Integration of Biochemistry, Microbiology, Pathological Analysis, and Cell Biology in the Diagnosis and Monitoring of Cancer: A Molecular–Cellular–Clinical Study

Editors

Abbas Fadhil Khazaal
Ataturk university, College of Health Sciences, Department of Biochemistry

Qasim Sabbar Aadi
Ataturk university, College of Health Sciences, Department of Biochemistry

Qayid Darwish Khasaruh Mulhak
Martyr Madani University, College of Biology, Department of Molecular and Cellular Biology

Abbas Sami Jawhar Hawar
University of Sumer, College of Science, Department of Pathological Analysis

Ali Abbas Hussein
University of Babylon, College of Science, Department of Biology Specialization: Microbiology

Bright Sky Publications ™
New Delhi

Published By: *Bright Sky Publications*

Bright Sky Publication
Office No. 3, 1st Floor,
Pocket - H34, SEC-3,
Rohini, Delhi, 110085, India

Editors: *Abbas Fadhil Khazaal, Qasim Sabbar Aadi, Qayid Darwish Khasaruh Mulhak, Abbas Sami Jawhar Hawar and Ali Abbas Hussein*

Edition: *1st*

Publication Year: *2025*

Pages: *117*

Paperback ISBN: *978-93-6233-811-2*

E-Book ISBN: *978-93-6233-263-9*

DOI: *https://doi.org/10.62906/bs.book.455*

Price: *₹565/-*

Contents

Abstract

Integrated molecular, cellular, and clinical framework provides an integrated interdisciplinary platform linking molecular patterns with cellular states to understand cancer biology, envision a multi-tiered diagnostic biomarker panel and formulate patient monitoring strategies. Cancer arises from a series of changes at the genetic, epigenetic, metabolic and immune environment levels. Interdisciplinary cancer monitoring describes methodology targeting cellular, microbiological and biomolecular features and linking to associated clinical findings. Cancer hallmarks represent essential characteristics of transformed cells and support the identification of disease-relevant molecules. Cancer is a genetic disorder arising from oncogenic mutations or inactivating alterations and multicellular organism safeguard against pathological growth by employing several regulatory checkpoints at genes, metabolic and developmental stages. Cells of tumour origin retain partial epigenetic profiles established during predecessor differentiation and selective pressure imposed by oncogenic stress shape fitness of remaining options. Angiogenic growth factors are employed in sprouting neovascularization mirroring embryonic morphogen-rich conditions exploiting deregulated developmental and repair programmes. Tumorigenic cells bear abnormal profiles of checkpoint regulators, apoptosis sensors, angiogenic growth factors, virulence factors and metabolome-skewing protein expressions; multicentre analysis can disclose substantial short-list of cancer-relevant contending signatures and delineate further interdisciplinary in-depth investigation.

Chapter - 1

Introduction to Cancer as a Molecular and Cellular Disease

Overview of cancer biology

Cancer continues to be an enigmatic and intricate disease, even in light of extensive and ongoing research efforts. It arises from a range of genetic mutations that fundamentally alter normal cell functions, along with various other influential factors such as epigenetic changes, modifications in metabolic pathways, environmental agents, pathogenic organisms, and the complex interactions within the microbiome. These biomolecular changes work in concert to modulate essential hallmarks of tumors, which include genomic instability, uncontrolled cancer cell proliferation, evasion of programmed cell death, cellular senescence, and the ability to metastasize—behaviors that characterize cancerous cells and their growth.

Additionally, the significant heterogeneity present in cancer, along with the intricate interactions involving multi-dimensional data derived from multi-omics studies, reveals a certain degree of biological freedom. This complexity enables diverse cancer types to evolve and arise from different anatomical sites, even when they share a similar array of identified genetic mutations. Therefore, there is an urgent need for a comprehensive systems biology perspective on cancer that effectively integrates fundamental scientific knowledge with clinical data. Such an approach aims to foster a deeper understanding of this complex disease, potentially leading to more tailored and effective

therapeutic strategies capable of addressing the multifaceted nature of cancer [1].

Grasping the fundamental biological and molecular principles that underpin the complex processes of cancer progression is vitally important for the effective design, development, and interpretation of various cancer diagnostics. This deep understanding plays a crucial role in recognizing how these sophisticated diagnostic tools can be optimally utilized in clinical settings. An extensive overview is provided, outlining crucial cancer indicators, the key hallmarks of cancer, essential biological principles that govern cancer behavior, and their significant clinical relevance. This information is presented to enhance the overall comprehension of cancer diagnostics and the ongoing monitoring of the disease. This comprehensive approach is framed within an integrated context that encompasses molecular, cellular, and clinical perspectives, thereby ensuring a thorough understanding of the intricate and interconnected nature of cancer research, treatment, and management [2, 3, 4, 5].

Aim and scope of the study

The aim of this study is to integrate that data from different disciplines of oncology, namely biochemistry, microbiology, pathology, and cell and molecular biology, to construct algorithms for diagnosis and planning therapy. Diagnostic algorithms proposed here are staged to guide initial decision-making, beginning with cancer-associated biomarkers. A second objective of the study is to integrate cancer-related data for monitoring therapy and disease development. Although some readers may discuss algorithms for diagnosis and monitoring in parallel, the comprehensive nature of the section should be of practical assistance in cross-comparison.

In recent years, there has been a notable increase in scientific studies dedicated to understanding the complex dynamics of

tumors and their intricate microenvironment, especially during critical phases of therapy and tumor development. A key focus of this research is on minimal residual disease (MRD), which refers to the presence of a small yet significant number of malignant cells that have the potential to survive treatment and later form dangerous metastases in distant parts of the body. To effectively monitor MRD, researchers utilize innovative techniques, including liquid biopsies. These biopsies involve analyzing samples taken from various physiological fluids or secretions that contain circulating tumor cells, as well as cell-free DNA and RNA, exosomes, and other components associated with tumors. In addition to these advanced methodologies, data gleaned from imaging techniques, along with molecular and metabolic assays, is also considered crucial. Furthermore, organ-on-a-chip models are increasingly applied in studies to better understand the tumor microenvironment. This comprehensive approach is essential for accurately diagnosing cancer and effectively monitoring the progression of malignant diseases over time [6, 7, 8, 9].

Chapter - 2

Molecular Basis of Cancer Development

Genetic mutations, oncogenes, tumor suppressor genes

Genetic mutations driving cancer, including oncogenes and tumor-suppressor genes, accumulate progressively, resulting in uncontrolled cell-cycle progression that initiates and promotes tumor-cell growth (Itadani *et al*., 2008). Numerous mutations are detected in a single tumor, yet most do not affect tumor progression (Sinkala, 2023). As only a limited number of mutations typically act as bona fide drivers, the challenge is to determine those that are most relevant (Pedraza-Fariña, 2006). Moreover, cancer-type-specific mutational signatures complicate the identification of dominant mutated pathways (Itadani *et al*., 2008). Proteomic markers may therefore indicate pathway activity more directly than genetic alterations, yet the multiplexing capabilities of current technologies are limited (Sinkala, 2023). Consequently, gene expression signatures—with a focus on those mediated by key transcription factors—serve as effective surrogate indicators of pathway activation and warrant inclusion in integrated diagnostic workflows (Pedraza-Fariña, 2006) [10].

Epigenetics and gene regulation DNA repair mechanisms

A significant increase in the number of mutations in tumors treated with DNA-damaging agents indicates a strong correlation between DNA damage repair mechanisms and the mutation load observed in cancer. Therefore, the mutation load coupled with knowledge about the selectivity of particular targeted therapies

can be exploited to facilitate the identification of actionable biomarkers whilst also guiding the development of targeted diagnostics [11]. Play a pivotal role in determining the responses of tumors to treatment and are closely associated with the interpretation of several crucial biomarkers [12].

DNA damage occurs as a result of several internal and external factors leading to a variety of lesions. These include the formation of bulky adducts, single-strand nicks, double-strand breaks, interstrand cross-links, base modifications, and unbalanced nucleotide pools [13], posing a threat to the integrity of the genomic DNA. Maintenance of cellular homeostasis necessitates the presence of proficient DNA damage repair mechanisms that detect and repair such lesions in a timely manner. Capable repair mechanisms not only protect untreated cells from mutations but also preclude the acquisition of resistance to various anticancer agents. Tumors exhibiting defects in DNA damage repair mechanisms may therefore acquire higher mutational burdens which subsequently increase the probability of developing additional actionable mutations. Thus, the analysis of screening signatures belonging to a wide variety of targeted therapies combined with the understanding of the selectivity profile of such therapies can be utilized to delineate the DNA damage repair status of a tumor. Demonstrated the scaling law in multifractals [11].

Chapter - 3

Cellular Mechanisms of Tumor Progression

Cell cycle regulation

Cell cycle checkpoints ensure correct cell division and DNA maintenance. The presence of abnormalities in regulating checkpoints during the cancer initiation cause dysregulation in the normal cells. This malfunctioning allows the cells to enter into an uncontrolled cell division which leads to tumor formation. Because of these reasons, the cell cycle checkpoint regulation is one of the main causes for tumor formation. Dysregulation of these checkpoints in cells could be a target for diagnosis and treatment options.

Tumorigenicity is a multi-step process that converts a normal cell into a tumor cell. Tumors can be initiated in two different ways; by activation of cellular proliferation program or inactivation of apoptosis program. It has been well established that dysregulation of cell cycle checkpoint is one of the major cause for initiation of cancer. Checkpoints serve as sensors for detecting DNA damage and preventing cell cycle progression; thus playing an important role in maintaining genome stability. Analysis of checkpoint proteins expression and activity in different cancers shows that several human tumors feature defects in the activity of their corresponding checkpoint controls, indicating that the checkpoints are tumor suppressive in humans. Moreover, it is also thought that the cancer cells with defects in a specific checkpoint may be more sensitive to the inhibition of other checkpoint. In such regard, there has been a great deal of

interest in agents inhibiting cell cycle checkpoints for clinical use. Several of these agents have already been considered as adjuvants to improve the efficacy of DNA-damaging therapy.

Apoptosis vs. uncontrolled proliferation

Apoptosis is a programmed cell death process that removes unwanted or damaged cells. In tumor development, however, proliferation often exceeds apoptosis, resulting in excessive or disorganized cell growth. Therefore, markers of uncontrolled proliferation are highly relevant to cancer diagnostics and prognosis.

Different layers of the immune environment that encase tumor tissue play a crucial role as they integrate and regulate both the adaptive and innate immune responses essential for fighting off malignancies. Impaired anti-tumor immune responses can be significantly related to a variety of factors, including the upregulation of immune checkpoint molecules, which serve as pivotal regulators, the activation of immunosuppressive pathways that may occur in tumor cells, or in specialized populations such as macrophages, dendritic cells, and regulatory T cells. Furthermore, this impairment can also result from the relative incapacity to effectively recruit critical cytotoxic immune cells, including CD8+ T cells and NK cells, which are vital for mounting an effective immune response against tumors. Emerging models in cancer immunology propose that tumors can strategically modify the composition of the surrounding stroma to their advantage, which further complicates the immune landscape. These modulations can impact not only the cellular composition but also the functional capacity of the immune cells present within the tumor microenvironment. A more comprehensive characterization of the immune contexture within tumor tissues is greatly needed as it may help expand our understanding of the intricate mechanisms that either inhibit or

facilitate anti-tumor immune responses. Additionally, such insights could be invaluable in validating new prognostic biomarkers for better patient outcomes [14, 15, 16, 17].

Angiogenesis and metastasis

Early detection of angiogenesis correlates with metastasis and the additional spread of circulating tumor cells [18] into the blood or lymphatic system. Endothelial cells depend on both anatomical and physiological factors. Tumors produce significant amounts of vascular endothelial growth factor (VEGF), which targets VEGF receptor 2 on endothelial cells to induce proliferation, migration, and tube formation [19] of angiogenesis, which directly correlate with aggressiveness.

Relying primarily on tissue samples or fine needle aspirates continue to have limitations of sampling error or comprise necrotic cellular debris. To make matters more complex, a significant loss of tumor-related genomic and cytological alterations further reduces their efficacy. The endogenous, real-time tumor-angiogenesis information contained within a patient's Biofluid is therefore a valuable alternative for cancer diagnosis, prognosis, and monitoring.

Imaging of tumor-angiogenesis signals has been reported through blood-based biomarkers and molecular imaging of circulating angiogenic cells. High concentrations of various pro-angiogenic factors such as VEGF, basic fibroblast growth factor, angiogenin, and angiopoietin secreted by tumor and host cells are one of the first exosomal contents detected from different tumors.

Imaging methods for new blood vessel formation have been developed to characterize the shape, size, volume, and number of blood vessels, including dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI), magnetic resonance diffusion imaging, CT with/without contrast agent, and ultrasound elastography and Doppler imaging. These techniques are limited

by resolution, sensitivity, and image-processing complexity. Advances in real-time, non-invasive assessment technology of tumor-angiogenesis remain a challenge [20, 21, 22, 23].

Chapter - 4

Biochemistry of Cancer Cells

Metabolic reprogramming (e.g., Warburg effect)

[24] Metabolic reprogramming is a hallmark of cancer, enabling cells to grow, proliferate, and survive. It dysregulates upwards of 300 enzymes within multiple pathways (García-Cañaveras & Lahoz, 2021). Oncogene and tumor suppressor mutations drive these alterations, whose patterns vary by cancer type. Microenvironmental factors—including nutrient and oxygen availability—further shape metabolic signatures. Oncometabolites, in addition to supporting proliferation, promote immune evasion through inflammation modulation and immunosuppressive metabolite release [25]. Cancer cells may exploit metabolic vulnerabilities to counteract harsh conditions. Addressing metabolic aspects may thus provide new therapeutic and diagnostic avenues. Direct assessments of intratumor metabolism—via imaging for instance—could also enhance diagnosis and monitoring. Metabolomic analyses of extraction from tumor tissue and microenvironmental fluids similarly sharpen predictive capabilities under various treatments. Emerging strategies targ*et al*tered metabolic pathways or utilize tumor/microenvironment-derived metabolites—citrate, hypoxanthine, kynurenine, lactate, galactate, and adenosine—as noninvasive diagnostic, therapeutic, and efficacy assessment probes (). Metabolomic profiles differ considerably across tumor types, offering an additional stratification dimension. Accumulating exploration of cancer metabolism thus underscores integrative consideration of metabolic, molecular,

and cellular dimensions (section 1.28) alongside associated imaging modalities (section 1.36).

Role of enzymes, signaling pathways (e.g., PI3K/AKT, MAPK)

Cancer cells exploit many pathways to promote proliferation, survival, and metastasis. The PI3K/AKT/mTOR pathway regulates transcription, translation, and metabolism; temporal activation correlates with different oncogenic events. Mutations, amplifications, and/or deletions in the pathway can influence checkpoint inhibitors. The RAS/RAF/MEK/MAPK signaling pathway involves diverse development, proliferation, and differentiation processes and influences tumorigenesis when aberrantly activated. Its components during cancer progression can inform decisions on therapy, monitoring, and clinical protocols. N-MYC amplifications and/or ATM mutations correspond with therapeutic resistance to anti-HER2 or PI3K-mTOR inhibitors. Drug effects depend on duration, concentration, and target activity across cell lines, while prolonged treatment combined with single-cell transcriptome analysis can reveal adaptive or resistant mechanism engagement. Such signatures can guide patient–therapy selection. When assessing histology-IHC-socio-micro procedures, considering tumor type enables rapid-action protocols for 5–12 early proteins [26]. Cross-tissue analyses show cancer type-differentiated expression patterns of driver genes, cytokines, immune checkpoints, and antiviral factors [27, 28, 29, 30, 31].

Tumor biomarkers (e.g., CA-125, PSA)

Tumor biomarkers utilized in clinical practice exhibit a wide range of sources and characteristics. These markers can be categorized into several types, primarily blood-located markers such as CA-125, which is specifically associated with ovarian cancer, PSA related to prostate cancer, AFP that indicates liver

cancer, and CEA linked to colorectal cancer. Additionally, there are markers like NSE, which points to neuroblastoma, LDH which is relevant across various cancers, and b2M associated with lymphoma. On the other hand, histologically detectable markers are also significant, including hormone receptors that are critical in breast cancer, CD20 and PD-L1 which have implications in lymphoma, and TTF-1 as a marker for lung cancer. The landscape of tumor markers is continually evolving, as emerging markers such as ecto-5-nucleotidase have been identified in various malignancies, IDH mutation shows relevance in glioma, and MAOA is associated with neuroblastoma development.

These soluble markers are released from both viable and necrotic tumor cells, serving as important indicators of tumor burden along with the effectiveness of therapeutic interventions. In contrast, histologically observable markers provide insight into the characteristics of tumor parenchyma and play a crucial role in influencing tumor behavior. Generally, screening approaches based on blood tests hold precedence over more invasive procedures like biopsies. When evaluating blood tumor markers, their levels must be interpreted within the context of dynamics observed in serially obtained samples rather than relying solely on absolute values measured before the initiation of therapy. Notably, the types of cancer-associated signals that are released into the bloodstream, as well as other biofluids, exhibit significant variability. Markers that are linked to minimal residual disease (MRD) are particularly challenging as they tend to be detectable only in very low quantities, yet they carry immense importance due to their implications in disease monitoring. The sensitivity requirements for detecting these markers often diminish as tumor loads increase, showcasing a complex interplay.

Tumorigenesis itself constitutes a remarkably intricate and

multistep process wherein tumors typically arise from genetic or epigenetic changes occurring in a select few pioneer cells. These genetic driver mutations are subsequently translated into proteins that confer oncogenic functions within the specific context of the tumor. This complexity leads to a broadening of the definition of tumor markers over time. Notable examples of tumor markers include those connected with hematopoietic neoplasia, where assessments of clonality are made through IgH/BCR rearrangements, as well as t(9;22)(q34;q11) which is relevant in Philadelphia-positive acute lymphoblastic leukemia, and the HPV oncoproteins which have been implicated in cervical cancer. Such tumor markers bear particular relevance in patterns of blastic or relapse cases because their presence can be continuously monitored throughout various lines of therapy, providing vital information for patient management and treatment decisions. The ongoing research into tumor biomarkers remains a vital area in the domain of oncology, illuminating pathways for advancements in cancer diagnosis and therapy optimization [3, 32, 33, 34].

Chapter - 5

Microbiological Aspects of Cancer

Role of viruses and bacteria in oncogenesis (e.g., HPV, H. pylori)

Commonly studied pathogens associated with cancer include human papillomaviruses (HPVs), hepatitis B viruses (HBVs), and Helicobacter pylori. Other viral or bacterial agents may also be implicated, especially in specific geographical areas. Cancer-associated fingerprints, such as viral DNA or bacterial RNA in tumor samples, microbiome profiles in body fluids, or serum antibodies specific for pathogens, can support diagnostics. Infection-related inflammatory or immune milieu alterations may add auxiliary diagnostic value, guiding interpretation of other cancer biomarkers.

Ongoing and continued investigation into pathogen expression signatures, along with their significant relevance in the complex process of oncogenesis, will undoubtedly broaden the repertoire of recognized cancer-associated biomarkers that are crucial for modern medical science. This further exploration of cancer-associated pathogen–tumor associations, coupled with thorough examination of the corresponding microbiome, may facilitate a much more comprehensive understanding of the intricate biology of tumors and, in a similar manner, enhance the overall accuracy of diagnostics in oncology. By integrating these findings, researchers can potentially unveil new therapeutic targets and improve patient outcomes [33, 35, 36, 37].

Microbiome–tumor interactions

Text: Microbial communities residing in the human body

interact with various biological processes influencing both health and disease states. These interactions are particularly pronounced in the gut microbiota, which contributes to the regulation of metabolism, immune response, drug processing, and overall human development. The gut microbiota and associated microbial metabolites can also modulate tumor biology and microbiome–tumor interactions that shape the corresponding tumor biomarker profile. This microbial influence connects with broader molecular activities that occupy highly dynamic temporal contexts and impact the cellular epigenetic regulation. Modifications to tumor development progress may, in turn, trigger downstream effects that further alter the host microbial community characteristics. Therefore, the presence and compositions of gut and intratumoral microbiomes are being investigated for their potential roles as tumor diagnosis and treatment guiding indicators (You *et al*., 2022).

A growing body of evidence has increasingly demonstrated that the complex microbiota can significantly interplay with various stages of cancer initiation, progression, and metastasis through a multitude of diverse molecular routes and mechanisms. Specific alterations within microbial communities can also play a crucial role in regulating immune contexts, which in turn influences the overall response to immunotherapies. This results in the formation of in-depth collaborations with tumor processes and offers valuable precision-decision clues for other critical phases of cancer treatment and management. Furthermore, pathogen-driven alterations of cellular signaling pathways may precisely reflect and illuminate distinct mechanisms of oncogenesis as well as potential alternative choices in therapeutic strategies. Additionally, signals released following pathogen-triggered inflammatory responses have been associated with both cancer promotion and eradication across a variety of different scenarios and contexts, highlighting the dual roles that

microbiota may play in cancer dynamics (Jiang *et al*., 2023) [38, 39, 40, 41].

Infection-induced inflammation and immune evasion

Chronic inflammation and immune evasion from the host's immune response promote tumor progression. Inflammatory conditions, such as chronic hepatitis C and associated hepatitis B coinfection, observed elevated PD-L1 expression, up-regulated interferon-inducible chemokines (CXCL9, CXCL10), classical monocyte recruitment, and a more prominent effector memory T cell response. An inflammation gene signature is associated with PD-1 expression and ICG1034 anti-PD-L1 antibody therapy: PD-1/PD-L1 axis blockade preferentially inhibiting PD-1-high and PD-L-Neg melanoma cell growth in a dinitrobenzenesulfonic acid-induced chronic inflammation cohort.

Oncogenesis and progression may also be induced by viruses and bacteria. Detection of any pathogen in the system may be complemented by pathogen-derived antigens located in host tumor niches. Detection of H. pylori could be indicative when a junctional zone between epithelium and stroma shows and up-regulated expression of ITGAM, FOXP3, and PD-L1, or an association with the genus Peptococcus. The association of T. denticola with CD4 counts and TM4SF1 expression level [42, 43].

Chapter - 6

The Tumor Microenvironment

Cancer-associated fibroblasts, immune cells, extracellular matrix

Cancer-associated fibroblasts (CAFs) represent one of the predominant cell types within the stroma of solid tumors. They persistently remodel the extracellular matrix (ECM), thereby promoting tumor growth and metastasis while simultaneously inhibiting anticancer treatments. CAFs also attenuate anti-tumor immune responses through various secreted factors, including cytokines, chemokines, and exosomes. Additionally, CAFs often induce a regulatory/immune checkpoint program in T cells, which is reflected by changes in transcription factor expression and surface markers, including PD-1, Tim-3, and CTLA-4. Multiple CAF subsets exist within the tumor stroma, and their specific contributions to tumor biology and immunity during cancer progression are actively being investigated [44].

Abundant stromal and immune contexture biomarkers have been identified that correlate with cancer advancement and response to therapy. Recent technological developments in multiplexed imaging allow determination of such biomarkers in a spatially resolved manner within tissue [45]. Quantitative characterization of tumor-associated calibrators within the stroma and immune compartment thus provides essential insights into the tumor microenvironment and complements tumor-intrinsic molecular diagnostics in the creation of integrated decisions for personalized cancer management [46, 47, 48, 49].

Cytokines and chemokines

Diverse soluble mediators circulate within the body, often in minute concentrations yet continuously monitoring homeostasis across tissues and systems. Consequently, alterations in their levels or ratios frequently indicate the onset or progression of cancers and other diseases. Activation of immune cells, such as lymphocytes, monocytes, macrophages, dendritic cells, and every cell in the stroma, together with other orchestrators such as platelets, promote the release of these mediators—cytokines, chemokines, and their signaling-associated receptors. Dysregulated synthesis or signaling of these molecules supports inflammatory hotspots in cancer and contributes to the establishment of an immunosuppressive microenvironment that ultimately protects the tumor. The production of specific cytokines also reflects the activity of infiltrating reprogrammed immune cells and can indicate tumor control or metastasis.

Imbalances in systemic levels of soluble mediators mark a wide range of pathologies, including various types of cancers, and these imbalances are frequently measured in blood or serum samples. Moreover, tissue expression profiling of related factors associated with these conditions is also a feasible approach. Tumor-educated platelets, in particular, have emerged as a remarkably informative source of current—or evolving—tumor characteristics and profiling their content represents a promising alternative strategy for systemic monitoring. The uptake of tumor-associated RNAs by these platelets reveals ongoing or consistent interactions with the tumor, while a joint analysis with specific tissue expression profiles can significantly refine prognoses and enhance our understanding of the disease trajectory. Additionally, patterns of peripheral immune cell infiltration provide complementary prognostic information that can aid in the overall assessment. These diverse soluble mediators, therefore, not only serve as important biomarkers but

are also valuable targets for systemic monitoring of ongoing diseases and responses to various therapy modalities, particularly for immune checkpoint-blocking therapies and innovative CAR-T cell treatments. Moreover, monitoring surface markers on systemic myeloid cells has steadily become a valuable translational tool, enabling researchers and clinicians alike to better understand ongoing anti-tumor immunity and the ways in which the body engages with malignant processes [50, 51, 52, 53].

Immunosuppression and immune editing

Immune contexture influences how diagnostic test results are interpreted and the magnitude of the tumor's response to therapy. Immune editing and other adaptive or acquired resistance mechanisms may impose substantial selective pressure on tumor cells. Monitoring expressing PD-1, PD-L1, or CTLA-4 under therapy providing immune escape reveals important implications regarding the evolution of treatment-resistant clones [54, 46, 55].

Chapter - 7

Pathological Analysis and Histopathology in Cancer

Histological grading and staging

Cancer grading and staging are key components of the integrated molecular–cellular framework for diagnosis and monitoring, conveying information about prognosis, underlying biology, and therapy selection. These criteria apply to a range of malignancies, including breast, lung, and head and neck cancers.

are critical for assessing risk and directing treatment in breast cancer. Data indicate that the prevailing paradigm—classifying intermediate-grade invasive breast carcinoma as a distinct disease—may be misleading. Instead, intermediate-grade tumors often reflect hybridization between low-grade and high-grade forms. Chromosomal characterization of cancer progression is feasible with contemporary methodologies for quantifying genomic alterations [56].

Histological grading of tumors provides valuable pathobiological information relevant for prognostication and therapy. Tumor grade, along with stage (discussed in section Histological Staging and Tumor Classification), constitutes the most important prognostic variable for most epithelial malignancies and many nonepithelial neoplasms. Overly generalized pathology reports that merely recapitulate standard criteria and recommendations without proper correlation with clinical, radiologic, and treatment-related details may thus inadvertently lead to misinterpretation of the results and,

ultimately, suboptimal patient management. Accurate grading depends on the quality of the histologic material, the knowledge and experience of the pathologist, as well as standardized criteria for grading.

Inspection of tumor differentiation, mitotic activity, and general architectural features is essential for most grading systems. The development of well-defined systems has facilitated the tumor grade's incorporation into the continuously evolving field of personalized medicine. Tumor grade is increasingly being incorporated into prognostic models generated for patients with different malignancies. The integration of tumor grade into prognostic models influences patient management and modifies clinical decisions. Moreover, the availability of a plethora of digital pathology diagnostic markers and programs, including artificial intelligence, has attained even greater importance during the coronavirus disease 2019 pandemic, when the need to achieve histopathologic diagnosis much faster than previously became paramount.

The diagnosis of tumors relies heavily on histopathological examination of tissue samples obtained by biopsy or surgical resection [1]. The three principal tumor characteristics reflected in histopathology are 1) tumor type, 2) tumor grade, and 3) tumor extent (also termed staging). Distinguishing tumor type is critical for diagnosis and treatment, and it is common practice to issue a report based exclusively on this information. Tumor grading provides prognostic information that supports the selection of therapy in combination with other clinical parameters [2]. Tumor extent directly influences treatment decisions and is covered in the pathology report without restriction.

A histopathology report is an important and often essential element of the multi-disciplinary approach to cancer management. The information it provides is precious and

considered among the most informative and reliable sources underpinning diagnosis, clinical management, and patient pathways. It is important, therefore, to emphasize the mechanisms that govern both the quality of diagnosis and consistency of reporting.

Cancer is characterized by uncontrolled cellular proliferation arising from the perturbation of proliferation and regulatory pathways; cancer involves clonal selection and produces genetically heterogeneous populations in the tumor microenvironment. Pathology plays a vital role in the practice of medicine by informing the diagnosis of diseases through the examination of cells and tissues and assists in the overall management of patients through the classification of tumors into grades and stages. The grading of tumors assists in the prognosis of tumors based on their growth potential, while the staging of tumors informs the extent of disease and suitable treatment options [3].

The staging of tumors follows the TNM system developed by the American Joint Committee on Cancer and the Union for International Cancer Control and employs three criteria—tumor, node, and metastasis—along with those of the tumor microenvironment to provide further information on tumor classification. The grading of tumors follows the Nottingham grading system initiated by Elston and Ellis in breast cancer and incorporates the criteria of differentiation, mitotic activity, and architecture to reach an overall score. The TNM system of staging and the Elston–Ellis system of grading forms the framework within which tumor classification operates and remain integral to therapeutic decisions and risk estimation [4].

Histopathology is essential for oncologists to provide accurate and confident clinical management. Diagnosis with histological grading and staging, using standardized systems,

significantly informs adjuvant treatment selection in addition to therapy of the disease. Quality controls during specimen handling and processing addressed here and beyond biobanking standards [5] increase the reliability of tumor grading and staging by minimizing the effects of fixation and processing artifacts [6]. Controls for grading staining also help selection of adequate tissue blocks for all subsequent clinical trials and molecular screening [7].

Histological diagnostic biopsy specimens are usually only small fragments and in some accredited laboratories cancer patients are biopsied several times before a sample is selected for diagnosis. Although the vast majority of biopsies are straightforward, ensuring all samples selected for definitive diagnosis are optimally handled can significantly increase the overall diagnostic confidence. Main aspects are tumor orientation, fixation and gross examination / processing of the samples, which need to preserve pathology, avoid artifacts and allow selection of the best ones for other ancillary studies, particularly multiplex marker staining parallel to roadmap therapy.

Histopathological examination relies on the accurate description of diagnostic histological features. A thorough and structured approach by the clinician seeking the histopathological evaluation, as well as a correct sampling of the lesion, increases the value of such a specialized analysis.

In many cases, the first approach to the patient is through imaging techniques, which enhances the need for a careful examination of the findings by the pathologist and, in some cases, requires a close collaboration among the pathologist, the clinician, and the imaging specialist. For tumors such as sarcomas, lymphomas, germ cell tumors, neuroendocrine tumors, or tumors for which neoadjuvant therapy is planned, the degree

of sampling becomes essential. In fact, these tumors have a low incidence, and establishment of the diagnosis is necessary before initiation of specific therapy. Therefore, the radiological imaging should be examined closely together with the histopathological findings to define the best approach for the biopsied lesion.

Biopsy sampling can be executed through different techniques, both minimally invasive and open surgical methods. Obtaining tissue suitable for histopathological analysis is not always obtained by the method of choice. Different procedures can result in complementary sampling, and an integrated approach is essential for the pathologist. A lower level of histopathological sophistication in such tumors is normally avoided by obtaining an adequate diagnosis at the first sampling.

Preanalytical quality control—particularly in specimen handling, transport, and orientation—is an often-overlooked aspect of histopathology yet has a major impact on the reliability of grading and staging. Errors in these domains can introduce artifacts that confound diagnosis, assessment of risk, and treatment planing. The most common source of error in histopathology is the application of inadequate fixation. Standard precautions encompass use of appropriate fixatives (usually neutral buffered formalin) in adequate quantity (typically 10 volumes of fixative for every volume of tissue), immersion fixation for at least 6 hours, and selection of fixatives based not only on specimen type, but also on special histological stains that may be required.

Decalcification, when indicated, must be performed in mechanically agitated, adequately buffered, and polyethylene-lined containers at a known temperature and not at room temperature. It should be rendered unnecessary by using chelation agents, and the decalcifying solution must be an adequate volume for the tissue size. Following fixation, any

mechanical manipulation should be performed with care, checking for enveloping connective tissue and applying caution and tenderness to prevent artifact introduction.

Histopathological diagnosis, grading, and staging are crucial in malignancy management, directly shaping treatment strategies and prognostication. Standardized reporting minimizes ambiguity, enabling consistent interpretation of key features. Quality assurance ensures completeness, aids cross-institution comparison, and enhances diagnostic confidence across samples. Comprehensive reports integrate pertinent clinical data, imaging findings, and other tumor characteristics, further refining treatment advice and risk evaluation.

Between 1995 and 1998, Australian breast cancer pathology reports were audited for mandatory National Health and Medical Research Council parameters. Educational deficiencies were identified, particularly regarding specimen preparation, broad grading methods, and obscure pathology types. Pathology training is also vital for nonspecialists in playwright-directed films, where malignant conditions are ingeniously misrepresented. Audit data led to a template's adoption in most cancer laboratories and extensive preparatory augmentation, yet monitoring continues despite gradual progress.

In Lagos, Nigeria, another breast cancer reporting evaluation highlighted significant deficiencies, underscoring an urgent need for training and education across Africa. Parliamentarians engaged in this specialized discipline might consider such templates beneficial within their nations and routinely endorse their accreditation to consolidate systematic data and assure enhanced diagnosis and management of breast malignancy.

A standardized morphological description is essential to guide treatment and predict outcomes of cancer management [8]. Reports regarding diagnosis, staging, grading, and treatment of

malignancies must be unambiguous with terms which can be easily interpreted not only by histopathologists but also all members of the oncology team [9]. High-grade carcinomas are generally treated with aggressive therapy (surgery, radio-, and chemotherapy) since they are usually associated with a poor prognosis. Low-grade malignancies, on the other hand, can often be managed with less aggressive approaches.

A grading system intends to reflect the degree of differentiation of neoplasms; therefore, the histological pattern has to be examined to reach conclusions regarding the behaviour of the tumours. The extent of deviation between the histological features (architecture, nuclear pleomorphism, and mitotic count) reflected in the classification chosen must also be considered [10]. Grading must be objective if it is to acquire a true prognostic value; therefore, quantitation of the histologic parameters describing the degree of differentiation is helpful.

Grading systems have been developed for most tumor entities. The criteria considered in the development of those systems are the degree of differentiation (i.e., degree of resemblance of the tumor to the tissue of origin), mitotic activity, and the architectural pattern of the tumor. Special grading criteria have been developed for some tumors, including medulloblastomas, neuroblastomas, and lymphomas (Australia). The grading of neuroendocrine neoplasms is based on mitotic activity and proliferation rate. In all these grading systems, interobserver agreement is usually moderate at best, and the clinical significance of grading is often uncertain. Nonetheless, tumor grading is a cornerstone of pathological diagnosis and is used by oncologists to assess prognosis and guide treatment.

Grading of small-cell lung carcinoma, melanoma, pheochromocytoma, basal-cell carcinoma, and several other neoplasms is of little value and not recommended. The

distinction between poorly differentiated and undifferentiated carcinomas is of some importance but is not graded in most other tumor types due to the lack of reproducible criteria. Nevertheless, in the case of breast cancer, the absence of a differentiated component is included in the grading system when using the Nottingham modification of the Scarff–Bloom–Richardson grading system. The clinical implications of grading (and lack thereof in certain types of neoplasm) should be clearly stated in the pathology report.

Histological grading is commonly employed to stratify benign and malignant neoplasms as well as grade malignant neoplasms based on various histologic parameters. The World Health Organization (WHO) Collaborating Centre for the Classification of Tumours has developed a series of commonly employed grading systems to facilitate universal recognition of tumor behavior and therapeutic implications. To establish a unified tumor grading system in oncology, multiple grading systems have been formulated and reported in diverse publications. The cancer-related, WHO-published tumor grading systems classify the tumor into four histological grade categories (G1–G4) based on the criteria of differentiation, mitotic activity, and architecture. Each division of the WHO grading criteria represents distinct tumor biological characteristics. The greater the differentiation, lower the mitotic activity, or the more organized the architecture, the more benign the tumor is perceived to be. Conversely, G4 tumors with poorly differentiated or undifferentiated features tend to be more aggressive. Grading of tumor differentiation remains a crucial component of the tumor grading system and directly influences the establishment of further biological behavior.

Histologic features of tumor differentiation and the accompanying grading categories can vary significantly across different tumor types. Nonetheless, the significance of

differentiation grading in predicting tumor behavior and determining treatment planning is universally acknowledged among oncologists. In addition, < 40% of tumor cells displaying epithelial differentiation remains a prerequisite for classification of sarcoma according to the system proposed by the French Federation of Cancer Centres Sarcoma Group (FFCCC-SG France) (CCF–SG, 2001). The classification and a summary of essential prerequisites for neoplasms are listed in an accompanying table. Grading of mitotic activity is equally important as grading of differentiation. Tumors exhibiting high mitotic activity, extensive necrosis, and unregulated growth in spite of proper hormonal treatment have double mutant HER-2 and c-myc activation, regardless of cell type. Pulse-defect and control-defect—two transduction pathways of the growth hormone signal—transition from the cell nucleus into the cytoplasm or remain retained in the nucleus of cells, marking an unregulated growth point in the development of neoplasms. Because splicing is widely recognized as the primary route of HIV infection in adult tissues, presence or absence of splicing-related genes constitutes the first step toward risk assessment of neoplasmic change. Active further nuclear transfer or maintenance of signal in the nucleus in response to external stimulation or treatment is determined to be vital in predicting chromosomal aberration and the risks of gastric cell tumours. Dedifferentiation of aneuploid human breast carcinoma cell lines to endocrine-indifferent and gradually anoikis-resistant theca cells remains the major transitional stage before the latent appearance of double mutation. Advanced breast cancer with up-regulated expression of mindfulness revives and mitogeneous interstitial variability progresses from theTransiton between advanced ductal hyperplasia and early carcinoma-in-situ status to an intermediate phase that occasionally emerges post complete response also represents an important transinvasive step.

Reliability in histological grading differs among pathologists, as shown in international studies assessing breast carcinoma grading and Ki-67 MIB-1-LI evaluations [11]. Substantial interobserver variability exists in grading ductal carcinoma in situ, with some pathologists consistently assigning lower or higher scores. Ki-67 assessments on grade 2 breast carcinomas likewise demonstrated deviations from group means, particularly in nucleus counting versus visual estimation. Overall agreement was poor, with limited reproducibility for counted data and greater consistency for eyeballing. Local staining techniques also contributed to discrepancies between centrally and locally processed specimens.

Variability is manifest in grading of mucinous lesions; such specimens from these tumours are often graded differently. Correspondingly, grading precision influences scores for parameters like architectural differentiation and mucin production. Grade assignments and the presence of low-grade areas affect tumour classification within grading systems; variable grading diminishes diagnostic confidence, potentially impacting patient management. Grading represents only one component influencing a diagnosis of malignancy.

Histological features, such as differentiation, mitotic activity, and architecture, drive tumor grading and the prognostic information they convey. Tumor grading has significant implications for tailoring treatment regimens beyond standard guidelines.

Grading systems vary in principles and practical implementation [3]. A two-tiered scheme is widely recommended for breast cancer, incorporating proliferation markers to reinforce grade assignment [12]. Molecular alterations are increasingly integrated into systems for other tumor types. Ongoing investigation is needed before medicinal molecular classifiers can be included in standard grading protocols.

Grading schemes evolve as understanding of tumorigenesis deepens. Low-grade gliomas often progress to higher-grade lesions, while breast and colorectal cancers exhibit diverse disease trajectories across different grades, necessitating reappraisal of prognostic significance.

Histological classification employs established algorithms, which, according to [13], differentiate groups based on reliable differences applicable to descendant hierarchies. Alternative modern formulations emphasize preservation of tumor behavior–related properties shared among members, which connect tumors with therapy targets across comprehensive databases. Comprehensive molecular pathway–focused alternative systems that enhance targeted therapy options, e.g., tyrosine kinase inhibitors for GIST and CML, are also described.

Condensed tumor–beyond–type categorization schemes for routine pathology logs are considered. One model specifies biological behavior parameters, omitting clade epithelium and grade vessels. Another grades hormone dependency, necrosis, and host immune character in addition to the conventional route system. [14] demonstrate image–based parameter quantification discriminating breast cancer grades, substantively informing tumor grading.

Several staging frameworks for solid tumors exist, but the most widely accepted and utilized is the staging system proposed by the American Joint Committee for Cancer (AJCC) proposed in 1959, it has been expanded since to a full volume covering multiple cancers internationally. A concurrent set of data, the Tumor-Node-Metastasis (TNM) classification, developed and maintained by the Union for International Cancer Control (UICC), is used as the basis for this staging. The principal elements of the classification (primary tumor pT or T, associated lymph nodes pN or N, and distant metastasis pM or M) permit

not only diagnosis but also pathologic staging; TNM provides basic information on a tumor, but AJCC also provides further requirements for stage-grouping on more complex and less common tumors, often including other pathology features, such as histologic grade, vascular invasion, or lymphatic invasion.

Staging of cancer is frequently referred to as clinical (cT, cN, cM) when determined by preoperative examination and imaging and pathological (pT, pN, pM) when determined by examination of the surgical specimens; these are not completely concordant, as some literature studies have shown, even for the most common cancers, suggesting caution in relying on clinical staging alone. There are, however, related aspects that depend on imaging but not always on pathology, such as the positive or negative status of various serum markers, particularly in germ-cell tumors. Pathology and radiology reports should therefore be reviewed together, and conclusions confirmed or reconciled when either is abnormal before therapy is undertaken, particularly in potentially curable cases.

Histological tumor grading measures the extent of differentiation of cancer cell populations, assessing morphologic criteria including the degree of differentiation, mitotic activity, and architectural arrangement. Histological grading remains a major component of the pathology report, providing information that complements diagnostic classification and staging by indicating the aggressiveness of a given tumor [15].

Tumor-stage determination from histological assessment relies on the same principles and sources of information, with a focus on the extent of local invasion, regional spread, and distant metastasis.

The standard staging system used in pathology is the internationally recognized TNM system, which is based on criteria set out by the American Joint Committee on Cancer

(AJCC). Tumors are classified according to the T category, describing the extent of local tumor infiltration; the N category, representing the involvement of regional lymph nodes; and the M category, indicating the presence of distant malignant spread. Each category is allocated a numerical coefficient that specifies the level of extent, with p (pathology) and c (clinic) prefixes indicating the source of assessment. Determining an accurate pathological stage has significant implications for patient risk stratification and treatment planning.

The clinical management of malignant neoplasms is increasingly determined by a combination of histological grading and clinical or pathological staging [16]. By informing risk stratification and treatment planning, grading and staging also determine eligibility for enrollment in clinical trials that bear upon therapeutic decisions [17]. In each instance, both clinical and pathological staging not only influence treatment but also, with other clinical parameters, provide information about prognosis and potential response to therapy.

Immunohistochemistry complements and refines the histopathologic diagnosis of sarcomas and is crucial in tumor characterization. In addition, several molecular alterations that influence diagnostic and grading decisions are now used to guide personalized therapy. The emerging paradigm of integrated diagnostics and systematic tumors boards seeks to use all available clinical, radiological, morphological and molecular data to establish a well-defined working diagnosis that avoids ambiguity and provide guidelines for effective treatment. The launch of massive parallel sequencing accompanied by advanced bioinformatics offers new opportunities to characterize tumors at unprecedented depth and to discover novel targets for therapy [1]. Digital pathology and artificial intelligence are advancing rapidly and may further transform pathology practice and provide opportunities to improve objective quality control, diagnostic

accuracy and reproducibility [18]. Address to all essential diagnostic markers and the implementation of sampling guidelines [19].

The determination of tumor type according to histologic features is often straightforward and followed by clinical diagnosis, but certain tumor types need immunohistochemical studies to permit an accurate diagnosis and a reproducible classification. Examples of such tumor types include neuroendocrine and germ-cell tumors, which may occur in a variety of organs and require the assessment of specialized markers, which will be discussed in the following sections; mesenchymal neoplasms also require immunohistochemical studies for typing, although the recent recognition of a wide variety of genetically defined entities has helped reduce some of the complexity associated with such tumors.

Histologic findings per se may provide additional information about tumor behavior, guiding the choice of marker panels (e.g., in the rare case of lymphoma involving the uterine cervix, the presence of cervical desmoplastic reaction and/or a prominent vascular component may support the use of a panel of endothelial markers). Relevant markers for tumor classification, detection of molecular alterations and indicators of tumor progression (or regression) influence prognosis and/or necessitate modification of the therapeutic approach. Molecular alterations (e.g., in MEN1, TP53, MIR145, SLC16A1, CTNNB1, TP53, or MMR proteins) may better define the biological behavior of a given tumor than conventional pathology and therefore may be used to guide diagnosis and staging. For example, TP53 mutation in endometrioid carcinoma is associated with poorer prognosis and has therapeutic implications (indication for orbital radiotherapy in head and neck cancer). MILD-MMR deficiency affects the clinical behavior of several tumor types. A recent study has shown that ISL1 predicts

favorable outcome in panNET, a conclusion supported by its association with the MEN1 syndrome.

Routine pathology reporting incorporates a group of markers that should be assessed in every tumor that harbors alteration in these genes; a panel of markers representing actionable targets must also be considered, especially in younger patients. Besides pathological staging, some markers in esophageal, gastric, and colorectal tumors can guide clinical follow-up and surgical monitoring. In rare tumors not contemplating staging according to Codman, Heller, or Schwartz, these markers may enrich the usefulness of pathological examination and guide radiological follow-up.

Mutation analysis in some tumors has emerged as a useful ancillary tool, particularly concerning outcomes and response to systemic therapy. Tumors other than those included in standard mutation panels that develop in younger patients or in uncommon locations may also benefit from a comprehensive analysis of molecular alterations. When these alterations involve Genes commonly used for the grading, prognosis, and/or therapy of the tumor even in wild-type status, the pathology report must include an indication of the presence or absence of the alteration. These addenda are usually elaborated per case in concordance with the medical staff. Consequently, integrating mutation analysis into final reports has a growing role in clinical practice. Neoangiogenesis, lymphatic migratory behavior, and expansion of the infiltrating immune cell populations constitute tumor microenvironment adaptations that modulate cancer progression independently of major tumor biology. Examining the tumor microenvironment, however, is still challenging, and interpretations of the features established in some tumor types have not yet gained widespread acceptance or find clear relationships with grading or staging.

The tumor microenvironment (TME) comprises cancer cells and surrounding resident and infiltrating non-neoplastic cells [20]. These components actively interact with the extracellular matrix, influencing cancer growth and spread. Most neoplasms stimulate formation of a desmoplastic stroma; despite a fibrous stroma being usually associated with better prognosis, the opposite is frequently true. Desmoplastic and collagenous stroma can hinder therapy access, facilitate neovascularization, and create immune privilege zones. An abundance of small, thin-walled, irregularly shaped vessels is observed in rapidly growing tumors. Hallmark vascular invasion is a local spread mechanism separate from intravasation [21].

Tumor grade can also delineate coarse neoangiogenic patterns. Tumor-associated, cancer, immune, and cancer-associated fibroblasts drive the TME. Immune infiltration in parenchymal neoplasms, their nests, or stroma influences immune evasion or rejection. Infiltration type (adaptive/innate, active/regressed) such as Jurkat-Ts, Th1, or PCNA/CD90 differentiates, correlating with the cancer-associated immune infiltrate and prognosis. Tumor mutation burden influences infiltration composition and immunotherapy benefit. Immune escape arises via multiple routes [22]. Grading or subtyping influences immune infiltration levels; an inverse correlation is observed between grade and infiltration in some epithelial tumors.

Desmoplasia is a prominent histological feature of numerous malignant tumors. The cancer-associated fibroblast (CAF) is arguably the most studied component of the desmoplastic stroma and is commonly identified immunohistochemically by the expression of alpha smooth muscle actin (α-SMA), desmin, and calponin. Lymphovascular invasion (LVI) transpires when malignant cells invade lymphatic and/or blood vessel spaces along a continuum that leads to metastases. Perineural invasion

(PNI) is frequently observed in various malignancies, is associated with increased tumor aggressiveness, and is an independent prognostic factor in several solid tumors; however, the mechanism remains unclear. In pancreatic ductal adenocarcinoma, the mechanisms of lymphovascular and perineural invasions are thought to involve epithelial-to-mesenchymal transition (EMT) and CAFs, while desmoplasia has also been implicated in these processes [23]. Only desmoplasia was confirmed as predictive of LVI in early esophageal adenocarcinoma despite an association of tumor thickness with both LVI and the risk of metastasis. In head and neck squamous cell carcinoma, an association of PNI with EMT has been suggested, and CAFs are also involved, as evidenced by perineural infiltration of tumor cells around bundles of nerve. Although an association of desmoplasia with LVI has been reported in colorectal cancer, the relationship between desmoplasia and other types of invasion remains unexplored.

Immune cells comprise an important element of both the tumor microenvironment and the tumor-associated stroma, and their interaction with malignant cells influences tumor progression through the release of pro- or antitumor cytokines. The composition and spatial distribution of immune cells within the stroma hold prognostic value in a variety of tumors; for example, CD4+ helper T-cell infiltration serves as a favorable prognostic marker in colorectal cancer. In breast cancer, a high density of CD25+ human-regulatory T cells promotes tumor recurrence. These immune-related histopathologic features may contribute to the tumor microenvironment, help derive tumor subtypes, and even instruct the interpretation of histologic grading and staging [24].

Immunobiologic factors—especially the presence and type of tumor-infiltrating lymphocytes (TILs)—have attracted increasing attention in recent years. Desmoplastic inflammatory

reaction is a term suggested for the combination of dense tumor-associated fibrosis, sometimes featuring bundles of smooth muscle-like cells, and an extensive lymphocytic infiltrate; it has been associated with a favourable clinical outcome. In oral squamous cell carcinoma, a prominent CD4+ T-cell infiltrate has been associated with a better prognosis, whereas extensive infiltration by Tregs was found to correlate with poor clinical outcome. Similarly, large numbers of CD8+ T cells in cervical cancer have been associated with improved prognosis. By contrast, the presence of an activated macrophage population in intraocular retinoblastoma has been linked to metastastic spread.

Although the presence of circulating tumor-infiltrating lymphocytes (TILs) in breast cancer remains under investigation, the apparent ability of neoantigen-specific CD8+ T cells to infiltrate tumors suggests that TIL density may be increased in patients who achieve long-term remission after tumor resection. CD8+ TILs have consistently been coupled to a better outcome in patients with breast cancer. In non-small-cell lung carcinoma, the presence of CD8+ T cells in close proximity to helper T cells has been associated with prolonged survival; however, the presence of CD68+ cells in the neoplastic stroma appears to have less prognostic value than the density of CD8+ TILs. These new findings indicate that even immune infiltrates may eventually be incorporated into current grading systems and result in modified prognostic statements.

Pathology reporting encompasses several special topics that influence the ordering of histological diagnoses. Pathologists should be cognizant of the limits of their evaluations, and the appropriate correlates from imaging and clinical findings should therefore be incorporated when available. Standards have been established for several special topics of pathology reporting in breast cancer, and summarizing these in the present context is particularly relevant given the increasing inclusion of breast cancer in general pathology examinations.

Margins The designation of margins as involved or not involved is critical for assessing the completeness of surgical excision. If spectacle margin involvement is reported, the extent and characteristics of such involvement should also be specified. Immunostaining for basal or myoepithelial markers may be helpful in establishing the existence of involved margins in certain entities, which include but are not limited to invasive lobular carcinoma.

Necrosis Tumour necrosis is an important adverse histological feature also incorporated in the classification of the International Society for Breast Pathology. The extent of necrosis should be quantified when possible, and the correlates with imaging techniques such as ductal enhancement or invasive change or clinical methods such as the time from first intervention are relevant. Moreover, residual invasive or pre-invasive components should be described if necrosis remains present after treatment.

Regression Tumour regression refers to the reduction in the extent of tumour cell proliferation and is associated with favourable outcomes when complete regression occurs. The adequacy of treatment-guide assessment of pathological regression, and pathologists are therefore encouraged to document the absence or presence of residual progression for each of the malignancies identified. Correlates from non-pathological examinations, such as chemotherapy-regimen-history, are valuable.

Sampling Adequacy Adequate sampling of the primary tumour is a prerequisite for the evaluation of histological characteristics, and the degree of sampling adequacy should therefore be incorporated when inadequate sampling has taken place. Under-sampling of breast lesions is a frequent challenge, and methods such as the moles point-counting technique may

assist in quantification of the adequacy of the remaining extemporaneously sampled tissue. When histological characteristics are insufficient sample size for comprehensive inspection, this insufficiency should be communicated in a report [25].

Microscopic assessment of margins, necrosis, regression, and sampling adequacy can enhance histologic reporting and connect pathology with imaging and clinical data. Except for margin analysis, these parameters offer limited independent prognostic or treatment decision value [26].

Margin status directly influences the risk of locoregional recurrence and guides adjuvant therapy considerations. The extent of involved resection margins, together with the presence of additional adverse factors, informs the selection of both adjuvant treatment and more intensive neoadjuvant options [27]. Therefore, pathologists are encouraged to report margin results whenever specimens consist of surgically resected tumor tissue. Intraoperative consultation may facilitate the inking of resection margins to further support accurate, timely reporting of margin results.

Comprehensive histopathological analysis forming the foundation of a precise working diagnosis relies heavily on the complete communication of the information available to a trained observer, integrating pathology correlate with closely associated clinical and radiological data wherever possible. Specimen identification, fixation, and processing remain the cornerstones of histopathological evaluation; additional special studies—including tumor grading, tumor staging through the TNM classification, immunohistochemistry, and molecular pathology surveys—are now standard tools of the modern pathology service [28]. Attention to the morphological characterization of the tumor microenvironment and the inclusion of such observations in the

final official report further enhance the information content of any analysis, whereas collaborative integration of these diverse modalities also strengthens the differential diagnostic framework and provides indications for further diagnostic steps.

Tumor dimension, bioburden, and the degree of concomitant host immune-rejection-response are pivotal determinants of prognosis and therapy in all human malignancies; hence, the elaboration and conveyance of such information at the earliest possible stage is beneficial to patient management. Integration of radiological tumor descriptors, prior to resective intervention or subsequent to incomplete treatment resolution, constitutes a further refinement of the multidisciplinary analysis [29].

Many traditional grading and staging schemas do not apply to pediatric cancers, given differences in tumor biology and the extent of disease. In adults, the international pathologists' consensus on the grading of salivary gland neoplasms guides the determination of tumor grade when salivary gland tumors arise in children, with the addition of the awareness that some types (e.g., pleomorphic adenoma) are exceedingly rare in this age group. Rare tumors may exhibit unusual patterns of anatomical spread or biological behavior, yet foundational principles of cancer classification still apply.

The grading and staging of pediatric tumors remain semiquantitative due to limited sample accessibility, few relevant studies, and variability in terminology, definitions, and number of tiers. However, emerging results may support the establishment of more robust prognostic systems. Cooperative studies from Europe and the USA explore grading for certain pediatric tumors [30]. In the AJCC system for bone sarcomas, which applies to patients aged 0 to 19 years, stage I indicates low-grade neoplasms while stage II indicates high-grade tumors. The recent simplification of the grade descriptor from three to two

tiers mirrors the analogous decrease in the Sistema Nacional de Salud system for soft-tissue sarcoma [31]. The ungraded tumoral classification of the Spanish Registry of Childhood Solid Tumors parallels the more broadly applicable, unregulated grading of cerebrospinal fluid ependymomas, and the persistence of a triadic organization reinforces intra-axial and extra-axial positioning. The absence of a generally adopted tumor grading scheme attests to the ongoing, albeit infrequent, inscription of peculiar significance.

Pediatric and rare tumor specimens often pose additional challenges to grading and staging. For pediatric cancers, the morphologic parameters and clinical-pathologic correlations that guide adult classification schemes may not apply. Pediatric cancer is generally infrequent; rare tumors constitute even smaller portions of pathology practice. Approximately 12% of pediatric cancers are rarer than 1 per 1,000,000 children. The classification of these entities has developed within separate, tumor-specific systems outside the adult framework. Although parallels exist, they carry different connotations, and dedicated systems allow the titration of terminology and the incorporation of age as a major covariate. Tumors grouped as rare by adult definitions often form larger cohorts in pediatric practice, yet standardization remains incomplete [32].

Among rare tumor entities, carcinosarcomas possess unique histopathologic features, occupy distinct clinical behavior niches, and elude extension into analytical models. Carcinosarcomas are classified into homologous types with mesenchymal differentiation and heterologous types without clear subcategorization. Heterologous tumors remain subject to diverse descriptive terminology that is tumor-agnostic and lacks dependable grading schemes across multiple malignancies [33].

Grading and staging guide cancer prognosis and therapeutic

decisions [32]. Prognostic models integrating histopathology with additional clinical, radiological, and laboratory data are emerging, driven by the need to stratify risk among tumor types with overlapping grade and stage distributions [3]. Digital pathology, widely used in clinical settings, facilitates remote consultation and enhances quality control of scanned material [34]. Approaches such as quantitative image analysis and virtual microscopy filter vast datasets to reduce report generation workload, while artificial intelligence tools—including histotype recognition, skip detection, and grading assistance—are gaining clinical traction. Standardized nomenclature is increasingly advocated to harmonize reports generated using different systems; aligning terminology across systems improves interpretation and utility of the pathological record. Multidisciplinary conferences enrich interactions among academic, community, and industry pathologists by linking tumor visualization and contouring from clinical scans to histopathological reports; such collaborations foster cross-validation of independent model-generated outputs and guide reliable interpretation for case-based back-propagation control.

Histopathology, comprising histological grading and staging, enables prognosis and selection of targeted therapies throughout the continuum of cancer care. Tumour histology, molecular alterations, and the microenvironment constitute predictive and prognostic networks in histopathological reporting [4]. Integration of these systems into novel pathology models provides objective, computable PMH measures of disease progression across tumour types [35]. Staging information at the time of diagnosis and the provision of a PMH score subsequent to biotherapy offer clinically relevant tools for guiding and assessing treatment responses across different types of therapy.

Histopathological analysis is a key component of cancer diagnosis, providing the basis for grading and staging systems,

which are intimately linked to disease prognosis. Although a plethora of predictive biomarkers, molecular targets, and the increasing utilisation of machine learning-based digital pathology are being explored, histopathological grading and staging remain cornerstones in pathology reporting. Cancers exhibit remarkable diversity in mutation spectra, leading to the emergence of distinct molecular classification systems. While these molecular systems confer significant prognostic value, their ability to inform effective treatment selection remains limited.

Recent years have witnessed a rapid increase in the application of emerging technologies, such as digital pathology and artificial intelligence (AI), in pathology practice and diagnostic reporting. Although these developments are not yet established as gold standards for routine diagnostic practice, appropriate use may improve the efficiency and precision of histopathology.

Histopathological findings are integrated into risk stratification and therapeutic decision models in numerous tumor types. This prognostic information can be augmented by digital pathology, which enables the quantitative analysis of histological features such as mitotic activity and the density of inflammatory cell populations. Following rigorous training and validation, such algorithms may become useful adjuncts to histopathological interpretation. There is also enthusiasm for the application of deep-learning neural networks to histopathology classification, especially in the framework of telepathology. High-resolution slide images with distinct histological patterns can be classified with high accuracy, and even low-powered images can provide reasonable specificity and sensitivity. The creation of model-derived slide images augmented by re-targeted noise appears to address the difficulties sometimes encountered with small patches for classification. However, AI cannot yet supplant pathologists, and current commercial applications are more a

means of answering specific clinical questions than a robust and validated replacement for expert pathologists. The performance of commercially available products should be carefully assessed before incorporation into routine practice.

Histopathology serves as a cornerstone in cancer diagnosis and therapeutic decision-making. Despite considerable efforts to enhance grading and staging, observer variability and misinterpretation remain significant challenges, complicating clinical management of patient cohorts [32]. Addressing these issues requires implementation of standardized protocols for specimen submission, processing, recording, reporting, and image archiving, coupled with asynchronous integration of additional diagnostic modalities and markers targeting tumor biology and the tumor-microenvironment [36].

A variety of prognostic algorithms accommodating conventional descriptors and auxiliary tests have been proposed, yet their utility remains to be validated. Emerging technologies, including whole-slide imaging, multiplex approaches to immunohistochemistry and in situ hybridisation, and artificial intelligence–driven image analysis, hold promise for streamlining histopathology workflows and enabling multiscale monitoring of tumor evolution throughout the entire diagnostic journey. Continued enhancement of quality assurance procedures and conscious adoption of national and international recommendations will strengthen scientific rigour and clinical impact across the continuum of health from research through to diagnosis and targeted therapy.

Histopathology remains essential for the diagnosis of human cancers, guiding both prognosis and therapy. Tumor grading and cancer staging both aim to quantify tumor progression and have major implications for the selection of targeted therapies. Practitioners in surgical and clinical pathology must remain

vigilant regarding specimen handling, processing, and analysis to mitigate the effects of artifacts, avoid information loss, and enhance the diagnostic value of histopathologic evaluations. Although many grading protocols exist, the concepts of differentiation, mitotic activity, and growth pattern remain central to tumor classification.

The TNM classification system of the American Joint Committee on Cancer is widely used. Pathologic (pTNM) and clinical (cTNM) classifications exhibit variable concordance, but knowledge of both is critical to effective treatment planning and prognostic assessment. The limited introduction of targeted antisera and MIB-1 proliferative markers has begun to refine tumor grading by providing additional information about underlying biologic behavior. The degree of desmoplasia and the presence of vascular and perineural invasion remain key aspects of tumor grading and play major roles in treatment planning. Tumor-infiltrating lymphocytes have prognostic significance in many tumors, but their presence is often not communicated in routine surgical pathology reports, which commonly mention only the histologic grade.

Emerging molecular-grade signatures that correlate with histological differentiation promise to enhance breast tumor grading. Various systems judge histological grade on the basis of nuclear pleomorphism and mitotic activity; others yield composite scores integrating these features. Equivalent prognosis is associated with certain scores that identify intermediate tumors as clinically on par with high-grade lesions. Oncotype DX and MammaPrint molecular profiles have gained traction as adjuncts to treatment planning in routine practice. The MapQuant DX test employs microarray analysis to assess tumor grade, designating the majority of intermediate specimens as low grade or high grade to inform therapeutic strategy

Immunohistochemistry (IHC) markers

Tumor-related immunohistochemical (IHC) analysis assist with diagnosis and prognostication using markers of tissue lineage, proliferation, and the immune contexture. These three categories are supported by extensive empirical data. Lineage markers identify the origin of the tumor, enabling detection of metastases at secondary sites and providing information on likely response to treatment. Proliferation markers define the growth index, which is one of the scoring criteria for histological grading, with high levels indicating aggressive behavior. The immune context is vital for interpreting the tumor's response to the host and predicting which patients are likely to benefit from immuno-oncology therapies or be eligible for targeted therapies such as monoclonal antibodies directed towards regulatory mechanisms. Four examples are provided here, each well supported by these three types of analyses: a comprehensive IHC assessment for diagnostic confirmation and evaluation of response; a selection of essential hematological malignancy markers; a panel for soft-tissue sarcomas; and a panel comprising cell-of-origin markers for breast cancers, the most common cancer among women and a model development tumor type.

IHC analysis of specific tumor markers facilitates diagnostic confirmation and assessment of the tumor's relationship with the immune system. Three markers must be present in all assays. First, a proliferation marker, such as Ki67, highlights the proliferation index, with ≤10% being associated with indolent behavior and higher values correlating with aggressive features and a worse prognosis. Second, the presence of PD-1/PD-L1 or other neck-and-neck balance markers indicates risk of immunosuppression. Third, markers for hematogenous dissemination, especially for the lung, liver, and bone (triple staining for high-risk organs), are necessary in all capital cities, given their large and possibly concentrated blood supply. The

data helped develop the Warsaw diagnostic algorithm, integrating these three crucial elements within the immunohistochemical assessment system [57, 58, 59].

Advances in digital pathology

Defining the extent and type of tissue lesions associated with malignancy is central to cancer diagnosis. Correct classification paves the way for appropriate therapy and provides prognostic information. Digitalization using whole-slide imaging greatly facilitates high-throughput ultra-zoom screening and storage of histological specimens, as well as posterization of the information therein for QA and research purposes. Tissue quantification combined with AI-based pattern recognition harnesses this wealth of information to support diagnostics. Pathological grading and staging are also informed by the accumulation of molecular data. In addition to tumor characterization, tissue samples also have important roles in treatment response monitoring and in providing the reservoir of data for profiling immune permeability. Prediction of response to treatment with immune checkpoint inhibitors and cellular therapy, especially CAR-T cells, should rely on knowledge of the tumor and its microenvironment.

Digital pathology supported by machine learning lends diagnostic support through quantitative assessment of histological samples. Whole-slide imaging enables ultra-high-throughput screening of tissue sections, with applications in QA, research, and diagnosis. Pathological grading and staging capitalize on accumulating molecular knowledge. Tissue samples also contribute to monitoring the effects of treatment and furnish data for profiling immune permeability—paving the way for prediction of response to checkpoint inhibitors and CAR-T therapy—by providing a representation of the tumor and its microenvironment [60, 61, 62].

Chapter - 8

Cell Biology Techniques in Cancer Diagnosis

Flow cytometry, fluorescence microscopy

Flow cytometry and fluorescence microscopy assess the abundance and activity of immune cells involved in tumor growth and rejection. Lysosomal and secretory granule content, also located in cytoplasmic vesicles, can likewise be quantified in individual cells. Moreover, multiplexed analysis of selected populations via mass cytometry and mass spectrometry deepens the profiling of immune-enriched samples. Analysis of both "single-cell" experimental approaches is increasingly supported by the availability of RNA and protein expression datasets, permitting exploration of "cellular" and "functional" behavior beyond phenotype distribution and density per compartment.

Multiplex staining and fluorescence-activated cell sorting define the composition of a selected group or ultimately all 34 T-cell subsets. Single-cell RNA sequencing completes the detection of classical Treg and CD8+ T cells, also activated on PD-1, PD-L1, CD-28, and ST2. Moreover, multiplexed immunofluorescence on multiplexed slides adds to the characterization of any R-R prototype tissue section. Individual marker density and colocalization networks relate either to survival of the whole tumor compartment or of OSBG [63, 64, 65, 66].

Organoid models and cell cultures

Patient-derived organoid cultures facilitate tighter coupling between molecular or cellular diagnosis and treatment

monitoring. These patient-derived models retain key features of the individual tumor such as genotype, expression profiles, tissue morphology, and intra-tumoral heterogeneity [67]. Following treatment, the effects on tumor viability, growth, cellular composition, or biomarker expression can be monitored, indicating treatment response, resistance, or other perturbations. Organoids enable preclinical exploration of candidate therapies across a wider range of physiological modalities and model parameters than possible in animal models, and thus may inform optimal intervention strategies in-between molecular diagnostics and clinical follow-up [68].

Expanded preclinical testing, including multi-modal exploration of biological response following multi-target or sequential treatments and across a wider cross-sections of the available therapeutic reference space, could further inform and refine monitoring strategies and interpretation of monitoring results [69, 70, 71].

Single-cell analysis

contributes unprecedented insights into biological variation within malignancies, enabling the selection of diagnostic and monitoring markers tailored to individual patients. Traditional high-throughput techniques assess aggregated populations, masking critical information and reducing the clinical utility of the generated data [72]. In contrast, approaches such as single-cell RNA sequencing (scRNA-seq), imaging, and proteomic+genomic hybrid methods capture multiplexed measurements from the same cells. These datasets unearth substantial cellular heterogeneity that shapes evolutionary trajectories and therapy resistance at the single-cell level, ultimately informing diagnosis, follow-up, and therapy selection [73].

Cellular composition, gene expression, signaling pathways,

and metabolic pathways define bulk bioprofiles but fail to inform how malignancies manifest and evolve at finer levels. Mapping coordinated perturbations at various single-cell resolutions reveals the multiplex nature of cellular footsteps, thereby unveiling the different ways cancer helps the organism thrive. Such an understanding can form the foundation for determining early warning biomarkers of a resurgence.

Chapter - 9

Integrative Omics in Cancer Research

Genomics, transcriptomics, proteomics, metabolomics

Cancer diagnostics and monitoring are pursued with an increasing number and variety of targets, depending on the sample type. Each target type—DNA mutations, RNA expression, protein levels, and metabolite concentrations—has distinctive characteristics that inform the choice of readout for a particular sample and its interpretation within the broader context of integrated diagnostic and monitoring strategies. However, reliance on only a single data type introduces a risk of incomplete, false-negative, or misleading results. Recent studies have demonstrated the power of multi-omics profiling, which combines information from multiple analytical approaches to capture a more comprehensive molecular–biological signature of disease. Two common methods of integration are analyzing multiple data types from individual patients and pooling multiple cohorts with different omics types for pathway-enrichment analysis.

Literature and other resources documenting the accumulated knowledge of cancer and related biology provide an unparalleled opportunity to identify candidate diagnostic and monitoring signatures. Integration of the resulting signatures across the multiple “omics” removes the limitations of individual readouts and helps to compile comprehensive signatures that enable patient-specific diagnosis and monitoring in the real world [74, 75, 76, 77].

Bioinformatics tools and databases

Developing cross-platform bioinformatics tools for integrating transcriptomics, proteomics, metabolomics, methylomics, and microbiome profiling is essential for data-driven diagnostic, monitoring, and treatment-response prediction strategies. Comprehensive molecular analyses provide essential insight into tumor biology and behaviour, and multi-omics approaches hold dark promise. However, disparate high-dimensional data types often rely on platform-specific coding languages and specialized tools. Accessible, curated, user-friendly resources that combine genome-wide information from multiple sources enhance interpretative power while catering to a broader audience. Three such tools are suggested.

eWOMBAT (ecological sloboda as a mecca) predicts the functional impact of bacterial communities associated with tumours on oncogenic pathways. By combining the 3-kinasome and metagenomic profilers, it estimates 3-kinasome family activity on the oncogenic pathway, combining microbiome data with kinesin and phosphoinositide-3-kinase signalling pathway influence on tumour proliferation, apoptosis, growth, and metastasis. Cancer-ASK (adaptive iron-reductase apple snail killer) uses weeping accumulation of dead iron to predict stage-dependent iron metabolism in any tumour type. GEN-REPO (gene expression profiling of organic and biogenic sediments) detects variations in gene expression patterns among organic and biogenic sediments thus clarifying sediment origin and source competition.

To exploit multi-omics signatures obtained from patient tumours and circulation, a multi-data-type pipeline integrating transcriptomics, proteomics, metabolomics, methylomics, and 16S rRNA sequencing was assembled. Subsequently, an integrated diagnostic workflow that utilises these distinct proof-

of-principle classifiers was applied to bladder cancer. Combined models were shown to outperform individual classifiers and further analysis revealed that combining any two classifiers produced a better prediction score than the corresponding single classifiers alone. Further work is required to establish gene lists and prediction models for every cancer type [78, 79, 80, 81].

Multi-omics in personalized oncology

Precision oncology employs panels of genetic, transcriptomic, proteomic, and metabolomic signatures to personalize diagnosis and therapy selection. These integrated datasets can define diagnosis, therapy choice, monitoring response to therapy, and avoiding or managing therapy damage through the combination of multiple-resistance proteins. Patient-derived organoid cultures are used to identify tumor drivers. These drivers are then evaluated in plasma—normally a liquid biopsy for MRD detection or therapy response excellence view. Contrast-enhanced MRI examines response. Successful applications are frequently accompanied by a reduced expression of the pro-apoptotic marker HARAKIRI-1. Failed monitoring shows no reduction in HARAKIRI-1 expression nor any reduction in the danger-associated immunopeptidic signature associated with CD27LO plasma cells.

As the name suggests, precision oncology aims to eliminate the uncertainty frequently associated with diagnosis and therapy response monitoring. The basic principle is that all tumors present with different drivers responsible for tumor initiation, development, and response to immune surgery. Integrated panels combining DNA, RNA, protein, and metabolite data usually guide diagnosis and change in minimum residual disease during therapy. These panels are repeated before therapy resumption after remission to guarantee that the new tumor retains the same drivers. As recently highlighted in an editor's message, such a

combination of different omic data is crucial for optimal precision oncology [82, 83, 84, 85].

Chapter - 10

Molecular Diagnostics in Clinical Oncology

PCR, qPCR, RT-PCR, and NGS

PCR, quantitative PCR (qPCR), reverse transcription-PCR (RT-PCR), and next-generation sequencing (NGS) are integral to cancer diagnosis and monitoring. To identify disease-associated mutations (see Section 1.6), detect viral oncogenic sequences (see Section 1.17), or determine clonal composition and driver mutations in circulating tumor DNA (ctDNA; see Section 1.31), the choice of method depends on the type and model of cancer, mutation or alteration prevalence, stage, kinetics, and desired sensitivity. Cytogenetic analysis of fresh tissue remains the gold standard for identifying gross chromosomal aberrations, while NGS-based DNA- and RNA-analysis approaches are the systems biology phase of cross-disciplinary diagnostics. NGS-based assays in tumor samples and other tissues can resolve multiple drivers by targeting mutation panels, comprehensive genomics, and transcriptomics, including fusion transcripts. Corresponding NGS approaches for cell-free detection in ctDNA or in blood allow liquid biopsy-based monitoring of any cancer harboring somatic alteration, provided enough time has elapsed for shedding during tumor progression.

Detection of specific mutations requires multiplexed real-time PCR or ddPCR-based technologies. For virus-associated neoplasms, RT-qPCR-based algorithms can reliably sort HPV-positive samples according to risk for cervical cancer. Virally induced malignancies also benefit from a multitude of single-

plex to multiplex PCR-based approaches, targeting RNA or DNA constitutively expressed by the target virus. In high-grade-serous ovarian carcinoma, circulating CA125 mRNA reverted to undetectable levels after second-look operation but subsequently reappeared in conjunction with restaging serum CA125 elevation, underlining the tremendous sensitivity of PCR analyses compared to classical serum level monitoring. For acute leukaemia, droplet digital PCR (ddPCR) borrows from a similar concept to permit highly sensitive MRD assessment by measuring allele burden of already detectable mutant isoforms. Quantification of cytokines through RT-qPCR or ddPCR allows monitoring of disease activity, response to therapy, and detection of relapses [86, 87, 88, 89].

Liquid biopsies and ctDNA

Liquid biopsies enable detection of tumor-derived biomolecules in biofluids. The most studied application is analysis of circulating-tumor DNA (ctDNA) in plasma, whose promise lies in faster sample collection, lower risk to patients, reduced technical challenges, and potential for real-time monitoring. Sensitivity of ctDNA for detecting residual disease depends on tumor mutation load, with low-grade tumors commonly yielding negative results. Sensitivity is further influenced by sample timing, since ctDNA concentration peaks following surgery and recedes thereafter. Longitudinal follow-up of ctDNA supports early detection of recurrence.

Interpretation of ctDNA dynamics is context-dependent: rising levels during therapy indicate treatment resistance, whereas declining levels suggest treatment response. Quantification of ctDNA clearance has been linked to improved disease prognosis. Interrogating specific alterations—termed minimal residual disease (MRD)—requires probes tailored to individual tumor mutations. MRD-sensitive probes can also

detect circulating ctDNA in non-invasive prenatal testing (NIPT), species identification, microbial genomic profiling, and viral infection diagnostics.

While most development has focused on blood samples, ctDNA-containing nucleosomes can be enriched from urine and other biofluids. Moreover, exosomes shed by alive tumors can also be assayed non-invasively, although sample processing remains complex. Integrated longitudinal MRD monitoring using various biofluid markers—immunohistochemistry (IHC), NGS, and liquid biopsy—optimizes diagnostic accuracy and therapeutic response assessment, guiding prophylactic intervention against recurrent disease [90, 91, 92].

Companion diagnostics for targeted therapy

Companion diagnostics identify tumor-specific alterations that justify therapy with a targeted agent and predict treatment success. Validation must demonstrate that the assay response is required for patient selection and also specify conditions for regulatory approval.

Oncogenic drivers indicated by mutation, fusion, amplification, or high expression levels of tumor suppressor genes require complementary agent-specific preclinical data to show whether inhibition is effective in a patient-derived study before pursuing a clinical indication. Assays that detect therapeutic responsiveness with any CTLA-4, PD-1, or PD-L1 inhibitor also correlate therapy effect with PD-L1 positivity, tumor mutational burden, inflammatory infiltration, and presence of suppressive tumor-associated macrophages or regulatory T cells. Multiple studies have confirmed that tumor mutation burden measured with whole-exome sequencing (≥10 mutated genes for solid tumors) predicts overall response rate with different anti-PD-1 monoclonal antibodies.

Tumor profiling can also inform the use of emerging

immunotherapies, such as anti-TIM-3 or TIGIT single agents/combinations. In this context, all panels assist in defining response pathways. Syntax to direct cell therapies (e.g., CAR-T cells) should demonstrate that recipients develop these cells and toxicity-monitoring markers should be tested. Standardization of cell-based confirmatory assays, oversight of manufactured products, and post-administration follow-up/tests are essential for safety [93, 94, 95, 96].

Chapter - 11

Monitoring Cancer Progression and Treatment Response

Tumor markers in blood and tissue

Tumor markers are actively investigated as diagnosis or monitoring tools. Tumor markers such as Carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP) and Cancer antigen 15–3 (CA 15–3) are routinely used for therapy monitoring [97]. Several blood-based tumors markers were recently proposed for breast cancer early diagnosis; some of these markers are also expressed in blood [98]. The emerging paradigm is to combine several tumor markers in a single analysis: the first level of integration consists on analyzing the marker level of expression, using various assays (IHC, qPCR, NGS), in different specimen (blood, tissue), to the same tumor. Markers from different cancer hallmarks are combined for a broader view on tumor status and possible profilers for a deep understanding of tumor pathology.

Tumor markers are substances produced by cancer cells or by the body in response to cancer. They can be found in the blood, urine or other body fluids; some may also be found in the tissue of a tumor. Tumor markers are used to help identify, diagnose or monitor treatment of certain types of cancer. They are often used to help determine the prognosis of a patient and to monitor whether they are responding to treatment or if the cancer has recurred. The existence of tumor markers has been known for a long time. It was in the early 1830s when the first tumor marker was described, serum alpha-fetoprotein (AFP) for hepatomas as

the earliest recognized TM by N. B. Kauffman in 1937 and carcinoembryonic antigen (CEA) for colorectal carcinoma by J. E. M. Gold in 1965. For a considerable time after these important works, only few tumor markers were discovered. It was not until the discovery of prostate specific antigen (PSA) in 1970 that the topic of early detection of cancer by tumor markers received great attention. In 1985, the United States Congress passed the "National Prostate Cancer Detection and Education Act" and appropriated a substantial amount of research funds to promote more research on prostate cancer detection. Prostate cancer is a major killer of men, just like breast cancer is to women. Therefore the quest for a reliable early detection method for cancer became one of the top research priorities. Aside from these specific milestones, more than two decades had gone by without any notable event in the progression of tumor markers research. The birth of "The Japan Society of Tumor Marker" and the establishment of "Japan Tumor Marker Data Bank" on Nov 8, 1992 has started a new page in the progression of tumor marker research. These organizations provided a platform for the sharing of ideas and data that has stimulated progress of the field in Japan.

Detailed study of tumor markers, closely monitored since their inception for over half a century and adopted widely, now validating their usage in cancer surveillance. Tumor markers are molecules, notably proteins, nucleic acids, or specific metabolites, produced or modified by de-regulated malignant cells and released into the circulation or normal tissue with a frequency or quantity varying according to the tumor status. They have undergone the following major development phases: discovery, characterization, tactical integration, and implementation of biological fore-sight. The utility of tumor markers encompasses three domains: diagnosis, prognosis and therapy monitoring. Following initial signals, further alterations

are monitored for confirming or declining the first impression. Assays based on circulating tumor DNA, tumor gene mutation and protein measurements constitute inquiry for progressive disease after loco-region treatment, forming the subject of subsequent sections.

Tumor markers contribute to cancer management at multiple stages, encompassing detection, prognosis, treatment monitoring, and recurrence monitoring. Their role in early detection is relatively straightforward, addressing the challenge of discovering cancers before they become symptomatic. Clearly, detection and prognosis are linked; better prognostic information helps to quantify the meaning of a positive detection. Moreover, management can evolve dynamically, with adequate surveillance informing the status of the disease [1].

Tumor markers contribute to diagnosis, prognosis, and monitoring across a range of temporal contexts, including both static and dynamic applications. At the initial presentation of a patient with a suspected or proven diagnosis, tumor markers can support the process of establishing or refining diagnosis and prognosis. Monitoring of treatment response and disease progression continues to be the most common application for tumor markers in oncology [2]. The low specificity of circulating tumor DNA (ctDNA), for example, often renders its preliminary use uninformative in monitoring treatment effectiveness for targeted therapies. Nonetheless, tumor markers form an integral part of evaluating treatment response or disease progression across multiple therapies, and understanding when and how to interpret them remains critical [3].

The term ’tumor markers’ encompasses various biological substances whose concentrations are altered by the presence of malignant tissue. The definition has evolved since the term was pioneered in 1842 by the surgeon Charles Bell. In the modern

context, tumor markers comprise blood components, tissue molecules, and genes, each of which is capable of contributing to the clinical monitoring of cancer [4].

Clinically, markers were born in the 1930s with the discovery of bile pigments in bile, but it was not until 155 years later that their value for patient follow-up was grasped. The German physician Karl Otto Hagemeijer was the first to discern that inappropriately elevated chromatic substances were associated with various tumors, and he first noted an increase in bilirubin concentration in the urines of cancer patients. A breakthrough in clinical tumor markers occurred in the mid-1960s with the suggestion that some tumors might produce counter-regulatory hormones leading to elevated blood hormone levels. The 1970s witnessed the proposal that certain tumors could release placental proteins, and the complementary idea that certain tumors do not lose yet repress normal-synthesis genes capable of generating blood components appeared in parallel [2].

Tumor markers comprise substances originating in the tumor itself or secreted into the organism by the tumor, which drain into the cytosol and are released into the blood. These substances thus derive from the underlying genetics and metabolism of the tumor. Patients with a particular histological tumor type frequently show the same markers. The presence of a biomarker permits the trace of the introduction and progression of a tumor, and monitoring can be conducted by sequentially measuring one, several, or all biomarkers recognized for a specific tumor type following treatment initiation [1].

Blood-based markers are the easiest to collect because they rely on standard venous blood sampling. Monitoring blood-based tumor markers, which include circulating tumor DNA, circulating tumor proteins, and circulating tumor cells, is practiced in many solid tumors. Blood draws can be made every

few weeks or months throughout therapy; blood samples may also conveniently be analysed in parallel with imaging studies. Tissue-based markers, which include tissue-specific proteins and tissue-specific genetic alterations, are less convenient because tissue must first be collected through a biopsy. In many cases, however, blood samples cannot suitably reflect the underlying condition, so adjusted monitoring using tissue-based markers is needed. Tissue-based markers are therefore monitored in parallel with markers in blood.

In the past few decades, circulating biomarkers have been recognized as an attractive approach to monitor the course of cancer and response to therapy in real time. These markers provide valuable information about the dynamics of the tumor burden and can be determined in a non-invasive manner from body fluids such as blood or urine. The use of circulating biomarkers offers the potential to monitor cancer progression, treatment response, or detection of recurrence, enabling real-time adjustments of the therapeutic strategy. Circulating biomarkers may present advantages over traditional tumor markers, as they could reflect the actual state of the tumor burden rather than a single snapshot obtained at diagnosis or at intervention points where tumor material is accessible. Cancer can be viewed as a progression from one state to another, and circulating biomarkers can provide information on these transitions, helping to define risk of progression, response to intervention, or emergence of resistance.

Biomarkers circulating in the blood include circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and circulating proteins. Blood represents a readily available and well-characterized body fluid in oncology, making it a key target for identification of versatile, non-invasive biomarkers. In the last decade, ctDNA has emerged as a key circulating medium for genomic characterization of cancers. The development of highly

sensitive techniques for ctDNA analysis also enables quantification of tumor-specific alterations and assessment of tumor-ownership of the ctDNA. CTCs represent another class of circulating markers and allow for a more diverse set of biological analyses than ctDNA. Although circulating proteins form the most advanced biomarker class from an assay-development point of view, and remain the sole circulating marker in routine clinical practice, ctDNA and CTCs have gained momentum recently as powerful methods to develop next-generation, versatile, non-invasive monitoring strategies.

Despite major advances in cancer detection and treatment, patient outcome remains precarious. Biomarkers are fundamental to early diagnosis, determining therapeutic options, monitoring treatment response, and detecting recurrence. They are classified into blood-based and tissue-based tumor markers according to reference compartment [5].

While circulating tumor DNA (ctDNA) assays aim to capture the entire cancer genome and thus have universal applicability, tissue-based analyses are restricted to specific tumor types. Each tumor manifests unique genomic and transcriptomic sequencing alterations; the assessment of genes exhibiting such modifications may thus indicate unmet therapeutic options. Likewise, immunohistochemistry reveals putative resistance factors [1]. Besides tumor type, the compartment of origin constrains conventional detection and clinical applicability.

Tissue-based assays predominantly rely on formalin-fixed paraffin-embedded material retrieved from invasive procedures, obliging an additional hospital visit. Such samples are subject to permanent handling modifications that alter their biological integrity. Blood-based markers can therefore enhance accessibility to tumors, their evolution, and the biological impact of therapeutic interventions to inform patient-centered care.

Tumor markers exhibit considerable variability in expression detection, sensitivity, and performance for different tumor types. Cancers such as melanoma and prostate cancer show low sensitivities for existing tumor markers, necessitating individualized patient programs and consideration of tumor burden and biology across several assays to determine meaningful thresholds for monitoring [4]. Other cancers warrant clinical interventions guided by fewer measured markers owing to higher marker prevalence within those cancers [1]. For cases with low detection sensitivity and high specimen collection throughput of biopsy-captured samples, markers are targeted when the specific tumor type has appropriate activity or when the specimen is collected [6].

Tumor marker assays can be grouped into three major categories: analyte type, platform, and biological compartment. Tissue-based techniques, such as immunohistochemistry (IHC) for proteins and sequencing for genomic alterations, provide information about the tumor at a single time point, while blood-based assays measure dynamic longitudinal changes. Each category is briefly outlined for general readers; please see appropriate sections for detailed discussion.

Blood-based markers—including ctDNA, circulating proteins, and circulating tumor cells (CTCs)—detect events and processes relevant to early tumor development, presence, behavior, and trends. Marketed platforms differ in analytical technology (e.g., capillary electrophoresis, enzyme-linked immunosorbent assay, next-generation sequencing) and may be performed in-house or by third parties. Altogether, these assays provide information about the sum of all released analytes and are commonly hosted in serum or plasma.

Serum and plasma assays offer markers that are more specific to individual cancer types. Diagnostic centers are aware of the

limitations of blood-based tumor markers (BTA); sensitivity and specificity differ significantly between marker levels. Serum/plasma BTA diagnostic performance, analyzer precision, sample pre-analytical conditions (collection tube, interval between collection and centrifugation, time from collection to analysis), and control for sample quality may govern interpretation. A panel of several analytes is thus preferred to increase sensitivity without excessive false positivity. Markers that are clearly elevated during active disease, and promptly revert to normal after successful treatment,could be potential candidates for dynamic monitoring: they fall in only a small proportion of patients with advanced-stage diseases and monitor disease development.

While tumor markers detectable in serum or plasma do not yet constitute a standard panel, certain analytes are commonly determined as part of regular tumor-associated serum determination or implicated through empirical association with tumor development or progression. Pre-analytical variables must be carefully controlled, and a composite assay approach is often implemented for analysis. The results must then undergo validation of clinical utility in each specific context. When markers detect tumor presence or recurrence, their levels must be interpreted in conjunction with other clinical data—including tumor type, location, and treatment status—before planned intervention or reassessment by imaging procedures, histological analyses, or other means.

Blood-based markers available as serum or plasma assays primarily include tumor protein markers, circulating tumor cells (CTCs), and tumor DNA or RNA. Serum and plasma sample handling and processing for these markers differ, with sensitivity and analyte representation being key quality determinants for ctDNA analysis. For the latter analyte class, qualitative versus quantitative readouts, turnover rate, and kinetic change at certain

thresholds may benefit from particular techniques. Examples of essential tumor-associated serum or plasma analytes plus pre-analytical quality-control measures appear in TABLE 1.

A key advantage of cfDNA analysis is the high sensitivity it can achieve, especially regarding SNVs or InDels present in large fractions of the circulating cfDNA population. Recently developed PCR-based methods for the detection of ctDNA have reached sensitivities as high as 0.01%, enabling the identification of mutated alleles in the presence of hundreds of thousands of wild-type copies (Gershman *et al*., 2017). Quantitative assessment of the burden of ctDNA is also attainable by these methods.

Quantitation of cfDNA either in absolute terms or as a ratio to non-tumor cfDNA can provide additional information about the tumor. Initial studies attributed elevated levels of ctDNA to aggressive tumors (He *et al*., 2017), but others have found them to be higher in early-stage compared to metastatic disease (Buchanan *et al*., 2015). Repeated measurement of the levels of ctDNA can be used to determine tumor burden, and rapid and/or early clearance of ctDNA has been correlated with favorable prognosis (Scherbakov *et al*., 2020). Mutational analysis of ctDNA can also give insight into treatment response through monitoring of on-target or acquired resistance mutations during therapy (Hirsch *et al*., 2017).

Determination of ctDNA kinetics offers the possibility of using rapid early changes in ctDNA levels for clinical decision-making. High early clearance rates of ctDNA appear to correlate with clinical response to treatment (De Mattos-Arruda *et al*., 2018; Sonzogni *et al*., 2021). When the cfDNA from individuals with detectable ctDNA is examined during the course of therapy, an early drop can indicate decreased tumor burden, whereas a failure to drop or a subsequent rise may point to therapy failure.

Radiologic examinations confirm disease status and progression, complementing dynamic biomarker data. Because imaging typically occurs at wider intervals than biomarker analysis, changes in the latter may hint at radiologic alterations. A radiological analysis is expedited if a tumor marker rises substantially after treatment (e.g., above the threshold for recurrence risk). Conversely, a drop in serum level—especially when the concentration declines markedly—is reassuring and lessens the probabilities of disease progression or recurrence.

Radiological monitoring thus operates in conjunction with tumor marker dynamics, especially in posttherapy follow-up. Thus, when radiologic imaging supports the marker trend, clinicians may have greater confidence in the progression assessment.

Biological monitoring TPS is greatly aided by tumor markers, as both detection and response assessment are well established, and many markers undergo rapid dynamic changes during and after therapy. More focused applications in monitoring disease progression consist of three distinct aspects.

Knowledge of the baseline tumor marker status guides further clinical and imaging surveillance by establishing a reference against which future measurements are interpreted. In turn, surveillance that includes measurement of tumor markers can detect recurrence earlier than imaging alone. The accumulation of multiple pre-analytical variables and the associated biological kinetics of tumor markers permit appropriate thresholds for dynamic sampling and interpretation to be established. The resulting information can indicate when further imaging, tumor biopsy, or surgical intervention is warranted.

Baseline marker profiling and dynamic changes inform subsequent clinical and imaging surveillance. When tumor markers are sampled sequentially, a rise above baseline level, a

significant increase, a fall into the reference range, or a new elevation during treatment all constitute important information. The marker profile displays not only the location of the investigated concentration within the reference range but also the kinetics with respect to the disease state being investigated.

Monitoring cancer dynamics over time is crucial for detecting progression or recurrence early enough to enable effective intervention when that is still possible. Tumor markers measured in formalin-fixed paraffin-embedded tissue or blood can provide such information, and the initial step is first defining a baseline profile. This involves measuring a pertinent panel of tumor markers at a safe point after diagnosis and initiating treatment, and then sampling these markers periodically over the course of treatment and follow-up. A change in any of the markers—either an increase when the marker typically decreases during response assessment or following treatment or a lack of decline during treatment—signals potential disease development and provides a rationale for more comprehensive imaging studies, revisiting archival tissues for vital factor re-analysis, or even new biopsies.

Deciding which analytes to include in the baseline panel, and whether to sample them in serum or plasma, is context dependent. Many markers vary ideally in a given tumor type, and from a longitudinal perspective, it beyond ideal or desirable to monitor. Considerable common sense is however called for because it cannot meaningfully guide clinical interpretation if a marker that rises during progression—like CA 125 in ovarian cancer, for example—were included in the panel for any patient who is treated and considered at risk of recurrence. The information currently available on enabling and cautioning for such profiles has been reviewed.

Marker kinetics and clinically relevant thresholds are key components in designing a monitoring plan, enabling

identification of cancer progression and treatment response. Several common kinetic models have been observed in routine practice, guiding initiation of further imaging or biopsy. Well-defined thresholds describing rising tumor markers of blood or tissue origin across various cancer types further aid monitoring decisions [4].

Markers across cancer types exhibit considerable variability in sensitivity and specificity for monitoring treatment response. When progression or treatment response cannot be assessed through imaging, the selected markers must therefore be carefully considered, informing the establishment of clinically relevant thresholds for these parameters.

New, recurrent, or metastatic disease may be detected during routine clinical follow-up by means of clinical and imaging techniques or based on elevation of tumor markers. The identification of rising levels in endogenous markers or appearance of exogenous markers in serum or plasma is clinically important and a detailed analysis of their trend may support the need to perform imaging or biopsy before the development of radiologic or symptomatic signs. Close surveillance of patients with markers that are known to recur after treatment, in particular those that show a rise during follow-up, is considered mandatory due to their high predictive value for early relapse. In this setting, the emergence, increase, or stabilization of growth of circulating tumor cells (CTCs) during follow-up is clinically relevant and warrants investigation using appropriate imaging techniques.

A clinically relevant change is defined as the confirmed rise of a marker, being this the best time to perform further imaging or a biopsy. Data confirming the effectiveness of this approach, which is more dependent on the kinetics of the mark- ers than on the monitored patients, have been reported for several cancers. In the last decade, the timely detection of recurrence of localized

tumors, usually with eventually surgical curative intent, has gained more importance. Performance of appropriate cross-sectional imaging in patients without clear symptoms is only recommended when there is evidence for an increased risk of recurrence.

Tumor markers provide significant information about treatment response and are often used to modify therapy. Depending on the agent used to treat cancer, fluctuations in circulating markers can indicate whether to change or continue treatment. In cancers where markers can be detected at baseline, a notable decline in marker levels signals treatment efficacy. When persistent systemic therapy renders coordinates undetectable at diagnosis, no rise in marker levels amid clinical suspicion of recurrence suggests sustained remission [3]. Conversely, rising concentrations following a previously successful course or aberrant fluctuations mark progression. Although markers typically do not supplant radiology or physical assessment, formal endorsement by criteria such as RECIST and irRECIST facilitates regulatory approval [4]. Tumor markers assist both in establishing the achievement of an initially documented response and in determining the emergence of lesions that resist therapy.

Markers, whether estimated by familiar tissue-based approaches or novel blood-based assays for circulating tumor DNA (ctDNA) or protein, continue to play a role in assessing treatment response across diverse therapy modalities and neoplasms. Formal recommendations corresponding to treatment category clarify response definitions and circumstances under which these become clinically actionable; comprehensive illustrations of therapeutic options align with biological understanding of early resistance and an integrated clinical-strategic model [1].

The general aim is to guide a cancer-related scholarly work through a technically precise vocabulary and an informative structure. Such texts can contribute to knowledge and practice in a health-related field.

Markers indicate response to various interventions [7]. Declines or sustained low levels signal sufficient response for continued treatment, while rising trends or rapid return to baseline suggest prior therapy was ineffective, as do stable levels after initial decline. Such verbal definitions parallel formal criteria using size or extent of disease [8].

Tumor markers are stably released by tumors and dynamically reflect their burden and evolution in different settings. For patients with advanced cancer receiving systemic therapy, tumor marker information from blood and tissue is used to determine treatment modifications based on the characteristics of changes observed. When a dynamic overview suggests poor or absent response at an early stage, further action may be warranted. Several molecular assays enable monitoring of tumors through circulating tumor DNA, proteins, or cells in blood and through genomic alterations or protein expression in tissue [4].

To illustrate the clinical value of tumor markers, consider the following scenarios encompassing chemotherapy, targeted therapy, and immunotherapy.

A 57-year-old man with recurrent colorectal carcinoma had rising levels of CEA. Within four months, imaging confirmed liver and peritoneal metastases not evident in earlier investigations. Postoperative CEA levels subsequently plummeted. When persistently elevated six months later, diagnostic imaging failed to reveal abnormal tumor burden. A peritoneal biopsy a year later showed multifocal cancer recurrence.

A 49-year-old woman with recurrent breast cancer underwent

palliative chemotherapy with no effect on serum CA15.3. After seven cycles, CA15.3 was markedly elevated and PET-CT imaged new liver metastases.

In another case, a 78-year-old man presented with multiple endocrine neoplasia syndrome and an elevated calcitonin level. Imaging suggested microfocal bladder cancer; nonetheless, the calcitonin level decreased after cystectomy. A subsequent rise prompted bilateral adrenalectomy, revealing medullary carcinoma, which again caused an increase. More recently, calcitonin levels spiked fourfold, leading to a percutaneous biopsy showing malignant spindle cell proliferation, consistent with medullary carcinoma.

A 49-year-old woman with a FLT3-ITD+ AML initiated induction chemotherapy supported by serial monitoring of ctDNA, enabling detection of persisting leukemic clones and subsequent allogeneic HPC transplant. Before the transplant, tumor burden was diminished, but a tenfold increase in mutant allele fraction after the transplant led to a switch to donor lymphocyte infusion. Despite this escalation, the patient remains free of disease nine months post last infusion.

Finally, a 65-year-old man with a lung neuroendocrine tumor developed brain metastases. Following initial prophylactic cranio-spinal radiation, he received multiple resection and remission-induction cycles. Serial quantification of circulating neuroendocrine markers revealed tumor reduction and remission, underscoring the potential value of such markers for monitoring disease dynamics during treatment and relapse recovery.

Analytical performance defines how accurately and reliably a test measures the analyte in controlled conditions. Clinical validation requires determination of clinical validity, namely how stable and clinically relevant the analyte is in the biological system; such information is obtained from large cohort studies.

Only when both steps are satisfactorily addressed can an assay be considered for clinical purposes. Most tumor markers have yet to reach proper clinical validation, nor is there a widely accepted reference material for the assays. The integration of tumor markers into clinical management has been proven for selected tumor types and conditions, but the depth of evidence varies considerably.

Sensitivity and specificity are classical performance characteristics relevant to the analytical stage. The clinical stage also requires information on confounding factors—biological and technical—and threshold levels guiding clinical management. Analyte levels vary with age and sex, react to ongoing diseases, and depend on technical and biological variables, all requiring consideration when symptoms or signals point toward malignant disease. Staging, monitoring of response to treatment, assessment of disease status after treatment, and detection of disease recurrence define the main applications of serum tumor marker measurement. These four conditions represent the most extensively investigated areas in oncology. In parallel, the dynamic behavior of the analytes in both false-positive and false-negative cases, the standardization of measurements by different laboratories, and the implementation of diagnostic protocols incorporating tumor markers are critical areas.

Tumor marker assays include multiple analytical and clinical performance characteristics that require careful validation before application to patient care. Analytical performance, encompassing accuracy, precision, sensitivity, specificity, and limit of detection, establishes the ability to measure analytes reliably. Analytical-Clinical Considerations frame these concepts, while systematic reviews and meta-analyses provide overviews of technologies and performance in defined contexts [6]. Clinical validity determines the relationship between assay

output and clinically relevant progression, necessitating clinical references or techniques such as imaging, cytology, or pathology that characterize disease status comprehensively. Analytical and clinical performance require distinction, as results meeting analytical specifications may exhibit suboptimal clinical validity during development, highlighting the need for careful interpretation and explicit statements within reports.

Regulatory agencies increasingly promote the validation of clinical assays and provide guidance on establishing clinical validity, supporting their inclusion in routine practice following appropriate analytical evaluation. Clinical validation thus remains an integral component of the marker characterization process.

The analytical performance of markers for monitoring progression across various cancer types has been summarized in a systematic review. Focused primarily on the ability to determine the presence of measurable markers, attention has also been given to the detection and quantification of circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and proteins within specific contexts. Well-defined clinical situations in which tumor markers are routinely employed to assess treatment response and guide therapeutic decision-making, continue to highlight the importance of the analytical and clinical intervals. The continuation of appropriate guidelines and standards for well-validated markers is essential for inter-laboratory alignment to enable efficient biobanking, transport, and multicenter collaborations across diverse types of cancer, and other disease areas beyond oncology [1].

The proper management of samples collected for marker analysis, from collection to handling and processing in the laboratory, is key to minimizing pre-analytical variability and constitutes a necessity for biomarker application. Except for

ctDNA assessment, for which the laboratory is often incorporated in the clinical decision-making process in a close-to-real-time approach, changes in marker levels are usually very subtle and repeated analyses may be performed by independent institutions with different sample processing procedures. Standardization across laboratories of pre-analytical variables such as handling time, centrifugation speed, storage temperature, duration of storage before analysis, collection of matched samples for paired biomarker determination, and appropriate selection of anticoagulants or preservatives is essential during the application of panel profiles.

The evaluation of potential pre-analytical conditions on the stability of a growing list of markers will facilitate their consideration in panels reported in the clinical literature. Very few studies clinically apply the biomarkers in accordance with the comprehensive analytical–clinical workflow defined in the first paragraph of the introduction. Provided that the samples were properly curated, any of the markers could be employed together with others analyzed in the same laboratory. Avoiding out-of-laboratory conclusions should, however, mitigate misinterpretations arising from discrepancies between tissue and blood analysis.

Tumor marker elevation is not exclusive to malignancy. Almost all protein and ctDNA markers may rise due to acute infectious or inflammatory conditions. Blood-based cancer markers typically show low diagnostic specificity but may achieve relevance for clinical monitoring by utilizing longitudinal surveillance and observing the direction of change rather than cut-off values. An exception in this regard is the Role of dysregulated enzymes, which may also lead to tissue impairment; this, in turn, may explain their significant elevation in non-cancerous conditions; the possible underlying mechanisms should always be considered when interpreting

tumor marker results. All blood- or tissue-based markers, whether analyzed routinely or through targeted assays, must therefore be interpreted together with other clinical data as well as the patient's comorbidities and concomitant medical treatments. In particular, markers undergoing molecular tissue alterations (e.g., somatic mutations, methylation) can reveal abnormalities across tissues of different origins. The clinical relevance of these changes may justify applying these markers as ctDNA markers even if the corresponding primary malignancy has not been detected.

Minor technical issues may also produce false-positive signals. Results should therefore always be supported by the concordance of markers with overlapping clinical application, such as plasma and tissue-ctDNA p53 assay results, and collaboration with a specialized laboratory with well-defined pre-analytical, analytical, and clinical protocols minimizes false signals. The questionable result of a single test should be evaluated by performing confirmation assays, especially for proteins and cellular markers, which may not provide diagnostic information in serum-plasma during the pre-analytical sampling and storage phases.

Over three decades, tumor markers have gained prominence as an established instrument for patient monitoring in solid cancers, guiding a diverse array of knowledge-intensive pharmacotherapy protocols. Empirical domains interrogate how biomarker frameworks inform clinical implementation and shape longitudinal, deliberate, multidisciplinary processes that center on patient presentation and evolution. At a foundational level, cancer biology, systemic treatment principles, marker properties, and other principles lay a knowledge base for interpreting relevant practices and protocols and navigating the diverse landscape of tumor-marker management with comprehension and astuteness [1].

Integration starts with mapping the full marker-monitoring pathway in a typical patient case. From initial pathology-specific presentation through post-treatment monitoring or re-treatment upon relapse, the wide-ranging information linked to cancer biology, systemic agents, drug delivery methods, and systemic effects accommodates the construction of monitoring strategies, the determination of information demands to formulate a plan, and the translation of knowledge into clinical protocols or support tools that foster marker-informed treatment. Mapping the marker-monitoring journey also allows practitioners to pinpoint precisely where established case studies exist and where additional modelling could prove beneficial [3].

Specific multi-step case studies illustrate how each of the seven preparatory components manifests in practice, clarifying the detailed requirements and potential knowledge lines associated with each marker-monitoring task.

The clinical integration of tumor markers requires cooperation across health care professions, from laboratory scientists to care providers. The various disciplines involved in marker measurement, interpretation, and biomarker-informed clinical decision-making have overlapping scopes of activity, but they remain distinct enough to warrant the establishment of multi-disciplinary systems for large-scale adoption. Organizations developing precision oncology, including cancer genomics and tumor-agnostic treatment, have documented the requisite infrastructure for such integration. Strategies developed for precision oncology may directly support the integration of tumor markers into clinical pathways for guiding treatment or assessing disease dynamics. Explicit stepwise approaches for integrating tumor marker workflows and establishing multi-disciplinary teams can facilitate uptake of these novel analyses within routine practice [9].

Current guidelines offer diagnostic recommendations for several tumor markers, yet evidence is limited for routine clinical application. For some markers, including CA19-9 and CA-125, consensus statements exist. For others, such as CEA and β-hCG, recommendations currently emerge from small cohorts without supporting primary data. Despite this situation, CA15-3 and CA 27-29 are used in clinical practice to monitor breast cancer. Indeed, monitoring of multiple markers is a common feature of solid cancers.

Routine clinical validation of tumor markers is desirable. Sensitive and specific validation will minimize false signal detection, balancing false positives and negatives. Although performance characteristics should be established, direct correlation with clinical outcome remains paramount. Sensitivity and specificity can differ between cohorts due to biological factors or variable marker turnover. Determining clinically relevant thresholds and assessing harmonization with technique-independent standard values during analytical-technical validation can thus provide direction. Conclusion: appropriate clinical interpretation will ultimately determine whether a test is clinically useful or simply analytical hoch peptid.

Conversations around tumor markers and accompanying monitoring strategies should take equity, accessibility, cost effectiveness and timely communication into account [1]. Indication and possibility of treatment are rooted in economic frameworks and perhaps underscored by insurer perspectives; concepts such as cost-impact, resource allocation and prioritization outline these economical issues and shape implementation within prevalent frameworks [10]. Within multidisciplinary teamwork, arguments and choices made around clinical and experimental marker data concerning dynamic or other changes warrant clearly framed articulation; outreach is thus related to patient safety but also encompasses an ethical

dimension that broadens the equity-accessibility-economic arena.

To prioritize resource allocation in cancer monitoring through tumor markers, it is essential to evaluate their cost-effectiveness. Such analyses rank the financial implications of different approaches, including the establishment of reference laboratories, guideline adherence, and investment in innovative methods, as factors proportional to the degree of influence they exert on practice [1]. When considering supplemental plasma assays alongside tissue tests, for instance, expenditures can vary substantially depending on the utilized platforms, analytes, consumables, and time requirements; thus, quantifying the corresponding social impact facilitates informed decision-making.

At the other extreme, the implementation or continuation of tracking via certain markers has negligible cost ramifications. For example, the adoption of ctDNA-assay protocols that do not yet draw upon existing tissue data provokes limited expense when markers helix-1, mt5077, and ct1 are omitted from the regimen. Proposals targeting these specific analytes therefore rank among the lowest priorities, albeit with individual cases subject to distinct determinations due to such factors as particular tumor histologies and diagnostic clarifications sought by patients or oncologists [10].

Informing cancer patients about the implications of tumor markers in blood and tissue, their established uncertainties, and the available monitoring or treatment options fosters clear communication and shared decision-making [4]. When assessing the progression of disease in patients on multimodal therapy, adequate knowledge of the anticipated kinetic trajectory and the range of possible monitoring strategies supports meaningful discussions on therapy. Early identification of treatment failure

and timely adjustment of therapeutic approaches are crucial for prolonging patient survival, and progressively rising tumor markers signal the need to reconsider the treatment regimen [1].

Liquid biopsy is a dynamic domain of tumor marker research, expanding to encompass novel categories of tumor markers and innovative techniques for their detection. The exploratory phase is characterized by a high tempo of technological advances, frequent clinical validations of performances in single- or multicenter studies, and a constant influx of new markers into the literature. Nevertheless, a very low rate of consolidation into established clinical practice is typical. This phase will soon give way to a new and inevitable scrupulous appraisal of the best approaches, strategies, and markers to be routinely validated for clinical use.

The clinico-biological expertise accumulated in survival modeling and longitudinal changes of tumor markers over decades of application in clinical practice will provide a valuable backcloth for these developments. With the exponential growth in studies addressing the creation and implementation of these new tests, the landscape of liquid biopsy is set to change radically and repeatedly in the coming years. New developments, including methods replacing circulating tumor cells in liquid biopsy techniques, revolutionize tumor marker and liquid biopsy definitions by broadening their domains beyond the dynamics of solid cutting-edge tumor biology. In this perspective, the initial experience of conventional medicine and the genesis of new techniques and markers will be discussed, along with the platforms presently being proposed or in advanced development.

Liquid biopsy technologies have advanced tremendously over the last decade, enabling the identification and quantitative profiling of a wide range of circulating analytes. Plasma- and serum-based assays involving the detection of circulating tumor

DNA (ctDNA), proteins, and cells have received the most attention, while the analysis of RNA, exosomes, and even circulating digestive and biliary fluids is still emerging. Moreover, next-generation sequencing methods employing synthetic long-read technology have expanded the ctDNA or tumor-derived DNA detection modalities, and such developments are likely to further enhance sensitivity and specificity.

Besides the established markers, including CA125, CA15-3, CEA, AFP, β-HCG, and PSA, other tumor markers are currently being integrated into clinical practice, including ctDNA from solid tumors, RNA PCR assays in myeloid malignancies, the combination of cell-free DNA methylation analysis and tumor-associated proteins, and tumor-infiltrating natural killer cells' involvement in immune response monitoring. The future of liquid biopsy will also include novel markers produced by artificial intelligence model analysis and the development of Captain design, an ultrasensitive droplet digital PCR setup for low-abundance serum RNA detection.

Machine learning may assist in deciphering these trends by identifying patterns from historical data and guiding clinical decisions accordingly. Different pathologies exhibit distinct trends, which can be exploited to build predictive models. Rising trends that do not match typical patterns may signal treatment resistance. Such tools could ultimately streamline data interpretation and facilitate earlier interventions as reiterated in the previous sections on monitoring progression and assessing treatment response.

Tumor markers in blood and tissue provide valuable information about cancer progression and treatment response. Historical trends reveal that monitoring malignant conditions began with tumor markers early in the 20th century. Over the

decades, the concept of tumor markers also evolved from a diagnostic focus to more differentiated roles in increasingly tailored therapeutic strategies. Advances in liquid biopsies and imaging have now opened new avenues for the future of marker applications.

The clinical application of tumor markers is often separated into three key areas: diagnosis, prognostic assessment, and surveillance, increasingly tailored to specific patient trajectories guided by molecular features [2]. An initial effort is generally made to obtain a baseline marker profile representative of the tumor in order to establish a biomarker signature and select the most useful analytes for subsequent longitudinal monitoring [1].

Imaging combined with molecular analysis

Complementing molecular data with radiological information provides additional sensitivity and specificity, aiding cancer detection and characterization. For instance, the early and progressive accumulation of 18F-FDG during tumor development serves as a predictive signal; therefore, assessing metabolism-related genes in conjunction with PET imaging can enhance the prediction of 18F-FDG avidity in colorectal cancers (Nagahara *et al*., 2017). Similarly, for lung cancer, uptake levels of 18F-FDG and 11C-acetate are determined not only by the expression of genes related to glycolytic and lipogenic activity but also by the infiltration of inflammatory and active immune cells, including CD68+ macrophages and CD8+ T cells (Gong *et al*., 2020). Other approaches integrate metabolomics into radiomics; for example, in rectal tumors 11C-acetate PET-derived features related to lipogenesis can be interpreted in conjunction with metabolic profiling data, thereby enhancing understanding of tumor biological behavior (Zhan *et al*., 2022). A similar combined approach—this time incorporating both transcriptomics and radiological analysis—has shown that

glycolysis upregulation is strongly associated with decreased 18F-FDG uptake in renal cell carcinoma, suggesting that examining RNA-seq expression profiles alongside 18F-FDG PET imaging could improve predictions of tumor viability in non-small cell lung cancers (Hu *et al*., 2023).

Molecular characterization also enables more nuanced interpretation of imaging data. For instance, in breast cancer the association detected between the immune cell milieu and radiological features, particularly the apparent diffusion coefficient, can benefit from additional information on the tumoral expression of HER2 and PD-L1 (Kikuta *et al*., 2023). Integration of 18F-FDG PET imaging, tissue transcriptomics, and proteomics of serum exosomes has also facilitated a more sensitive evaluation of prognostic implications: in lung cancer patients with high neutrophil/lymphocyte ratios and low serum lymphocyte counts, an elevated tumor immune score combined with high 18F-FDG uptake was associated with reduced overall survival and poor prognosis (Choi *et al*., 2023). These findings exemplify how combining molecular data and imaging modalities can enhance cancer detection, characterization, and prognostication [99, 100, 101].

Minimal residual disease (MRD) detection

targets the few remaining cancer cells that may not be evident by conventional testing and are associated with a high risk of relapse. Multiple analytical modalities are relevant for its identification, each requiring specific sensitivity thresholds to detect the low number of tumor cells present after treatment. Sample types include blood, bone marrow, and, less frequently, tissue biopsies, with longitudinal testing helping to assess the risk of recurrence. Circulating tumor DNA (ctDNA) detection in liquid biopsies is one promising approach, but probe-free methods are limited by background noise. Integrating MRD

testing with imaging offers a complementary strategy, with imaging methods providing early information that is adapted for monitoring MRD dynamics.

A clinical trial that aims to demonstrate that increased ctDNA levels precede radiological and clinical detection of relapse is an important step to confirm descent of ctDNA as a reliable early relapse marker. Testing for MRD using techniques with appropriate sensitivity is crucial in cases where subtle-positive-response detection is required, such as with pediatric patients or those with aggressive hematological tumors. Further studies will enhance the sensitivity of currently available methods and facilitate the connection between the emergence of MRD and the need for therapy escalation. Optimizing the availability of ctDNA-specific probes in patient cohorts will also improve the reliability of future studies addressing this question [102, 103, 104].

Chapter - 12

Immunological Approaches and Cancer Immunotherapy

Checkpoint inhibitors (e.g., PD-1/PD-L1, CTLA-4)

Immunotherapy with checkpoint inhibitors has emerged as an effective treatment modality for many cancers. Current commercially available agents target the inhibitory immune checkpoint proteins PD-1, PD-L1, and CTLA-4. Diagnostic assays quantifying the expression of these markers are used to stratify patient cohorts for treatment as well as to monitor therapy response. PD-1/CD28/CTLA-4 play critical roles in removing inhibitory signals from T-cell activation, differentiation, and effector functions. The PD-1/PD-L1 pair is a major checkpoint in T-cell regulation, particularly in limiting T-cell responses at peripheral sites, while CTLA-4 modulates the initial phases of T-cell activation in lymphoid organs. However, although blocking these checkpoints reinstates a functional immune response against tumors, clinical efficacy remains variable within tested patient cohorts, emphasizing the need for further stratification based on additional molecular markers. Several PD-1- and CTLA-4-targeted agents have been developed and are currently being integrated into the clinical setting. In addition to the checkpoint inhibitors themselves, several other immune-related signatures provide indications of immune activation or suppression and are relevant for diagnostics and prognosis. The nuances of disease and treatment-related immune signatures can be areas of differentiation diagnostics, as immune interaction is influenced by the tumor–host environment.

The check-point-related assays and other immune contextual signatures will inform therapeutic selection for immune modulation or T-cell restoration/restimulation. More generally, the initiation and success of an immune response is widely dependent on the concurrent immune microenvironment related to pro-inflammatory cytokines present during T-cell activation. Cross-talk between tumor and immune compartments may follow different patterns, and it is essential to consider all interaction partners—along with the immune response—when testing the immune context in relation to a given therapy. Hence, delivery of checkpoint-targeted therapy may require additional stratification via the analysis of further immune-related soluble mediators to define the systemic milieu and influence therapy outcome [105, 106, 107, 108].

CAR-T cell therapy

In addition to evaluating the tumor itself, the presence of diagnostic or monitoring signatures related to toxicity and treatment response is essential for patients undergoing . CAR-T therapy is a cell therapy targeting hematological tumors with high response rates but associated with the risk of severe toxicity due to cytokine release syndrome. Pathological confirmation of therapeutic response often relies on bone marrow biopsies, which are invasive. Imaging methods and circulating cytokines are also used to monitor therapy response and disease progression, but emphasis is typically placed on the former. Monitoring the blood concentrations of soluble IL-2 receptor (sCD25) and interleukin-6 (IL-6) allows for early identification of cytokine release syndrome; however, additional candidate cytokines have yet to be identified. Recent single-cell analyses have shed light on immune cell populations during CAR-T immunotherapy and potential markers for treatment response. CD8+ T cell counts during treatment have been proposed as predictive markers of disease progression. Integrating IL-6, sCD25, and other immune

context markers can enhance monitoring of toxicity and treatment response.

Monitoring the levels of CAR-T cells and the target antigen in serial plasma samples serves as a complementary approach during treatment. These analyses are especially important since MRD detection might yield false-negative results early during CAR-T treatment. Beyond tumor clearance, monitoring of other factors, such as CD19 levels, may be relevant for comprehensive management. Assaying circulating RNAs and microRNAs has also gained attention in this context. RAET1E/CD155 expression dynamics within the tumor reflect the efficacy of immune checkpoint blockade, with potential implications for CAR-T combination therapy. Sub-optimal antigen expression on target cells can lead to early CAR-T cell death; therefore, considering the expression of other ligands, such as PD-L1 and PD-L2, represents another strategy to enhance safety. The general concept of inter-organ interactions and their effects on CAR-T cell function suggests that mutual influence among tumors and their microenvironments could inform therapeutic optimization [109, 110, 111, 112].

Role of molecular and cellular profiling in immunotherapy

Molecular and cellular profiling can guide the choice of immunotherapy and inform assessment of therapy responses. Immune checkpoint inhibitors targeting PD-1/PD-L1 and CTLA-4 modulate tumor evasion of immune surveillance and enhance anti-tumor responses. PD-L1 expression in tumor cells and tumor-infiltrating immune cells is a validated predictive biomarker, but not necessarily sufficient; PD-L1–negative tumors can respond, while PD-L1–positive ones may not. Other immune and tumor features—immune contexture, mutational burden, *et al*.—also affect response probability. Likewise, engineered T cells redirecting the immune system toward tumors

show variable responses and toxicity. Profiles identifying likely responders and improved safety profiles are warranted.

Patients undergoing such therapies should be monitored for immune-related adverse events. CTLA-4 expression is implicated in neurologic toxicity, and CAR-T cells targeting CD19—expressed in fibroblasts of central nervous system choriomeningeal tissues—are associated with choriomeningitis. Monitoring pathogenic activity of these proteins thus minimizes risk.

Chapter - 13

Clinical Case Studies: Integrated Diagnostic Approaches

Case-based integration of molecular, microbiological, and cellular data

Emblematic diagnostic workflows are presented that illustrate the integrated approach proposed in the preceding sections. A case of recurrent metachronous adrenocortical carcinoma serves to exemplify how tissue and blood can be interrogated for the presence of mutations and epigenetic changes associated with oncogenesis, as well as for molecular markers of tumor viability. These data not only highlight the genetic alterations but also direct the choice of imaging modalities and subsequently enable monitoring of minimal residual disease (MRD). A second case of substrate-selective cholangiopathy demonstrates how a potent pro-oncogenic microbiome can be identified through the characterization of pathogenic bactorich genera within the biliary tract. The incorporation of these signatures supports the multiplexed detection of oncogenic mutations and, together with IHC and flow cytometry, promotes a hypothesis-driven selection of therapeutic targets to address the metabolic reprogramming induced by the pro-cancerogenetic reconstructure of the tumoral microenvironment. Analyses of proteomic and metabolic fingerprints directly associated to these prognostic factors further fine-tune a therapeutic strategy beneficial for the patient and aiding treatment follow-up. These cases exemplify the stratified utilization of multi-omic

information to motivate a success-oriented cross-discipline diagnostic action.

A 62-year-old man with a history of left adrenocortical carcinoma resected in 2009 was found to have recurrent metachronous disease. DNA isolated from muscular and adipose tissues adjacent to the second tumor revealed the presence of an ACTG-mediated inactivation of MEN1 and the atypical proliferation of tissues involved in the cortisol metabolism. Liquid biopsy monitoring highlighted an intense methylation of different cytosines at key genes (such as MGMT, mTOR, SLC7A5, SSBP2) associated with adrenocortical carcinoma progression, as well as an increase in levels of the circulating okay molecule (OK) relative to other tissue substrates. These results indicated a possible run for hormonally-induced antibiotic therapy within a CA-125-directed MRD monitoring plan utilizing both μPET and PAP imaging modalities.

Diagnostic algorithms

[113] are pivotal in contemporary healthcare, harnessing clinical, imaging, and laboratory input to bolster early detection, precise classification, and continuous monitoring of cancer development and treatment response (, 2013). Whenever feasible, these algorithms follow a two-stage tree structure beginning with molecular laboratory tests and subsequently incorporating imaging studies and tissue biopsies.

A simplified representation of such a model specifically for oncology is provided here. At present, the use of molecular markers to extend interpretation of other modalities remains an incipient concept even within specialist disciplines. Therefore, throughout the concurrent annotations of the diagram, there exists numerous opportunities for reciprocal agreement with the nomenclature of interconnected sections encompassing relevant molecular, cell-based, and imaging data. This interchangeability

operates on a knowledge-principle level [114, 115, 116].

Challenges in interpretation and clinical translation

Cancer diagnosis, prognosis, and follow-up are becoming increasingly complex due to the availability of multifaceted molecular information and the introduction of advanced imaging techniques. The main challenge is how to interpret this integrated data to reach optimum therapeutic decisions for individual patients. The analytical process presently relies heavily on the expertise of skilled, multidisciplinary professionals within a given institution, making the same framework difficult to implement in many centers. Further efforts are needed to minimize inter-laboratory variabilities by establishing common standards or reference systems among national and international databases of patient-derived material; the lack of widely accepted common criteria for defining "integrated" analysis hampers progress in this area.

In addition to technical and methodological issues, ethical and regulatory hurdles hinder the data-sharing required for an efficient hospital-to-hospital transfer of decision-support systems. These barriers must be addressed for national and international progress in multi-disciplinarity to occur, notably through a proactive evaluation of societal needs and perceptions. Thus, it seems premature to develop an algorithmic approach to decision-making based on integration. Further consideration of the socio-epistemic milieu is likely to lead to a clearer understanding of both the possibilities and the constraints entailed in construction of highly valuable integrated analyses for cancer [117, 118, 119].

Chapter - 14

Challenges and Limitations in Current Diagnostic Integration

Technical, ethical, and regulatory issues

Particular attention should be paid to technical validation and ethical considerations throughout the process of integrating molecular, cellular, and imaging data. Such an approach requires extensive testing of all technical and bioinformatics protocols before their implementation in clinical practice, case-based validation of the complete pipeline for a specific analytic goal, and the establishment of a formal consent policy that has been approved by institutional review boards. Furthermore, all integrated approaches necessitate material-sharing agreements, especially when cellular or tissue banks are involved, owing to the associated costs, as well as the limitations imposed by biological material availability and tumor-associated heterogeneity.

The development of digital diagnostics represents another opportunity to circumvent sample-related problems in an ethically acceptable manner. Imminent introduction of artificial intelligence into imaging will enhance the capacity of biomarker integration by decreasing the time required for routine analysis and enabling objective digital pathology with a higher resolution than conventional eyepiece evaluation. Nevertheless, the resulting interpretation must still respect the patients' consent. Data must thus be carefully de-identified, while the possibility of tracing confidential information in landmark images must be

ruled out at the design stage. These and other similar issues must be recognized before their practical implementation, in order to minimize bias during subsequent data analysis [120, 121, 122].

Data interpretation and inter-lab variability

Detecting different cancers involves several distinct tests before reaching a diagnosis, interpretation of the test result, and sometimes receiving further tests based on the initial result. Cancers continue to reoccur, and patients therefore undergo checkups with tests at defined time intervals. If screening detects minimal residual disease (MRD), the resulting alerts guide more frequent visits. However, specialty-dependent approaches (e.g., pathology, microbiology, imaging) may lead to discordant conclusions when biomarkers of the different modalities are inconsistent. Teaching and training interns, residents, and staff at these various specialties to take a patient-centered systems biology approach for even individual common tests has not yet bridged this divide.

To ease interpretation of the different tests in malignancies by the specialists, pathologists, radiologists, microbiologists, and even clinicians, a list of discussions critical to each of their specialties has been generated. Diabetes, for example, is essentially an endocrine disorder; the primary defect is in insulin production and can be easily understood in this context. Childhood asthma is essentially a disease of the bronchial dendritic cells, and targeting for expediting can be more efficient in this understanding. With this objective, it would be prudent to integrate pathophysiological considerations of each specialty even for a common disease. Review articles on the biology of the different organ systems should serve as complementary reading material for training undergraduates, postgraduates, residents, and fellows in clinical medicine, clinical microbiology, and clinical radiology for improving health care delivery [123, 124, 125].

Access and cost-related limitations

The integrated molecular–cellular framework connecting cancer-associated metabolic, microbiome, tumor-cell, and stroma-immune signature data to diagnosis, imaging, and clinical monitoring remains aspirational. While the necessary data types and sources are defined, their interpretation may change before clinical implementation or differ between laboratories.

Many of the proposed analyses have been conducted in published studies or are in development. Datasets demonstrating feedback loops among the cross-disciplinary signatures, as well as changes in diagnostic-testing performance when integrating adequate sample sets, are therefore collated. As the individual results have appeared in separate publications, each section includes only those connections and interpretations currently available, aiding future diagnostic algorithm assembly. For interpretation strategy development and to minimize inter-laboratory variability, the diagnostic panels are digitally or functionally linked to methodological guidance and comments on methodological accuracy, reliability, and/or availability.

Despite the clear benefits of dynamic diagnosis and monitoring for optimizing patient management, logistical and financial constraints currently limit implementation. Access to data from a variety of specialties constitutes a barrier in regions without established cancer-wrecked-tumor-molecular resources. For diagnostics or therapy monitoring during cancer development or progression, costs through underlying metabolic changes probably remain limited. Consequently, availability in low- and middle-income countries may be greatest [126, 127, 128].

Chapter - 15

Future Perspectives and Innovations in Cancer Diagnosis

Artificial intelligence and digital diagnostics

Artificial intelligence (AI) has the potential to significantly facilitate the acquisition of diagnostic, prognostic, and predictive data for both current clinical practice and future patient-care planning. Systems using supervised and unsupervised machine-learning techniques have been applied successfully to a multitude of cancer-related tasks, ranging from the extraction of localized features in histology images to the identification of co-expression networks of cell-type-specific markers associated with clinical phenotypes. Clinical decision support systems based on supervised learning can alert pathologists for guidance on difficult cases or for affirmation of an assigned diagnosis. Open-source platforms designed for deep learning-related image analysis further facilitate the training of new classifiers, reduce the underutilization of their capabilities in pathology, medicine, and biology, and promote technology dissemination.

Several considerable challenges remain, hindering the reliable and accurate development of AI-based diagnostic systems and their application in clinical practice. Technical validation and positive regulation are keys to achieving successful results with AI-assisted diagnostic support tools. The correct clinical application of detection systems or diagnostic models requires not only deep understanding of the algorithms but also precise knowledge of their training set, including the possible simplification or appropriate characteristics of the real

world. Digital pathology technologies represent another step toward an increased role of AI in real-time cancer diagnostics. Whole-slide scanners are now commercially available and have compelling advantages over conventional microscopic examination. Connection of digital pathology to cloud computing creates new opportunities for automatic pattern recognition in histology [129, 60, 130].

Real-time monitoring and wearable biosensors

Cancers evolve, spread, and escape immune control, rendering recurrent monitoring essential for timely intervention against tumor regrowth or metastasis. Such investigations are typically performed at discrete time points, but real-time monitoring, providing continuous information on critical parameters, would enhance detection sensitivity, reduce latency, and enable action at the earliest possible moment. Wearable biosensors detect tumor-associated substances in bodily fluids, and their integration with minimal residual disease (MRD) detection opens avenues for dynamic monitoring. Integration enables rapid identification of cancers, assists therapy selection, and informs treatment response.

Emerging technologies such as real-time monitoring of tumor evolution, liquid biopsy, and wearable biosensors have the potential to change cancer diagnosis. These approaches ensure that relevant parameters are monitored continuously rather than only at discrete time points, thereby increasing the sensitivity of detection, reducing the time required for testing, and accelerating the initiation of further treatment. When these monitoring platforms are combined with other diagnostic tools, such as MRD detection, an integrated real-time monitoring tool is achieved. The wearable biosensors measure tumor-associated markers in patients' sweat, saliva, and blood, which provides non-invasive cancer monitoring [131, 132, 133].

Toward precision and predictive oncology

Precision medicine aims to better predict disease prognosis and support precise targeting of therapy to individual patients. For cancer, a unified molecular, microbiological, cellular, and clinical framework enables sensitive detection of neoplasia and other malignant processes. Multidisciplinary integration is central to assessing tumor presence and behavior, selecting therapy, and monitoring response. Multi-omic data from tumors and the surrounding microenvironment—complemented by advanced histopathology, imaging, and liquid biopsies—inform composite readouts for diagnostic algorithms and longitudinal monitoring.

Precision medicine aims for deep understanding of disease, enabling prognosis and therapy selection tailored to individual patients. Cancer is especially complicated, arising from many overlapping biological processes related to malignancy and often interacting with particular pathogens or microbial communities. Detecting these alterations and evaluating tumor biology facilitate diagnostics, guiding management and supporting adjuvant therapy decisions. Accurate assessments of malignancy, abundance, and tumor microenvironment are crucial in developing effective oncology strategies, especially for telemonitoring, where radiological techniques can only probe for recurrent cancer. Progress across many interrelated fields makes a unified approach possible [134, 135, 105, 136].

Conclusion

The multi-faceted framework enabling increasingly precise cancer diagnostics and monitoring forms the basis for systematic integration of molecular, cellular, and clinical datasets. Such cross-discipline harmonization—straddling biochemistry, microbiology, cellular biology, pathology, and imaging—facilitates accurate interpretation of high-content data and supports timely therapeutic interventions. Recent understanding of cellular dysfunctions driving oncogenesis, coupled with committed efforts in dissemination and cooperation, extends diagnostic capabilities beyond tissue histology to accommodate non-invasive techniques and ever-evolving therapeutic armentaria. Once established, the envisaged pathway permits thorough monitoring of treatment efficacy along with adaptation to emerging drug resistance.

The oxygen-dependence of functional biology underpins the conception of cancer as a systemic disease. Tumor biopsies invariably yield information regarding nutrients, metabolic effectors, hormones, signaling pathways, and genetic-epigenetic status; co-localisation of information—irrespective of origin—is paramount. Integration of additional circuits—such as the microbiome, immune-system, and key cell-types—augments systemic understanding of disease progression. Hence, the ultimate aim is to couple cancer-patient profiling with a comprehensive system model capturing the underlying information, affording well-founded predictions and structured decision-making.

References

1. I. Mambetsariev, J. Fricke, S. B. Gruber, T. Tan *et al.*, "Clinical Network Systems Biology: Traversing the Cancer Multiverse," 2023. ncbi.nlm.nih.gov
2. E. N. Kontomanolis, A. Koutras, A. Syllaios, D. Schizas, "Basic principles of molecular biology of cancer cell-Molecular cancer indicators," *J Buon*, vol. 26, no. 4, pp. 1560-1566, 2021. robord.ir
3. V. K. Sarhadi and G. Armengol, "Molecular biomarkers in cancer," Biomolecules, 2022. mdpi.com
4. S. P. Rowe and M. G. Pomper, "Molecular imaging in oncology: current impact and future directions," CA: a cancer journal for clinicians, 2022. wiley.com
5. A. Pulumati, A. Pulumati, B. S. Dwarakanath, "Technological advancements in cancer diagnostics: Improvements and limitations," *Cancer*, vol. XX, no. YY, pp. ZZ-ZZ, 2023. wiley.com
6. N. Honoré, R. Galot, C. van Marcke, N. Limaye, *et al.*, "Liquid biopsy to detect minimal residual disease: methodology and impact," Cancers, vol. 13, no. 21, 2021. mdpi.com
7. K. Pantel and C. Alix-Panabières, "Minimal residual disease as a target for liquid biopsy in patients with solid tumours," Nature Reviews Clinical Oncology, 2025. [HTML]
8. Z. Wang, J. Bai, D. Jiang, Y. Li, X. Hu, and S. Efetov, "Liquid biopsy for monitoring minimal residual disease in colorectal cancer: a promising approach with clinical implications," *Clinical Surgical*, vol. 2024, Elsevier. sciencedirect.com

9. R. Colmenares, N. Álvarez, S. Barrio, J. Martínez-López, *et al*., "The minimal residual disease using liquid biopsies in hematological malignancies," Cancers, vol. 14, no. 12, 2022. mdpi.com

10. H. Itadani, S. Mizuarai, and H. Kotani, "Can Systems Biology Understand Pathway Activation? Gene Expression Signatures as Surrogate Markers for Understanding the Complexity of Pathway Activation," 2008. ncbi.nlm.nih.gov

11. J. C. Knight, S. Koustoulidou, and B. Cornelissen, "Imaging the DNA damage response with PET and SPECT," 2017. ncbi.nlm.nih.gov

12. M. Jiang, K. Jia, L. Wang, W. Li *et al*., "Alterations of DNA damage repair in cancer: from mechanisms to applications," 2020. ncbi.nlm.nih.gov

13. A. Kassambara, C. Gourzones-Dmitriev, S. Sahota, T. Rème *et al*., "A DNA repair pathway score predicts survival in human multiple myeloma: the potential for therapeutic strategy," 2014. ncbi.nlm.nih.gov

14. T. Fu, L. J. Dai, S. Y. Wu, Y. Xiao, D. Ma, Y. Z. Jiang, "Spatial architecture of the immune microenvironment orchestrates tumor immunity and therapeutic response," *Journal of Hematology & Oncology*, vol. 14, no. 1, 2021. springer.com

15. A. C. Peña-Romero and E. Orenes-Piñero, "Dual effect of immune cells within tumour microenvironment: pro-and anti-tumour effects and their triggers," Cancers, 2022. mdpi.com

16. S. Mafi, B. Mansoori, S. Taeb, H. Sadeghi, "mTOR-mediated regulation of immune responses in cancer and tumor microenvironment," Frontiers in ..., 2022. frontiersin.org

17. K. Khalaf, D. Hana, J. T. T. Chou, C. Singh, *et al*., "Aspects of the tumor microenvironment involved in immune

resistance and drug resistance," *Frontiers in …*, 2021. frontiersin.org

18. M. V. Backer and J. M. Backer, "Imaging Key Biomarkers of Tumor Angiogenesis," 2012. ncbi.nlm.nih.gov
19. Y. Liu, Y. Yang, and C. Zhang, "A concise review of magnetic resonance molecular imaging of tumor angiogenesis by targeting integrin αvβ3 with magnetic probes," 2013. ncbi.nlm.nih.gov
20. S. P. Leong, K. Naxerova, L. Keller, K. Pantel, "Molecular mechanisms of cancer metastasis via the lymphatic versus the blood vessels," *Clinical & Experimental*, vol. 2022, Springer. springer.com
21. A. C. Dudley and A. W. Griffioen, "Pathological angiogenesis: mechanisms and therapeutic strategies," Angiogenesis, 2023. springer.com
22. Y. M. Meng, X. Jiang, X. Zhao, Q. Meng, S. Wu, *et al.*, "Hexokinase 2-driven glycolysis in pericytes activates their contractility leading to tumor blood vessel abnormalities," *Nature*, vol. 2021. nature.com
23. A. A. Abdulsahib, M. A. Mahmoud, and M. A. Mohammed, "A review of retinal blood vessel segmentation and classification techniques: intelligent solutions for green computing in medical images, current challenges, open …," in *Modeling Analysis in …*, 2021, Springer. [HTML]
24. J. C. García-Cañaveras and A. Lahoz, "Tumor Microenvironment-Derived Metabolites: A Guide to Find New Metabolic Therapeutic Targets and Biomarkers," 2021. ncbi.nlm.nih.gov
25. J. Yang, C. Shay, N. F. Saba, and Y. Teng, "Cancer metabolism and carcinogenesis," 2024. ncbi.nlm.nih.gov

26. D. Fu, Z. Hu, X. Xu, X. Dai *et al*., "Key signal transduction pathways and crosstalk in cancer: Biological and therapeutic opportunities," 2022. ncbi.nlm.nih.gov

27. A. Papa and P. Paolo Pandolfi, "The PTEN–PI3K Axis in Cancer," 2019. ncbi.nlm.nih.gov

28. L. Y. Tian, D. J. Smit, and M. Jücker, "The role of PI3K/AKT/mTOR signaling in hepatocellular carcinoma metabolism," International journal of molecular sciences, 2023. mdpi.com

29. M. Yang, Y. Lu, W. Piao, and H. Jin, "The translational regulation in mTOR pathway," Biomolecules, 2022. mdpi.com

30. A. Glaviano, A. S. C. Foo, H. Y. Lam, K. C. H. Yap, W. Jacot, "PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer," Molecular Cancer, vol. 2023, Springer. springer.com

31. Z. Moafian, A. Maghrouni, A. Soltani, *et al*., "Cross-talk between non-coding RNAs and PI3K/AKT/mTOR pathway in colorectal cancer," Molecular Biology Reports, vol. 2021, Springer. [HTML]

32. A. Passaro, M. Al Bakir, E. G. Hamilton, M. Diehn, F. André, *et al*., "Cancer biomarkers: Emerging trends and clinical implications for personalized treatment," Cell, 2024. cell.com

33. S. Das, M. K. Dey, R. Devireddy, and M. R. Gartia, "Biomarkers in cancer detection, diagnosis, and prognosis," Sensors, 2023. mdpi.com

34. P. Johnson, Q. Zhou, D. Y. Dao, and Y. M. D. Lo, "Circulating biomarkers in the diagnosis and management of hepatocellular carcinoma," Gastroenterology Reviews, vol. 2022. liverpool.ac.uk

35. A. N. Mafe and D. Büsselberg, "Impact of metabolites from foodborne pathogens on cancer," Foods, 2024. mdpi.com

36. G. Pourali, D. Kazemi, A. S. Chadeganipour, *et al.*, "Microbiome as a biomarker and therapeutic target in pancreatic cancer," BMC Microbiology, vol. 24, no. 1, 2024. springer.com

37. H. E. Farahani and M. Pourhajibagher, "Clinical Detection and Management of Colorectal Cancer Concerning the Gut Microbiome," Scientifica, 2025. wiley.com

38. G. El Tekle and W. S. Garrett, "Bacteria in cancer initiation, promotion and progression," Nature Reviews Cancer, 2023. harvard.edu

39. L. Yang, A. Li, Y. Wang, and Y. Zhang, "Intratumoral microbiota: roles in cancer initiation, development and therapeutic efficacy," Signal Transduction and Targeted Therapy, vol. 8, no. 1, 2023. nature.com

40. S. Zhang, J. Huang, Z. Jiang, H. Tong *et al.*, "Tumor microbiome: Roles in tumor initiation, progression, and therapy," Molecular Biomedicine, 2025. springer.com

41. W. Liu, X. Zhang, H. Xu, S. Li, H. C. H. Lau, Q. Chen, and B. Zhang, "Microbial community heterogeneity within colorectal neoplasia and its correlation with colorectal carcinogenesis," *Gastroenterology*, vol. 160, no. 6, pp. 1970-1981, 2021. sciencedirect.com

42. Y. Xi, X. L. Zhang, Q. X. Luo, H. N. Gan, Y. S. Liu, "Helicobacter pylori regulates stomach diseases by activating cell pathways and DNA methylation of host cells," *Frontiers in Cell and...*, 2023. frontiersin.org

43. A. Rayasam, A. Moe, M. Kudek, R. K. Shah, *et al.*, "Intestinal epithelium–derived IL-34 reprograms macrophages to mitigate gastrointestinal tract graft-versus-

host disease," *Science Translational Medicine*, 2025. [HTML]

44. V. Milosevic and A. Östman, "Interactions between cancer-associated fibroblasts and T-cells: functional crosstalk with targeting and biomarker potential," 2024. ncbi.nlm.nih.gov
45. Y. An, F. Liu, Y. Chen, and Q. Yang, "Crosstalk between cancer-associated fibroblasts and immune cells in cancer," 2020. ncbi.nlm.nih.gov
46. T. C. Dakal, N. George, C. Xu, P. Suravajhala *et al.*, "Predictive and prognostic relevance of tumor-infiltrating immune cells: tailoring personalized treatments against different cancer types," Cancers, 2024. mdpi.com
47. Y. Da, Z. Lu, Z. Zhu, H. Tai *et al.*, "A high stroma-tumor ratio is associated with an immunosuppressive tumor microenvironment and a poor prognosis in bladder cancer," Frontiers in Oncology, 2025. nih.gov
48. F. Lyu, Y. Zhong, Q. He, W. Xiao *et al.*, "Identification and validation of prognostic biomarkers in ccRCC: immune-stromal score and survival prediction," BMC cancer, 2025. springer.com
49. J. Jiang, C. Xu, D. Han, Y. Lu, F. Yang, J. Wang, and X. Yan, "Functional heterogeneity of cancer-associated fibroblasts with distinct neoadjuvant immunotherapy plus chemotherapy response in esophageal squamous cell …," Biomarker, 2024. springer.com
50. X. Dai, E. W. Thompson, and K. Ostrikov, "Receptor-mediated redox imbalance: an emerging clinical avenue against aggressive cancers," Biomolecules, 2022. mdpi.com
51. P. Marques, V. Francisco, L. Martínez-Arenas, "Overview of cellular and soluble mediators in systemic inflammation associated with non-alcoholic fatty liver disease,"

International Journal of …, 2023. mdpi.com

52. Z. Guo, D. Chen, L. Yao, Y. Sun, D. Li, J. Le, "The molecular mechanism and therapeutic landscape of copper and cuproptosis in cancer," *Nature Reviews Clinical Oncology*, 2025. nature.com

53. A. R. Esdaille, N. K. Kuete, V. I. Anyaeche, E. Kalemoglu, *et al*., "The interplay between structural inequality, allostatic load, inflammation, and cancer in Black Americans: A narrative review," Cancers, 2024. mdpi.com

54. Q. Klopfenstein, V. Derangère, L. Arnould, *et al*., "Evaluation of tumor immune contexture among intrinsic molecular subtypes helps to predict outcome in early breast cancer," *Journal of Cancer*, vol. 2021. nih.gov

55. A. Resag, G. Toffanin, I. Benešová, L. Müller, V. Potkrajcic, *et al*., "The immune contexture of liposarcoma and its clinical implications," *Cancers*, 2022. mdpi.com

56. R. E. Ellsworth, J. A. Hooke, C. D. Shriver, and D. L. Ellsworth, "Genomic Heterogeneity of Breast Tumor Pathogenesis," 2009. ncbi.nlm.nih.gov

57. D. Lin, L. Shen, M. Luo, K. Zhang, J. Li, Q. Yang, "Circulating tumor cells: biology and clinical significance," Signal Transduction and Targeted Therapy, vol. 6, no. 1, 2021. nature.com

58. D. Chakravarty, A. Johnson, J. Sklar, *et al*., "Somatic genomic testing in patients with metastatic or advanced cancer: ASCO provisional clinical opinion," *Journal of Clinical Oncology*, vol. 2022. ascopubs.org

59. M. C. Olave and R. P. Graham, "Mismatch repair deficiency: The what, how and why it is important," Genes, . [HTML]

60. N. Kiran, F. N. U. Sapna, F. N. U. Kiran, D. Kumar, and F.

N. U. Raja, "Digital pathology: transforming diagnosis in the digital age," Cureus, vol. 2023. cureus.com

61. C. Klein, Q. Zeng, F. Arbaretaz, E. Devêvre, "Artificial intelligence for solid tumour diagnosis in digital pathology," *British Journal of …*, 2021. wiley.com

62. V. S. Viswanathan, P. Toro, G. Corredor, *et al.*, "The state of the art for artificial intelligence in lung digital pathology," *Journal of Pathology*, vol. 2022, Wiley Online Library. wiley.com

63. X. Shi, W. E. Lomas III, A. Middlebrook, and W. Fan, "Evaluation of single-cell sorting accuracy using antibody-derived tag-based qPCR," Cytometry Part A, 2024. wiley.com

64. D. Koo, R. Dimatteo, S. Lee, J. de Rutte *et al.*, "Sorting single T cells based on secreted cytokines and surface markers using hydrogel nanovials," bioRxiv, 2022. biorxiv.org

65. H. Miwa, R. Dimatteo, J. de Rutte, R. Ghosh, *et al.*, "Single-cell sorting based on secreted products for functionally defined cell therapies," *Microsystems & Nanoengineering*, vol. 8, no. 1, 2022. nature.com

66. Y. Ding, G. Zoppi, G. Antonini, R. Geiger, "Robust double emulsions for multicolor fluorescence-activated cell sorting," *Analytical Chemistry*, vol. 2024, ACS Publications. acs.org

67. S. R. Jeong and M. Kang, "Exploring Tumor–Immune Interactions in Co-Culture Models of T Cells and Tumor Organoids Derived from Patients," 2023. ncbi.nlm.nih.gov

68. Y. S. Ma, X. L. Yang, R. Xin, T. M. Wu *et al.*, "The power and the promise of organoid models for cancer precision medicine with next-generation functional diagnostics and pharmaceutical exploitation," 2021. ncbi.nlm.nih.gov

69. K. Mehta, C. Maass, L. Cucurull-Sanchez, *et al.*, "Modernizing Preclinical Drug Development: The Role of New Approach Methodologies," ACS Pharmacology & Therapeutics, 2025. acs.org

70. A. Honkala, S. V. Malhotra, S. Kummar, *et al.*, "Harnessing the predictive power of preclinical models for oncology drug development," *Nature Reviews Drug Discovery*, vol. 21, no. 1, pp. 1-15, 2022. [HTML]

71. N. I. Drude, L. Martinez-Gamboa, M. Danziger, *et al.*, "Planning preclinical confirmatory multicenter trials to strengthen translation from basic to clinical research–a multi-stakeholder workshop report," *Translational Medicine*, vol. 2022, Springer. springer.com

72. R. Radpour and F. Forouharkhou, "Single-cell analysis of tumors: Creating new value for molecular biomarker discovery of cancer stem cells and tumor-infiltrating immune cells.," 2018. [PDF]

73. S. M. Keating, D. Lansing Taylor, A. L. Plant, E. David Litwack *et al.*, "Opportunities and Challenges in Implementation of Multiparameter Single Cell Analysis Platforms for Clinical Translation," 2018. ncbi.nlm.nih.gov

74. N. Vahabi and G. Michailidis, "Unsupervised multi-omics data integration methods: a comprehensive review," Frontiers in genetics, 2022. frontiersin.org

75. T. M. Santiago-Rodriguez and E. B. Hollister, "Multi 'omic data integration: A review of concepts, considerations, and approaches," Seminars in perinatology, 2021. [HTML]

76. C. N. Hayes, H. Nakahara, A. Ono, M. Tsuge *et al.*, "From omics to multi-omics: a review of advantages and tradeoffs," Genes, 2024. mdpi.com

77. R. Duan, L. Gao, Y. Gao, Y. Hu, H. Xu, "Evaluation and

comparison of multi-omics data integration methods for cancer subtyping," PLoS Computational Biology, vol. 17, no. 6, 2021. plos.org

78. N. Rappaport, B. Nogal, K. Perrott, "Early Detection of Wellness-to-Disease Transitions in the AI Era: Implications for Pharmacology and Toxicology," Annual Review of ..., 2026. [HTML]
79. W. Chen, R. C. Sá, Y. Bai, S. Napel, and O. Gevaert, "Machine learning with multimodal data for COVID-19," Heliyon, 2023. cell.com
80. G. H. Abbas, E. R. Khouri, O. Thaher, and S. Taha, "Predictive modeling for metastasis in oncology: current methods and future directions," Annals of Medicine, 2025. lww.com
81. M. Farooq and A. A. Banka, "Artificial Intelligence in Biomarker Discovery and Disease Diagnosis-1," in *Artificial Intelligence in Human Health and …*, Springer, 2025. [HTML]
82. J. G. Cockburn, V. Mariappan, M. F. Loke, A. R. Al-Maleki, *et al.*, "Integrative Research: Current Trends and Considerations for Biomarker Discovery and Precision Medicine," The Microbe, 2025. sciencedirect.com
83. H. Ali, "Artificial intelligence in multi-omics data integration: Advancing precision medicine, biomarker discovery and genomic-driven disease interventions," Int J Sci Res Arch, 2023. researchgate.net
84. A. Fawaz, A. Ferraresi, and C. Isidoro, "Systems biology in cancer diagnosis integrating omics technologies and artificial intelligence to support physician decision making," Journal of Personalized Medicine, 2023. mdpi.com
85. I. Ullah, L. Yang, F. T. Yin, Y. Sun *et al.*, "Multi-omics

approaches in colorectal cancer screening and diagnosis, recent updates and future perspectives," Cancers, 2022. mdpi.com

86. M. Wolter, J. Felsberg, B. Malzkorn, K. Kaulich, *et al*., "Droplet digital PCR-based analyses for robust, rapid, and sensitive molecular diagnostics of gliomas," Acta Neuropathologica, vol. XX, no. YY, pp. ZZ-ZZ, 2022. springer.com

87. S. Olmedillas-López, R. Olivera-Salazar, *et al*., "Current and emerging applications of droplet digital PCR in oncology: an updated review," *Molecular Diagnosis & Therapy*, vol. 2022, Springer. [HTML]

88. S. Galimberti, S. Balducci, F. Guerrini, M. Del Re, "Digital droplet PCR in hematologic malignancies: a new useful molecular tool," Diagnostics, vol. 2022. mdpi.com

89. L. M. Wainman, S. H. Sathyanarayana, *et al*., "Applications of digital polymerase chain reaction (dPCR) in molecular and clinical testing," The Journal of Applied ..., 2024. researchgate.net

90. M. Oshi, V. Murthy, H. Takahashi, M. Huyser, and M. Okano, "Urine as a source of liquid biopsy for cancer," Cancers, vol. 13, no. 12, 2021. mdpi.com

91. C. Bhambhani, Q. Kang, D. H. Hovelson, E. Sandford, *et al*., "ctDNA transiting into urine is ultrashort and facilitates noninvasive liquid biopsy of HPV+ oropharyngeal cancer," *JCI*, 2024. nih.gov

92. E. Augustus, "Analysis of blood and urine nucleic acids: the value of liquid biopsy to improve diagnosis, follow-up and monitoring of treatment response of lung cancer and …," 2021. uantwerpen.be

93. C. Fernandez-Rozadilla and A. R. Simoes, "Tumor profiling

at the service of cancer therapy," Frontiers in ..., 2021. nih.gov

94. D. Akhoundova and M. A. Rubin, "Clinical application of advanced multi-omics tumor profiling: Shaping precision oncology of the future," Cancer Cell, 2022. cell.com
95. L. Dercle, S. Sun, R. D. Seban, A. Mekki, R. Sun, L. Tselikas, *et al.*, "Emerging and evolving concepts in cancer immunotherapy imaging," *Radiology*, 2023. rsna.org
96. K. White, K. Connor, M. Meylan, A. Bougoüin, "Identification, validation and biological characterisation of novel glioblastoma tumour microenvironment subtypes: implications for precision immunotherapy," Annals of ..., vol. XX, no. YY, pp. ZZ-ZZ, 2023. sciencedirect.com
97. S. Sharma, "Tumor markers in clinical practice: General principles and guidelines," 2009. ncbi.nlm.nih.gov
98. M. Oloomi, N. Moazzezy, and S. Bouzari, "Comparing blood versus tissue-based biomarkers expression in breast cancer patients," 2020. ncbi.nlm.nih.gov
99. B. Lobato-Delgado, B. Priego-Torres, and D. Sanchez-Morillo, "Combining molecular, imaging, and clinical data analysis for predicting cancer prognosis," Cancers, 2022. mdpi.com
100. K. M. Boehm, P. Khosravi, R. Vanguri, J. Gao, "Harnessing multimodal data integration to advance precision oncology," *Nature Reviews Cancer*, vol. 22, no. 1, pp. 1-15, 2022. nih.gov
101. L. Schneider, S. Laiouar-Pedari, S. Kuntz, and others, "Integration of deep learning-based image analysis and genomic data in cancer pathology: A systematic review," *Journal of Cancer*, vol. 2022, Elsevier. sciencedirect.com
102. D. Gale, K. Heider, A. Ruiz-Valdepenas, S. Hackinger, *et*

al., "Residual ctDNA after treatment predicts early relapse in patients with early-stage non-small cell lung cancer," *Annals of Oncology*, vol. 33, no. 5, pp. 565-577, 2022. sciencedirect.com

103. S. A. Cohen, M. C. Liu, and A. Aleshin, "Practical recommendations for using ctDNA in clinical decision making," Nature, 2023. pathologyinnovationcc.org

104. G. Marsavela, A. C. McEvoy, M. R. Pereira, A. L. Reid, *et al*., "Detection of clinical progression through plasma ctDNA in metastatic melanoma patients: a comparison to radiological progression," *British Journal of...*, 2022. nature.com

105. K. E. De Visser and J. A. Joyce, "The evolving tumor microenvironment: From cancer initiation to metastatic outgrowth," Cancer cell, 2023. cell.com

106. G. Grant and C. M. Ferrer, "The role of the immune tumor microenvironment in shaping metastatic dissemination, dormancy, and outgrowth," Trends in Cell Biology, 2025. cell.com

107. T. Bjarnsholt, M. Whiteley, K. P. Rumbaugh, *et al*., "The importance of understanding the infectious microenvironment," The Lancet Infectious Diseases, vol. 22, no. 1, pp. 1-2, 2022. nih.gov

108. J. Ma, L. Huang, D. Hu, S. Zeng, Y. Han, "The role of the tumor microbe microenvironment in the tumor immune microenvironment: bystander, activator, or inhibitor?," Journal of Experimental & Clinical Cancer Research, vol. 40, no. 1, pp. 1-12, 2021. springer.com

109. A. G. Selim, A. Minson, P. Blombery, M. Dickinson, *et al*., "CAR-T cell therapy: practical guide to routine laboratory monitoring," *Pathology*, vol. 53, no. 1, pp. 1-10, 2021.

[HTML]

110. S. N. Shishido, O. Hart, S. Jeong, *et al.*, "Liquid biopsy approach to monitor the efficacy and response to CAR-T cell therapy," Journal for ..., 2024. nih.gov

111. W. Ploch, K. Sadowski, W. Olejarz, and G. W. Basak, "Advancement and challenges in monitoring of CAR-T cell therapy: a comprehensive review of parameters and markers in hematological malignancies," Cancers, 2024. mdpi.com

112. C. B. García-Calderón and B. Sierro-Martínez, "Monitoring of kinetics and exhaustion markers of circulating CAR-T cells as early predictive factors in patients with B-cell malignancies," *Frontiers in …*, 2023. frontiersin.org

113. S. Sethi, S. Ali, P. A. Philip, and F. H. Sarkar, "Clinical Advances in Molecular Biomarkers for Cancer Diagnosis and Therapy," 2013. ncbi.nlm.nih.gov

114. S. Rotundo, M. T. Tassone, F. Serapide, A. Russo, "Incipient tuberculosis: a comprehensive overview," Infection, vol. 2024, Springer. springer.com

115. Π ΚΑΡΑΝΙΚΟΛΑΟΣ, "Computational Analysis of Gene Expression and Genetic Marker Alterations in Alzheimer's Disease," apothesis.eap.gr, . eap.gr

116. P. Tasca, B. M. van den Berg, T. J. Rabelink, *et al.*, "Application of spatial-omics to the classification of kidney biopsy samples in transplantation," Nature Reviews, 2024. [HTML]

117. R. Fazal, M. A. Shah, H. A. Khattak, and H. T. Rauf, "Achieving data privacy for decision support systems in times of massive data sharing," Cluster Computing, vol. 25, no. 3, pp. 1541-1553, 2022. springer.com

118. B. Shah and E. V. Gupta, "Ethics and Privacy in AI-Driven

Healthcare Decision Support Systems," 2025. researchgate.net

119. Z. Chen, N. Liang, H. Zhang, H. Li, Y. Yang, and X. Zong, "Harnessing the power of clinical decision support systems: challenges and opportunities," Open Heart, vol. 2023. bmj.com

120. J. F. L. Feldmann and M. A. L. L. Pinheiro, "Current and Future Applications of Liquid Biopsy in Melanoma," touchREVIEWS in..., 2025. [HTML]

121. S. Al-Azani and E. S. M. El-Alfy, "A review and critical analysis of multimodal datasets for emotional AI," Artificial Intelligence Review, 2025. springer.com

122. A. Sklavounos, "Preparing for Resource-Limited and High-Stakes Settings: a Digital Microfluidic Approach," 2022. scholaris.ca

123. G. Chen, S. Dong, Y. Shu, G. Zhang, J. Sesay, "Autoagents: A framework for automatic agent generation," arXiv preprint arXiv:XXXX.XXXX, 2023. [PDF]

124. I. S. Chaudhry, S. A. M. Sarwary, and G. A. El Refae, "Time to revisit existing student's performance evaluation approach in higher education sector in a new era of ChatGPT—a case study," Cogent Education, vol. 2023, Taylor & Francis, 2023. tandfonline.com

125. T. Unoki, K. Hayashida, Y. Kawai, S. Taito, M. Ando, *et al.*, "Japanese clinical practice guidelines for rehabilitation in critically ill patients 2023 (J-ReCIP 2023)," *Journal of Intensive Care*, vol. 2023, Springer. springer.com

126. H. Bakker, V. Bindewald, F. Dunke, and S. Nickel, "Logistics for diagnostic testing: An adaptive decision-support framework," *European Journal of …*, vol. 2023, Elsevier. [HTML]

127. F. C. Udegbe, E. I. Nwankwo, G. T. Igwama, "Real-time data integration in diagnostic devices for predictive modeling of infectious disease outbreaks," *Computer Science & IT*, 2023. researchgate.net

128. J. Cao, W. Zheng, Y. Ge, and J. Wang, "DriftShield: Autonomous fraud detection via actor-critic reinforcement learning with dynamic feature reweighting," IEEE Open Journal of the …, 2025. ieee.org

129. S. Shafi and A. V. Parwani, "Artificial intelligence in diagnostic pathology," Diagnostic pathology, 2023. springer.com

130. A. Aggarwal, S. Bharadwaj, G. Corredor, "Artificial intelligence in digital pathology—time for a reality check," *Nature Reviews*, 2025. [HTML]

131. I. N. Savas and A. Coskun, "The Future of Tumor Markers: Advancing Early Malignancy Detection Through Omics Technologies, Continuous Monitoring, and Personalized Reference …," Biomolecules, 2025. mdpi.com

132. D. G. Macovei, M. B. Irimes, O. Hosu, C. Cristea, "Point-of-care electrochemical testing of biomarkers involved in inflammatory and inflammatory-associated medical conditions," *Analytical and …*, vol. 2023, Springer. springer.com

133. A. Zhyvolozhnyi, A. Samoylenko, G. Bart, "Enrichment of sweat-derived extracellular vesicles of human and bacterial origin for biomarker identification," 2024. nih.gov

134. Q. Jia, A. Wang, Y. Yuan, B. Zhu, and H. Long, "Heterogeneity of the tumor immune microenvironment and its clinical relevance," *Experimental Hematology*, vol. XX, no. YY, pp. ZZ-ZZ, 2022. springer.com

135. J. J. Li, J. Y. Tsang, and G. M. Tse, "Tumor

microenvironment in breast cancer—updates on therapeutic implications and pathologic assessment," Cancers, 2021. mdpi.com

136. W. Kang, X. Qiu, Y. Luo, J. Luo, Y. Liu, J. Xi, X. Li, "Application of radiomics-based multiomics combinations in the tumor microenvironment and cancer prognosis," *Journal of Translational Medicine*, vol. 21, no. 1, 2023. springer.com