological follow-up parameters, underscoring their importance in predicting disease prognosis. The development of an innovative predictive model, integrating both conventional and new biomarkers, improved the accuracy of prognosis for advanced colon cancer.

1. Histopathological Evaluation of Colon Cancer

1.1. Conventional Biomarkers

1.1.1. TNM Staging

The TNM pathological staging system, developed by AJCC and UICC in 1950, represents a standardized and reliable framework for assessing the extent of colon cancer, essential for predicting prognosis and determining treatment strategies [9,14,24–26]. The 8th edition of the AJCC/UICC Staging Manual from 2017, along with ESMO and NCCN guidelines, provides updated information on tumor invasion, lymph node involvement, and the presence of metastases, guiding therapeutic and monitoring decisions [9,26–29].

The pT stage in the TNM system assesses the degree of tumor invasion in colon cancer, ranging from pT1 (invasion of the submucosa) to pT4b (invasion of adjacent organs), with a variable prognosis from favorable to unfavorable depending on the depth of invasion [26–29]. The 8th edition of the AJCC-TNM manual faces challenges in distinguishing between pT3 and pT4a stages, suggesting the need for subdividing pT3 to improve the accuracy of prognosis and treatment [9,30,31]. The pN stage in the TNM system indicates the involvement of regional lymph nodes, ranging from pN1 (1 – 3 positive nodes) to pN2 (4 or more positive nodes), with subdivisions based on the presence of metastases or tumor deposits in the lymph nodes [26]. According to current ESMO and NCCN guidelines, pN1a, pN1b, and pN1c have a more favorable prognosis, while pN2a and pN2b are associated with an unfavorable prognosis [27–29]. The challenges of this staging include correctly identifying micrometastases, which have a significant negative prognostic impact and require the use of IHC for detection [9,29]. The pM stage in the TNM system assesses distant metastases, where pM1a indicates metastases to a single organ, pM1b indicates multiple metastases to different organs, and pM1c indicates peritoneal metastases, with prognosis varying from favorable to unfavorable according to ESMO and NCCN guidelines [26,28,29].

The integration of molecular biology into the evaluation of colon cancer, complementary to TNM staging, has improved the accuracy of prognosis prediction and facilitated the development of personalized therapeutic strategies [9,24,30–32]. However, the current TNM system has limitations in adequately capturing the biological variability of tumors and in HP evaluation practices, highlighting the need for continuous updates to more accurately reflect the complexity of colon cancer and improve clinical management of patients [9,30,31].

1.1.2. Histological Subtype and Grade of Tumor Differentiation

Colon cancer predominantly manifests as adenocarcinomas (ADK), with conventional, non-specific (NOS) ADK representing 90 – 95% of cases, characterized by large glands and cells with oval nuclei and numerous mitotic figures [33–35]. According to the 2019 WHO classification, common *histological subtypes* include mucinous ADK, which accounts for approximately 10% of cases and responds poorly to treatment in metastatic stages, and serrated ADK, which constitutes 10 – 15% of cases and is frequently located in the right colon [33–36].

Rare histological subtypes of colon cancer include micropapillary and adenomatoid ADK, signet-ring cell carcinomas, adenosquamous, sarcomatoid, and undifferentiated NOS carcinomas, each with distinct characteristics and variable prognoses [35]. Micropapillary ADK and signet-ring cell carcinoma are aggressive subtypes, often diagnosed at advanced stages and associated with an unfavorable prognosis [33,35].

The degree of tumor differentiation, used as a HP biomarker since the 1920s and classified into three grades (well, moderately, and poorly differentiated), has significant prognostic value independent of disease stage. However, according to the CAP classification, it is a category 2A marker with limited clinical relevance, as therapeutic decisions are primarily based on TNM staging [14,37–40]. The degree of differentiation of colonic tumors, reflecting the level of glandular "maturation," is a numeric indicator of malignancy based on microscopic differences from normal cells. Tumors are classified into well-differentiated (G1), moderately differentiated (G2), and poorly differentiated (G3), each with a different prognosis [14,38,41]. According to the 2019 WHO guidelines, the grading of colon cancer has been refined, classifying it into low-grade tumors (G1 and G2) and high-grade tumors (G3). It is also recommended that this grading be performed on surgical resection specimens, with a separate evaluation of the tumor invasion front (TIF) [14,35].

For an accurate assessment of the degree of differentiation in colon cancer, it is essential to consider tumor heterogeneity, particularly spatial heterogeneity [14]. The separate evaluation of the degree of differentiation between the tumor center and the TIF, according to the current WHO guidelines, allows for more accurate patient stratification and provides additional prognostic information complementary to TNM staging, thereby contributing to the optimization of oncological management in colon cancer [14,35].

1.1.3. Perineural, Lymphovascular Invasion and Tumor Budding

Perineural invasion (PNI) and lymphovascular invasion (LVI) are essential HP biomarkers for prognostic evaluation in colon cancer, indicating aggressive tumor behavior and an unfavorable prognosis, independent of disease stage, according to current guidelines [9,10,26,39]. PNI in colon cancer, defined by the presence of tumor cells in direct contact with nerve bundles, is associated with an aggressive tumor phenotype, frequently observed in advanced stages, facilitating regional dissemination and having an incidence ranging from 9% to 42%, according to a study by Dawson et al. [9,10,26,35,39]. Tumors located in retroperitoneal segments have a higher incidence of PNI, and its presence is often correlated with LVI and lymph node metastases, suggesting the need for chemotherapy [9,39]. LVI, which refers to the penetration of tumor cells into blood and lymphatic vessels, is an independent prognostic factor associated with an increased rate of local recurrence and reduced overall survival (OS) [10,26]. According to current guidelines, it is recommended to separately evaluate lymphatic invasion and extramural venous invasion (EMVI) and intramural venous invasion (IMVI), with EMVI being a strong predictor of unfavorable prognosis but challenging to evaluate histopathologically, although elastin staining techniques have improved detection sensitivity [9,26,32,35]. Preoperative imaging evaluation through magnetic resonance imaging (MRI) is the only reliable method for detecting vascular invasion, particularly EMVI [32].

Tumor budding (TB), characterized by the presence of isolated cells or small clusters of neoplastic cells (≤ 4 dedifferentiated cells) at the TIF, is facilitated by epithelial-mesenchymal transition (EMT), which allows malignant cells to detach and migrate, contributing to LVI, PNI, and distant dissemination [10,14,32,42,43]. TB is an independent prognostic marker recognized by current guidelines and integrated into the standard evaluation of colon cancer, with its reporting standardized in 2016 using a three-tier scoring system for metastasis risk stratification, evaluated by examining at least 10 microscopic fields at the TIF, though diagnostic methods require further standardization due to variability in interobserver agreement [9,27,29,33,35,43,44]. TB is a significant biomarker of aggressiveness, associated with G3 tumors with an "offensive" TIF, facilitating PNI and LVI, and correlating with an increased risk of lymphatic dissemination and the development of stenosing tumors [10,14,32,44]. Additionally, intratumoral budding (ITB), present in approximately 17 – 20% of cases, is associated with an aggressive TIF, and recent studies support the inclusion of ITB evaluation in the HP assessment of colon cancer due to its significant impact on patient survival [14,32,44].

1.2. New Prognostic and Prediction Biomarkers

1.2.1. Tumor Heterogeneity

In the past two decades, research on tumor heterogeneity, especially in colon cancer, has highlighted its molecular complexity and gradual oncogenetic progression, emphasizing the role of genetic and epigenetic changes in the processes of cellular growth, differentiation, and apoptosis, granting tumor cells a survival advantage [14,16,42,45–47]. Advances in DNA sequencing technologies have challenged the linear model of cancer evolution and revealed that colon tumors exhibit molecular heterogeneity influenced by genetic, epigenetic, and non-genetic factors such as lifestyle and the gut microbiome, reflecting a dynamic polyclonal evolution [15–17,42,45,47]. In this context of dynamic polyclonal evolution, three models have been described to explain the origin and implications of tumor heterogeneity [16]. The first model focuses on the presence of two types of cells in the tumor: tumor stem cells (TSCs), capable of initiating and sustaining carcinogenesis, and non-stem tumor cells (NSTCs), which do not directly contribute to cancer development [16]. The second model suggests tumor development through the gradual accumulation of genetic mutations in a single dominant cell, resulting in clusters of clones that evolve either through survival competition or via a cooperative mechanism known as "branched evolution" [16,42,47]. The third model, known as the "Big Bang" model, proposes that the tumor rapidly accumulates a large number of genetic mutations, establishing its complexity and behavior from the outset without a specific dominant clone [15,16,46]. Each model influences tumor heterogeneity, affecting treatment resistance and survival rates [17,42]. In the context of colon cancer, two essential forms of heterogeneity are emerging: inter-tumoral and intra-tumoral heterogeneity [17,45]. *Inter-tumoral heterogeneity*, characterized by diverse genetic profiles, HP features, and clinical behaviors, has led to the development of a new molecular classification (CMS) that divides tumors into four distinct categories based on the involved molecular mechanisms and clinico-pathological characteristics, influencing prognosis and treatment response [8,15,17,23,45,46]. Colon cancer exhibits significant *intra-tumoral heterogeneity*, influenced by genetic, epigenetic, and non-genetic factors, leading to morphological diversification (e.g., heterogeneity in tumor differentiation) and the presence of distinct clonal populations [15,17,45,46]. Additionally, the concept of *spatial heterogeneity*, which examines the distribution of clonal populations in different regions of the tumor, is essential for understanding tumor behavior in both the primary site and metastatic sites [15,17,45].

1.2.2. Tumor Invasive Front and Tumor Stroma

The area known as the "tumor invasion front" (TIF) is crucial for understanding the complex interactions between the colon tumor and the host, decisively influencing cancer progression through local invasion and metastatic dissemination processes [32,43]. Characterized by physical and functional relationships between tumor cells and the TME, the TIF provides crucial information regarding the risk of recurrence and survival rates, facilitating the development of personalized therapeutic strategies [35,43,48]. The TIF is an important prognostic biomarker in colon cancer, as variations in the "zonal architecture" and degree of differentiation influence tumor invasion and aggressiveness [43,49]. Evaluating differentiation at the TIF allows for a more comprehensive understanding of tumor dynamics and the identification of high-risk patients who might benefit from more aggressive adjuvant treatments [15,48,49]. PDCs, described in the 5th edition of the WHO guidelines and used as markers of the TIF, are composed of five or more tumor cells, are thought to evolve from TB, have a similar morphology, and are associated with the process of EMT [32,35,49]. PDCs have been recently integrated into the HP evaluation of colon cancer and are recognized as more reliable prognostic biomarkers than TB [48,49]. The recognition and detailed evaluation of the TIF, a reliable prognostic biomarker highlighting the dynamic interactions between tumor cells, the TME, and the host, are essential in the diagnosis and management of colon cancer, providing pathologists with an accurate estimate of tumor behavior and supporting the personalization of therapeutic strategies [9,15,43,48,50]. The TME, composed of immune cells, fibroblasts, blood vessels, and ECM, plays a crucial role in tumor progression through its dynamic interaction with tumor cells [51,52]. A key element of the TME is *the stroma*, predominantly composed of cancer-associated fibroblasts (CAFs) and elements of the ECM. CAFs, through their structural contribution and secretion of pro-angiogenic and pro-proliferative factors, facilitate metastasis through the process of EMT [18,51–56]. The characteristics of the stroma at the TIF significantly influence prognosis and therapeutic response, making the HP evaluation of the stroma/tumor ratio (STR) and stroma typology essential for predicting these factors in colon cancer [18,51,53,54,56]. An increased STR and a desmoplastic stroma rich in CAFs are associated with an unfavorable prognosis and treatment resistance, while intermediate and immature stroma types, characterized by "scar-like" collagen and a myxoid ECM, indicate heightened tumor aggressiveness [53,54,56]. In conclusion, the STR and stroma typology function as bidirectional biomarkers, decisively impacting both prognosis and therapeutic efficacy [18,53,54].

1.2.3. Tumor-Induced Inflammation and Tumor-Infiltrating Lymphocytes

Inflammation induced by colon tumors involves pro-tumor immune responses through the disruption of the intestinal barrier and activation of NF-κB and STAT3 pathways, and anti-tumor responses through the IFN-γ pathway and pro-inflammatory cytokines that recruit effector cells to destroy tumor cells [57–59]. The TME modulates the balance between pro-tumor and anti-tumor inflammation through cytokines and chemokines, influencing tumor progression or regression; thus, a detailed understanding of the functions of the involved immune cells is crucial for prognostic evaluation and optimizing therapeutic strategies in colon cancer [57,59].

Tumor-associated macrophages (TAMs) are correlated with an unfavorable prognosis in colon cancer, as the predominance of M2 macrophages, which promote tumor growth, angiogenesis, and suppression of adaptive immunity, is associated with increased tumor aggressiveness, in contrast to M1 macrophages, which are involved in host defense and anti-tumor activity [57–61]. *Tumor-associated neutrophils (TANs)* in colon cancer have an ambivalent role in the TME, with N1-TANs exerting anti-tumor effects through cytotoxic substance secretion, while N2-TANs promote tumor proliferation through various mechanisms, with their polarization determined by TME cytokines and chemokines and influenced by TGF-β and interferon-β signaling [58–60,62,63]. *Tumor-infiltrating lymphocytes (TILs)* also play an ambivalent role in the immune response, where B lymphocytes can have both anti-tumor effects by activating CD4+ helper T cells and CD8+ cytotoxic T cells, and pro-tumor effects by maintaining an inflammatory TME, while T cells and NK cells vary in function depending on subtype and disease stage, with their abundance being associated with a better prognosis due to effective immune surveillance [63–65]. *Tumor-infiltrating eosinophils (TIEs)*, frequently observed in colon tumors, exhibit significant anti-tumor effects through the direct destruction of tumor cells and modulation of other immune cell activities, being recruited to the TME by the chemokine CCL11/eotaxin-1 and mediating an anti-tumor effect via IFN-γ signaling, independent of CD8+ cytotoxic T cells [66]. Immune cells in the colon cancer TME play a crucial role in disease progression, yet their assessment is not yet included in current clinical guidelines, lacking a standardized methodology for evaluation. Detailed analysis of immune cell phenotypes and interactions can enhance prognostic prediction and enable the development of personalized treatments [57–59,63,64,66,67]. Studies show that the presence of an extensive tumor inflammatory infiltrate, dominated by anti-tumor cells, significantly increases OS and progression-free survival (PFS) rates [65].

2. Molecular Evaluation of Colon Cancer

2.1. Conventional Molecular Biomarkers

The integration of molecular biology into the oncologic management of colon cancer has highlighted essential biomarkers with prognostic and predictive roles [10,23,68,69]. Current ESMO and NCCN guidelines recommend assessing the DNA mismatch repair (MMR) system, microsatellite instability (MSI), RAS gene mutations, and mutational status of the BRAF gene to provide critical information about prognosis and therapeutic response [10,23,27–29,68,69].

The MMR system, composed of heterodimeric protein complexes MLH1-PMS2 and MSH2-MSH6, corrects DNA replication errors in chromosomal microsatellites, preventing inconsistencies and ensuring accurate DNA resynthesis [23,70,71]. Inactivation of an essential gene in this system, through germline mutations or epigenetic modifications, results in MMR deficiency and leads to high microsatellite instability (MSI-H), which is implicated in the initiation of colon cancer [23,70]. Current studies indicate that the dMMR/MSI-H phenotype is associated with a favorable prognosis in sporadic colon cancer, owing to a robust anti-tumor immune response, and is linked to Lynch syndrome in hereditary cases [23,70–73]. Tumors with proficient MMR system (pMMR) and microsatellite stability (MSS) have an unfavorable prognosis due to the absence of a protective immune infiltrate [23,72,73]. According to international oncology guidelines, assessing MMR/MSI in stages II and III is essential for decisions regarding adjuvant chemotherapy, and in stage IV for determining the benefit of immunotherapy [27–29,70,73,74].

Proteins encoded by *RAS oncogenes* (KRAS, NRAS) are GTPases that regulate the MAPK signaling pathway, and point mutations in these genes are frequently encountered in colon cancer [10,23,70,74,75]. These mutations cause uncontrolled cellular growth, leading to the formation of bulky tumors and also enabling malignant cells to proliferate in low glucose concentrations [10]. RAS mutations are essential predictive biomarkers for resistance to anti-EGFR treatment, negatively influencing OS and PFS rates; guidelines recommend genotyping these mutations in the metastatic stage [23,70,76–78]. In colon carcinogenesis, another important somatic missense mutation occurs in the *BRAF gene*, which encodes RAF proteins in the MAPK pathway, and in approx. 90% of cases, this mutation appears in exon 15, codon 600 (BRAF V600E mutation) [10,23,74,78,79]. This mutation serves as a negative prognostic biomarker in advanced stages of colon cancer, with an average OS rate of under 12 months [23,28,29,69,76,77,80].

2.2. CDX2 as a New Prognostic Biomarker

CDX2 plays a crucial role in maintaining cellular homeostasis in the adult intestinal tract, controlling genes involved in differentiation, proliferation, cell adhesion, cell cycle, and apoptosis, activated by the Wnt/β-catenin, MAPK, HNF, and GATA signaling pathways, and inhibited by transcription factors SOX2 and SOX9 [12,21–23,81–83]. CDX2 acts as a tumor suppressor by inhibiting the Wnt/β-catenin pathway and maintaining the integrity of the intestinal barrier, and its absence leads to major abnormalities in intestinal structure and function [12,21,23,81–83]. In colon cancer, loss of CDX2 expression, often due to epigenetic changes, results in loss of intestinal epithelial differentiation, abnormal mucin secretion, and uncontrolled cell proliferation, contributing to a pro-tumoral microenvironment [83–85].

The expression of CDX2 protein is frequently altered in colon cancer (10 – 30% of cases) and is recognized by numerous researchers as a significant independent prognostic biomarker [21,23,81]. Negative expression of CDX2 is correlated with advanced TNM stages due to its essential role in cell cycle control and regulation of the Wnt/β-catenin signaling pathway, disruption of which leads to cellular proliferation and migration, favoring invasion and metastasis [12,20,21,23,81,86–89]. Tumors with negative CDX2 expression are frequently of G3 type, located in the right colon, and are associated with the serrated pathway and BRAF V600E mutation [12,20,81,83,86,88,89]. LVI is also more common in tumors with negative CDX2 expression due to the disruption of genes involved in cell adhesion, which are normally regulated by CDX2 [21,86,89]. Ultimately, loss of CDX2 expression is frequently observed in aggressive histological subtypes of colon cancer, such as mucinous ADK and signet ring cell carcinomas, due to its crucial role in regulating mucin production [23,84].

Loss of CDX2 expression, closely linked to activation of EMT and amplification of the TGF-β and WNT/β-catenin pathways, enhances the migratory and invasive capacities of colon tumor cells, facilitating their migration as TB cells or PDCs [23,87,89,90]. There is bidirectional communication between TME and CDX2 protein expression that influences tumor progression [12,83]. Additionally, loss of CDX2 expression is associated with a high level of TAMs and dMMR/MSI-H status, but it has a negative prognostic significance only in tumors with pMMR/MSS phenotype [12,89,91]. Numerous studies have demonstrated that negative CDX2 expression is associated with a negative prognosis in colon cancer due to its correlation with several unfavorable pathological and molecular parameters [23].

3. Work Hypothesis and General Objectives

Colon cancer represents an extremely heterogeneous pathology, both molecularly and morphologically, aspects that complicate both the diagnosis and treatment of this disease. Recent research over the last decade has highlighted the limitations of the conventional panel of HP and molecular biomarkers in accurately predicting patient prognosis, emphasizing the necessity of identifying and integrating additional biomarkers to provide a deeper and more comprehensive understanding of tumor behavior.

The work hypothesis of this study suggests that integrating new HP and molecular biomarkers will enable a more precise evaluation of tumor behavior in colon cancer, facilitating efficient risk stratification and guiding therapeutic decisions, ultimately impacting patient prognosis through correlation with conventional markers and oncological follow-up parameters, as well as through the identification of more reliable prediction models.

The first objective of this study is to evaluate a series of new HP and molecular biomarkers currently under international research and validation and to analyze their interaction, as well as their relationship with demographic, clinical parameters, and conventional HP and molecular biomarkers. The study evaluates new HP biomarkers including tumor differentiation heterogeneity, tumor stroma, overall tumor immune infiltrate, TILs, differentiation patterns in TIF and PDCs, alongside the newly analyzed molecular biomarker - the IHC expression of the CDX2 protein in the tumor, in the TIF, and in the PDCs, within the context of intratumoral and spatial heterogeneity.

The second objective is to examine the correlation between both conventional and new biomarkers with patient prognosis, analyzing the relationship between each biomarker and oncological follow-up parameters (disease progression, type of progression - metastasis/recurrence, and PFS rate).

The third and final objective of this study is to create two prediction models: the first utilizing conventional biomarkers and the second integrating both conventional and new biomarkers, aiming to conduct a comparative analysis of these models regarding the prognosis of the participants included in the study. This endeavor aims to assess the potential of new biomarkers to significantly enhance the accuracy of prognostic prediction when integrated into the conventional panel, thereby opening new perspectives for optimizing the oncological management of patients with colon cancer.

4. General Research Methodology

4.1. Inclusion and Exclusion Criteria. Materials and Methods

The inclusion criteria for this study were carefully designed to ensure the integrity and comparability of results. Cases of colon cancer undergoing elective surgery for primary tumor excision without prior IHC evaluations of CDX2 expression were included, and only cases with complete medical data and high-quality tissue samples were considered. No demographic restrictions were imposed to ensure broad representativeness of results. The study focused on molecular biomarkers KRAS and MMR/MSI, excluding the BRAF gene due to limited accessibility. Additionally, only preoperatively untreated patients were included to maintain tumor condition unaffected. Last but not least, the study focused only on pT3 and pT4 tumors to accurately reflect the clinicopathological and molecular relationships in aggressive tumors.

Exclusion criteria were established to ensure a homogeneous study group, eliminating cases with rectal tumors due to significant differences from colon tumors in therapeutic protocols and biological behavior. Preoperative radiotherapy, commonly used in rectal cancer, can alter tumor status and influence results. Additionally, cases with metachronous or synchronous tumors were excluded to assess the direct impact of biomarkers on primary colon tumors. Moreover, cases with a history of genetic predisposition to cancer were eliminated, focusing the study solely on sporadic colon cancer to avoid genetic influences that could distort the results.

Materials and Methods: This retrospective study, approved by the Ethics Committee of Colțea Clinical Hospital in Bucharest (protocol 34/14.12.2023), analyzed 97 cases of advanced colon cancer selected according to pre-established criteria. Medical data were anonymously collected from the archives of the Oncology, Surgery, and Pathology departments of the hospital, including demographic, clinical, HP, molecular, and oncological follow-up parameters. Pathological samples (97 H&E slides and FFPE tissue blocks) were retrieved from the Pathology department of Colțea Clinical Hospital. The analysis of the H&E slides and processing of the FFPE blocks to obtain IHC-CDX2 stained slides were conducted at the OncoTeam Diagnostic laboratory in Bucharest. The data obtained in this study were entered into a database using Microsoft® Excel® 2021 MSO (version 2404 Build 16.0.17531.20152) and were statistically analyzed (descriptive and inferential) using Minitab® (version 22.1) and OpenEpi (version 3.01) software.

4.2. Analysis of New Biomarkers

The evaluation of *tumor differentiation* in the context of intratumoral and spatial heterogeneity was performed through semi-quantitative analysis of the distribution of differentiation grades (G1, G2, G3) in the tumor center and at the level of TIF, quantifying the percentage of each region relative to the total tumor area. Based on these analyses, four distinct, recurrent categories of heterogeneous differentiation were identified: category A - tumor with G1 and G2 regions, category B - homogeneous G2 tumors, category 3 - tumor with G2 and G3 regions, and category D - "mosaic" tumor with G1, G2, and G3 regions. The *evaluation of TIF* revealed four distinct patterns of differentiation grade distribution, named F1 (G1 and G2), F2 (G2 and G3), F3 (homogeneous G2), and F4 (homogeneous G3). The composition and quantity of *tumor stroma* were rigorously and semi-quantitatively assessed according to the criteria of the Glasgow Microenvironment Score (GMS), analyzing the total percentage of STR and classifying the stroma based on the percentages of mature and immature stroma. Based on these evaluations, cases with homogeneous stroma (100% mature stroma) and heterogeneous stroma (a mixture of immature and mature stroma) were identified. *Tumor-associated inflammation* was assessed through a semi-quantitative analysis of the total percentage of immune infiltrate using the adapted Klintrup-Mäkinen score (a component of the GMS score). The semi-quantitative *analysis of TILs* was conducted by estimating the total percentage of lymphocytes within the overall immune infiltrate present in the colon tumor. To verify whether tumor inflammation is strictly associated with the tumor, the evaluation included an *observational analysis of bacterial superinfection* and a semi-quantitative *analysis of tumor necrosis*, classified into 4 scores (0 – 3) based on the extent of necrosis. The *PDCs* were analyzed semi-quantitatively, similar to the evaluation method of the TB (internationally validated score). Considering the lack of a standardized method for evaluating CDX2 expression via IHC, an associated study was conducted alongside this research, involving the development of a new semi-quantitative scoring system for CDX2 expression on a sample of 43 advanced colon tumors [92]. The study identified 3 distinct *categories of heterogeneous IHC CDX2 expression* and demonstrated a significant association between these categories and TB scores, as well as tumor differentiation categories: the 1st category with strong and moderate expression, the 2nd with negative and moderate expression, and the 3rd with "mosaic" expression (strong, moderate, and negative). The analysis of CDX2 expression was broadened in this study to both the level of TIF (6 patterns named CDF) and the level of TB (positive, mosaic, and negative expression), through an observational analysis of IHC CDX2 expression patterns.

5. Study 1: Correlations between Conventional and Novel Biomarkers

5.1. Introduction. Materials and Methods.

The central hypothesis of this study asserts that the new HP and molecular biomarkers, including tumor differentiation categories, STR, stromal types, the K-M score, TILs, the presence of bacterial superinfection, tumor necrosis score, tumor differentiation patterns in TIF, the PDCs score, categories of IHC expression of the CDX2 protein at the tumor level, as well as in TIF and TB, exhibit a significant correlation both with conventional HP biomarkers (histological subtype, tumor differentiation grade, TB score, LVI, and PNI) and with conventional molecular biomarkers (KRAS, MMR/MSI). This correlation leads to the generation of specific morphological patterns capable of influencing tumor behavior in advanced colon cancer. Additionally, the study suggests the existence of a significant interrelationship between new biomarkers.

The first objective of this study is to analyze the distribution of HP and molecular biomarkers, both conventional and new, within the analyzed cohort consisting of 97 cases of advanced colon tumors. Additionally, it aims to examine the distribution and relationship of these biomarkers with demographic parameters (sex and age) and clinicopathological parameters (oncological stages, general and specific localization of the tumor, length and thickness of the tumors, as well as their stenosing nature). *The second objective* aims to analyze the relationship between each new HP and molecular biomarker and conventional HP and molecular biomarkers, in order to highlight the specific interdependencies and interactions among their phenotypes. Through this approach, the goal is to elucidate morphological patterns that indicate a certain tumor behavior. *The third objective* focuses on highlighting the intratumoral spatial heterogeneity through a detailed examination of the relationship between the morphology of the central region of the tumor and that of the TIF. This analysis will include an assessment of tumor differentiation and the IHC expression of the CDX2 protein in these distinct regions. Additionally, the correlation between the IHC expression of the CDX2 protein in the main tumor mass and in TB from the TIF will be explored.

For this study, data from 97 participants were used to create a comprehensive database for statistical analysis. The first objective of the study was accomplished through descriptive statistical analysis, while the second and third objectives involved inferential statistical analyses (e.g., One-Way ANOVA, Chi-Square, and Pearson correlation tests) using software such as Minitab® and OpenEpi.

5.2. Results and Discussions

The descriptive analysis of the study cohort shows a balanced distribution by gender, with a slight female predominance (51.55%), and a mean age of 65 years, indicating a predominantly elderly population. Almost half of the patients are in stage II (46.39%), with the majority of tumors being stenosing (72.16%) and of moderate size (mean length of 4.94 cm, mean thickness of 1.96 cm), distributed almost equally between the right and left colon. The tumors are predominantly conventional ADK (67.01%), with a significant presence of LVI (20.61%) and PNI (29.90%), and a predominance of the pMMR/MSS phenotype (83.61%) and KRAS mutations (47.17%), indicating aggressive tumor behavior. Analysis of the new HP biomarkers reveals significant tumor differentiation heterogeneity, with Category C (G2, G3) representing 38.14% of cases, and G2 being most prevalent. TIF is predominantly homogeneous (G2) in 52.58% of cases, while tumor stroma is mostly heterogeneous (93.81%), with a mean STR of 34.12%. The K-M score indicates minimal to mild inflammation (scores 0 – 1) in the majority, with low average TILs (14.88%), and approx. 40% of tumors exhibit moderate necrosis. PDCs score aligns statistically with the TB score, indicating high proliferative activity. Statistical analysis shows moderate correlations between tumor differentiation categories and TIF patterns ($p = 0.0001$), with G2 and G3 grades predominantly observed in TIF. Additionally, moderate correlation is seen between differentiation categories B, C, and D and high TB score (3) ($p = 0.000$), suggesting aggressive tumor behavior.

The inferential analysis revealed a weak correlation between the type of stroma and oncological stages ($p = 0.025$), with homogeneous stroma exclusively present in stage II, and a significant influence of stroma type on tumor length ($p = 0.033$), tumors with heterogeneous stroma having a greater average length. Additionally, a significant correlation was found between the type of stroma and the TB score ($p < 0.0000001$), with tumors scoring high (2 and 3) exclusively having heterogeneous stroma, and a moderate correlation with differentiation categories ($p = 0.010$). The percentage of STR increases progressively from stage II to stage IV ($p = 0.012$), associated with stenosing tumors ($p = 0.029$), heterogeneous differentiation G2-G3 ($p = 0.001$), mucinous histological subtype ($p = 0.033$), elevated TB score ($p = 0.000$), and PNI ($p = 0.014$). Conversely, a lower average percentage of STR correlates with the dMMR/MSI-H phenotype ($p = 0.000$). Tumors with a high K-M score (3 - moderate to strong inflammation) show a high percentage of STR and longer lengths, while the immune response (K-M score 2) is significantly influenced by the dMMR/MSI-H phenotype. Low lymphocytic infiltration is associated with PNI, whereas a high percentage of TILs (>20%) correlates with

the dMMR/MSI-H phenotype and necrosis, indicating a pro-inflammatory TME. Extensive necrosis (N3) is associated with strong inflammation and bacterial overgrowth. Additionally, there's a strong correlation between the PDCs score and the TB score, confirmed statistically ($p = 0.000$).

Statistical analysis revealed that 75.26% of cases exhibit a mosaic expression of CDX2 (strong, moderate, and negative), while 18.56% show negative and moderate expression. Tumors with strong and moderate CDX2 expression are exclusively found in stage II, while tumors with negative and moderate expression are present in all oncological stages, with their frequency increasing as the disease progresses. Category 1 tumors mostly show strong CDX2 expression (88.33%), Category 2 predominantly displays negative expression (63.06%), and Category 3 exhibits a mixed distribution of strong (47.12%) and moderate (41.71%) expressions, with variable negative expression (5 – 65%). Pattern analysis within Category 3 CDX2 revealed that Pattern 3A (strong and moderate intensities) was the most common (50.68%), followed by Pattern 3B, dominated by moderate expression (34.25%), while Patterns 3C and 3D, characterized by negative expression, were rare. Statistical analysis of the distribution of CDX2 expression patterns in TIF revealed the predominance of CDF1 (mosaic expression) and CDF3 (moderate and negative expression), each with a frequency of 38.14%, suggesting an aggressive tumor behavior. Statistical analysis of CDX2 expression in TB showed that the mosaic pattern prevails (43.30%), with negative CDX2 expression in 34.02% of cases, confirming its influence on tumor proliferation. The correlation between CDX2 categories and TB expression types is moderate, with most cases in CDX2 category 3 (74.32%) correlating with a BD3 score (60.27%), indicating pronounced proliferative activity. Colon tumors with negative and moderate CDX2 expression have a high average length (6.08 cm) and a balanced distribution between pMMR/MSS and dMMR/MSI-H phenotypes, while most category 3 tumors are associated with pMMR/MSS. There's also a moderate association between CDX2 categories and differentiation, indicating variability according to tumor differentiation heterogeneity. Statistical analysis reveals a strong association between CDX2 categories and stromal type, with tumors expressing CDX2 strongly and moderately having homogeneous stroma, while those with negative and mosaic CDX2 expression have heterogeneous stroma. Tumors with negative and moderate CDX2 expression exhibit a higher STR, and there's a weak association between extensive necrosis and negative and mosaic CDX2 categories.

6. Study 2: Correlations between Biomarkers and Disease Evolution

6.1. Introduction. Materials and Methods.

The central hypothesis of this study is that the panel of conventional HP and molecular biomarkers is insufficient for accurately predicting the prognosis of colon cancer, as it does not take into account tumor heterogeneity. We propose supplementing the conventional panel with new biomarkers that better reflect this heterogeneity, such as tumor differentiation categories, stroma, and tumor-associated immune cells, differentiation patterns in TIF, PDCs score, and IHC expression of CDX2 protein (in the tumor, in TIF, and in TB). Integration of these additional biomarkers could significantly improve prognostic prediction, providing a more precise risk stratification and guiding therapeutic decisions in a more efficient manner, thus contributing to treatment personalization and enhancing patient survival.

The first objective of this study is to investigate the correlations between conventional and newly proposed biomarkers and cancer progression, based on detailed oncological data of each participant included in the study. The analysis will involve evaluating each biomarker, both conventional and new, in relation to oncological follow-up parameters. These parameters include the presence or absence of disease progression, the type of progression (metastasis or local recurrence), progression-free survival interval (PFS rate, measured in days), types of metastases, and their number per patient. *The second objective of this study* is to assess the predictive capacity of the conventional HP and molecular biomarkers panel compared to an extended panel, which includes the newly proposed HP and molecular biomarkers. This approach aims to determine to what extent the integration of new biomarkers can improve the accuracy of prognostic prediction. Through this approach, new perspectives are opened up in the HP and molecular evaluation of colon tumors, with the potential to optimize the oncological management of patients with advanced colon cancer.

Out of the initial 97 cases included in the study, only 72 were analyzed in this study because not all participants were recorded in the oncological database of the hospital from which the cases were retrieved.

The study database integrated clinical and oncological follow-up parameters, with the results of both conventional and new biomarker analyses subjected to rigorous statistical analysis, including descriptive and inferential analyses (correlation tests and logistic regression tests) using Minitab® and OpenEpi software, to compare the predictive capacity of conventional and new HP and molecular biomarkers in colon cancer.

6.2. Results and Discussions

The descriptive analysis shows that approx. 56% of cases exhibited disease progression, mostly through metastasis (45.83%). The average duration of the PFS rate was approximately 432 days (15 months). Metastases were most commonly located in the liver (41.67%) and lungs (21.17%), with the majority of cases having a single metastasis (37%), while multiple metastases (up to 4) were rarely observed (19%). The analysis indicates a significant variation in PFS based on the number of metastases, with patients having a single metastasis exhibiting a shorter PFS rate compared to those with four metastases, suggesting the influence of the number and location of metastases on clinical outcomes. The disease progression rate significantly increases with the advancement of oncological stage, underscoring the need for more aggressive therapeutic strategies and rigorous monitoring in advanced stages ($p = 0.001$). Statistical analysis did not identify significant correlations between disease progression and demographic and clinical parameters (location, dimensions, and stenosing nature). Regarding conventional HP biomarkers, it was observed that mucinous ADK have a weak association with progression through metastasis ($p = 0.037$, Cramer = 0.091). On the other hand, tumors with LVI and PNI significantly correlate with disease progression ($p = 0.020$, $p = 0.003$), and additionally, tumors with PNI are more prone to metastasis ($p = 0.000$) compared to those with LVI (marginal $p = 0.061$). Regarding tumors concurrently presenting LVI and PNI, a significant association with disease progression was observed ($p = 0.026$). Despite the aforementioned correlations, LVI and PNI do not provide a robust predictive estimate of PFS ($p = 0.070$, $p = 0.108$). The TB score proved to be the strongest predictor of prognosis, showing a significant association with disease progression ($p = 0.000$, Cramer = 0.260). Regarding conventional molecular biomarkers, only MMR/MSI status demonstrated a significant correlation with disease progression ($p = 0.018$), with patients exhibiting dMMR/MSI-H phenotype having an exclusively favorable prognosis. In clinical settings, colon tumors are typically classified according to a single grade of differentiation. However, statistical analysis indicates that this method is inefficient, as there is no significant association between the assigned grades of differentiation and disease progression ($p = 0.399$). In contrast, the use of differentiation categories, reflecting tumor heterogeneity, has proven to be much more effective in predicting prognosis ($p = 0.007$). Regarding the TME, the study found a statistically significant correlation between the type of stroma and disease progression ($p = 0.021$), with patients having homogeneous tumors exhibiting an exclusively favorable prognosis. Additionally, it was found that tumors with a high STR (approx. 43%) often exhibit disease progression ($p = 0.000$). The

analysis revealed a significant correlation between tumors with low K-M scores (0 and 1) and disease progression (p = 0.000). Moreover, patients with a high percentage of TILs (>19%) have a favorable prognosis compared to those with low lymphocytic infiltration (approx. 11%) (p = 0.000). Statistical analysis of the CDX2 biomarker reveals a significant correlation between CDX2 expression categories and disease progression (p = 0.030), indicating that tumors with predominantly strong expression are associated with a favorable prognosis, supported by the role of the CDX2 protein in tumor suppression [50]. Although there is no significant correlation between CDX2 categories and PFS rate, tumors with mosaic expression exhibit a higher PFS rate (approx. 468 days) compared to predominantly negative ones (approx. 291 days). Metastatic progression is significantly associated with CDX2 categories (p = 0.042), with tumors predominantly expressing negative CDX2 having the highest metastatic rate (66.67%). These data highlight the potential of CDX2 categories to identify patients at high risk of metastasis. Regarding the expression patterns of CDX2 in category 3, no significant correlation with disease progression was identified, possibly due to the small size of the sample. In the context of intratumoral spatial heterogeneity, statistical analysis has revealed a strong correlation between CDX2 expression patterns in TIF and disease progression (p = 0.00002165), as well as between tumor differentiation patterns in TIF and disease progression (p = 0.00006174). Patterns of altered CDX2 expression (moderate and negative) and poor differentiation (G2, G3) in TIF are associated with disease progression. Additionally, a strong correlation was identified between CDX2 expression patterns in TB and disease progression (p = 0.000), with mosaic and negative expression associated with aggressive proliferation. These results underscore the importance of separately assessing TIF from the central region of the tumor, as TIF morphology provides precise information about tumor behavior. The comparative analysis of two logistic regression models for prognostic prediction demonstrates the superiority of Model 2, which integrates both conventional and new biomarkers. Model 1, based solely on conventional biomarkers, identifies only the TB score as a significant predictor (p = 0.001). In contrast, Model 2 highlights several biomarkers with significant predictive impact, most of which are new, such as STR (p = 0.000), TILs (p = 0.005), differentiation categories (p = 0.047), CDX2 expression patterns in TB (p = 0.048), and in TIF (p = 0.054). The superior performance of Model 2 is confirmed by an R-sq index of 89.17% and an R-sq (adj) of 81.24%, compared to the lower values of Model 1. These results underscore the ability of Model 2 to capture the complexity and variability of factors influencing disease progression, providing a more accurate prediction of prognosis in colon cancer and confirming the need for the addition of new biomarkers for accurate prediction.

7. Conclusions and Personal Contributions

This study aimed to address a series of scientific research objectives to contribute to a comprehensive understanding of the HP and molecular alterations in advanced colon cancer. Within this context, the objectives were formulated to allow for a detailed and rigorous exploration of the subject, utilizing appropriate methods and tools to generate relevant and significant data. The analysis of the obtained results clearly demonstrated that the proposed objectives were substantially achieved.

The study fulfilled the first objective by evaluating new biomarkers and analyzing their interaction with demographic, clinical parameters, and conventional biomarkers, demonstrating their relevance in the prognosis of advanced colon cancer. *The second objective*, regarding the correlation of biomarkers with oncological follow-up parameters, showed that the new biomarkers offer significantly superior correlations compared to conventional ones. *The third objective*, creating and comparing two prediction models, highlighted the superiority of the extended model that includes the new biomarkers, providing a much more precise prognosis prediction (R-sq = 89.17%, adjusted R-sq = 81.24%), confirming the hypothesis that integrating new biomarkers improves prediction accuracy and optimizes oncological management.

Further research should involve robust multicenter studies with larger samples to validate and generalize the findings and ensure the robustness of the results and a better understanding of inter-tumoral variability. Standardizing methodologies for the evaluation and interpretation of the proposed biomarkers, including CDX2 protein, is essential to facilitate their integration into clinical practice and to develop personalized and effective treatments, given their demonstrated efficiency.

This study makes significant contributions to the clinical practice of oncology and pathology by addressing intra-tumoral and spatial heterogeneity in evaluating colon cancer biomarkers. Developing innovative assessment systems based on categorical scores and including TIF as a distinct entity enables a more precise prediction of tumor behavior and patient prognosis, demonstrating a more accurate correlation with disease progression compared to conventional methods. The introduction of the CDX2 protein as an independent molecular prognostic biomarker, cost-effective and accessible, along with the improved prognostic prediction model, highlights the potential of new biomarkers to optimize oncological management in advanced colon cancer.

Selective Bibliography

[1] International Agency for Research on Cancer. GLOBOCAN World Cancer Fact Sheet. Lyon: 2022.

[2] International Agency for Research on Cancer. GLOBOCAN World Incidence, Males and Females, Colon Cancer Fact Sheet. Lyon: 2022.

[3] International Agency for Research on Cancer. GLOBOCAN High Income vs Low Income Mortality, Males and Females, Cancer Fact Sheet. Lyon: 2022.

[4] International Agency for Research on Cancer. GLOBOCAN Europe Cancer Fact Sheet. Lyon: 2022.

[5] International Agency for Research on Cancer. GLOBOCAN Europe Incidence and Mortality, Males and Females, Colon Cancer Fact Sheet. Lyon: 2022.

[6] International Agency for Research on Cancer. GLOBOCAN Romania Cancer Fact Sheet. Lyon: 2022.

[7] International Agency for Research on Cancer. GLOBOCAN Romania Mortality, Both Sexes, Cancer Fact Sheet. Lyon: 2022.

[8] Buikhuisen JY, Torang A, Medema JP. Exploring and modelling colon cancer inter-tumour heterogeneity: opportunities and challenges. *Oncogenesis*, 9(7):66, 2020. https://doi.org/10.1038/s41389-020-00250-6.

[9] Dawson H, Kirsch R, Messenger D, Driman D. A review of current challenges in colorectal cancer reporting. *Arch Pathol Lab Med*, 143:869–82, 2019. https://doi.org/10.5858/arpa.2017-0475-RA.

[10] Chen K, Collins G, Wang H, Toh JWT. Pathological features and prognostication in colorectal cancer. *Curr Oncol,* 28:5356–83, 2021. https://doi.org/10.3390/curroncol28060447.

[11] Testa U, Castelli G, Pelosi E. Genetic alterations of metastatic colorectal cancer. *Biomedicines,* 8:1–29, 2020. https://doi.org/10.3390/biomedicines8100414.

[12] Badia-Ramentol J, Gimeno-Valiente F, Dur E, Martínez-Ciarpaglini C, Linares J, Iglesias M. The prognostic potential of CDX2 in colorectal cancer : Harmonizing

biology and clinical practice. *Cancer Treat Rev*, 121:102643, 2023. https://doi.org/10.1016/j.ctrv.2023.102643.

[13] Koveitypour Z, Panahi F, Vakilian M, Peymani M, Forootan FS. Signaling pathways involved in colorectal cancer progression. *Cell Biosci,* 9(1):1–14. 2019. https://doi.org/10.1186/s13578-019-0361-4.

[14] Sagaert X, Vanstapel A, Verbeek S. Tumor Heterogeneity in Colorectal Cancer: What Do We Know So Far? *Pathobiology*, 85:72–84, 2018. https://doi.org/10.1159/000486721.

[15] Molinari C, Marisi G, Passardi A, Matteucci L, De Maio G, Ulivi P. Heterogeneity in colorectal cancer: A challenge for personalized medicine? *Int J Mol Sci*, 19(12):3733, 2018. https://doi.org/10.3390/ijms19123733.

[16] Blank A, Roberts DE, Dawson H, Zlobec I, Lugli A. Tumor heterogeneity in primary colorectal cancer and corresponding metastases. Does the apple fall far from the tree? *Front Med*, 5:234, 2018. https://doi.org/10.3389/fmed.2018.00234.

[17] Ramón y Cajal S, Sesé M, Capdevila C, Aasen T, De Mattos-Arruda L, Diaz-Cano SJ, et al. Clinical implications of intratumor heterogeneity: challenges and opportunities. *J Mol Med*, 98:161–77, 2020. https://doi.org/10.1007/s00109-020-01874-2.

[18] Colangelo T, Polcaro G, Muccillo L, D'Agostino G, Rosato V, Ziccardi P, et al. Friend or foe?: The tumour microenvironment dilemma in colorectal cancer. *Biochim Biophys Acta Rev Cancer*, 1867:1–18, 2017. https://doi.org/10.1016/j.bbcan.2016.11.001.

[19] Chen Y, Zheng X, Wu C. The Role of the Tumor Microenvironment and Treatment Strategies in Colorectal Cancer. *Front Immunol*, 12:792691, 2021. https://doi.org/10.3389/fimmu.2021.792691.

[20] Issac R, Masih D, Ranjan M. Primary adenocarcinoma of colon: A clinicopathological study with the prevalence and correlation of CDX2 biomarker expression. *Indian J Cancer*, 60(1):45–50, 2023. https://doi.org/10.4103/ijc.ijc_760_21.

[21] Çalık İ, Çalık M, Özercan İH, Dağlı AF, Artaş G, Türken G, et al. Decreased CDX2 expression adversely affects the prognosis of patients with colorectal cancer. *Dicle Tıp Dergisi*, 47(1):57–65, 2020. https://doi.org/10.5798/dicletip.706005.

[22] Asgari-Karchekani S, Karimian M, Mazoochi T, Taheri MA. CDX2 Protein Expression in Colorectal Cancer and Its Correlation with Clinical and Pathological Characteristics, Prognosis, and Survival Rate of Patients. *J Gastrointest Cancer,* 51(3):844–849, 2019. https://doi.org/10.1007/s12029-019-00314-w.

[23] **Ilie-Petrov A-C**, Cristian D-A, Diaconescu AS, Chitul A, Blajin A, Popa A, et al. Molecular Deciphering of Colorectal Cancer: Exploring Molecular Classifications and Analyzing the Interplay among Molecular Biomarkers MMR/MSI, KRAS, NRAS, BRAF and CDX2 - A Comprehensive Literature Review. *Chirurgia*, 119:136, 2024. https://doi.org/10.21614/chirurgia.2024.v.119.i.2.p.136.

[24] Banias L, Jung I, Chiciudean R, Gurzu S. From Dukes-MAC Staging System to Molecular Classification: Evolving Concepts in Colorectal Cancer. *Int J Mol Sci*, 23(16):9455, 2022. https://doi.org/10.3390/ijms23169455.

[25] Lea D, Haland S, Hagland HR, Soreide K. Accuracy of TNM staging in colorectal cancer: A review of current culprits, the modern role of morphology and stepping-stones for improvements in the molecular era. *Scand J Gastroenterol,* 49:1153–63, 2014. https://doi.org/10.3109/00365521.2014.950692.

[26] American Joint Committee of Cancer. *AJCC Cancer Staging Manual*. Eighth Edition. American College of Surgeons, Chicago, 2018.

[27] Argilés G, Tabernero J, Labianca R, Hochhauser D, Salazar R, Iveson T, et al. Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology,* 31:1291–305, 2020. https://doi.org/10.1016/j.annonc.2020.06.022.

[28] Cervantes A, Adam R, Roselló S, Arnold D, Normanno N, Taïeb J, et al. Metastatic colorectal cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Annals of Oncology*, 34:10–32, 2023. https://doi.org/10.1016/j.annonc.2022.10.003.

[29] Benson AB, Adam M, Chen Y-J, Ciombor KK, Cohen S, Deming D, et al. *NCCN Guidelines.* Version 1.2024. Colon Cancer Continue NCCN Guidelines Panel Disclosures, Plymouth Meeting, 2024.

[30] Foersch S, Lang-Schwarz C, Eckstein M, Geppert C, Schmitt M, Konukiewitz B, et al. pT3 colorectal cancer revisited: a multicentric study on the histological depth of invasion in more than 1000 pT3 carcinomas—proposal for a new pT3a/pT3b subclassification. *Br J Cancer*, 127:1270–8, 2022. https://doi.org/10.1038/s41416-022-01889-1.

[31] Merkel S, Brunner M, Geppert CI, Grützmann R, Weber K, Agaimy A. Proposal of a T3 Subclassification for Colon Carcinoma. *Cancers*, 14(24):6186, 2022. https://doi.org/10.3390/cancers14246186.

[32] Athanasakis E, Xenaki S, Venianaki M, Chalkiadakis G, Chrysos E. Newly recognized extratumoral features of colorectal cancer challenge the current tumor-node-metastasis staging system. *Ann Gastroenterol,* 31:525–34, 2018. https://doi.org/10.20524/aog.2018.0284.

[33] Johncilla M, Yantiss RK. Histology of Colorectal Carcinoma: Proven and Purported Prognostic Factors. *Surg Pathol Clin*, 13:503–20, 2020. https://doi.org/10.1016/j.path.2020.05.008.

[34] Remo A, Fassan M, Vanoli A, Bonetti LR, Barresi V, Tatangelo F, et al. Morphology and molecular features of rare colorectal carcinoma histotypes. *Cancers*, 11(7):1036, 2019. https://doi.org/10.3390/cancers11071036.

[35] Nagtegaal ID, Arends MJ, Odze RD. *WHO Classification of Tumors*. 5th Edition. International Agency for Research on Cancer, Lyon, 2019.

[36] Lee C-T, Huang Y-C, Hung L-Y, Chow N-H, Su P-F, Ho C-L, et al. Serrated adenocarcinoma morphology in colorectal mucinous adenocarcinoma is associated with improved patient survival. *Oncotarget,* 8(21):35165–35175, 2017. https://doi.org/10.18632/oncotarget.16820.

[37] Zlobec I, Dawson HE, Blank A, Bokhorst JM, Berger MD, Nagtegaal ID, et al. Are tumour grade and tumour budding equivalent in colorectal cancer? A retrospective analysis of 771 patients. *Eur J Cancer,* 130:139–45, 2020. https://doi.org/10.1016/j.ejca.2020.02.007.

[38] Ueno H, Ishiguro M, Nakatani E, Ishikawa T, Uetake H, Matsui S, et al. Optimal Criteria for G3 (Poorly Differentiated) Stage II Colon Cancer Prospective Validation in a

Randomized Controlled Study (SACURA Trial). *Am J Surg Pathol*, 44(12):1740–1750, 2020. https://doi.org/10.1097/PAS.0000000000001570.

[39] Chen K, Wang H, Collins G, Hollands E, Law IYJ, Toh JWT. Current Perspectives on the Importance of Pathological Features in Prognostication and Guidance of Adjuvant Chemotherapy in Colon Cancer. *Current Oncology,* 29:1370–89, 2022. https://doi.org/10.3390/curroncol29030116.

[40] Ueno H, Hase K, Hashiguchi Y, Shimazaki H, Tanaka M, Miyake O, et al. Site-specific Tumor Grading System in Colorectal Cancer Multicenter Pathologic Review of the Value of Quantifying Poorly Differentiated Clusters. *Am J Surg Pathol*, 38(2):197–204, 2014. https://doi.org/10.1097/PAS.0000000000000137.

[41] Johncilla M, Chen Z, Sweeney J, Yantiss RK. Tumor Grade Is Prognostically Relevant Among Mismatch Repair Deficient Colorectal Carcinomas. *Am J Surg Pathol*, 42(12):1686–1692, 2018. https://doi.org/10.1097/PAS.0000000000001161.

[42] Neelakantan D, Drasin DJ, Ford HL. Intratumoral heterogeneity: Clonal cooperation in epithelial-to-mesenchymal transition and metastasis. *Cell Adh Migr*, 9:265–76, 2015. https://doi.org/10.4161/19336918.2014.972761.

[43] Gerashchenko TS, Novikov NM, Krakhmal NV, Zolotaryova SY, Zavyalova MV, Cherdyntseva NV, et al. Markers of cancer cell invasion: Are they good enough? *J Clin Med,* 8(8):1092, 2019. https://doi.org/10.3390/jcm8081092.

[44] Cho SJ, Kakar S. Tumor budding in colorectal carcinoma: Translating a morphologic score into clinically meaningful results. *Arch Pathol Lab Med*, 142(8):952–957, 2018. https://doi.org/10.5858/arpa.2018-0082-RA.

[45] Punt CJA, Koopman M, Vermeulen L. From tumour heterogeneity to advances in precision treatment of colorectal cancer. *Nat Rev Clin Oncol,* 14:235–46, 2017. https://doi.org/10.1038/nrclinonc.2016.171.

[46] Büttner J, Jöhrens K, Klauschen F, Hummel M, Lenze D, Saeger W, et al. Intratumoral morphological heterogeneity can be an indicator of genetic heterogeneity in colorectal cancer. *Exp Mol Pathol,* 104:76–81, 2018. https://doi.org/10.1016/j.yexmp.2018.01.007.

[47] Chan DKH, Buczacki SJA. Tumour heterogeneity and evolutionary dynamics in colorectal cancer. *Oncogenesis*, 10:53, 2021. https://doi.org/10.1038/s41389-021-00342-x.

[48] Riffet M, Dupont B, Faisant M, Cerasuolo D, Menahem B, Alves A, et al. New histoprognostic factors to consider for the staging of colon cancers: tumor deposits, invasive tumor infiltration and high-grade budding. *Int J Mol Sci*, 24(4):3573, 2023. https://doi.org/10.3390/ijms24043573.

[49] Konishi T, Shimada Y, Lee LH, Cavalcanti MS, Hsu M, Smith JJ, et al. Poorly differentiated clusters predict colon cancer recurrence: An in-depth comparative analysis of invasive-front prognostic markers. *Am J Surg Pathol*, 42(6):705–714, 2018. https://doi.org/10.1097/PAS.0000000000001059.

[50] Wu L, Yan J, Bai Y, Chen F, Xu J, Zou X, et al. Spatially-resolved transcriptomics analyses of invasive fronts in solid tumors. *bioRxiv*, 10(21):465335, 2021. https://doi.org/10.1101/2021.10.21.465135.

[51] Sandberg TP, Stuart MPME, Oosting J, Tollenaar RAEM, Sier CFM, Mesker WE. Increased expression of cancer-associated fibroblast markers at the invasive front and its association with tumor-stroma ratio in colorectal cancer. *BMC Cancer*, 19(1):284, 2019. https://doi.org/10.1186/s12885-019-5462-2.

[52] Sullivan L, Pacheco RR, Kmeid M, Chen A, Lee H. Tumor Stroma Ratio and Its Significance in Locally Advanced Colorectal Cancer. *Curr Oncol*, 29:3232–41, 2022. https://doi.org/10.3390/curroncol29050263.

[53] Gao J, Shen Z, Deng Z, Mei L. Impact of Tumor–Stroma Ratio on the Prognosis of Colorectal Cancer: A Systematic Review. *Front Oncol*, 11:738080, 2021. https://doi.org/10.3389/fonc.2021.738080.

[54] Flam J, Gugić D, Benšić M, Tomić S, Rajc J. High tumor stroma proportion is a worse prognostic factor in colorectal cancer. *Acta Clin Croat*, 56:73–9, 2017. https://doi.org/10.20471/acc.2017.56.01.11.

[55] Najafi M, Mortezaee K, Majidpoor J. Stromal reprogramming: A target for tumor therapy. *Life Sci*, 239:117049, 2019. https://doi.org/10.1016/j.lfs.2019.117049.

[56] Ueno H, Kanemitsu Y, Sekine S, Ishiguro M, Ito E, Hashiguchi Y, et al. Desmoplastic pattern at the tumor front defines poor-prognosis subtypes of colorectal cancer. *Am J Surg Pathol*, 41(11):1506–1512, 2017. https://doi.org/10.1097/PAS.0000000000000946.

[57] Schmitt M, Greten FR. The inflammatory pathogenesis of colorectal cancer. *Nat Rev Immunol*, 21:653–67, 2021. https://doi.org/10.1038/s41577-021-00534-x.

[58] Zheng W, Wu J, Peng Y, Sun J, Cheng P, Huang Q. Tumor-Associated Neutrophils in Colorectal Cancer Development, Progression and Immunotherapy. *Cancers,* 14(19):4755, 2022. https://doi.org/10.3390/cancers14194755.

[59] Wang K, Karin M. Tumor-Elicited Inflammation and Colorectal Cancer. *Adv Cancer Res*, 128: 173–96, 2015. https://doi.org/10.1016/bs.acr.2015.04.014.

[60] Mizuno R, Kawada K, Itatani Y, Ogawa R, Kiyasu Y, Sakai Y. The role of tumor-associated neutrophils in colorectal cancer. *Int J Mol Sci,* 20(3):529, 2019. https://doi.org/10.3390/ijms20030529.

[61] Inagaki K, Kunisho S, Takigawa H, Yuge R, Oka S, Tanaka S, et al. Role of tumor-associated macrophages at the invasive front in human colorectal cancer progression. *Cancer Sci*, 112:2692–704, 2021. https://doi.org/10.1111/cas.14940.

[62] Wikberg ML, Ling A, Li X, Öberg Å, Edin S, Palmqvist R. Neutrophil infiltration is a favorable prognostic factor in early stages of colon cancer. *Hum Pathol*, 68:193–202, 2017. https://doi.org/10.1016/j.humpath.2017.08.028.

[63] Jakubowska K, Koda M, Grudzińska M, Kisielewski W, Lomperta K, Famulski W. Neutrophil infiltration combined with necrosis in the primary tumor is a useful prognostic indicator for three-year disease-free survival time in patients with colorectal cancer. *Oncol Lett,* 23(6):199, 2022. https://doi.org/10.3892/ol.2022.13320.

[64] Berntsson J, Nodin B, Eberhard J, Micke P, Jirström K. Prognostic impact of tumour-infiltrating B cells and plasma cells in colorectal cancer. *Int J Cancer*, 139:1129–39, 2016. https://doi.org/10.1002/ijc.30138.

[65] Bai Z, Zhou Y, Ye Z, Xiong J, Lan H, Wang F. Tumor-Infiltrating Lymphocytes in Colorectal Cancer: The Fundamental Indication and Application on Immunotherapy. *Front Immunol,* 12:808964, 2022. https://doi.org/10.3389/fimmu.2021.808964.

[66] Reichman H, Itan M, Rozenberg P, Yarmolovski T, Brazowski E, Varol C, et al. Activated eosinophils exert antitumorigenic activities in colorectal cancer. *Cancer Immunol Res,* 7:388–400, 2019. https://doi.org/10.1158/2326-6066.CIR-18-0494.

[67] Ye L, Zhang T, Kang Z, Guo G, Sun Y, Lin K, et al. Tumor-infiltrating immune cells act as a marker for prognosis in colorectal cancer. *Front Immunol*, 10:2368, 2019. https://doi.org/10.3389/fimmu.2019.02368.

[68] Ahluwalia P, Ballur K, Leeman T, Vashisht A, Singh H, Omar N, et al. Incorporating Novel Technologies in Precision Oncology for Colorectal Cancer : Advancing Personalized Medicine. *Cancers*, 16(3):480, 2024. https://doi.org/10.3390/cancers16030480.

[69] Koulis C, Yap R, Engel R, Jardé T, Wilkins S, Solon G, et al. Personalized Medicine—Current and Emerging Predictive and Prognostic Biomarkers in Colorectal Cancer. *Cancers,* 12(4):812, 2020. https://doi.org/10.3390/cancers12040812.

[70] Baraibar I, Ros J, Mulet N, Salvà F, Argilés G, Martini G, et al. Incorporating traditional and emerging biomarkers in the clinical management of metastatic colorectal cancer: an update. *Expert Rev Mol Diagn*, 23:231–41, 2023. https://doi.org/10.1080/14737159.2020.1782194.

[71] Li K, Luo H, Huang L, Luo H, Zhu X. Microsatellite instability: a review of what the oncologist should know. *Cancer Cell Int,* 20:16, 2020. https://doi.org/10.1186/s12935-019-1091-8.

[72] Nojadeh JN, Sharif SB, Sakhinia E. Microsatellite instability in colorectal cancer. *EXCLI J*, 17:159–168, 2018. https://doi.org/10.17179/excli2017-948.

[73] Toh JWT, Phan K, Reza F, Chapuis P, Spring KJ. Rate of dissemination and prognosis in early and advanced stage colorectal cancer based on microsatellite instability status: systematic review and meta-analysis. *Int J Colorectal Dis*, 36:1573–96, 2021. https://doi.org/10.1007/s00384-021-03874-1.

[74] Afrăsânie VA, Marinca MV, Alexa-Stratulat T, Gafton B, Păduraru M, Adavidoaiei AM, et al. KRAS, NRAS, BRAF, HER2 and microsatellite instability in metastatic colorectal cancer - practical implications for the clinician. *Radiol Oncol,* 53(3):265–74, 2019. https://doi.org/10.2478/raon-2019-0033.

[75] Maffeis V, Nicolè L, Cappellesso R. RAS, Cellular Plasticity, and Tumor Budding in Colorectal Cancer. *Front Oncol*, 9:1255, 2019. https://doi.org/10.3389/fonc.2019.01255.

[76] Koncina E, Haan S, Rauh S, Letellier E. Prognostic and Predictive Molecular Biomarkers for Colorectal Cancer: Updates and Challenges. *Cancers,* 12:1–25, 2020. https://doi.org/10.3390/cancers12020319.

[77] Chung C. Predictive and prognostic biomarkers with therapeutic targets in colorectal cancer: A 2021 update on current development, evidence, and recommendation. *J Oncol Pharm Pract*, 28:10781552211005525, 2021. https://doi.org/10.1177/10781552211005525.

[78] Bellio H, Fumet JD, Ghiringhelli F. Targeting BRAF and RAS in Colorectal Cancer. *Cancers*, 13(9):2201, 2021. https://doi.org/10.3390/cancers13092201.

[79] Caputo F, Santini C, Bardasi C, Cerma K, Casadei-Gardini A, Spallanzani A, et al. BRAF-Mutated Colorectal Cancer: Clinical and Molecular Insights. *Int J Mol Sci*, 20(21):5369, 2019. https://doi.org/10.3390/ijms20215369.

[80] Molina-Cerrillo J, San Román M, Pozas J, Alonso-Gordoa T, Pozas M, Conde E, et al. BRAF Mutated Colorectal Cancer: New Treatment Approaches. *Cancers*, 12(6):1571, 2020. https://doi.org/10.3390/cancers12061571.

[81] Bae JM, Lee TH, Cho NY, Kim TY, Kang GH. Loss of CDX2 expression is associated with poor prognosis in colorectal cancer patients. *World J Gastroenterol*, 21:1457–67, 2015. https://doi.org/10.3748/wjg.v21.i5.1457.

[82] Tomasello G, Barni S, Turati L, Ghidini M, Pezzica E, Passalacqua R, et al. Association of CDX2 Expression With Survival in Early Colorectal Cancer: A Systematic Review and Meta-analysis. *Clin Colorectal Cancer*, 17:97–103, 2018. https://doi.org/10.1016/j.clcc.2018.02.001.

[83] Balbinot C, Armant O, Elarouci N, Marisa L, Martin E, de Clara E, et al. The Cdx2 homeobox gene suppresses intestinal tumorigenesis through non-cell-autonomous mechanisms. *J Exp Med*, 215:911–26, 2018. https://doi.org/10.1084/jem.20170934.

[84] Gan GL, Liu J, Chen WJ, Ye QQ, Xu Y, Wu HT, et al. The Diverse Roles of the Mucin Gene Cluster Located on Chromosome 11p15.5 in Colorectal Cancer. *Front Cell Dev Biol*, 8:514, 2020. https://doi.org/10.3389/fcell.2020.00514.

[85] Singh MP, Rai S, Pandey A, Singh NK, Srivastava S. Molecular subtypes of colorectal cancer: An emerging therapeutic opportunity for personalized medicine. *Genes Dis,* 8:133–45, 2021. https://doi.org/10.1016/j.gendis.2019.10.013.

[86] El-Refaey HA, Bedeer AE, Shoeir HT, Ghoraba HM. Histopathological and Immunohistochemical Study of the Prognostic Significance of Cox2 and CDX2 Expression in the Available Cases of Colorectal Carcinoma. *Med J Cairo Univ*, 87:71–80, 2019. https://doi.org/10.21608/mjcu.2019.52323.

[87] Tóth C, Sükösd F, Valicsek E, Herpel E, Schirmacher P, Tiszlavicz L. Loss of CDX2 gene expression is associated with DNA repair proteins and is a crucial member of the Wnt signaling pathway in liver metastasis of colorectal cancer. *Oncol Lett*, 15(3):3586–3593, 2018. https://doi.org/10.3892/ol.2018.7756.

[88] Slik K, Turkki R, Carpén O, Kurki S, Korkeila E, Sundström J, et al. CDX2 loss with microsatellite stable phenotype predicts poor clinical outcome in stage II colorectal carcinoma. *Am J Surg Pathol,* 43(11):1473–1482, 2019. https://doi.org/10.1097/PAS.0000000000001356.

[89] Olsen J, Espersen MLM, Jess P, Kirkeby LT, Troelsen JT. The clinical perspectives of CDX2 expression in colorectal cancer: a qualitative systematic review. *Surg Oncol*, 23:167–76, 2014. https://doi.org/10.1016/j.suronc.2014.07.003.

[90] Hansen TF, Kjær-Frifeldt S, Eriksen AC, Lindebjerg J, Jensen LH, Sørensen FB, et al. Prognostic impact of CDX2 in stage II colon cancer: results from two nationwide cohorts. *Br J Cancer,* 119:1367–73, 2018. https://doi.org/10.1038/s41416-018-0285-5.

[91] Chewchuk S, Jahan S, Lohnes D. Cdx2 regulates immune cell infiltration in the intestine. *Sci Rep*, 11:1–12, 2021. https://doi.org/10.1038/s41598-021-95412-w.

[92] **Ilie-Petrov A-C**, Cristian D-A, Grama FA, Chitul A, Blajin A, Popa A, et al. Evaluation of the Immunohistochemical Scoring System of CDX2 Expression as a Prognostic Biomarker in Colon Cancer. *Diagnostics,* 14:1023, 2024. https://doi.org/10.3390/diagnostics14101023.

List of Published Works

1) **Ilie-Petrov, A.-C.**, Cristian, D.-A., Grama, F.A., Chitul, A., Blajin, A., Popa, A., Mandi, D.-M., Welt, L., Bara, M.A., Vrîncianu, R., and Ardeleanu, C.M. „Evaluation of the Immunohistochemical Scoring System of CDX2 Expression as a Prognostic Biomarker in Colon Cancer". *Diagnostics*. 2024; Vol. 14, no. 10: 1023. An ISI-indexed journal, with an impact factor of 3.6. https://doi.org/10.3390/diagnostics14101023 (original research article derived from Chapter 2).
2) **Ilie-Petrov, A.-C.**, Cristian, D.-A., Diaconescu, A.S., Chitul, A., Blajin, A., Popa, A., Mandi, D.-M., Negreanu, R., Vieru, C., Vrîncianu, R., and Ardeleanu, C.M. „Molecular Deciphering of Colorectal Cancer: Exploring Molecular Classifications and Analyzing the Interplay among Molecular Biomarkers MMR/MSI, KRAS, NRAS, BRAF and CDX2 - A Comprehensive Literature Review". *Chirurgia*. 2024; Vol. 119, no. 2: 136–155. An ISI-indexed journal, with an impact factor of 0.6. https://doi.org/10.21614/chirurgia.2024.v.119.i.2.p.136 (literature review derived from Chapter 2).