

SIGNIFICANCE OF BIOMARKERS IN INTESTINAL DISORDERS AND CANCERS

Abstract

This chapter explores the magnitude of biomarkers in understanding, diagnosing, and treating intestinal cancers and disorders. It mainly focuses on intestine-related cancers like colorectal cancer, small intestine cancer, and intestinal disorders, such as inflammatory bowel disease (IBD). The specific biomarkers related to intestinal cancers (with a particular emphasis on colorectal cancer) and disorders (especially IBD) are discussed. Furthermore, genetic biomarkers like KRAS, BRAF, TP53, and APC mutations are examined, along with epigenetic biomarkers such as DNA methylation and histone modifications. Additionally, protein biomarkers like CEA, Ki-67, and p53 are highlighted, each offering valuable insights into disease development, prognosis, and treatment response. Future perspectives and challenges surrounding biomarkers in intestinal cancers and disorders are then explored. Precision medicine, liquid biopsies, multi-omics integration, early detection and screening, prognostic and predictive biomarkers, and challenges related to validation, standardization, cost, accessibility, ethical considerations, biomarker combinations, and the heterogeneity of intestinal cancers and disorders are addressed.

Keywords: Inflammatory bowel disease; Multi-omics; Diagnosis; Epigenetics; Disease development; Biopsy.

Authors

Muskan Verma

Clinical Biochemistry Lab
Department of Biochemistry
School of Chemical and Life Sciences
Jamia Hamdard, New Delhi-110062, India.

Dr. Asghar Ali

Clinical Biochemistry Lab
Department of Biochemistry
School of Chemical and Life Sciences
Jamia Hamdard, New Delhi-110062, India.
asgharali@jamiahamdard.ac.in

Dr. Mohan Kamthan

Clinical Biochemistry Lab
Department of Biochemistry
School of Chemical and Life Sciences
Jamia Hamdard, New Delhi-110062, India.
mohan.kamthan@jamiahamdard.ac.in

I. INTRODUCTION

Biomarkers, also known as biological markers, are crucial in modern medicine and clinical research. They serve as objective indicators that can be accurately and consistently measured, allowing us to assess various biological processes, disease states, and treatment responses. The definitions of biomarkers from various sources highlight their broad scope and significance. According to the National Institutes of Health (NIH), biomarkers are characteristics that indicate a normal biological process, pathogenic processes, or responses to therapeutic interventions [1]. The International Programme on Chemical Safety, in collaboration with World Health Organization (WHO), defines biomarkers as structures, substances, or processes that can be measured in the body or its products which predict disease incidence or outcomes. A broader definition considers the effects of treatments, interventions, and environmental exposures, recognizing the complex interactions between biological systems and potential hazards.

Biomarkers encompass a wide range of measurements, from basic physiological indicators like blood pressure and pulse to more complex laboratory tests from tissues and blood. Biomarkers have a previous history in clinical practice, representing the most objective and quantifiable medical signs that modern laboratory science allows us to measure reproducibly [2]. In clinical research, biomarkers, especially laboratory-measured ones, are increasingly prevalent. However, establishing the significance and implications of biomarker findings in clinical settings remains a critical challenge [3].

Biomarkers play a crucial role in the detection, diagnosis, prognosis, and management of various diseases, including intestinal cancer. Intestinal cancer, including colon and rectum cancers (colorectal cancer), is a significant health concern worldwide [3]. The use of biomarkers in intestinal cancer provides valuable insights and benefits for both patients and clinicians. Some key significance for studying biomarkers in intestinal cancer is that biomarkers can be indicative of the presence of cancer even at its early stages when symptoms may not be apparent. Screening for specific biomarkers can aid in the early detection and diagnosis of intestinal cancer, enabling prompt intervention and potentially better treatment outcomes. Certain biomarkers can help in predicting the aggressiveness of cancer and the likelihood of disease progression. This information can guide clinicians in tailoring personalized treatment plans and surveillance strategies for individual patients. Biomarkers can provide insights into the molecular characteristics of cancer, making it easier to select appropriate treatments. Targeted therapies that specifically address the identified biomarkers can improve treatment efficacy while reducing unnecessary side effects. They can serve as indicators of treatment response. Regular monitoring of biomarker levels during and after treatment can help assess the effectiveness of the chosen therapy and allow for timely adjustments if necessary. Some biomarkers can indicate the risk of cancer recurrence after initial treatment. Identifying patients at high risk of recurrence can prompt more intensive monitoring and follow-up care. They can provide insight into why certain patients may not respond well to standard treatments [2]. Understanding the underlying reasons for therapeutic resistance can aid in the development of alternative or combination therapies. Studying biomarkers in intestinal cancer can lead to a better understanding of the disease's underlying biology and its molecular mechanisms. This knowledge can drive the development of novel targeted therapies and improve overall treatment strategies. Biomarkers can potentially contribute to cost-effective healthcare by aiding in targeted screening, minimizing

unnecessary interventions, and optimizing treatment choices based on individual patient profiles.

The purpose and scope of this chapter are to explore the role and significance of biomarkers in the context of intestinal-related cancers and disorders. The chapter aims to provide a comprehensive understanding of how biomarkers can aid in the diagnosis, prognosis, treatment selection, and monitoring of patients with intestinal cancers (with a particular emphasis on colorectal cancer) and various intestinal disorders (IBD, IBS, and gastrointestinal stromal tumors). Overall, the chapter aims to serve as a valuable resource for researchers, clinicians, and healthcare professionals involved in the study, diagnosis, and management of intestinal cancers and disorders. It seeks to advance knowledge in the field of biomarkers and promote their practical application for the benefit of patients' health and well-being.

II. BIOMARKERS

Biomarkers can be categorized into different types based on their characteristics and applications. One essential group is diagnostic biomarkers (Table 1), which are used to identify the presence or absence of specific diseases or conditions, aiding in early detection and accurate diagnosis [2]. Prognostic biomarkers provide information about the likely outcome or progression of a disease, offering insights into the patient's prognosis and enabling tailored treatment plans (Table 1). Predictive biomarkers, on the other hand, help in personalized medicine by predicting the response to a particular treatment or therapy, allowing healthcare professionals to select the most effective approach for individual patients [3]. Another significant category is surrogate biomarkers, which serve as measurable indicators of a treatment's effect and can accelerate the drug development process by substituting for clinical endpoints in clinical trials. Pharmacodynamic biomarkers evaluate the biological response to a drug, helping researchers understand its mechanism of action and effects on target tissues or pathways (Table 1). Safety biomarkers play a crucial role in monitoring potential adverse effects during clinical trials, ensuring the safety and efficacy of interventions [4]. In addition to these, proximity biomarkers measure the spatial proximity of molecules within cells or tissues, providing valuable information about molecular interactions and signalling pathways [5]. Imaging biomarkers, obtained through medical imaging techniques, offer visual representations of disease-related changes in tissues or organs, aiding in diagnosis and monitoring disease progression. Genomic biomarkers analyze genetic variations or mutations associated with diseases, facilitating personalized medicine and targeted therapies [1]. Lastly, proteomic biomarkers analyze proteins in body fluids or tissues, contributing to a deeper understanding of disease mechanisms and potential therapeutic targets. The diversity of biomarkers and their applications make them invaluable tools in advancing medical research, diagnosis, and treatment across various fields of medicine.

Table 1: Some Selected Biomarkers with their Significance

	Biomarkers	Significance	Examples	References
A.	Diagnostic biomarkers	These biomarkers play a role in identifying or verifying the existence of a disease or condition, and	Troponin is a biomarker for diagnosing heart attacks (myocardial	[1]

SIGNIFICANCE OF BIOMARKERS IN INTESTINAL DISORDERS AND CANCERS

		they can also aid in categorizing different subtypes of the disease.	infarction). Elevated levels of troponin in the blood indicate damage to the heart muscle	
B.	Monitoring biomarkers	These biomarkers are measured repeatedly to evaluate the state of a disease, exposure to medical treatments or environmental substances, or to detect the impact of therapies.	Monitoring blood pressure is a simple and essential biomarker used to assess cardiovascular health and manage conditions like hypertension	
C.	Response/ Pharmacodynamic biomarkers	Biomarkers that change in response to exposure to medical products or environmental agents. These biomarkers play a crucial role in clinical practice and early stages of therapeutic development, as they aid in evaluating the effectiveness of treatments and establishing appropriate dosages.	Ki-67 is a biomarker used in cancer research to measure cell proliferation. It is often used to assess the response of tumors to chemotherapy or other anti-cancer treatments	[4]
D.	Predictive biomarkers	These Biomarkers identify individuals or specific groups with a higher likelihood of responding favorably or unfavorably to medical products or environmental agents. Establishing the validity of predictive biomarkers necessitates thorough clinical studies to showcase their practicality.	In colorectal cancer, the presence of certain KRAS mutations indicates resistance to anti-EGFR therapies like cetuximab and panitumumab	[1]
E.	Prognostic biomarkers	These are indicators that assess the probability of clinical events, recurrence of disease, or progression of disease in patients with a particular medical condition. These biomarkers are different from susceptibility/risk biomarkers, which are associated with the transition from health to	The Gleason score is a prognostic biomarker used in prostate cancer. It helps predict the aggressiveness of cancer and the likelihood of disease progression	[5]

		disease, and predictive biomarkers, which are linked to the effects of interventions.		
F.	Safety Biomarkers	These biomarkers are measured before or after exposure to environmental agents or medical interventions to indicate the presence, or extent of toxicity as an adverse event. Monitoring safety biomarkers is critical in ensuring that a given therapy can be safely sustained	Biomarkers like Alanine Transaminase (ALT) and Aspartate Transaminase (AST) are used to assess liver function and detect drug-induced liver injury	[3]
G.	Susceptibility /Risk Biomarkers	These biomarkers indicate the potential for developing a disease or medical condition in individuals who do not currently have a clinically apparent disease or condition. These biomarkers are foundational for epidemiological studies that assess the risk of disease development.	PRS is a composite score that combines multiple genetic variants to assess an individual's overall genetic risk for a particular disease	[1], [2]

III. ANATOMY AND FUNCTION OF THE INTESTINE

The intestine, also known as the gastrointestinal tract or the gut, is a vital component of the digestive system responsible for processing and assimilating nutrients from the food we consume. It is an elongated, tube-like organ that stretches from the stomach to the anus and is divided into two main sections: the small intestine and the large intestine[6]

1. Small Intestine: The small intestine, which is lengthier and narrower, measures approximately 20 feet in adults. It comprises three segments: the duodenum, jejunum, and ileum. Each segment has a specific role in the digestive process:

- **Duodenum:** The first segment of the small intestine, around 10 inches long, receives partially digested food from the stomach, bile from the liver via the bile duct, and digestive enzymes from the pancreas through the pancreatic duct. These substances aid in further breaking down the food into simpler nutrients for absorption [5].
- **Jejunum:** Positioned in the middle of the small intestine and measuring approximately 8 feet, the jejunum serves as the primary site for nutrient absorption. It

assimilates breakdown products of carbohydrates, proteins, and fats into the bloodstream for utilization by the body [6].

- **Ileum:** The final segment of the small intestine, approximately 12 feet long, continues the process of nutrient absorption, with a particular focus on vitamin B12 and bile salts. The ileum also houses lymphoid tissue, contributing to the gut's immune function [7].

2. Large Intestine: The large intestine, wider and shorter than the small intestine, spans about 5 feet in length. It starts at the end of the ileum and extends to the anus. The primary functions of the large intestine are as follows [8]

- **Cecum:** Located at the beginning of the large intestine where it connects with the small intestine, the cecum is pouch-like in structure. It houses the vermiform appendix, a small finger-like projection that plays a role in immune function [8].
- **Colon:** The colon, the longest part of the large intestine, is divided into four segments: the ascending colon, transverse colon, descending colon, and sigmoid colon. Its main responsibilities include further water and electrolyte absorption from the indigestible food, consolidation of waste into feces, and transportation of feces toward the rectum [8].
- **Rectum and Anus:** The rectum serves as the terminal portion of the large intestine, storing feces until they are ready for elimination. The anus, an external opening, expels feces from the body during defecation [8].

3. The Function of the Intestine: The crucial part of the digestive system is the intestine which is responsible for many important functions as digestion is the breakdown of complex molecules like carbohydrates, and fats into smaller molecules that can be absorbed [6], [8]. The small intestine is the main site of nutrient absorption. Nutrients such as glucose, amino acids, fatty acids, vitamins, and minerals are absorbed through the intestinal walls into the bloodstream and lymphatic system [9]. Also, secrete various digestive enzymes and mucus to aid in the digestive process. Enzymes help break down the food further, while mucus lubricates and protects the intestinal lining from the acidic contents passing through. The large intestine (colon) is responsible for reabsorbing water and electrolytes from the remaining indigestible food matter after it has passed through the small intestine [7]. As food moves through the large intestine, water and electrolytes are gradually reabsorbed, and the remaining waste materials become more solid, forming feces. The intestines host a complex ecosystem of beneficial bacteria known as gut microbiota [10]. These microbes play a vital role in fermenting undigested food components, producing certain vitamins (like B vitamins and vitamin K), and maintaining a healthy gut environment [6]. They also contribute to the overall function of the immune system. And both the small and large intestines exhibit rhythmic contractions called peristalsis. These muscular contractions help propel the food and waste materials through the digestive tract. It also plays an essential part in the immune system. They contain specialized immune cells that help defend against harmful pathogens and prevent them from entering the bloodstream.

The anatomy and function of the intestine are crucial for proper digestion, nutrient absorption, waste elimination, and supporting the body's immune system for maintaining overall health. These functions of the intestine are vital for maintaining proper nutrient balance, supporting overall health, and eliminating waste from the body.

IV. INTESTINAL CANCER AND OTHER DISORDERS

1. Intestine-Related Cancers: Intestine-related cancers encompass a group of malignant diseases that originate within the digestive tract, specifically in the intestines. The gastrointestinal (GI) system consists of two main segments, namely the small intestine and the large intestine (colon and rectum). Cancers that may arise in these areas include:

- **Colorectal Cancer (CRC):** This is the most prevalent type of intestine-related cancer. It begins in the colon or rectum and typically starts as abnormal growths called polyps on the inner lining of the intestines. Over time, some of these polyps can transform into cancerous tumors[11].
- **Small Intestine Cancer:** Less common than colorectal cancer, this type of cancer can develop in various parts of the small intestine, including the duodenum, jejunum, and ileum (subsequent parts of the small intestine)[12].
- **Anal Cancer:** Originating in the tissues of the anal canal, which is the final part of the digestive tract between the rectum and the anus[13].
- **Gastrointestinal Stromal Tumors (GISTs):** Although not as prevalent as colorectal cancer, GISTs are a type of soft tissue sarcoma that can develop in the walls of the digestive tract, including the stomach and small intestine.

Early detection through regular screenings like colonoscopies is crucial in identifying and treating intestine-related cancers at an early and becoming more manageable. Figure 1 depicts the major intestinal cancers.

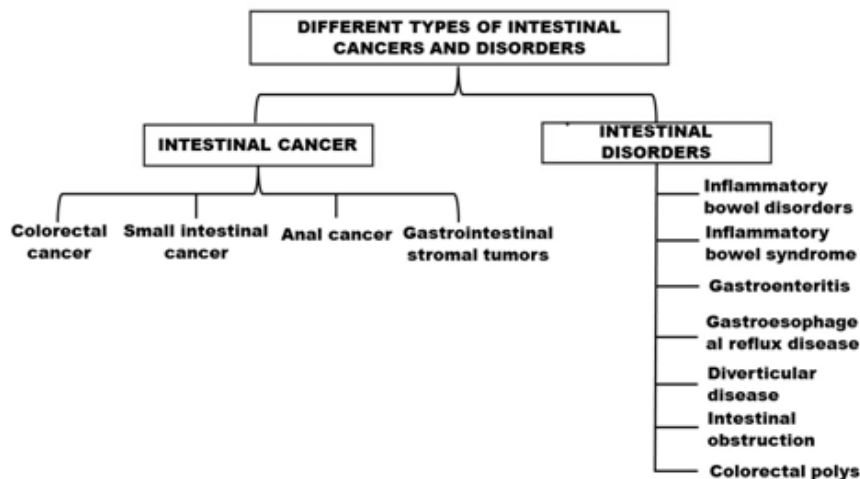


Figure 1: Different types of intestinal cancers and disorders

2. Intestinal Disorders: Intestinal disorders encompass a diverse range of medical conditions and health issues that impact the gastrointestinal (GI) tract, specifically the intestines. The GI tract plays a crucial role in digesting and absorbing nutrients and comprises both the small intestine and the large intestine. Several common intestinal disorders include:

- **Inflammatory Bowel Disease (IBD):** IBD is a group of chronic inflammatory conditions that affect the intestines, with the main types being Crohn's disease and ulcerative colitis. These conditions lead to inflammation and damage to the intestinal lining, resulting in symptoms like abdominal pain, diarrhea, weight loss, and fatigue [14].
- **Irritable Bowel Syndrome (IBS):** IBS is a functional GI disorder characterized by abdominal pain, bloating, and changes in bowel habits (diarrhea, constipation, or both). While it doesn't cause permanent damage to the intestines[15]
- **Gastroenteritis:** Also known as the stomach flu or food poisoning, gastroenteritis is an inflammation of the stomach and intestines. Viral or bacterial infections usually cause it and lead to symptoms such as diarrhea, vomiting, nausea, and abdominal cramps [16].
- **Gastroesophageal Reflux Disease (GERD):**it is a chronic condition where the acid in the stomach frequently flows back into the esophagus, causing heartburn and irritation. Over time, it can lead to esophageal damage and other complications.
- **Celiac Disease:**It is an autoimmune disorder that gets triggered by the ingestion of gluten, a protein found in wheat, barley, and rye. This condition prompts an immune response that damages the small intestine lining, causing malabsorption of nutrients and various gastrointestinal symptoms [17].
- **Diverticular Disease:** Diverticular disease occurs when small pouches (diverticula) form in the colon's walls. In some cases, these pouches can become inflamed or infected, resulting in diverticulitis, with symptoms like abdominal pain, fever, and changes in bowel movements[18]
- **Intestinal Obstruction:** Intestinal obstruction refers to a blockage that hinders the normal flow of contents through the intestines. It can be caused by factors like adhesions, hernias, tumors, or inflammation, and requires prompt medical attention.
- **Colorectal Polyps:** Colorectal polyps are noncancerous growths that can develop in the colon or rectum. Although most polyps are harmless, some may progress to colorectal cancer if not treated.

These examples represent only a portion of the intestinal disorders (Figure 1), as numerous other conditions can impact the GI tract. The symptoms and severity of these disorders can vary significantly, and accurate diagnosis and management often necessitate evaluation by a healthcare professional. Treatment approaches may involve dietary adjustments, medications, lifestyle changes, and, in certain cases, surgical intervention.

V. BIOMARKERS IN INTESTINAL CANCERS

Intestine cancer biomarkers are biological indicators that can provide valuable information about the presence, stage, prognosis, and treatment response of cancers that originate in the intestines, particularly colorectal cancer (Figure 2). These biomarkers can be detected in blood, tissue, or stool samples and play an important role in early detection, personalized treatment, and monitoring of intestinal cancer patients. Some promising intestine cancer biomarkers include:

1. **Carcinoembryonic Antigen (CEA):** Carcinoembryonic Antigen (CEA) is a glycoprotein that was originally identified in fetal gut tissue but is also found in small quantities in healthy adults. It is a member of the immunoglobulin super family and is encoded by the

CEACAM5 gene. The significance of CEA lies in its association with certain types of cancers, particularly colorectal cancer. When cancerous tumors develop in the gastrointestinal tract or other CEA-expressing tissues, the tumor cells often produce CEA at abnormally high levels. As a result, CEA can be detected in the blood, making it a potential biomarker for monitoring cancer progression, treatment response, and recurrence [12].

- **Diagnostic Utility:** Screening for colorectal cancer is not typically recommended as a standalone screening tool for colorectal cancer due to its limited sensitivity and specificity. However, in some cases, it may be used in combination with other tests, such as fecal occult blood testing (FOBT) or colonoscopy, to improve the accuracy of diagnosis. Monitoring cancer treatment after the diagnosis of colorectal cancer. CEA levels can be measured over time to monitor the patient's response to treatment. A decline in CEA levels after surgical removal of the tumor or during chemotherapy may indicate a positive treatment response. Following cancer treatment, CEA levels can be regularly monitored to detect cancer recurrence. A rising trend in CEA levels may indicate the reappearance of cancer cells before clinical symptoms become apparent [19].
 - **Limitations:** While CEA is a valuable tool in certain clinical contexts but it has several limitations like in various non-cancer conditions elevated CEA levels lead to false-positive results. It can also provide false positives, particularly in the early stages of the disease. Therefore, CEA alone cannot be relied upon for diagnosis. It can also interfere due to Factors such as smoking, certain medications, and other medical conditions that can influence CEA levels, leading to inaccurate interpretations.
- 2. Fecal Occult Blood Test (FOBT):** The Fecal Occult Blood Test (FOBT), also known as the Fecal Immunochemical Test (FIT) or Hemoccult test, is a screening test used to detect the presence of small amounts of blood. This hidden or occult blood may be an early sign of various gastrointestinal conditions, most notably colorectal cancer [20].
- **Types of FOBT:** Guaiac FOBT (gFOBT) is the traditional form of FOBT that has been used for several decades. It detects the peroxidase activity of heme and the other one is the Fecal Immunochemical Test (FIT) which is a modern and preferred version of FOBT. It uses antibodies specific to human Hb to detect blood in the stool.
 - **Diagnostics Utility:** FOBT is commonly used as a primary screening test for colorectal cancer, particularly in individuals at average risk for the disease, typically starting at age 50. It can also detect bleeding from precancerous polyps in the colon or rectum. Identifying and removing these polyps through colonoscopy can prevent their progression to colorectal cancer. For individuals diagnosed with colorectal cancer, to monitor the response to treatment it can be used. A decline in the presence of occult blood in the stool may indicate a positive response to therapy. After colorectal cancer treatment, FOBT can be used to monitor for cancer recurrence. A rise in the presence of occult blood in the stool may suggest the possible reappearance of cancer cells.
 - **Limitations:** The limitation of this test includes false positive results when there is blood in the stools due to reasons other than CRC like hemorrhoids, ulcers, IBD, and

many more. It can also lead to false negative results because not always blood in stool is detected in the presence of cancer. Certain food and medications can affect the results of FOBT. Despite these limitations, FOBT remains an essential tool in colorectal cancer screening programs due to its non-invasive nature, cost-effectiveness, and ability to identify early signs of bleeding in the gastrointestinal tract. However, to enhance the effectiveness of colorectal cancer screening, newer tests like stool DNA tests and colonoscopies are being used in conjunction with FOBT to improve sensitivity and specificity [21].

3. Microsatellite Instability (MSI): Microsatellite Instability (MSI) is a molecular characteristic observed in certain types of cancer cells that reflects the accumulation of errors in the replication of repetitive DNA sequences known as microsatellites. Microsatellites are short, repeated sequences of nucleotides found throughout the human genome. In normal cells, the process of DNA replication and repair ensures the stability of microsatellites. However, in cancer cells with MSI, the DNA repair machinery is impaired or defective, leading to the accumulation of errors or mutations in the microsatellites [22]. This instability is referred to as Microsatellite Instability (MSI). The primary cause of MSI is the loss of function of one or more key proteins involved in the DNA mismatch repair (MMR) pathway. The MMR system is responsible for correcting errors that occur during DNA replication, including those in microsatellites. **Diagnostics utility:** MSI testing is an essential component in the diagnosis of Lynch syndrome (hereditary non-polyposis colorectal cancer or HNPCC). Lynch syndrome is an inherited genetic disorder characterized by an increased risk of developing colorectal cancer, endometrial cancer, and other cancers. MSI status serves as a prognostic marker in certain types of cancers. For example, in colorectal cancer and endometrial cancer, MSI-high (MSI-H) tumors are associated with a more favorable prognosis compared to microsatellite-stable (MSS) or microsatellite-low (MSI-L) tumors. It can influence treatment decisions in colorectal cancer and other cancers. For example, MSI-H colorectal cancer patients with advanced disease may be considered for immune checkpoint inhibitor therapy as a first-line or later-line treatment option, especially when standard chemotherapy is not effective. This testing can aid in distinguishing between different types of cancer that may have overlapping histological features. For example, MSI-H status can help differentiate between sporadic colorectal cancer and Lynch syndrome-associated colorectal cancer [19].

- **Limitations:** MSI testing requires tumor tissue samples, which may not always be available or feasible to obtain, especially in cases where a biopsy is not possible due to the tumor's location or patient-related factors. Its status can be heterogeneous within the same tumor, meaning that different areas of the tumor may have varying levels of microsatellite instability. It can lead to false positive and negative results also. It cannot differentiate both somatic and germline mutations. Additional genetic testing is necessary to determine if Lynch syndrome or hereditary cancer is present. Despite these limitations, MSI testing remains a valuable tool in cancer diagnosis and treatment decision-making, especially in colorectal cancer and endometrial cancer [12].

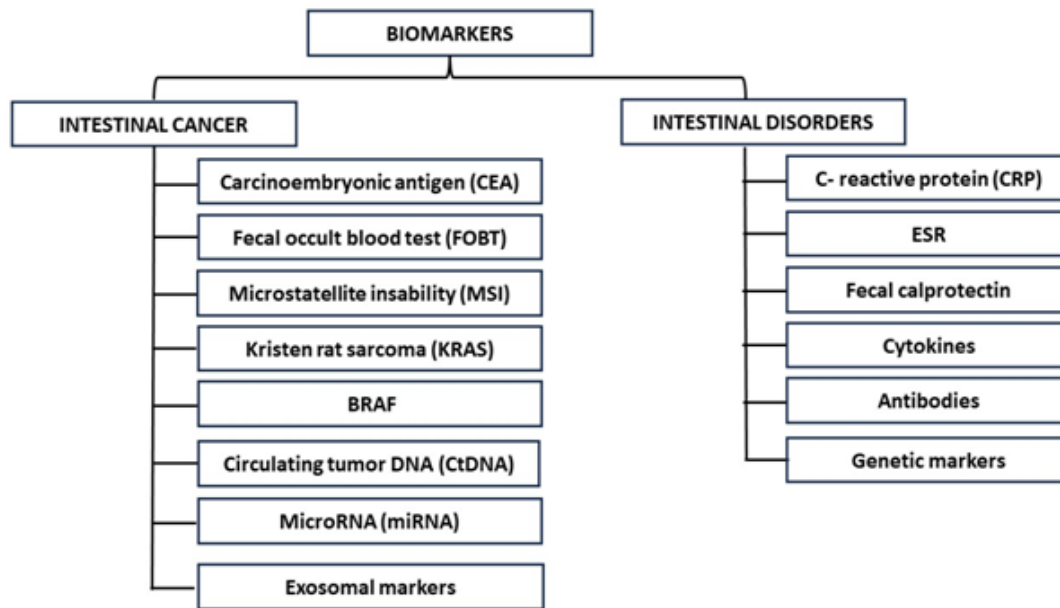


Figure 2: Types of Biomarkers in intestinal cancers and disorders

4. **KRAS Mutation:** KRAS (Kirsten Rat Sarcoma) is a proto-oncogene that encodes a GTPase protein involved in the regulation of cell growth and differentiation. It is an essential part of the RAS/RAF/MEK/ERK signaling pathway, which plays a crucial role in controlling cell proliferation, survival, and differentiation. However, when certain mutations occur in the KRAS gene, it can lead to the activation of this pathway and promote uncontrolled cell growth, contributing to cancer development.
- **Diagnostic Utility:** it helps in the sub typing and diagnosis of cancer KRAS testing is commonly used in cancer diagnosis to identify specific mutations in the KRAS gene. For example, in colorectal cancer, KRAS mutations are a common molecular alteration, and testing can aid in the diagnosis and sub typing of the disease. In some cancers, the presence of specific KRAS mutations may have prognostic significance. For example, in colorectal cancer, KRAS mutations at specific codons have been linked to poor prognosis and increased risk of disease recurrence. This testing is a critical predictive biomarker in various cancers. For instance: In colorectal cancer, patients with KRAS mutations are less likely to respond to anti-EGFR therapies (cetuximab and panitumumab). It is also crucial in guiding treatment decisions, especially in colorectal cancer[12].
 - **Limitation:** Tumors are often heterogeneous, meaning that different regions within the same tumor may have different genetic profiles. KRAS testing on a single tissue sample may not fully represent the entire tumor's genetic makeup, leading to potential inaccuracies in determining the presence or absence of KRAS mutation. KRAS mutations can occur at different codons and result in various alterations in the protein structure. Not all KRAS mutations have the same clinical significance, and some may respond differently to targeted therapies[23]. Therefore, the specific type and location of the KRAS mutation need to be considered when interpreting the test results. This testing is a valuable tool in oncology and has significantly improved our

understanding of cancer biology and treatment selection. Integrating KRAS testing results with other clinical and molecular information is crucial for making well-informed treatment decisions for patients with KRAS-mutated cancers. Continued research and advancements in targeted therapies hold promise for improving outcomes in patients with KRAS-driven cancers.

- 5. BRAF Mutation:** BRAF (v-Raf murine sarcoma viral oncogene homolog B) is a proto-oncogene that encodes a protein kinase involved in the RAS/RAF/MEK/ERK signalling pathway. This pathway plays an important role in regulating cell growth, differentiation, and survival. However, when specific mutations occur in the BRAF gene, it can lead to the constitutive activation of the BRAF protein, promoting uncontrolled cell growth and contributing to cancer development. The most common and clinically significant mutation in the BRAF gene is the V600E mutation, where a valine (V) is replaced by a glutamic acid (E) at position 600. This mutation results in the activation of the BRAF protein, leading to cell proliferation and survival

 - **Diagnostic Utility:** BRAF testing is commonly used in cancer diagnosis to identify the presence of specific BRAF mutations, particularly the V600E mutation. It aids in determining the molecular profile of the tumor and guiding treatment decisions. In some cancers, the presence of the BRAF V600E mutation may have prognostic significance. For example, in melanoma, patients with the BRAF V600E mutation may have a different disease course and prognosis compared to those without the mutation[23]. The presence of the BRAF V600E mutation is a predictive biomarker for targeted therapies that specifically inhibit the activity of the mutant BRAF protein. Examples include drugs like vemurafenib and dabrafenib, which are used to treat BRAF V600E-mutated melanoma and other cancers.
 - **Limitation:** BRAF mutations are not limited to the V600E mutation. Other mutations in the BRAF gene can also occur, leading to different alterations in the BRAF protein's activity. The clinical significance and response to targeted therapies may vary depending on the specific BRAF mutation present. While targeted therapies against the BRAF V600E mutation have shown remarkable initial responses, some patients may develop resistance to these treatments over time[19]. The presence of BRAF mutations can interact with other genetic alterations in cancer cells, influencing treatment response and disease behavior. BRAF mutation testing is a valuable tool in oncology for diagnosing and characterizing specific cancers, predicting treatment response, guiding treatment decisions, and determining patient prognosis. Integrating BRAF mutation testing results with other clinical and molecular information is crucial for optimizing personalized treatment approaches for patients with BRAF-mutated cancers [12], [19].
- 6. Circulating Tumor DNA (ctDNA):** Circulating Tumor DNA (ctDNA) is a subset of cell-free DNA (cfDNA) found in the bloodstream of cancer patients. It originates from tumor cells and carries genetic information specific to the tumor. As cancer cells grow, die, and undergo apoptosis, they release ctDNA into the bloodstream. ctDNA carries somatic mutations, copy number alterations, and other genetic changes that are characteristic of the tumor from which it originates.

- **Diagnostic Utility:** It helps in early cancer detection ctDNA analysis holds promise for early cancer detection and monitoring of high-risk individuals. By detecting specific tumor-related mutations in the blood, ctDNA testing can potentially identify cancer at earlier stages when treatment outcomes are better. It is minimally invasive testing. After primary treatment, ct DNA can be used to detect minimal residual disease (MRD), which refers to the presence of small tumor cell populations that may not be detected by conventional imaging or clinical evaluation. Also, predict the risk of cancer recurrence. Persistent or rising levels of ct DNA after treatment may indicate a higher risk of disease relapse[12].
 - **Limitations:** The sensitivity of ctDNA testing can vary depending on the type and stage of cancer. In some cases, the amount of ctDNA released into the bloodstream may be too low to be detected, leading to false-negative results. The ctDNA may not fully represent the entire tumor's genetic profile, especially in tumors with significant heterogeneity. This could result in incomplete or misleading information about the tumor's genetic makeup. This test can provide false-Positive Results. The testing can be expensive, and cost considerations may limit its widespread use in routine clinical practice. Despite these limitations, ctDNA testing holds great promise as a powerful tool in cancer diagnosis, monitoring, and personalized treatment. Ongoing research and technological advancements are expected to address some of the current limitations and expand the clinical applications of ctDNA testing in oncology[11].
7. **Micro RNAs (miRNAs):** Micro RNAs (miRNAs) are small, non-coding RNA molecules that have a significant role in the post-transcriptional regulation of gene expression. They are involved in the control of various cellular processes, including cell proliferation, differentiation, apoptosis, and metabolism. miRNAs function by binding to specific messenger RNA (mRNA) molecules, leading to either degradation of the mRNA or inhibition of its translation into proteins. This regulation ultimately influences the expression levels of target genes, impacting various cellular functions [11].
- **Diagnostic Utility:** It helps in the diagnosis of cancer the miRNAs have shown potential as biomarkers for cancer diagnosis. Altered expression patterns of specific miRNAs have been observed in various cancer types, making them attractive candidates for early cancer detection. The expression levels of certain miRNAs have been associated with the prognosis and clinical outcome of cancer patients. High or low expression of specific miRNAs may correlate with a more aggressive disease or better treatment response. miRNAs can serve as predictive biomarkers for response to specific therapies. The expression levels of certain miRNAs may indicate whether a patient is likely to respond well to a particular treatment. It is minimally invasive testing[11].
 - **Limitations:** miRNAs can have tissue-specific expression patterns, which may limit their utility as universal cancer biomarkers. The expression of certain miRNAs can vary among different cancer types. Some miRNAs have relatively small sizes, making them more prone to degradation during sample processing and storage, potentially affecting the accuracy of results. The analysis requires specialized techniques, such as real-time PCR (RT-PCR) or next-generation sequencing, which may be more complex and expensive than traditional molecular tests. Ongoing research and advancements in

miRNA analysis techniques are likely to enhance their clinical utility and contribute to more personalized and effective treatment approaches[12].

8. Exosomal Markers: Exosomes are small extracellular vesicles released by cells into the bloodstream and other bodily fluids. They play a crucial role in cell-to-cell communication by transferring various molecules, including proteins, lipids, and nucleic acids, between cells. Exosomes are involved in several physiological and pathological processes, and their cargo reflects the status of the cells of origin. Exosomal markers refer to specific proteins, nucleic acids, or other molecules found within exosomes, which can be used as indicators of certain cellular or disease states.

- **Diagnostic Utility:** In cancer diagnosis, exosomal markers have shown promise as potential biomarkers for cancer diagnosis. Specific proteins or nucleic acids enriched in exosomes can indicate the presence of tumors and assist in early cancer detection. It can be isolated from easily accessible body fluids, such as blood, urine, and saliva, making exosomal markers attractive for non-invasive and convenient diagnostic testing. Also works as a prognostic indicator[19], [23].
- **Limitations:** Exosomes are a heterogeneous population of vesicles, and their composition can vary depending on the cell type, physiological state, and disease condition. This heterogeneity can make the identification of specific exosomal markers challenging[11], [12]. The isolation of exosomes from complex biological fluids can be technically challenging and may lead to contamination or loss of exosomal markers during the isolation process. The specificity and sensitivity of exosomal markers as diagnostic or prognostic biomarkers may vary depending on the disease and the marker being assessed. Despite these limitations, exosomal markers represent a rapidly advancing field in biomarker research.

VI. BIOMARKERS IN INTESTINE-RELATED DISORDERS

Several biomarkers have been studied and utilized in IBD management. Some of the common IBD biomarkers include:

- 1. C-reactive protein (CRP):** C-reactive protein (CRP) is a protein produced by the liver in response to inflammation and tissue damage in the body. It is a member of the acute-phase proteins, a group of proteins that increase in concentration in the blood in response to inflammation, infection, or injury. CRP is an important biomarker in the context of Inflammatory Bowel Disease (IBD)[24].

Significance: CRP is an indicator of Inflammation. CRP levels can be used to monitor the response to treatment in IBD. A reduction in CRP levels over time suggests that the treatment is effectively managing inflammation and controlling the disease[25]. Conversely, persistently high CRP levels despite treatment may indicate inadequate disease control and the need for therapy adjustments. Persistently elevated CRP levels, especially if they consistently remain high, may indicate an increased risk of disease flares or exacerbations in IBD patients. Rising CRP levels during treatment may suggest an increased risk of treatment failure or the need for more aggressive therapies[24]. CRP is a nonspecific marker of inflammation, meaning that it does not provide information

about the specific location or cause of inflammation within the gastrointestinal tract. It can be elevated in various other inflammatory conditions and infections as well. CRP levels may not rise immediately after the onset of inflammation in IBD; it can take several hours or even days to increase. In some individuals with IBD, CRP levels may remain within the normal range despite active inflammation [26]. Conversely, CRP levels may be elevated in other conditions, even without apparent inflammation in IBD. However, its nonspecific nature and other limitations should be considered when interpreting CRP results. CRP testing should be combined with other clinical and laboratory assessments to provide a comprehensive evaluation of disease activity and guide treatment decisions in individual IBD patients [27].

2. Erythrocyte Sedimentation Rate (ESR): ESR is a widely used laboratory test in the assessment of various inflammatory conditions, including Inflammatory Bowel Disease (IBD).

- **Significance:** In the context of IBD, the ESR test holds importance in many ways like in detecting inflammation because ESR is a nonspecific marker of inflammation but in IBD, the intestinal lining undergoes chronic inflammation due to an abnormal immune response [26]. An elevated ESR level suggests the presence of ongoing inflammation in the body. In IBD, disease activity can vary over time, with periods of active inflammation (flares) and periods of remission. ESR levels can be used as one of the indicators of disease activity. Monitoring changes in ESR levels over time can help evaluate the response to treatment. Elevated ESR levels, especially if they consistently remain high, can be a predictor of potential disease flares or exacerbations in IBD patients. Rising ESR levels during treatment may indicate an increased risk of disease relapse [28].
- **Limitations:** ESR is a nonspecific marker of inflammation, meaning it does not provide information about the specific location or cause of inflammation within the gastrointestinal tract. Levels can vary between individuals, and some patients with IBD may have normal ESR levels despite active inflammation. It may not rise immediately after the onset of inflammation in IBD; it can take several hours or even days to increase. Similarly, it may remain elevated for some time after the inflammation has resolved.

3. Fecal Calprotectin: Fecal calprotectin is a biomarker that is increasingly used in the diagnosis and management of Inflammatory Bowel Disease (IBD). Calprotectin is a protein complex released by activated neutrophils during inflammation.

- **Significance:** Fecal calprotectin is highly specific for inflammation in the gastrointestinal (GI) tract. In IBD, the intestines are inflamed, leading to increased shedding of calprotectin into the stool. Therefore, elevated fecal calprotectin levels indicate ongoing inflammation within the GI tract. Calprotectin levels are generally low in non-inflammatory conditions of the gut. Elevated levels of fecal calprotectin help differentiate IBD from functional gastrointestinal disorders, such as irritable bowel syndrome (IBS), where inflammation is absent. Fecal calprotectin levels correlate well with the activity of IBD. During active disease flares, calprotectin levels increase, and during remission or successful treatment, levels decrease. Regular

monitoring of calprotectin aids in assessing disease activity and treatment response[14], [27].

- **Limitations:** It is not specific to IBD because fecal calprotectin is not specific for differentiating between Crohn's disease and ulcerative colitis. While elevated levels indicate intestinal inflammation, they do not specify the type of IBD or its location. Calprotectin levels can be elevated in other gastrointestinal conditions, infections, or acute illnesses. Therefore, transient elevations may not always be attributed solely to IBD. Certain dietary components or medications, can affect calprotectin levels and potentially lead to false results. Despite these limitations, fecal calprotectin is a valuable tool in the management of IBD. It aids in early detection, monitoring disease activity, guiding treatment decisions, and assessing response to therapy[14].
4. **Cytokines:** Cytokines are small signalling proteins secreted by various immune cells that play a crucial role in regulating the immune response and inflammation. In the context of Inflammatory Bowel Disease (IBD), including Crohn's disease and ulcerative colitis, cytokines have both diagnostic and therapeutic importance:
- **Significance:** Cytokines work as a diagnostic biomarker where specific cytokines are associated with the pathogenesis of IBD. Measurement of certain cytokines in the blood or inflamed tissues can help in the diagnosis and differentiation of IBD from other gastrointestinal disorders. It can be used in assessing disease activity cytokine levels, particularly in the inflamed intestinal tissues or in the blood, and can correlate with disease activity in IBD patients. Elevated levels of pro-inflammatory cytokines often indicate active inflammation and their measurement aids in assessing disease severity. Cytokines are critical in driving the inflammatory response in IBD. Targeting specific cytokines with biological therapies has become an essential component of IBD treatment. For example, anti-TNF (tumor necrosis factor) agents have revolutionized the management of IBD by neutralizing the effects of TNF-alpha, a pro-inflammatory cytokine[14].
 - **Limitations:** The cytokine networks in IBD are complex, with multiple cytokines involved in different stages of inflammation and immune response. Their profiles can vary widely between individual IBD patients due to differences in disease subtype, location, and severity. The expression of cytokines can vary locally within the inflamed gut tissues and systemically in the bloodstream. Assessing tissue-specific cytokine levels may require invasive procedures like biopsies. They play a significant role in the pathogenesis and management of Inflammatory Bowel Disease [27].
5. **Antibodies:** Antibodies play a significant role as diagnostic and prognostic markers, as well as guiding treatment decisions.
- **Significance:** It works as a diagnostic Biomarker where antibodies against specific microbial or self-antigens can aid in the diagnosis and differentiation of various forms of IBD. For example, distinguishing between Crohn's disease and ulcerative colitis can be done with the help of anti-Saccharomyces cerevisiae antibodies (ASCA) and anti-neutrophil cytoplasmic antibodies (ANCA). Disease course can be predicted in

Certain antibodies that have been associated with specific disease phenotypes and outcomes in IBD. For instance, the presence of certain antibodies may be linked to a higher risk of complications or a more aggressive disease course[14], [27].

- **Limitations:** There is a lack of specificity in some antibodies, such as ASCA and ANCA, while helpful in distinguishing Crohn's disease from ulcerative colitis, may not be specific enough for definitive diagnosis or sub typing of IBD. It is a complex and heterogeneous disease, and the presence or absence of specific antibodies may not always correlate with disease activity or clinical outcomes. Some antibody tests can be expensive and may not be widely available in all healthcare settings, limiting their routine use as diagnostic or prognostic tools. Antibodies play a vital role in the management of IBD, serving as diagnostic markers, guiding treatment decisions, and predicting treatment response. However, their clinical utility is limited by factors such as lack of specificity, disease heterogeneity, and the potential for secondary loss of response to biological therapies[29].

6. Genetic Markers: Genetic markers, particularly specific genetic variants or mutations, play a crucial role in developing and understanding Inflammatory Bowel Disease (IBD).

- **Significance:** Certain genetic indicators are linked to an elevated likelihood of developing IBD. Genome-wide association studies (GWAS) have pinpointed numerous genetic locations that contribute to IBD susceptibility. These markers provide insights into the genetic underpinnings of IBD and illuminate the fundamental processes involved in disease progression. These biomarkers are employed to evaluate an individual's vulnerability to IBD. Specific genetic variations are correlated with an escalated risk of IBD, offering an understanding of the genetic facet of the ailment. The identification of precise genetic markers can assist in the early detection of IBD, particularly when symptoms are not yet severe or evident[24]. Timely diagnosis can prompt intervention and enhance disease management. These biomarkers can illuminate the biological pathways and mechanisms engaged in IBD advancement. This comprehension can guide investigations into targeted treatments and potential drug targets. It can also aid in forecasting how individuals might react to specific therapies. This information can steer healthcare professionals in shaping treatment strategies for improved outcomes and diminished adverse effects.
- **Limitations:** Inflammatory Bowel Disease (IBD) is an intricate and multifaceted disorder influenced by a blend of genetic, environmental, and immunological factors[14]. Although certain genetic indicators have been detected, they only elucidate a fraction of the vulnerability to the disease, leaving numerous factors shrouded in uncertainty. Some genetic markers linked to IBD risk also exist in the general population without the ailment. This lack of precision can complicate their utilization as definitive diagnostic tools[22]. While genetic markers can furnish a probabilistic evaluation of disease susceptibility, they lack the precision to definitively predict the development of IBD in an individual. The interplay of environmental elements and the intricate interactions among multiple genes hold significant sway. Several genetic markers connected to IBD susceptibility have been pinpointed in particular populations or regions. This may curtail their relevance and precision in diverse populations. While genetic markers offer insights into potential pathways

implicated in IBD, translating these insights into effective therapies is intricate. The advancement of therapies necessitates a more profound comprehension of the mechanics of the disease beyond genetic markers alone[14]. The risk and severity of IBD are molded by an array of environmental influences like diet, lifestyle, and exposure to microorganisms. Genetic markers by themselves might not fully encompass these influences. The domain of IBD genetics is swiftly advancing, ushering in new genetic markers that are being uncovered and refined. Staying current with the latest research and assimilating novel information can be formidable. To conclude, genetic markers assume a crucial role in comprehending the genetic groundwork of IBD, evaluating disease susceptibility, and conceivably guiding treatment approaches. Nonetheless, the limitations of genetic markers, particularly the intricate genetic structure of IBD and the sway of environmental aspects accentuate the necessity for a holistic methodology that amalgamates genetic data with clinical, environmental, and other pertinent information to attain a more accurate and individualized comprehension of the ailment.

VII. FUTURE PERSPECTIVES AND CHALLENGES

Future perspectives and challenges regarding biomarkers of intestinal cancers and intestinal disorders hold significant promise and potential advancements in the field of personalized medicine and improved patient outcomes. However, several challenges need to be addressed to fully realize the clinical utility of these biomarkers. Here are some future perspectives and challenges:

- 1. Future Prospective:** Biomarkers have the potential to enable precision medicine approaches, where treatments can be used for an individual's unique genetic and molecular profile. Identifying specific biomarkers associated with drug responsiveness will lead to more targeted and effective therapies for intestinal cancers and disorders. Liquid biopsies, tests that are based on blood and detect circulating tumor DNA and other biomolecules, offer non-invasive ways to monitor disease progression, treatment response, and minimal residual disease in intestinal cancers[23].The development and validation of liquid biopsies may transform cancer management by providing real-time information and reducing the need for invasive procedures (Figure 3).Integrating data from various omics technologies, such as genomics, epigenomics, transcriptomics, proteomics, and metabolomics, will enhance our understanding of the complex molecular mechanisms underlying intestinal cancers and disorders. This multi-dimensional approach can lead to the identification of novel biomarkers and therapeutic targets.Biomarkers that facilitate early detection of intestinal cancers and disorders can significantly improve patient outcomes. Developing robust and sensitive biomarkers for screening programs will help identify at-risk individuals and initiate timely interventions.Biomarkers that predict disease prognosis and treatment response can guide clinical decision-making and aid in selecting the most appropriate therapeutic options for patients with intestinal cancers and disorders.



Figure 3: Showing future perspectives of Biomarkers

- 2. Challenges:** The discovery of promising biomarkers requires rigorous validation in large, diverse patient cohorts to ensure their accuracy, sensitivity, and specificity. Biomarkers must also be reproducible across different laboratories and clinical settings[30]. Standardization of biomarker assays and methodologies is essential to ensure consistent and comparable results. Variability in testing methods and platforms can affect the reliability of biomarker data. Ethical issues related to the use of biomarkers, such as privacy concerns, informed consent, and data sharing, need to be addressed to protect patients' rights and promote responsible biomarker research. In many cases, a single biomarker may not be sufficient for comprehensive diagnosis or prediction. Combining multiple biomarkers or integrating different types of biomolecular data may improve diagnostic accuracy and precision. Implementation of biomarker-based approaches in clinical practice may face challenges related to cost, accessibility, and infrastructure requirements. Ensuring affordable and accessible biomarker testing is crucial for widespread adoption. Intestinal cancers and disorders are heterogeneous diseases with diverse molecular subtypes. Identifying biomarkers that are universally applicable across all subtypes remains a challenge.

Addressing these perspectives and challenges will advance the field of biomarkers for intestinal cancers and disorders, ultimately leading to more precise diagnoses, personalized treatment strategies, and improved patient outcomes.

VIII. CONCLUSION

Identifying and validating biomarkers for intestinal cancers and disorders have opened new avenues for early detection, accurate diagnosis, and personalized treatment strategies. Through advancements in genetic, epigenetic, and protein biomarker research, we have gained deeper insights into the complex molecular mechanisms driving these diseases. They offer non-invasive and cost-effective ways to monitor disease progression [31]. Additionally, liquid biopsies and multi-omics integration present exciting possibilities for real-time monitoring and improved disease management. However, as we move forward, we must address the challenges of validation, standardization, and cost-effectiveness to ensure the reliable and widespread use of these biomarkers in routine clinical practice. By harnessing the power of biomarkers, we can advance toward precision medicine, and tailoring therapies. Collaborative efforts among researchers, clinicians, and stakeholders are essential to overcome these obstacles and bring biomarker-driven approaches to the forefront of intestinal cancer and disorder management.

REFERENCES

- [1] K. Strimbu and J. A. Tavel, "What are biomarkers?," *Current Opinion in HIV and AIDS*, vol. 5, no. 6. pp. 463–466, Nov. 2010. doi: 10.1097/COH.0b013e32833ed177.
- [2] R. Mayeux, "Biomarkers: Potential Uses and Limitations."
- [3] R. M. Califf, "Biomarker definitions and their applications," *Exp Biol Med*, vol. 243, no. 3, pp. 213–221, Feb. 2018, doi: 10.1177/1535370217750088.
- [4] R. Mayeux, "Biomarkers: Potential Uses and Limitations."
- [5] R. M. Califf, "Biomarker definitions and their applications," *Exp Biol Med*, vol. 243, no. 3, pp. 213–221, Feb. 2018, doi: 10.1177/1535370217750088.
- [6] J. T. Collins, A. Nguyen, and M. Badireddy, *Anatomy, Abdomen and Pelvis, Small Intestine*. 2023.
- [7] P. P. Lopez, S. Gogna, and A. Khorasani-Zadeh, *Anatomy, Abdomen and Pelvis: Duodenum*. 2023.
- [8] P. Kahai, P. Mandiga, C. J. Wehrle, and S. Lobo, *Anatomy, Abdomen and Pelvis: Large Intestine*. 2023.
- [9] A. Barsouk, P. Rawla, A. Barsouk, and K. C. Thandra, "Epidemiology of Cancers of the Small Intestine: Trends, Risk Factors, and Prevention," *Medical sciences (Basel, Switzerland)*, vol. 7, no. 3. NLM (Medline), Mar. 17, 2019. doi: 10.3390/medsci7030046.
- [10] S. R. Chaudhry, M. N. P. Liman, and D. C. Peterson, *Anatomy, Abdomen and Pelvis: Stomach*. 2023.
- [11] M. Vacante, A. M. Borzi, F. Basile, and A. Biondi, "Biomarkers in colorectal cancer: Current clinical utility and future perspectives," *World Journal of Clinical Cases*, vol. 6, no. 15. Baishideng Publishing Group Co, pp. 869–881, Dec. 01, 2018.
- [12] M. Vacante, A. M. Borzi, F. Basile, and A. Biondi, "Biomarkers in colorectal cancer: Current clinical utility and future perspectives," *World Journal of Clinical Cases*, vol. 6, no. 15. Baishideng Publishing Group Co, pp. 869–881, Dec. 01, 2018.
- [13] P. F. Engstrom et al., "Anal Carcinoma," *Journal of the National Comprehensive Cancer Network*, vol. 8, no. 1, pp. 106–120, Jan. 2010, doi: 10.6004/jnccn.2010.0007.
- [14] K. Wagatsuma, Y. Yokoyama, and H. Nakase, "Role of Biomarkers in the Diagnosis and Treatment of Inflammatory Bowel Disease," *Life*, vol. 11, no. 12. MDPI, Dec. 01, 2021. doi: 10.3390/life11121375.
- [15] W. D. Chey, J. Kurlander, and S. Eswaran, "Irritable Bowel Syndrome," *JAMA*, vol. 313, no. 9, p. 949, Mar. 2015, doi: 10.1001/jama.2015.0954.
- [16] R. J. Gilkin, "The spectrum of irritable bowel syndrome: A clinical review.," *Clin Ther*, vol. 27, no. 11, pp. 1696–709, Nov. 2005, doi: 10.1016/j.clinthera.2005.11.012.
- [17] G. Caio et al., "Celiac disease: a comprehensive current review," *BMC Med*, vol. 17, no. 1, p. 142, Dec. 2019, doi: 10.1186/s12916-019-1380-z.
- [18] I. Parzanese et al., "Celiac disease: From pathophysiology to treatment," *World J Gastrointest Pathophysiol*, vol. 8, no. 2, p. 27, 2017.
- [19] C. V. Olovo, X. Huang, X. Zheng, and M. Xu, "Faecal microbial biomarkers in early diagnosis of colorectal cancer," *Journal of Cellular and Molecular Medicine*, vol. 25, no. 23. John Wiley and Sons Inc, pp. 10783–10797, Dec. 01, 2021. doi: 10.1111/jcmm.17010.
- [20] B. A. Alves Martins, G. F. de Bulhões, I. N. Cavalcanti, M. M. Martins, P. G. de Oliveira, and A. M. A. Martins, "Biomarkers in Colorectal Cancer: The Role of Translational Proteomics Research," *Frontiers in Oncology*, vol. 9. Frontiers Media S.A., Nov. 27, 2019. doi: 10.3389/fonc.2019.01284.
- [21] N. S. S. Kuiken, E. H. H. M. Rings, N. M. A. Blijlevens, and W. J. E. Tissing, "Biomarkers and non-invasive tests for gastrointestinal mucositis," *Supportive Care in Cancer*, vol. 25, no. 9. Springer Verlag, pp. 2933–2941, Sep. 01, 2017. doi: 10.1007/s00520-017-3752-2.
- [22] M. Vacante, A. M. Borzi, F. Basile, and A. Biondi, "Biomarkers in colorectal cancer: Current clinical utility and future perspectives," *World J Clin Cases*, vol. 6, no. 15, pp. 869–881, Dec. 2018, doi: 10.12998/wjcc.v6.i15.869.
- [23] B. A. Alves Martins, G. F. de Bulhões, I. N. Cavalcanti, M. M. Martins, P. G. de Oliveira, and A. M. A. Martins, "Biomarkers in Colorectal Cancer: The Role of Translational Proteomics Research," *Frontiers in Oncology*, vol. 9. Frontiers Media S.A., Nov. 27, 2019. doi: 10.3389/fonc.2019.01284.
- [24] X. Guo et al., "Gut Microbiota Is a Potential Biomarker in Inflammatory Bowel Disease," *Frontiers in Nutrition*, vol. 8. Frontiers Media S.A., Jan. 21, 2022. doi: 10.3389/fnut.2021.818902.
- [25] A. C. Porter et al., "Biomarkers of crohn s disease to support the development of new therapeutic interventions," *Inflammatory Bowel Diseases*, vol. 26, no. 10. Oxford University Press, pp. 1498–1508, Oct. 01, 2020. doi: 10.1093/ibd/izaa215.
- [26] K. Wagatsuma, Y. Yokoyama, and H. Nakase, "Role of Biomarkers in the Diagnosis and Treatment of Inflammatory Bowel Disease," *Life*, vol. 11, no. 12. MDPI, Dec. 01, 2021. doi: 10.3390/life11121375.

- [27] P. Chen et al., “Serum Biomarkers for Inflammatory Bowel Disease,” *Frontiers in Medicine*, vol. 7. Frontiers Media S.A., Apr. 22, 2020. doi: 10.3389/fmed.2020.00123.
- [28] K. Wagatsuma, Y. Yokoyama, and H. Nakase, “Role of Biomarkers in the Diagnosis and Treatment of Inflammatory Bowel Disease,” *Life*, vol. 11, no. 12. MDPI, Dec. 01, 2021. doi: 10.3390/life11121375.
- [29] P. Chen et al., “Serum Biomarkers for Inflammatory Bowel Disease,” *Frontiers in Medicine*, vol. 7. Frontiers Media S.A., Apr. 22, 2020. doi: 10.3389/fmed.2020.00123.
- [30] R. K. Montgomery and D. T. Breault, “Small intestinal stem cell markers,” *Journal of Anatomy*, vol. 213, no. 1. pp. 52–58, Jul. 2008.
- [31] B. A. Alves Martins, G. F. de Bulhões, I. N. Cavalcanti, M. M. Martins, P. G. de Oliveira, and A. M. A. Martins, “Biomarkers in Colorectal Cancer: The Role of Translational Proteomics Research,” *Frontiers in Oncology*, vol. 9. Frontiers Media S.A., Nov. 27, 2019. doi: 10.3389/fonc.2019.01284.