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REVIEW

The Age of Molecular Biomarkers: Cancer in the Era of Personalized Medicine. What Do Pathologists in Developing Countries Need to Know and Understand?

Zubair Ahmad[1], Shabina Rahim[2], Maha Zubair[3], Jamshid Abdul-Ghafar[4]

[1]Department of Pathology, Sultan Qaboos Comprehensive Cancer Care and Research Centre, Muscat, Oman; [2]Pathology Department, Royal Medical Services, Military Hospital, Manama, Bahrain; [3]Department of Internal Medicine, LSU Health Shreveport, Shreveport, LA, USA; [4]Department of Pathology and Clinical Laboratory, French Medical Institute for Mothers and Children (FMIC), Kabul, Afghanistan

Correspondence: Jamshid Abdul-Ghafar, Department of Pathology and Clinical Laboratory, French Medical Institute for Mother and Children (FMIC), Kabul, Afghanistan, Email jamshid.jalal@fmic.org.af

Abstract: The complexity of cancer care is continuously increasing in the era of personalized medicine and there is a paradigm shift in the diagnosis and management of cancer. This is the era of precision oncology whose main objective is to identify cancer patients who are candidates for specific targeted therapies. This advanced approach to cancer care formulates treatment strategies for cancer patients based on the specific molecular characteristics of a malignant tumor which are identified through advanced molecular testing. This is the age of advanced prognostic and predictive biomarkers. Targeted therapies represent a groundbreaking shift in cancer therapy and are the cornerstone of precision oncology. Termed "tumor agnostic therapy", these drugs can treat different cancer types across multiple organs which demonstrate the same molecular alterations. A targeted drug in a specific cancer may be effective in another non-related cancer if the same genomic alteration is present. Pathologists need to appreciate these radical and exciting changes and adapt their practices as they will be required to be collaborative clinicians in the new era with a role in diagnosis, prognostication, and treatment of cancer. Pathologists need to become familiar with the ever-expanding number of new biomarkers and their crucial role in cancer care. They need to understand and adapt to new technologies such as Next Generation Sequencing and Comprehensive Genomic Profiling, liquid biopsies, DNA and transcriptome studies etc. They also need to familiarize themselves with tumor agnostic therapies, and concepts such as tumor heterogeneity and resistance to therapy. They can no longer be just morphologists but assume a central role in cancer care. Pathologists in developing countries and resource limited settings who may not currently have access to advanced molecular techniques need to be aware of and understand these fundamental shifts in cancer care and especially their role in the new era. The major changes in cancer care in the era of personalized medicine are discussed in this review mainly for the benefit of pathologists working in LMICs.

Keywords: cancer, pathologists, developing countries

Introduction

The classical approach to cancer is undergoing a paradigm shift. We are witnessing a radical change in the diagnosis, prognostication and management of cancer. The complexity of cancer care is continuously increasing in the new era of "precision medicine" or "molecular guided medicine". This change is happening owing to two of the most impressive technical advances in recent history. One is the remarkable development of DNA and protein detecting technologies that can detect a variety of minute and precise changes at the molecular level in enormous numbers at ever increasing speed. These include DNA sequencing, microarray technologies, comparative genomic hybridization, digital PCR, mass spectrometry and others, and each generates data in enormous and unprecedented amounts – "big data". The other is the tremendous increase in our capacity to store, process, and transmit this data, which is made possible by exponential

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growth in computer power, internet bandwidth, and so called "cloud computing". "Informatics" as it is called is now a recognizable subdiscipline. Even more remarkably, these increases in capability have been accompanied by marked reduction in costs leading to increased application in clinical practice. The combination of artificial intelligence (AI) and big data is enabling clinicians and pathologists involved in cancer care to acquire a clearer and more holistic understanding of different cancers. This data-driven approach augmented by machine-learning not only sheds light on cancer but also on the dynamic interplay between cancer, treatment, and the patient, promoting a more optimal therapeutic decision-making process. Following the human genome project and the subsequent ENCODE (The Encyclopedia of DNA Elements) project, it became possible to see the complete picture of the human genome that governs all human anatomy and function, and which is disrupted by cancer. Understanding these intricate mechanisms is essential for accurate diagnosis and optimum therapy of various cancers. This advanced approach to cancer care formulates treatment strategies for cancer patients based on the specific molecular characteristics of a malignant tumor. Personalized medicine aims to find the uniqueness in each patient's pathophysiology, tailoring treatment and prevention plans to suit individual patients, thus achieving optimal results- personalized medicine or precision oncology- and this in turn needs to be supplemented by personalized or precision pathology. The main objective of precision oncology is to identify cancer patients who are candidates for specific targeted therapies. This personalized approach has transformed the oncological therapeutic scenario and prognostic evolution. Tremendous amount of medical data is now routinely available for every patient. In addition to genetic and genomic data, there may be information on proteomics, epigenomics, metabolomics, transcriptomics etc (collectively called "omics"), whole slide images (WSI), and therapeutic regimens. Together, these form a comprehensive and huge database which allows for detailed and continuous analysis of the patient's physical condition, from which personalized strategies can be devised to achieve a better outcome. Clinical guidelines now increasingly recommend broad molecular profiling of malignant tumors. The genomic, transcriptional and epigenetic changes that occur in any cancer make each patient unique. Deeper insights into tumor biology resulting from molecular profiling allows the best therapy to be chosen so that the right therapy can be given to the right patient at the right time. Ultimately, there is hope for an era where cancers cease to be direct causes of death but rather become waxing and waning illnesses treated effectively at home with improved quality of life and personalized care. An already existing example is chronic myeloid leukemia (CML). The success of genomic abnormalities providing practical treatment targets and disease monitoring tools was heralded by the identification of the Philadelphia chromosome, t (9; 22) (q34; q11) in CML. This translocation creates a gene fusion BCR-ABL1, that enables unrestricted activity of the BCR-ABL1 tyrosine kinase resulting in uncontrolled proliferation. CML cases with this event can now be targeted by tyrosine kinase inhibitors such as imatinib with excellent results.

Molecular Biology of Cancer

The genetic alterations resulting in cancer allow uninhibited growth and metastasis of cells. Implicated are mutations in oncogenes (KRAS and MYC) which are linked with multiple cancers and tumor suppressor genes (TP53, BRCA1 and 2) which allow cancer cells to bypass these regulatory controls and grow uncontrollably. Mutations in DNA repair genes (MLH1, MSH2) lead to loss of function, in turn leading to genetic instability-an enduring feature of cancer. The development of targeted therapies has been made possible by understanding the molecular pathways of cancer. For example, the discovery of EGFR mutations in lung cancer led to the development of EGFR inhibitor drugs, which has significantly enhanced the survival rates for cancer patients with EGFR mutations.

The exciting changes in cancer care are discussed below in detail. Breast cancer, non-small cell lung cancer (NSCLC), gastric cancer, colorectal cancer, cervical cancer etc. are used as examples to explain and illustrate these fundamental changes.

The Challenge for Pathologists

This new scenario has created both a challenge and an opportunity for pathologists: to adapt to, benefit from, participate in, and contribute to the dynamic era of precision oncology and precision pathology. As Juan Rosai wrote:

> Future discoveries will not likely be made by morphologists ignorant of molecular biologic findings, or by biologists unaware or scornful of morphologic data, but by those willing and capable of integrating them through a team approach.

Technological advancements and innovative research methodologies paving the way for emerging trends and continuous evolution in pathology practice include application of AI resulting in increased diagnostic accuracy and enabling personalized treatment plans leading to better patient outcomes. The pathologist can be at the center of this revolution in cancer care. Traditional pathology services utilizing manual procedures will increasingly take a back seat and the pathologist can have a lead role in the diagnosis, prognostication, and treatment of cancer. Predictive molecular biomarkers, which are measures of the likelihood of response or lack of response to a particular cancer therapeutic drug, are becoming essential tools in the optimal management of cancer. Based on these predictive molecular assays, selection of cancer patients for any specific therapy will be based equally on two factors: increasing benefit from the given therapy and reducing and avoiding toxicities resulting from ineffective therapies. The "power" of the predictive assays can be gauged from the fact that in the past, an average of 25% cancer patients responded to their treatment. Today, up to 80% responded to targeted therapies.

The pathologist of today has a critical role in the interpretation of both molecular and IHC based predictive markers. Some of these biomarkers are both prognostic and predictive. For example, HER2, which is available both as an IHC marker and FISH procedure, has important prognostic as well as predictive roles. HER2 positive cancers are aggressive cancers with high risk of metastasis and shorter survival compared to HER2 negative cancers in the absence of therapy. However, it is also an important predictive biomarker of response to anti-HER2 targeted therapies. It is also associated with greater response to chemotherapeutic agents and poorer response to endocrine therapies.

A biomarker refers to any biological marker found in blood, body fluids, or tissues that signals the presence of normal or abnormal biological processes or diseases including cancer.

A cancer biomarker is specifically used for early detection of cancer. Biomarkers also provide valuable insights into its likely progression including chances of recurrence and expected outcome of treatment. Cancer biomarkers play a crucial role in outlining the prognosis of any cancer independently of any treatment (prognostic biomarkers), or in predicting how a cancer will respond to a specific treatment (predictive biomarkers). For lung cancer, EGFR mutations and ALK rearrangements predict response to tyrosine kinase inhibitors (TKIs) such as Erlotinib/Geftinib. BRCA 1 and 2 mutations in breast and ovarian cancer indicate eligibility for PARP inhibitors like Olaparib while HER2 indicates eligibility for Trastuzumab. Thus, predictive biomarkers help in selection of targeted therapies. Thus, they are vital for confirming cancer diagnosis, predicting disease progression, and tailoring therapeutic modalities. Low levels of ctDNA and CTCs following therapy indicate that therapy is working, while high levels indicate resistance to therapy. Tumor mutational burden (TMB) measures the number of mutations in a cancer and predicts the success of immunotherapy and better survivability in response to drugs such as Immune checkpoint inhibitors (discussed below) in colorectal carcinoma (CRC), non-small cell lung carcinoma (NSCLC), urinary bladder carcinoma, malignant melanoma (MM) etc. Epigenetic markers such as hypermethylation of tumor suppressor genes like SEPT9 help in the early detection of CRC. Tumor-educated platelets (TEPs) are rapidly emerging and highly promising biomarkers. In ovarian cancer, FDA approved OVA1 test which detects five protein biomarkers in blood. Ideal cancer biomarkers should be straight-forward, reproducible, reliable, cost-effective methods of detection, and should clearly demonstrate that their use results in better treatment outcomes.

Immune biomarkers serve as indispensable tools in cancer therapy, offering insights that guide treatment decisions and increase the effectiveness of Immuno-therapies. They identify patients who are more likely to benefit from Immune checkpoint inhibitors. Thus, they go beyond cancer prognostication and play a pivotal role in shaping personalized targeted therapy. Examples include PD-L1, MSI and MMR, TILs etc.

These breakthroughs are laying the groundwork for early detection screening tools for various cancers and could significantly reduce mortality from cancer.

Innovations in Technologies for Early Detection of Cancer

Recent advances in the field of omics technologies such as genomics, epigenomics, transcriptomics, proteomics etc have accelerated the discovery of novel biomarkers for early detection of cancer and advancing the precision and accessibility

of biomarker detection. Screening for specific mutations in KRAS, EGFR, and TP53 or using oncology gene panels to detect mutations which drive the development and progression of cancer has so far been the most direct approach. ctDNA is a standout example, a non-invasive biomarker that detects fragments of DNA shed by cancer cells into the blood-stream, has shown promise in detecting many common cancers including lung, breast, colon etc at the pre-clinical stage, offering a window for intervention before symptoms appear.

ctDNA, microRNA (miRNA), exosomes etc are detected in liquid biopsies and constitute minimally invasive, highly sensitive markers for early cancer detection, therapy response, real-time monitoring of tumor evolution, and detection of minimal residual disease (MRD). Multi-cancer early detection tests (MCED) like Gallen use methylation patterns to identify more than fifty types of cancer from a single blood sample.

CRISPR-Cas 9 screening is a revolutionary ground-breaking new technique which is being used to identify and validate novel biomarkers with high efficiency. It is a gene editing technique which precisely edits specific regions of DNA and its ability to alter the genomic mutations responsible for cancer or increase the ability of the immune system to eliminate cancer cells. It is adaptable and flexible, easy to use, and affordable. Its discovery has given us the ability to find, validate and develop drug targets, and thus allow personalized gene therapies and advanced screening techniques, all of which are very promising for treatment of cancer (and other diseases).

Stool DNA testing: Cologuard is an FDA approved method for colorectal carcinoma (CRC) screening that identifies DNA mutations.

Molecular guided therapies are more effective as they directly address the cause of cancer. They have fewer side effects compared to conventional anti-cancer drugs and minimize harm to healthy cells. New data indicates that they are associated with better treatment responses and survival rates. These therapies represent a groundbreaking shift in cancer treatment and are increasingly based on the concept of "tumor agnostic therapy". Traditional targeted drugs treat a cancer with a specific genomic alteration that is present in a specific organ or tissue (test one drug in one cancer). On the other hand, a "tumor agnostic therapy" treats any kind of cancer regardless of the anatomic location or the cell of origin from which the cancer developed, provided that the cancer has the same genomic alteration which is targetable by an approved drug for a different cancer. In other words, driver genes and mutations are shared across different types of cancers. A target drug in a specific cancer may be effective in another non-related cancer if the same genetic alteration is present. Agnostic cancer therapy is now FDA approved. The agnostic approach shifts the focus from treating cancer based on its tissue type to targeting the underlying molecular drivers. In 2004, there were only 9 FDA approved anti-cancer drugs. This number increased to 44 by the end of 2014 and to above 90 by the end of 2023.[1–15]

Tumor Agnostic Therapy

Tumor agnostic therapy (pan-cancer therapy) is now the cornerstone of precision oncology. It has emerged as a major companion to conventional chemo and radiation therapy. It is a precision oncology treatment approach, a type of targeted cancer therapy which uses drugs or other substances that target specific genetic mutations and/or molecular biomarkers within a cancer, irrespective of where the cancer originated in the body. In other words, it treats cancer based on the cancer's genetic features without regard to the cancer type or where the cancer started in the body. It uses the same drug to treat all cancer types that have the genetic mutation or biomarker targeted by that drug. Tumor agnostic therapies represent a paradigm shift by altering the traditional means of characterizing cancers based on their origin or location. Instead, they target the specific genetic anomalies responsible for causing cancer. The watershed moment for tumor agnostic therapies came in 2017 with the FDA's historic approval of pembrolizumab, an immune checkpoint inhibitor.

This milestone, the combination of genomics and immunology as an immunotherapeutic agent, which was approved based on genomic biomarkers, specifically MSI-high or MMR deficiency was ground-breaking. Pembrolizumab was approved for treating MSI-H or MMR deficient solid cancers. Pembrolizumab later received another tumor agnostic approval solid cancers with high TMB. Approval of NTRK inhibitors such as Larotrectinib and entrectinib which combat NTRK gene fusions prevalent in various pediatric and adult cancer types (NTRK gene fusion-positive solid tumors such as thyroid, lung, salivary glands), further underscored the potential of these therapies. FDA approvals of targeted therapies such as dabrafenib for BRAF V600E variant-positive solid tumors, RET gene fusion-positive solid tumors, and an antibody-drug conjugate for HER2 positive (IHC 3+ expression) cancers, have offered newfound hope to patients

with advanced solid cancers harboring particular biomarkers. Pemigatinib was approved for FGFR1-rearranged myeloid or lymphoid neoplasms. Biomarker-guided therapies have revolutionized the clinical management of cancer. These therapies can be particularly beneficial for patients with rare cancers or when traditional treatments are not effective. It is essential to ensure that patients with rare cancers or those with specific molecular alterations have access to these therapies. Concurrently with the significant advances in drug development, innovative next-generation sequencing (NGS) technologies are facilitating the identification of patients with targetable genetic aberrations in real-time and enabling novel molecularly stratified clinical trials. Although very successful in many cancers, these have only been partially successful in some cancers. CNS neoplasms are a prime example. Technology has substantially enhanced the genetic characterization and classification of CNS neoplasms (a highly heterogeneous group of more than 120 different types), but in contrast to other solid cancers, targeted and biomarker-informed treatment options are limited, and current systemic therapeutic strategies still largely rely on conventional chemotherapy. The Phase III INDIGO trial demonstrated the efficacy of the dual IDH1/2 inhibitor vorasidenib in IDH-mutant gliomas, which led to a priority review by the FDA and highlighted the need for molecular profiling and precision medicine in CNS neoplasms. Yet, most clinical trials that assess biomarker-stratified therapies are still not available for CNS neoplasms and furthermore, a large proportion of targeted drugs do not achieve significant concentrations in the CNS compartment due to the blood-brain barrier. Thus, many therapies that have been successful in other cancers have so far not been effective in patients with CNS neoplasms, despite these neoplasms sharing similar alterations.

Thus, tumor agnostic approvals provide access to highly active drugs to meet the needs of patients with rare cancers. Recently, several dugs gained FDA approval in the absence of specific cancer indication (tissue/tumor agnostic) and patient age restriction (ag(e)nostic). These therapies are even more critical for pediatric cancers which are rare. In other words, these precision drugs need to be accessible across the age spectrum.

Between 1998 and 2022, 198 new oncology drugs received FDA approval and most were molecularly targeted precision drugs. Emerging cancer biomarkers which will hopefully be followed with specific agnostic drugs include ALK, MET, ROS1, PIK3CA, AKT, KRASG12C, HER2 mutations, HER2 low/ultralow, B7-H3, TILs to name a few.[16–23]

Tumor Heterogeneity and Resistance to Therapy

Drug resistance is the biggest hurdle to targeted cancer therapy and is the leading cause of treatment failure. It is primary or secondary. The former is due to the presence of intrinsic mechanisms in cancer cells which exist at the beginning of therapy, while secondary resistance means drug resistance occurring later in the course of treatment with cancers that were initially responsive to therapy becoming insensitive later. Cancer cells employ different mechanisms to resist the targeting agent. For example, in EGFR-mutant NSCLC, secondary resistant mutations on the target kinase domain emerge to diminish the binding affinity of first- and second- generation inhibitors. Sequential monotherapies may temporarily address the problem of acquired drug resistance but are limited by the ability of cancer cells to adapt and develop new resistance mechanisms against the targeting drugs. The principal cause of drug resistance in cancer is tumor heterogeneity.

It is important to understand the concept of "tumor heterogeneity". Tumor heterogeneity is defined as "the differences between tumors of the same type in different patients, the differences between cancer cells in the same tumor, or the differences between a primary and secondary tumor". Thus, it refers to the presence of distinct subpopulations of cells with diverse genetic and phenotypic profiles within a single tumor (intra-tumor) or between cancers of the same type in different patients (inter-tumor), and refers to the differences between cancers of the same type in different patients, the difference between cancer cells within a single tumor, or differences between a primary cancer and its metastasis. Cancers are dynamic and become more heterogeneous with the passage of time and this heterogeneity represents a significant challenge in their management. Tumor heterogeneity can be "spatial" or "temporal". The former can be intra or inter tumor heterogeneity and encompasses clonal heterogeneity (sub clones 1, 2 and 3). It is now evident that cancers are composed of populations of cells with distinct molecular and phenotypic features-a phenomenon termed "intra-tumor heterogeneity" which indicates spatial heterogeneity within a single tumor. Inter-tumor heterogeneity denotes heterogeneity between primary and metastatic tumor. Temporal heterogeneity: Cancers evolve and adapt to environmental challenges such as immune surveillance and treatment pressures. With tumor progression, resistant clones

become dominant while sensitive clones are suppressed or eliminated. Considerable intra-tumoral heterogeneity has been shown in cancers of the head and neck, breast, ovary, prostate, urinary bladder, kidney, liver, pancreas, NSCLC, CNS neoplasms, sarcomas and hematological malignancies with important therapeutic challenges and implications. Intra tumor heterogeneity can be genetic or non-genetic. Intra - and inter- tumor heterogeneity are important obstacles which need to be overcome when designing the best therapeutic strategies for cancer patients. Liquid biopsies may be able to track these changes and can help to better tailor cancer therapies.

Actionable alterations among cancers include CCND1 and 2, FGFR1, 2 and 3, MDM2, EGFR, ERBB2, CDK4 and 6, c-myc, KIT, FGF4, KDR, CCNE, RICTOR, NOTCH, CCNE, AR, AKT3, FLT3, VEGF, SRC, TOP2, CDKN2A, JAK3, LYN, NRAS etc. With targeted therapy, it is important to distinguish between founding or trunk mutations and progressor or secondary mutations. Targeted therapy should target the founding mutation. Heterogeneity within the tumor sample and the co-presence of multiple subclones in a cancer should be noted by pathologists using morphology, immunohistochemistry, and molecular sequencing. It is imperative that this heterogeneity is fully explained to the oncologist, so that therapy decisions are tailored to target all subclones. In breast cancer, for example, the realization of heterogeneity and the differences in the expression of various biomarkers and the observed differences in response to therapy have resulted in extensive efforts to better define the characters of each breast cancer subtype. It is now generally agreed that breast cancer is not a single disease, and not all breast cancer patients can benefit from the same therapy. Thus, pathologists are now required not only to provide diagnosis, but also to study the molecular characterization of each individual breast cancer case and play a significant role in the treatment planning of breast cancer patients. Their role is changing from morphologists to molecular pathologists.[24–31]

Molecular Profiling of Cancer and Cancer Biomarkers

Molecular profiling has already changed the classification of several cancers including breast and lung cancer. Precision oncology is the age of molecular biomarkers. These are displacing morphological classification and grading of cancers. Advances in sequencing technology allow the identification of clinically and therapeutically relevant genomic alterations creating opportunities for personalized cancer care (targeted therapies). Molecular biomarkers provide a definite link between the mechanism of tumor development and the mechanism of action of therapeutic anti- cancer drugs. They play a crucial role in cancer detection, diagnosis, prognosis, predicting response to therapy, identifying patients who are most likely to benefit from specific treatments, and monitoring cancer recurrence after treatment. Early screening of cancers is the most powerful public health tool that enables early detection, reduces annual incidence, provides a higher chance of treatment, improves patient response to therapy, and prolongs patient survival times especially for cancers with high mortality. In addition to invasive and expensive screening methods such as endoscopy, CT scan and tissue biopsy, noninvasive and cost-effective screening based on biomarkers from body fluids including blood, stool, saliva, and urine is becoming increasingly common. To date, thousands of these biomarkers including proteins, cytokines, metabolites, hormones, microRNA and circulating DNA have been explored and several of them are now used in the early screening of cancers. So-called “classical” biomarkers such as AFP, CEA, CA125, CA19-9, PSA and LDH have traditionally been used in the clinical screening of various types of cancers. However, a broad range of novel biomarkers have been explored recently, which include microRNA and other RNAs, microbial proteins, circulating tumor DNA, and circulating tumor cells. Many of these have shown great potential for clinical screening in clinical trials. Some of these biomarkers from body fluids may be difficult to detect owing to short circulation times, very low density etc. Synthetic biomarkers including small-molecule, DNA-based, mammalian cell-based, and bacterial cell-based sensors have been developed to amplify tumor signals, thus enhancing the sensitivity and efficiency of early-stage cancer detection. New screening tests based on these new techniques will be available in oncology clinics soon.[6–9] Testing for multiple biomarkers is a more effective screening strategy for accurate early cancer detection demonstrating high sensitivity and better clinical correlation. Combining traditional and new screening methods can result in easier, faster, more accurate, and more specific cancer diagnosis. The transition from serial to parallel testing enables the simultaneous identification of multiple markers. Clinical assays are being developed to non-invasively detect the chosen biomarker in cancer tissue or body fluids. Despite biases in study designs and technological issues, traditional and new biomarker assays are now applied in clinical studies for screening (eg, serum PSA in prostate cancer), diagnosis (eg, Bence-Jones protein in myeloma,

identifying EGFR mutation in suspected lung cancer without histological confirmation), prognosis (hCG in testicular cancer, hormone receptor status in breast cancer), and predicting treatment response (eg, gene signatures for immunotherapy in various cancers such as ALK gene rearrangements guiding treatment in lung cancer). The ideal cancer biomarker should be cost-effective, easy and reliable to assess with high sensitivity and specificity. Additionally, it should demonstrate remarkable detectability at early stages and the capacity to accurately reflect tumor burden, enabling continuous monitoring of cancer evolution during treatments. However, low diagnostic specificity is a significant limitation in the clinical applicability of some biomarkers, especially the older blood-based ones like CEA, CA125, and CA15-3. This is due to their potential expression by non-cancerous tissues. Thus, there is a risk of misinterpretation with patients undergoing incorrect or unnecessary treatment. Healthcare systems may be reluctant to invest in or integrate biomarkers into routine screenings and diagnostics if there are concerns about their cost-effectiveness. However, new and improved biomarkers are enhancing the rapidity and accuracy of cancer diagnosis and treatment and contributing to economic efficiency by minimizing unnecessary interventions and optimizing healthcare resource allocation. This applies to immune biomarkers, which today serve as indispensable tools in cancer therapy, providing information that guides treatment decisions and enhances the effectiveness of immunotherapies. By evaluating factors such as PD-L1 expression, tumor-infiltrating lymphocytes (TILs), and molecular signatures within the tumor microenvironment, clinicians can predict and monitor responses to immunotherapy. Biomarkers like MSI and MMR deficiency help identify patients more likely to benefit from immune checkpoint inhibitor therapy. Thus, the role of immune biomarkers extends beyond mere prognostication. They play a pivotal role in designing personalized and targeted treatment approaches and their integration into clinical practice promises to optimize cancer treatment strategies and improve patient outcomes. The same biomarker might have different roles in different cancers, and with different drugs eg HER2 mutations, amplifications, and overexpression represent specific biomarkers for lung, breast, and gastric cancer respectively, requiring different testing methods. Conversely, the agnostic approach represents a paradigm shift in this scenario, which applies to two scenarios: the same biomarker across cancer types (eg, NTRK gene rearrangements), and biomarker agnostic use of targeted drugs. This latter approach is at the basis of the emergence of antibody-drug conjugates (ADCs) across different types of cancer (as discussed earlier). ADCs use target antigens already present on cancer cells but not on normal cells, to deliver cytotoxic drugs more selectively within tumor sites. Precise stratification of cancer patients based on prognostic and predictive biomarkers is allowing the selection of more effective therapies for individual patients. One successful example is to distinguish the type of breast cancer by the expression of HER2, ER, and PR in breast cancer tissue. These biomarkers help to identify triple-negative breast cancer (TNBC) which is the most aggressive type of breast cancer with poor prognosis and limited treatment options, improving the management and treatment options in these patients. Other biomarkers include TP53 mutations (frequently found in various cancers and associated with tumor development and progression), EGFR mutations (targeted by specific therapies in lung cancer), KRAS mutations (common in several cancers, can affect treatment response), BRCA1/BRCA2 mutations (associated with increased risk of breast and ovarian cancers which can impact treatment decisions), Microsatellite instability (MSI) and/or mismatch repair (MMR) deficiency (these indicate a tumor's ability to repair DNA errors, can predict response to immunotherapy) etc. MSI is the condition of genetic hypermutability (predisposition to mutations) that results from impaired DNA mismatch repair (MMR). MSI and MMR are closely linked. MMR deficiency and resultant MSI may be caused by somatic or germline anomalies. MMR-d and MSI are prognostic and predictive biomarkers in oncology. However, studies have shown that there are many heterogeneous phenomena in patients with MSI cancers in terms of immunotherapy, prognosis, and chemotherapy sensitivity. Current testing relies on IHC in cancer tissue for detecting presence or absence of MMR proteins, and PCR based molecular assays for MSI detection. Deficiencies in MMR lead to MSI. MMR proteins form a complex that recognizes, binds to, and excises the mismatched DNA, allowing the correct sequence to be inserted. When MMR is deficient, these errors accumulate, especially in microsatellite regions (short repetitive DNA nucleotide sequences), causing MSI. Unrepaired mistakes within repetitive DNA sequences (microsatellites) alter the length of the microsatellite sequences, resulting in bands of different sizes (novel microsatellite fragments) than expected in a DNA sample. MSI is often observed in cancers with MMR deficiency, especially in cancers associated with Lynch syndrome (hereditary nonpolyposis colorectal cancer) and in some sporadic cancers. The presence of MSI represents phenotypic evidence that MMR is not functioning normally. MMR is a crucial DNA repair pathway

which corrects errors that spontaneously occur during DNA replication such as single base mismatches or short insertions and deletions. MSI status (MSI-high, MSI-low, or microsatellite stable) is used as a biomarker in cancer diagnosis, prognosis, and treatment. MSI-high cancers often respond well to certain immunotherapies. Both MMR and MSI deficiency are important considerations in the management of various cancers. In the testing for MMR protein expression, the most used protein markers are MLH1, MSH2, MSH6 and PMS2. These markers help in identifying MMR-deficient (MSI-H) tumors. MSI is associated with colon (15–20%), gastric (15%), endometrial (30%), and less frequently ovarian, hepatobiliary tract, urinary tract and skin cancers, and malignant brain tumors. Colorectal cancers with MSI are usually found in right colon, are poorly differentiated, have high mucinogens and TILs, and show better prognosis than MSI low or MSI stable colon cancers. MSI-H (high) status raises the possibility of Lynch Syndrome and confirmation requires testing germline DNA. Lynch Syndrome is associated with MSI and increases the risk for the cancers mentioned above. Apart from real time PCR (many kits are commercially available), AI is also being explored to predict MSI from the histological appearance of tumors. Digital pathology can be submitted to machine learning techniques and predictions about MSI can be made without any molecular testing. However, AI methods have not yet been incorporated into routine clinical care.[32–37]

Immunotherapy in Cancer

Immunotherapy is an innovative cancer treatment currently enjoying immense popularity that uses the body's own immune system to fight cancer cells. It is one of the most remarkable recent advancements in cancer treatment and modulates the immune system to attack cancer cells by either boosting the ability of the immune system to recognize and destroy cancer cells or by providing immune system components for attacking cancer. Immunotherapy is a type of cancer therapy which stimulates the body's own immune system to find and destroy the cancer cells. The T lymphocyte, especially its capacity for antigen-directed cytotoxicity, has become a central focus in the fight against cancer. Immunotherapy is mainly used to strengthen the immune system by regulating the immune microenvironment, so that immune cells can attack and destroy cancer cells at several points. It is not a single treatment but a class of treatment with various approaches, including immune checkpoint inhibitors, CAR T-cell therapy, and cancer vaccines. It can involve using substances like cytokines or modified immune cells (like in CAR T-cell therapy) to directly attack cancer cells. The immune system detects cancer cells by using "checkpoint" proteins, which allow an immune response to be switched on and off. Cancer cells use these "checkpoints" to avoid being detected by the immune system. Cancer cells can sometimes also evade the immune system by suppressing immune responses. Immunotherapy can help overcome these mechanisms, allowing the immune system to effectively target cancer cells. Immune checkpoint inhibitors (ICIs) constitute a breakthrough in immunotherapy and are a type of immunotherapy drugs that block proteins (checkpoints) produced by cancer cells that prevent the immune system from attacking cancer cells, allowing the immune system especially T cells to recognize and destroy cancer cells more effectively. Monoclonal antibodies (MABs) are lab-made proteins that can bind to specific targets on cancer cells, either directly killing them or "flagging" the immune system to destroy the abnormal cells. These include checkpoint inhibitors such as pembrolizumab and nivolumab. Some, like trastuzumab, can slow the growth of cancer cells by blocking parts of the cell which enable them to grow. Radioimmunotherapy uses antibodies to deliver radiotherapy to cancer cells by attaching radioactive molecules to antibodies. Such antibodies can also be used to diagnose some cancers by pinpointing where cancer cells exist in the body. The antibody may carry chemotherapeutic drugs directly to cancer cells. Nonspecific immunotherapies refer to cytokines, which are signaling molecules that regulate the immune system and help the immune system destroy cancer cells. These are typically given in combination with chemo or radiation therapy and include interferons which can help the immune system to slow the growth of cancer cells, and interleukins such as interleukin-2 which can increase the production of white cells and antibodies to fight cancer. CAR T-cell (Chimeric Antigen Receptor T cell) therapy is defined as a specialized form of immunotherapy that genetically modifies the patient's own T lymphocytes to recognize, target, and destroy cancer cells. It involves modifying a patient's T cells in the lab, then infusing them back into the patient. Cancer vaccines work by training the immune system to recognize and attack cancer cells. They are either prophylactic which prevent cancer cells from developing and are useful for cancers known to be caused by infections, such as the HPV (human papilloma virus)

vaccine; and therapeutic which use the immune system to fight existing cancer cells. Sipuleucel-T is an example of a therapeutic vaccine used to treat prostate cancer.

Immunotherapy has shown success in treating various cancers (15 and growing) including melanoma, and lung, urinary bladder and renal cancers. It is often used in advanced or metastatic cancers, or when other treatments have not been effective. It can, however, be used at different stages of cancer treatment, sometimes as a first-line therapy. It is usually well tolerated, and side effects are usually successfully managed. Its best use depends on the specific type and stage of cancer, as well as the individual patient's overall health. New and improved drugs are continually being developed. Immunotherapy can be given in cycles orally, intravenously, topically or intravesical. It can be given in the clinic or hospital. The frequency of treatment depends on the type of immunotherapy and the type of cancer.[38–42]

Tumor Mutational Burden (TMB), MSI and MMR

TMB is defined as the total number of mutations found in the DNA of cancer cells. It measures the total number of somatic (non-inherited or acquired) mutations per mega base of DNA in a cancer cell, and can be a predictive biomarker for response to immunotherapy or how well certain cancer types might respond to immunotherapy, specifically ICIs. Cancers with low TMB scores have lower number of genetic mutations, which means that the body's immune system is less likely to identify cancer cells. Higher TMB generally indicates more mutations, potentially leading to a greater number of unique abnormal tumor proteins (neoantigens) being produced by the tumor. These neo-antigens can be recognized by the immune system (mainly T lymphocytes) which then kill the cancer cells expressing these neoantigens, potentially making cancers with high TMB more susceptible to ICIs. However, while high TMB is associated with a better response to immunotherapy, not all cancers respond the same and the specific TMB level that indicates a positive response can vary between different cancer types. TMB has also shown promise as a tumor-agnostic biomarker, meaning that it can be useful across different cancer types, though its use is still to be standardized. TMB is typically measured through NGS of tumor tissue. Both MSI and TMB represent the production of new antibodies and studies have shown that many patients with MSI-H also have high TMB levels. Thus, ICIs can be considered in treating patients with MSI-H cancers who also have high TMB. Malignant melanoma and NSCLC were the first cancers to be successfully treated by ICIs. These cancers often have high TMB. In 2017, FDA approved the use of ICIs for cancers with MMR deficiency. Further studies have confirmed that the relationship between TMB and effectiveness of immunotherapy holds true across many cancer types. Among those receiving ICIs, patients having cancers with high TMB live longer. Newer studies have shown that high TMB by itself does not always mean better prognosis for the patient. It is only favorable if such cancers are treated with ICIs. Otherwise, high TMB is associated with a worse outcome. These studies led to the approval of pembrolizumab for all cancers exceeding a certain TMB level. This was called a tissue-agnostic approval, meaning it applied to any solid tumor with this genetic feature, regardless of type. A TMB of ten mutations per mega base in DNA of cancer cells was approved by FDA as cutoff. Cancers with TMB higher than ten usually have better outcomes than cancers with TMB below ten although it does not hold true for all cancers. Thus, although cancer immunotherapy holds great promise and has shown good responses in many cancer patients, these responses are not uniform in all patients or all types of cancers. There is an ongoing clinical need for objective diagnostic biomarkers to identify patients who will respond to immunotherapies. Cancer cells that do not express abnormal proteins may not be recognized or killed by T cells. Thus, TMB is both a prognostic and a predictive biomarker. It can be tested in primary or metastatic cancer cells. TMB testing in patients undergoing immunotherapy allows oncologists to know which therapy is appropriate. Studies have shown that TMB numbers are a useful biomarker for immunotherapy treatments for patients with advanced cancers. In the future, it may be possible to measure TMB levels from a blood sample (liquid biopsy).[43–47]

PD-L1 (Programmed-Death Ligand-1) Testing in Cancer

PD-L1 expression can indicate a potential response to immunotherapy targeting the PD-1/PD-L1 pathway. It is a protein on the surface of some normal cells as well as some cancer cells that helps them evade the body's immune system. PD-1 and PD-L1 are directly involved in cancer immune regulation and are considered one of the most relevant inhibitory checkpoints. PD-L1 acts as a "brake" by interacting with PD-1 on T lymphocytes, preventing them from attacking the cells expressing PD-L1. Binding PD-1 to its ligand PD-L1 activates downstream signaling pathways, and inhibits T cell

activation. Reactive expression of PD-L1 is a favorable event for a PD-L1 expressing cancer cell, as it can specifically inactivate the T cells that are attacking the cancer cells expressing PD-L1. Cancer cells can exploit this mechanism and develop an evasion strategy by expressing high levels of PD-L1, essentially hiding from the immune system. Thus, PD-L1 expression is an immune evasion mechanism and is generally associated with poorer prognosis. PD-L1 testing is often performed on tumor samples to determine the level of PD-L1 expression. This information can help oncologists decide if a patient is likely to respond to PD-1/PD-L1 targeted immunotherapy. Tumors with high PD-L1 expressions are more likely to respond to these immunotherapies. By blocking PD-L1 or PD-1, these therapies can release the brakes on the immune system, allowing T cells to recognize and attack cancer cells. PD-L1 IHC scoring is currently the basis for cancer patients to receive properly targeted therapies. In NSCLC, PD-L1 is evaluated using a tumor proportion score (TPS). A tumor with TPS less than 1% is considered to have no PD-L1 expression, a tumor with TPS of 1–49% is considered to have PD-L1 expression, while a tumor with TPS equal to or greater than 50% is considered to have high PD-L1 expression. In squamous cell carcinomas of cervix and head and neck, as well as in gastric/gastroesophageal junction adenocarcinomas, PD-L1 expression is determined by using combined positive score (CPS) which is the number of PD-L1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. Cancers having CPS less than 1 are considered to have no PD-L1 expression while those with CPS equal to or more than 1 are considered to have PD-L1 expression. In urothelial carcinoma and TNBC, PD-L1 expression is determined by using CPS. Tumors with CPS less than 10 are considered to have no PD-L1 expression while those with CPS equal to or greater than 10 are considered to express PD-L1. A positive PD-L1 test result is generally considered a good sign, indicating a higher chance of response to certain immunotherapies. PD-L1 expression is also suggested as a predictive biomarker of response to anti-PD-1/PD-L1 therapies. Innovative drugs targeting the PD-1/PD-L1 axis are very promising. In the last decade, several therapeutic monoclonal antibodies targeting both PD-1 (Pembrolizumab and Nivolumab) and PD-L1 (Atezolivumab, Avelumab) were developed. These drugs represent milestones of modern cancer immunotherapy and are nowadays used as the main therapeutic option for many kinds of cancers, often achieving far better results than conventional chemotherapy. However, several patients do not respond while potentially experiencing serious adverse drug reactions, so patients who will receive immunotherapy need to be chosen carefully.[48–53]

HER2 Positive Breast Cancer Testing and Targeted Therapies

Until recently, clinical decisions for the management of cancer were based on the tissue or organ affected, the histology of the tumor and the tumor stage. Today, cancer management decisions are increasingly being made based on the results of molecular profiling of the tumor. We will analyze this in detail using breast, lung, gastric and colorectal cancers as examples.

In 2000, breast cancer management decisions were made based on breast carcinoma subtypes which include: Luminal A-like (ER positive, PR positive, HER2 negative and Ki-67 proliferation index: low); Luminal B-like-HER2 negative (ER positive, HER2 negative and at least one of the following: PR negative or low, Ki-67 proliferation index: high; Luminal B-like-HER2 positive (ER positive, HER2 overexpressed or amplified, PR any, Ki-67 proliferation index any); HER2-positive non luminal (HER2 overexpressed or amplified, ER negative and PR negative); and Triple negative. Today, clinical decisions in breast cancer in many cases are increasingly based on genomic drivers. These include ER signaling; P13K/AKT/mTOR; Growth factor receptors: ERBB2, EGFR, FGFR1; Cell cycle regulators: CCND1, CDK4, RB1; DNA repair genes: BRCA 1 and BRCA 2. Targeted therapies can be used at different stages of breast cancer and represent a more precise approach than chemotherapy, which targets all rapidly dividing cells. By targeting the above molecules, the drugs can interfere with cancer cell division, spread, and ability to receive growth signals. Common targeted therapies in breast cancer include the following: HER2-targeted therapies. These are used for HER2 positive breast cancers (15–20% or 1 in 5 of all breast cancers) and target the HER2 protein which is overexpressed (extra copies of HER2 gene). The most prominent example is trastuzumab (Herceptin); CDK4/6 Inhibitors. These drugs block CDK4 and CDK6 proteins which are involved in cell cycle progression, PARP Inhibitors. These drugs target PARP proteins which are involved in DNA repair and are used for breast cancers with specific mutations, such as BRCA1 and BRCA2 mutations; PI3K Inhibitors. These drugs target the PI3K protein which is involved in cell growth and survival. They may be used for advanced hormone receptor-positive, HER2 negative breast cancers with a PIK3CA gene mutation;

Antibody-drug Conjugates. These are targeted drugs which combine a targeted monoclonal antibody with a potent chemotherapy drug to destroy cancer cells while sparing healthy tissues. The antibody binds to a specific target on the cancer cell, and the attached drug is then delivered directly into the cancer cell; Immunotherapies like pembrolizumab. These can be used in early-stage breast cancer to reduce the risk of recurrence after surgery and other treatments, as well as advanced or metastatic breast cancer to help slow the progression of cancer and improve survival and can be an option when other treatments are no longer effective. Targeted therapies for HER2-positive breast cancer, which specifically target the HER2 protein, are highly effective and have significantly improved outcomes for patients with these cancers. HER2 (human epidermal growth factor receptor 2) is a targetable transmembrane glycoprotein receptor protein of the EGFR family that plays a crucial role in regulating growth, differentiation and repair in normal cells. Aberrant HER2 signaling is implicated in various cancers, especially breast, gastric and ovarian cancers where overexpression or amplification correlates with aggressive behavior, higher risk of metastasis, and poor prognosis. HER2 gene is amplified, leading to an overproduction of the HER2 protein which promotes the growth of cancer cells. HER2-positive breast cancers tend to grow and spread and metastasize faster. However, they are much more likely to respond to targeted therapies like Trastuzumab which target and block the HER2 protein, preventing it from sending signals that promote cancer cell growth and division. The evolution of HER2 targeting drugs has significantly transformed the treatment landscape for HER2-positive breast and gastric cancers. Thus, testing for HER2 status by IHC (score 3+ means high level of HER2 protein) and by FISH (extra copies of the HER2 gene) in cases which are equivocal on IHC (score 2+), is crucial for determining whether Trastuzumab (Herceptin) should be given. If HER2 by FISH is positive in a case which was equivocal on IHC, the cancer is considered HER2-positive. If the IHC result is 0 with no membrane staining, the cancer is considered HER2-negative. These do not respond to drugs targeting HER2 protein. If IHC result is 0 with membrane staining meaning HER2 staining in more than 0% but no more than 10% tumor cells, the cancer is considered HER2-ultralow or 0+. These cancers may respond to antibody-drug conjugates. If the IHC is 1+ OR if the IHC is 2+ but FISH is negative, the cancer is considered HER2-low. These cancers may also respond to antibody-drug conjugates. If the IHC is 2+ but FISH is positive OR IHC is 3+, the cancer is HER2-positive, and these are treated with drugs targeting HER2. Trastuzumab was the first targeted therapy developed for HER2-positive breast cancer. Pertuzumab (Perjeta) is a similar monoclonal antibody drug which is often used in combination with trastuzumab and chemotherapy. Trastuzumab emtansine is an antibody-drug conjugate that combines trastuzumab with a chemotherapy drug called DM1, delivering the chemotherapy directly to cancer cells. Enhertu is used to treat HER2-low breast cancer which cannot be removed by surgery or metastasized, or patients who have received a prior chemotherapy for metastatic disease, or when cancer returned during or within six months of completing adjuvant chemotherapy. It is also used to treat HR positive, HER2-low OR HR-positive, HER2-ultralow breast cancer that cannot be removed by surgery or that has metastasized and one or more hormonal treatments for metastatic disease have already been given. Tyrosine kinase inhibitors that prevent HER2 from sending growth signals, are particularly effective in treating HER-2 positive breast cancer that has spread to the brain. Targeted therapies in breast cancer are often used in combination with chemotherapy, surgery, and radiation therapy, especially in early-stage, HER2-positive breast cancer. Targeted therapies are also used in metastatic HER2-positive breast cancer, often in combination with chemotherapy, and may be used in different sequences depending on the specific situation. Newer targeted therapies and combinations, including immunotherapies, are being developed to further improve outcomes.

Triple-negative breast cancers (TNBCs) are HER2, ER, PR negative. Hormone therapy and drugs that target HER2 are not helpful in treating these cancers. Triple-positive breast cancers are HER2, ER, PR positive and these are treated with hormones as well as drugs which target HER2. Over the past few years, the indications for HER2-targeted therapy have expanded beyond breast and gastric and gastroesophageal junction cancer to include various other solid cancers. HER2 overexpression is also found in many cases of extrahepatic cholangiocarcinoma, and cancers of gall bladder, cervical, uterine, urinary bladder and testis.

The anti-HER2 antibody-drug conjugate trastuzumab deruxtecan has become the new standard of care for patients with treatment-refractory HER2-positive gastric and GEJ cancer, HER2-mutant NSCLC or any HER2-overexpressing (IHC 3+) solid tumor owing to its potent antitumor activity and impressive clinical efficacy. Since trastuzumab in combination with chemotherapy was established as the standard of care for patients with previously untreated advanced-

stage HER2- positive gastric/GEJ cancer, addition of an immune checkpoint inhibitor (the anti-PD-1 antibody pembrolizumab) to this regimen has presented a new first-line treatment option for this cancer.[54–64]

HER2 Positive Gastric Cancer

This refers to a type of stomach cancer with overexpression or amplification of HER2 protein by the cancer cells. Approximately 10–20% of gastric cancers are HER2-positive, and this subset can benefit from trastuzumab. HER2-positive gastric cancers are often intestinal type and located at the gastroesophageal junction. Trastuzumab combined with chemotherapy, has become a standard first-line treatment for HER2-positive metastatic gastric cancer. Other therapies targeting HER2 protein including antibody-drug conjugates and tyrosine kinase inhibitors, are also being investigated. Immunotherapy is also being explored in combination with HER2-targeting therapies, showing promising results in preclinical models. HER2 testing is crucial for identifying gastric cancer patients who can benefit from HER2-targeted therapies. The prognosis for HER2-positive gastric cancer has improved with the introduction of HER2-targeted therapies.[65–67]

Hormone Receptor-Positive Breast Cancer Testing and Targeted Therapies

ER and PR are proteins found on some breast cancer cells that bind to the hormones estrogen and progesterone respectively and become activated and change the expression of certain genes, stimulating cancer cell growth. If breast cancer cells express either or both receptors, they are called ER/PR positive or hormone receptor positive. All breast cancers are now grouped into the following biomarker-defined subtypes/groups based on ER and ERBB2 (HER2) in order to determine the best treatment approach: ER-positive/HER2-negative, ER-positive/HER2-positive, ER-negative /HER2-positive, and ER-negative/HER2-negative. About 3 of 4 (80%) breast cancers are ER and PR positive (at least one if not both). ER+/PR+ cancers have a more favorable prognosis than ER+/PR-, ER-/PR+, or ER-/PR- subtypes. The cancer cells have receptors for estrogen and progesterone which means that the cancer relies on these hormones to grow. The hormone receptor status of the tumor is an important consideration as hormone therapy is often a key treatment option. If a breast cancer has one or both hormone receptors, certain drugs can be used to prevent estrogen and/or progesterone from attaching to the receptors, which in turn prevents the cancer cells from growing and spreading. According to ASCO/CAP guidelines for ER/PR testing, validated IHC performed on tumor tissue remains the standard for predicting which patients will benefit from hormonal therapy, and no other assays are recommended for this purpose. The test is sensitive enough to identify a hormone-positive cancer even if only 1% of tested cells have a hormone receptor. If 1% or more cells stain with ER and PR, the test is interpreted as positive. However, it is interpreted as low positive if 1–10% of cells stain. Breast cancer samples with 1% to 100% tumor cell nuclei positive should be interpreted as ER positive. There is limited data on benefits of hormonal therapy for low positive cancers. Such cancers are reported using a new reporting category, ER low positive. A cancer is considered ER negative if less than 1% or 0% of cancer cell nuclei are immunoreactive. The status of controls should be reported for cancers showing 0% to 10% staining. Similar principles apply to PgR testing, which is used primarily for prognostic purposes in the setting of ER-positive breast cancer. Testing of DCIS for ER is recommended to determine potential benefit of hormonal therapies to reduce risk of future invasive breast cancer, while testing DCIS for PgR is considered optional. These cancers appear to have a better prognosis than ER/PR-negative cancers, as they are more responsive to hormone therapy which may be used in combination with surgery or as a standalone treatment. A common option is tamoxifen, a drug that blocks hormone receptors on the cancer cells. The goal is to lower the level of estrogen and/or progesterone in the body and thus inhibit the growth of the cancer. Common hormone therapies include aromatase inhibitors, selective estrogen receptor modulators (SERMs), and drugs like fulvestrant. Certain targeted therapy drugs can make hormone therapy even more effective, although these drugs might also add to the side effects. CDK4/6 inhibitors block cyclin dependent kinases (CDKs) especially CDK4 and CDK6 in the cells and are approved to treat some hormone-receptor positive, HER2 negative breast cancers. They help stop cancer cells from dividing and slow cancer growth. For women with early-stage breast cancer that has spread to the lymph nodes and has a high chance of recurring after surgery, these can be given as adjuvant treatment along with tamoxifen or an aromatase inhibitor. They can be used by themselves in women with advanced breast cancer who have previously been treated with hormone therapy and chemotherapy. For women with

stage II or III breast cancer that has a high chance of coming back after surgery, these drugs can be given with an aromatase inhibitor as adjuvant treatment. mTOR inhibitors block mTOR, a protein in cells that normally helps them grow and divide. They may also stop tumors from developing new blood vessels, which can help limit their growth. These drugs seem to help hormone therapy drugs work better. They are used in post-menopausal women with advanced hormone receptor-positive, HER2-negative breast cancer, and are used with an aromatase inhibitor when cancer grows while being treated or when cancer started growing shortly after treatment was stopped. PI3K inhibitors block PI3K protein in cancer cells helping to stop them from growing. They can be used to treat women with advanced hormone receptor-positive, HER2- negative breast cancer with a PIK3CA gene mutation that has grown during or after treatment with hormone therapy. They are used along with hormone drugs such as fulvestrant. AKT inhibitors block formation of the AKT protein, which is part of a signaling pathway inside cancer cells that help them grow. They can be used along with fulvestrant to treat advanced hormone receptor-positive, HER2-negative breast cancer, if the cancer cells have any changes in the AKT genes on testing and if the cancer has grown during or after treatment with hormone therapy. Antibody-drug conjugates (ADCs) attach to the Trop-2 protein on breast cancer cells (some breast cancer cells have more Trop-2 which helps them to grow and spread quickly) and bring the chemo directly to them. ADCs can be used to treat advanced hormone receptor-positive, HER2-negative breast cancer patients who have already received hormone therapy and chemotherapy. Targeted therapy for cancers in women with BRCA gene mutations: PARP inhibitors. These inhibit PARP proteins which normally help repair damaged DNA inside cells. Because tumor cells with a mutated BRCA gene already have trouble repairing damaged DNA, blocking the PARP proteins often leads to death of these cells. They can be given to women with a BRCA mutation with early-stage HER2-negative breast cancer after surgery who have been treated with chemotherapy (before or after surgery) and are at high risk of recurrence. PARP inhibitors can be used to treat advanced or metastatic, HER2-negative breast cancer in women with a BRCA mutation who have already received chemotherapy. If the cancer is hormone receptor-positive, they can also be used following hormone therapy. TNBCs account for approximately 15% of breast cancers diagnosed worldwide, are more common in women younger than 40 years of age and women with BRCA1/2 gene mutations and are more aggressive than other types. No approved targeted therapies are available, although immunotherapy (in combination with chemotherapy) is available for advanced tumors that express PD-L1.[68–75]

Tumor Infiltrating Lymphocytes (TILs), TNBC, HER2 Positive Breast Cancer, BRCA1 and BRCA2

Pathologists must report tumor infiltrating lymphocytes (TILs) in breast cancer. TILs are primarily T lymphocytes which enter the tumor microenvironment and are crucial for an anti-tumor response, playing a key role in destroying cancer cells. With the rise of immunotherapies able to stimulate antitumor immune response, recent studies have shown that three transcriptome-based subtypes of breast cancer exist. These include the following: Immune-high which are characterized by the highest expression of TILs and PDL1. TNBC and HER2 positive breast cancers are mostly immune-high and represent potential responders to immunotherapies; Immune-medium and Immune-low which show little to no TILs and are unresponsive to immunotherapies. ER and PR-positive breast cancers usually fall into the immune-medium and immune-low groups. TILs have emerged as an important immunological biomarker related to the antitumor response in breast cancer including metastatic TNBC. Increased TILs are shown to be associated with a better response to neoadjuvant chemotherapy and improved survival. Thus, TILs are both predictive (adjuvant chemotherapy) and prognostic (higher levels are associated with a better prognosis in TNBC with a longer disease-free and overall survival). According to the recommendations for assessment of TILs in breast cancer proposed by the International Working Group for TILs in Breast Cancer, TILs are evaluated on H and E-stained slides, and the pathological stromal TILs present in the connective tissue stroma surrounding the cancer are assessed. The scoring methodology specifically excludes cancer cells, necrotic areas, and areas affected by crush artifacts. The final score is the percentage of the total stromal area occupied by TILs. Accordingly, three different groups can be identified: low TILs (0–10% immune cells in stromal tissue within the tumor), intermediate TILs (11–40%), and high TILs (greater than 40%).[76–80]

Only a small percentage of women with breast cancer are born with a mutated BRCA gene which is a normal gene present in all the cells of the body. Patients with breast cancer will need to be tested for BRCA mutations before starting targeted therapy with PARP inhibitors. BRCA1 and BRCA2 are tumor suppressor genes which produce proteins that prevent cells from growing too rapidly and are crucial for repairing DNA damage and maintaining the stability of cells. A mutation in either of these genes leads to unchecked cell growth. BRCA1 and 2 mutations are inherited and can be passed from either parent. BRCA1 and 2 mutations significantly increase lifetime risk for breast cancer (45–85%). The risk of cancer later developing in the contralateral breast is also significantly increased. They are also strongly linked to an increased risk of ovarian cancer. Less commonly, they can increase the risk of developing other cancers including pancreas, prostate and malignant melanoma. Thus, these inherited mutations can increase risk of cancers in both females and males. Cancers associated with BRCA1 and 2 mutations often develop at a younger age. These mutations are diagnosed in a proband by identification of a heterozygous germline pathogenic variant in BRCA1 or BRCA2 on molecular genetic testing using a blood or saliva sample. This testing can include a BRCA1 and BRCA2 gene panel and use of a multigene panel. BRCA1 and 2 testing is typically recommended for individuals with a strong personal history of breast cancer at or before age 50 years or multiple or bilateral breast cancers or TNBC or breast plus pancreatic or prostate cancer or male breast cancer), ovarian (including fallopian tube and primary peritoneal), pancreatic, or prostate cancer; or strong family history (one or more close relatives with breast or ovarian cancer, especially at a young age; or a history of multiple related cancers such as breast and pancreatic on the same side of the family; or a family member with a known BRCA1 or 2 mutation), or for those with Ashkenazi (Eastern European) Jewish ancestry. Genetic counselling and testing are recommended.[76–80]

Molecular Alterations in Lung Cancer and Targeted Therapies

Targeted therapies are particularly effective for advanced non-small cell lung cancer (NSCLC) with specific gene mutations and are designed to identify and attack specific proteins or pathways that drive cancer cell growth, such as EGFR, ALK, ROS1, BRAF and MET. By targeting these specific molecules, these drugs can interrupt signals that tell cancer cells to grow and divide, effectively slowing down or blocking cancer growth and progression. Targeted therapies are most used for advanced NSCLC that has spread or recurred after initial treatment. Lung cancer patients undergo biomarker testing to identify these mutations and determine if they are eligible for targeted therapy. Targeted therapy in NSCLC can be used alone or in combination with chemotherapy or other treatments, like immunotherapy. Examples of targeted therapies in NSCLC include the following: EGFR inhibitors. These drugs block the EGFR protein, which is overexpressed in some lung cancers, and are frequently used in advanced NSCLC harboring specific EGFR mutations. They can also be used in other cancers where EGFR signaling is implicated; TKIs. These are targeted therapies which act by specifically targeting and blocking EGFR protein which is found on the surface of cancer cells and plays a crucial role in cell growth and division. EGFR is overactive in some cancers leading to uncontrolled cell growth and development of cancer. Its inhibition by TKIs can slow down cancer cell growth and spread. EGFR TKIs have demonstrated improvements in patient-free survival (PFS), overall response rate (ORR), and quality of life compared to conventional chemotherapy in some settings. However, not all EGFR mutations are sensitive to all TKIs, and resistance to TKIs can develop over time; ALK inhibitors. These drugs target and block the abnormal ALK fusion protein found in a subset of NSCLC patients, inhibiting cancer growth. ALK-positive lung cancer is a specific type of NSCLC (mostly adenocarcinoma) which is often highly responsive to ALK inhibitors. It develops when the ALK (anaplastic lymphoma kinase) gene which is normally present in the body and plays a role in embryonic development is abnormal due to fusion with another gene, most commonly EML4, forming a fusion gene. This fusion creates an abnormal ALK protein that drives cancer cell growth and spread. ALK-positive lung cancer is typically found in younger people (median age at diagnosis is 52 years) and never smokers (or those who smoke very little). Testing the tumor tissue or blood (liquid biopsy) for ALK gene rearrangements is crucial for early diagnosis and timely treatment with these targeted drugs, leading to better outcomes; ROS1 inhibitors. These drugs target ROS1 gene rearrangements, another driver of lung cancer growth; BRAF inhibitors. These target BRAF mutations and may be used in combination with other targeted therapies. BRAF mutations occur in about 1–5% of NSCLC mainly adenocarcinomas, and the most common mutation, found in more than 50% of BRAF-mutated NSCLC, is the BRAF V600E mutation. BRAF and MEK inhibitors are effective treatments for advanced

NSCLC with the BRAF V600E mutation. KRAS-mutated NSCLC cells harbor a mutation in the KRAS gene. This mutation is a common oncogenic driver in NSCLC, especially adenocarcinomas, and can affect the response of the cancer to targeted therapies and may be associated with resistance to some. Newer targeted therapies target the KRAS G12C mutation, offering potential treatment options. KRAS mutations are found in approximately 25% lung adenocarcinomas and 3% squamous cell carcinomas. The most common is the G12C mutation. While KRAS mutations have historically been associated with a poorer prognosis, new targeted therapies are changing this outlook. Lung cancer cells can develop resistance to targeted therapies over time, requiring adjustments to treatment strategies. Many clinical trials are ongoing to explore new targeted therapies and improve outcomes for NSCLC patients.[81–97]

Molecular Alterations and Targeted Therapies in Colorectal Cancer

HER2

Overexpression is seen more in right sided cancers, in those with higher TMB, more advanced stage, and lymph node metastases. It is prognostic-HER2 amplification and mutations are prognostic-associated with poorer prognosis, and predictive-HER2 alterations are predictive biomarkers for resistance to anti-EGFR therapies.

BRAF

Mutations are more common with right-sided cancers, and mucinous adenocarcinomas, and are prognostic-associated with poorer prognosis, as well as predictive-these patients can be given chemotherapy plus anti-VEGFR antibody (bevacizumab). BRAF inhibitors are not satisfactory. Immunotherapy is more effective than traditional therapy. BRAF V600E mutation and MSI often occur together.

KRAS

Mutations are common, more in right-sided poorly differentiated cancers, associated with advanced stage, and distant metastases. Prognostic-associated with poor prognosis, and predictive-poor response to chemotherapy combined with anti-EGFR therapy. KRAS inhibitor drugs which inhibit KRAS-related signaling pathways are being explored.

MSI

More common with right-sided, mucinous, poorly differentiated cancers with high TMB. MSI-H CRCs demonstrate a favorable prognosis to Immune checkpoint inhibitors such as anti-PD1/PDL1 etc.

MSS

MSS stable CRCs constitute 85–90% of all CRCs, are more commonly left-sided, well differentiated, advanced stage, low TMB cancers with low TME (low immune cell infiltrate within tumor micro-environment), and are associated with poor prognosis marked by low survival rates and resistance to chemotherapy. Immunotherapy is useful and can be enhanced by means of dual immunotherapy.

Consensus Molecular Subtype (CMS 1-4)

Type 4 has worse prognosis, while type 1 is treated with immune checkpoint inhibitors.

ctDNA

Detection is associated with high recurrence risk and is predictive for emergence of acquired resistance to treatment. It is also predictive for treatment response. Detection after six months of chemotherapy is predictive of better outcomes.

RNAs

Including microRNAs, circular RNAs etc are prognostic, and predictive for development of chemo-resistance. Circular RNAs are being explored as therapeutic targets.

POLE/POLD1

Mutations are seen more with right-sided, early stage cancers and are associated with a better prognosis. They are biomarkers for therapy with PD1/PDL1, and also respond well to ICI therapy.

RET

RET oncogene fusions initiate downstream pathways such as RAS/MAPK etc stimulating tumorigenesis, seen in less than 1% CRCs, and are both prognostic and predictive.

Currently, status of KRAS, BRAS and MSI is important in predicting resistance in CRC. Routine testing of these genes is important. MSI status has the highest relevance as even advanced CRCs have a chance of long-term survival following immunotherapy. Targeted anti-HER2 drugs are coming up.[98–100]

Molecular Biomarkers and Targeted Therapies in Cervical Cancer

Molecular biomarkers are critical for early detection, prognosis and treatment of these HPV-related cancers.

Screening

Screening for high-risk HPV DNA/mRNA is essential. Primary screening with HPVE6/E7 mRNA detects active viral oncogene expression. p16/NK4a/Ki67 dual stain is a cytological marker for HSIL/CIN3 offering higher specificity than HPV testing alone. DNA methylation panels: Methylation of genes such as PAX-1, ZNF582, FAM A4, miR124-2 represent markers for progression to cancer. In normal cervical epithelium, CDC6 staining is absent or limited to basal proliferative layer. A linear increase in CDC6 staining is seen in areas exhibiting HPV changes.

Prognostic and Therapeutic

PDL1 guides ICI (pembrolizumab) therapy. VEGF and MMP-2/9 correlate with angiogenesis, advanced stage, and poor prognosis. PIK3CA, TP53, and KRAS mutations are also prognostic and predictive. SCC antigen is present more in dysplastic squamous epithelium. Raised serum SCC levels at initial diagnosis correlate significantly with tumor stage with higher levels indicating more advanced stage. Cervical cancer patients with plateau SCC levels indicate failure after radiotherapy. SCC levels are also useful for monitoring early detection of recurrence following primary treatment. SCC is thus both a prognostic and predictive biomarker.

Liquid biopsies with testing for CTCs, cfDNA, miRNA etc will be important in the near future for non-invasive surveillance.

These molecular approaches, especially DNA methylation and p16/Ki67 are supplementing and gradually replacing traditional cytology, and reducing unnecessary colposcopies by identifying true high-risk HPV disease. Molecular biomarker-based approach is very important in cervical cancer as it allows more accurate and sensitive detection of pre-cancerous lesions and early-stage cancers, allowing timely intervention and reducing mortality. HPV DNA, p16, Ki67 etc can identify high-risk lesions with greater precision than traditional screening methods alone improving patient outcomes. Molecular biomarkers are playing a pivotal role in revolutionizing prevention of cervical cancer by enabling advanced early detection, personalized risk assessment, tailored prevention strategies, targeted treatment, and better patient survival.

Early-stage, localized cervical carcinoma is treated by surgery or radiotherapy, while advanced, later-stage cancer is treated with a combination of chemoradiotherapy. However, targeted drugs and immunotherapy are increasingly being used for advanced, metastatic disease and are proving useful for advanced/metastatic and recurrent cancer when other treatments are not effective. Immune checkpoint inhibitors (monotherapy or in combination with chemotherapy) are showing significant improvement in prognosis in clinical trials. Following KEYNOTE-158 trial, ICIs are increasingly used in recurrent and metastatic cancers. Examples of ICIs and immunotherapy drugs used in cervical cancer include pembrolizumab, cemiplimab, and bevacizumab.

Other approaches such as therapeutic vaccination (in combination with ICIs), CAR-T cell therapy and tumor-infiltrating lymphocytes are currently being investigated.[101–106]

p16

The tumor suppressor protein p16 inhibits cancer development by regulating the cell cycle. The p16 gene (CDKN2A) is frequently inactivated through mutations or epigenetic silencing leading to uncontrolled cell proliferation and development of cancer. p16 is a part of the Ink4 family of CDK inhibitors and works by binding to and inactivating complexes of cyclin D, CDK4 and CDK6 (cyclin-dependent kinases). This inhibition prevents the phosphorylation of the retinoblastoma protein (Rb). By keeping Rb unphosphorylated, p16 keeps the cell in the G1 phase and prevents it from entering the S phase. The resulting cell cycle arrest allows time for DNA repair mechanisms to function, reducing the accumulation of mutations. Loss or inactivation of p16 is often an early and critical step in the development of many cancers, such as those in the head and neck, esophagus, lung, liver, pancreas, and urinary bladder.

The frequent alteration of p16 gene in cancer makes it a valuable biomarker for diagnosis and prognosis in certain types of cancer. Pathologists are expected to report p16 by IHC. It is also a product of HPV carcinogenesis, and a strong and diffuse pattern of staining is considered a highly sensitive surrogate marker for the identification of HPV-driven cancers such as squamous cell carcinomas of cervix, head and neck and anal canal. Immunostaining has been shown to have high association with the presence of HPV-16 DNA.[107–109]

Claudins

Claudins are proteins that play a crucial role in the formation of tight junctions between cells (cell adhesion and signaling hubs) by binding to multiple signaling molecules. They are structural components of tight junctions, and their dysregulation is increasingly recognized in various cancers. Claudins can act as both tumor suppressors and promoters. Specific claudins, like claudin-18.2, are being investigated as potential therapeutic targets due to their overexpression in certain cancers and their accessibility on cancer cell surfaces. Claudins contribute to the development and progression of cancer by affecting cell growth, proliferation, survival, differentiation, migration, and chemoresistance. In many solid tumors, claudin −1 is overexpressed and can drive remodeling of the extracellular matrix, forming a dense collagen barrier around the growth which helps shield the cancer from the immune system and immunotherapies, leading to resistance to immune and chemotherapies with poorer outcomes for patients. Antibodies targeting claudin-1offer a promising approach for treating various solid cancers by breaking down barriers to chemotherapy and selectively killing cancer cells. Claudins are involved in different steps of the metastatic cascade, including cancer cell migration, intravasation, and extravasation. Other claudins like claudin-6 can act as tumor suppressors. Altered claudin expression patterns can serve as biomarkers to predict prognosis and potentially guide treatment decisions. Claudin-18.2 is overexpressed in gastric and GEJ adenocarcinomas and is a promising target for therapies like monoclonal antibodies, antibody-drug conjugates and cellular immunotherapies, particularly in advanced and metastatic cases. It is normally found within the tight junctions of gastric epithelial cells. In gastric adenocarcinoma, it is overexpressed and exposed on the cell surface due to disrupted cell junctions. In the Cancer Genome Atlas, gastric cancer is classified into four new molecular subtypes, and claudin-18.2-ARHGAP fusion is present in the genomically stable type. Dysregulation of claudins-1, −3, −4, −6, −7 can be targeted for therapeutic intervention. Antibodies targeting claudin-1 can potentially trigger cancer cell death, disrupt collagen barriers around tumors, and improve the effectiveness of immunotherapies. These antibodies specifically target exposed claudin-1 on cancer cells and upon binding, they cause cancer cell death through antibody-dependent cell-mediated cytotoxicity. In gastric cancer, claudin 18.2 is a key target, and therapies combining monoclonal antibodies with chemotherapy show promise. Therapies such as zolbetuximab, a monoclonal antibody, specifically targets claudin 18.2 by binding to it. Recent studies and trials have shown that zolbetuximab, when combined with chemotherapy, can improve PFS and OS in patients with claudin −18.2 positive, HER2-negative locally advanced or metastatic gastric/GEJ adenocarcinoma. Zolbetuximab is now FDA approved for use in combination with chemotherapy for these patients. Thus, the discovery of claudin-18.2 as a targetable protein in gastric cancer and the subsequent development of zolbetuximab, represent a significant advancement in the treatment of gastric adenocarcinoma. This is very exciting since advanced gastric cancer has a high frequency of recurrence and metastasis with poor prognosis despite improvements in diagnostic ability and treatment strategies. Claudins-3 and −4 are implicated in pancreatic cancer progression, and C. perfringens enterotoxin (CPE) can potentially target these for treatment. Claudin-1

and −4 expression can vary significantly between different breast cancer subtypes, impacting prognosis. Claudin −3, −5, and −11 are also being recognized as promising therapeutic targets for breast cancer. Claudin −3 and −4 are highly expressed in ovarian cancer, and claudin-4 is associated with tumor aggressiveness. Expression of claudin-1 is implicated in colorectal cancer progression and chemoresistance. Claudin-1 and −2 can contribute to chemoresistance in lung cancer.[110–122]

Selected examples of new and emerging biomarkers:

- ctDNA
- Circulating tumor cells (CTCs)
- MicroRNAs
- PD-L1
- Tumor-educated platelets (TEP)
- Tumor Mutational Burden (TMB)
- In breast cancer, DNA methylation in promoter regions of tumor suppressor genes such as SOX7, BRMS1, etc correlate with increased metastases and poor prognosis.
- In prostate and colorectal cancer, methylation changes in angiogenesis genes such as VEGF and SFRP2 have been detected.
- In adult AML, NPM1 and EBPA mutations are associated with better prognosis, while RUNX1 and FLT3-ITD mutations are associated with a worse prognosis.
- OVA1 testing in ovarian cancer.
- Immunotherapy biomarkers. These include oncolytic virus therapies, ICIs, cancer vaccines, cytokine therapies, cancer immunotherapy, adoptive cell transfer like CAR-T, monoclonal antibodies etc.
- Epigenetic markers, for example hypermethylation of tumor-suppressor genes like SEPT9 for detection of colorectal cancer.
- AI and nanobiosensors are becoming crucial tools for cancer diagnosis, especially when combined together, and can detect biomarkers associated with various types of cancer at very early stages with high sensitivity and accuracy, and will likely play a major role in improving the prognosis of various cancers.

The Oncotype DX 21 Gene Breast Recurrence Score Test

Developed by Thermofisher, this is a precision oncology genomic test for newly diagnosed early-stage (I and II) ER, PR positive HER2 negative, axillary lymph node negative breast cancer. It is described in detail to illustrate the usefulness of genomic profiling in predicting prognosis and guiding treatment in cancer. It assesses the expression of 21 genes (16 cancer-related genes and 5 reference genes) using reverse transcription PCR (rt-PCR). It provides a recurrence score (RS) result, predicts ten-year risk of recurrence with endocrine therapy alone and predicts the benefit of adding chemotherapy. It provides a quantitative ER score to help assess the magnitude of hormonal therapy benefit along with additional supporting information such as PR and HER2 scores. It is included in all major breast cancer treatment guidelines such as National Comprehensive Cancer Network (NCCN). Many studies have validated the utility of this test in predicting RS in ER positive breast cancer and predicting outcome in patients undergoing chemotherapy. It can be performed on FFPE blocks unlike older tests which required fresh frozen tissue. It is the only test proven to predict likelihood of chemotherapy benefit. It analyses a sample of tumor tissue to see the activity of certain genes which affect the outcome. It is also called a "21-gene signature". It also helps in deciding whether patients require radiation therapy.

Patients Over 50 years

A score between 0 and 25 means a low risk of the tumor returning if patient gets hormonal treatment alone. Patients with this score will probably not benefit from getting chemotherapy. A score between 26 and 100 means a higher risk of cancer returning. Both hormonal therapy and chemotherapy are required.

Patients 50 years or Younger

A score of 15 or less means a low risk of cancer returning if patient gets hormonal treatment alone. These patients probably will not benefit from adding chemotherapy. A score between 16 and 20 means a low to medium risk of cancer returning with hormonal treatment alone. There may be a small benefit of receiving chemotherapy, but the benefits may not outweigh the risks of side effects. A score between 2 and 25 means there is medium risk of cancer returning with hormonal treatment alone. The benefits of chemotherapy outweigh the risks of side effects. A score between 26 and 100 means a high risk of cancer returning. Both hormonal therapy and chemotherapy will be required.[123–125]

Genomic Alterations in Cancer

Cancer is a disease of the genome, and we need to genotype it for its optimal management. It is no longer seen as one homogeneous disease, but a collection of many diseases, each driven by unique genomic characteristics. Routine genomic testing is increasingly being used in oncology for better treatment of cancer patients. According to current knowledge, about 57% of malignant tumors harbor actionable oncogenic events which can potentially be targeted therapeutically. The list is expanding continuously at a rapid pace. Even oncologists cannot grasp the full spectrum of these actionable molecular events. In addition, there are some driver mutations which occur in tumor suppressor genes or oncogenes and contribute to cancer progression. A continuous evolution of molecular guided therapies is occurring, and these therapies are proving to be effective even in refractory cancers.

Four classes of genomic alterations are believed to drive cancer growth: base substitutions and single nucleotide variants (SNVs); insertions and deletions (indels); copy number alterations; rearrangements (gene fusions). All four classes may be clinically relevant as they provide information about the molecular basis underlying the cancer. A cancer can have multiple types of mutations in more than one gene.

Since genomic alterations play an essential role in the development of cancers and since new targeted therapies target these alterations, it has become extremely important to detect all and not miss any genomic alterations in any cancer for its optimal management. Conventional molecular testing methods can detect some genetic alterations, but can miss others. For example, FISH and PCR can detect copy number alterations and rearrangements, but can miss indols and substitutions. Single gene testing is not enough as it can miss important genetic alterations. It has been used for the identification of single genes, such as EGFR or KRAS testing for lung cancer. PCR has been used for testing EGFR, while FISH is used for detecting ALK and ROS1 rearrangements in NSCLC. It has historically been viewed as a cost-effective approach, typically due to simpler workflows and producing rapid results. However, recent studies show advantages and cost-savings in employing a multi-gene testing approach. Since multiple gene alterations are implicated in cancers, single gene testing results in the potential loss of information from patient samples. For example, acquiring biopsies, especially for certain cancers such as NSCLC is usually challenging. Approximately 30% of single-gene tests fail due to inadequate biopsy samples or inadequate DNA for testing. Such inadequacies and failure to yield enough information about a patient's cancer type limits the clinician's ability to make optimal decisions about care.

Next Generation Sequencing (NGS)

Also known as massively parallel or high-throughput (the ability of a process to rapidly and efficiently handle and process a large volume of data or material within a specific timeframe) sequencing, it is an advanced, revolutionary transformative new technology used to determine the nucleotide order (sequencing) of millions of DNA and RNA fragments simultaneously and detect variants/mutations. It allows massive parallel sequencing of various lengths of DNA or RNA encompassing hundreds and thousands of genes or even whole genome in a short period of time. Using NGS, an entire human genome can be sequenced within a single day. NGS is now being increasingly used for diagnosis, prognosis, therapy decisions and follow up of cancer patients. The capacity of its massive parallel sequencing offers new opportunities for personalized precision oncology. Recent advances have focused on faster and more accurate sequencing, reduced costs, and improved data analysis. NGS can identify a much higher percentage of actionable genomic alterations across different tumor types compared to classic diagnostic modalities. The advent of NGS has

brought about a paradigm shift in cancer care enabling the development of targeted therapies, precision medicine approaches, and improved diagnostic methods.[126–128]

Hotspot cancer testing refers to a highly sensitive NGS method that detects and analyzes to the analysis of specific DNA sequences (hotspots) within genes known to be frequently mutated in cancer. These tests are used for diagnosis, personalized treatment planning, and assessing cancer risk by identifying recurring, actionable somatic mutations that drive cancer development and growth. In other words, they are high density areas of cancer cells. Cancer "hotspots" are physical structures on outer membranes of malignant cells which can provide the immune system access to the tumor. These are specific locations within genes known to be frequently mutated in cancer. Most cancers contain genetic "hotspots"-areas of DNA which are likely to be mutated. For example, more than 50% of all cancers harbor a mutation in the P53 gene. When TP53 gene is mutated, pre-cancerous cells are likely to grow uncontrollably. However, TP53 mutations while being advantageous to cancer growth can also make a cancer cell more noticeable to the patient's immune system, leaving malignant cells more open to attack. This can make cancer therapies, especially immunotherapies, more effective. Advances in molecular techniques have led to the use of multiplex panels with mutational hot-spot testing targeting several actionable mutations. Certain hotspot mutations can be highly specific to certain cancers, aiding in diagnosis. Targeted therapies can be developed to specifically target cancer cells with specific hotspot mutations. Identifying inherited hotspot mutations can indicate an increased risk of developing certain cancers (cancer risk assessment). However, hot-spot tests cannot detect all four major classes of genomic alterations and can miss indols, copy number alterations, rearrangements etc.

Several comprehensive and robust Hotspot panel assays are now commercially available and are designed to detect clinically relevant hotspot mutations in solid malignant tumors. They are usually compatible with fresh frozen as well as FFPE tissue samples and feature high sensitivity and throughput detecting clinically relevant hotspot variants down to 5%. Some examples of commercially available Hotspot panels include EntroGEN NGS Targeted Hotspot Panel, Genewiz Hotspot Panel, Atila Hotspot Cancer Panel etc. These panels target regions of known cancer genes that have been well characterized as mutational hotspots. The small size of the target region results in a high depth of sequencing coverage per run. They provide a cost-effective and efficient method for discovering rare somatic mutations and important cancer drivers. For example, the EntroGEN hotspot panel covers multiple genes and exons in multiple cancers including BRAF in lung and colorectal cancer and malignant melanoma; EGFR in lung cancer and glioma; ERBB2 in lung and breast cancer, MET, KRAS and BRAF in lung cancer, BRAF and NRAS genes in melanoma, KIT and PDGFRA genes in GIST etc.[129–131]

Whole genome sequencing (WGS) determines the complete DNA sequence of an organism's genome at a single time, identifying the order of all nucleotides. It provides the most comprehensive view of an individual's genetic makeup, including both protein-coding and non-coding regions. It can help treatment decisions tailoring therapies to specific cancer patients. It can be performed by extracting DNA from blood, saliva, or tissue. However, although the laboratory requirements are like conventional molecular tests, the amount of data is vast and requires a comprehensive computational and storage infrastructure to facilitate data processing within a clinically relevant timeframe. There are still unresolved issues associated with the clinical application.

Exome or whole exome sequencing (WES), is a high-throughput NGS method used to analyze the DNA sequence of all protein-coding regions of a person's genome, known as the exome. It specifically targets and sequences the protein-coding regions which comprise only 1–2% of the total DNA but contain almost 85% of known disease-carrying genomic alterations. It can simultaneously test all genes. It can be used when a patient's family history or symptoms suggest a genetic cause. It can be an efficient way to identify possible cancer-causing mutations in over 18,000 genes.[132–134]

The failure to detect many clinically important genomic alterations by conventional molecular methods including Hotspot NGS meant that there was an imperative need to develop newer, more efficient NGS methods to allow more comprehensive molecular profiling of cancers which can detect most genetic alterations without missing any. This led to the development of Comprehensive Gene Profiling (CGP). The ability of CGP to detect clinically relevant genetic alterations missed by other molecular tests has been demonstrated by multiple studies across multiple cancer types. In a comprehensive characterization of cancer driver genes and mutations, a recent TCGA analysis of more than 11,000 tumors identified 299 cancer driver genes. This study has provided very relevant information for research labs and drug companies to enrich the portfolio of potentially effective targeted anti-cancer therapies.

Conventional molecular diagnostics (FISH, RT-PCR etc) cannot address the increasing complexity of therapeutically relevant genomic information. IHC interpretation of molecular alterations (surrogate IHC markers), while very useful in resource limited settings, is qualitative and subjective and can give false negative and false positive results. It requires dedicated tissue and limits the testing for multiple targeted drugs. IHC may be used as a screening diagnostic method, but confirmatory gene fusion studies may still be required. FISH also requires dedicated tissue which limits testing for multiple targeted therapies, and interpretation of results can be challenging. FISH also has significant false negative and false positive rates. To detect gene fusions at multiple locations such as the three NTRK genes, multiple FISH tests would need to be run. RT-PCR, although fast and relatively inexpensive, cannot detect novel fusion partners. It is designed to identify only known translocation partner breakpoints.

Comprehensive genomic profiling (CGP) has transformed molecular pathology by enabling high-throughput sequencing of DNA and RNA. Unlike conventional sequencing methods, it can process millions of DNA fragments simultaneously, providing detailed information about genetic mutations and alterations that drive cancer development and progression, gene expression levels, and epigenetic modifications. It is an NGS approach that simultaneously assesses hundreds of cancer-related genes, both common and rare, in a single test to detect all four major classes of genomic alterations. Thus, by detecting hundreds of cancer biomarkers in a single test, it identifies clinically actionable genomic alterations to help select the most appropriate targeted drugs for each patient. This detailed genetic information is crucial for understanding the underlying mechanisms of cancer, resulting in more precise classification and better prediction of their behavior, for identifying biomarkers and for developing more effective, targeted therapies. It enables the identification of oncogenes and tumor suppressor genes. One of the most impactful applications of CGP in cancer is its role in guiding targeted therapies. By identifying specific genetic alterations that drive a particular cancer, CGP provides a basis for developing and selecting therapies that specifically target these genetic changes (precision oncology). Thus, CGP is important in development of new drugs allowing for increased precision in treating cancers based on their specific genetic mutations, thereby improving patient survival. Thus, new drugs that inhibit the function of mutated oncogenes or restore the activity of inactivated tumor suppressor genes can be developed. Digital platforms can integrate these genetic profiles with imaging data, creating a streamlined framework that supports treatment planning and real-time monitoring, essential for managing resistance to therapies. For example, the identification of BRAF V600E mutations in melanoma has led to the development of BRAF inhibitors, which have significantly improved outcomes for melanoma patients with this mutation. Personalized treatment plans improve the effectiveness of therapy, minimize adverse effects, and reduce the likelihood of resistance. For example, patients with colorectal cancer harboring KRAS mutations may not benefit from certain anti-EGFR therapies, and thus alternative treatments can be pursued based on their NGS results. Identifying mutations in the EGFR gene in NSCLC can predict response to EGFR-inhibitors, whereas mutations in the BRCA1 or BRCA2 genes in breast and ovarian cancers indicate susceptibility to PARP inhibitors. Thus, by sequencing tumor DNA from biopsy samples, clinicians can track the genetic evolution of cancers. This real-time monitoring can detect emerging resistance mutations, allowing for timely adjustments to treatment strategies. However, CGP is still not routinely used in all clinical settings. Several studies published between 2000 and 2023 have shown substantial evidence of the benefits of integrating CGP into routine care practice demonstrating that CGP is important in development of new drugs allowing for increased precision in treating cancers, thereby improving patient outcomes. CGP analyzes hundreds of genes simultaneously and multiple molecular biomarkers with one test. Thus, it enables a more complete evaluation of cancer mutations and can overcome the challenges associated with other techniques. It identifies all major groups of genetic alterations in cancer cells including base substitutions, insertions, deletions, copy number alterations, and rearrangements. It can identify specific genetic mutations that are known to be associated with cancer growth and response to certain treatments, as well as genomic instability signatures such as MSI, TMB and homologous recombination deficiency, which can help predict a tumor's response to immunotherapy and which are thus critical for initiating targeted therapy. It can be performed on tissue or less invasive liquid biopsies (blood samples). CGP is becoming increasingly important as the number of targeted anti-cancer therapies against genomic alterations including hard to detect rare mutations in the tumor agnostic setting is increasing. It can also help determine if a patient is eligible for clinical trials testing new targeted therapies.

While whole exon and genomic sequencing can also detect all four main classes of genomic alterations, calculate TMB and determine the MSI status of the tumor, its sensitivity is low, turnaround time is greater than four weeks, and amount of information provided is not manageable. Hotspot NGS testing cannot detect all four classes of genomic alterations, cannot calculate the TMB and cannot determine the tumor's MSI status. However, coverage depth of Hotspot testing is high, turnaround time is less than four weeks, and amount of information provided is manageable.

CGP in comparison ticks all the boxes. It uses less specimen, has high sensitivity, turnaround time is less than four weeks, and amount of information provided is manageable. Thus, CGP saves time and precious samples and provides all relevant information to decide optimal therapy. Many previous hurdles for implementing CGP are being gradually overcome with a decrease in sequencing cost, and availability of both open-source and commercial bioinformatics tools. These changes are helping in making CGP available to greater number of cancer patients. Whereas many cancers can share common mutations, an individual tumor can harbor different mutations. Thus, a comprehensive tissue agnostic approach of screening a wide array of mutations, is helpful in gaining a broader understanding of cancer progression and allowing more precise therapeutic intervention. With its ability to detect multiple chromosomal alterations that can be missed by other techniques, CGP will hopefully become widely incorporated into cancer care and help ensure that patients benefit from the latest therapeutic innovations and receive the best targeted personalized treatment.

Single gene testing in NSCLC partially or completely misses up to 35% of ALK rearrangements (FISH), and 17% EGFR alterations (Hotspot testing) while 21% EGFR alterations are missed by prior IHC and PCR tests. According to various studies, 50 to 69% of actionable targetable alterations are difficult to detect and can be missed by Hotspot testing without supplemental FISH. Up to 65% actionable genomic alterations are missed by conventional molecular tests. On the other hand, CGP detects all four classes of clinically relevant genomic alterations in NSCLC included in the NCCN guidelines (ALK, EGFR, BRAF, HER2, RET, MET, ROS1, KRAS). Thus, CGP has proven utility in directing treatment and improving patient outcomes in advanced NSCLC. Prior IHC, PCR and other NGS methods miss up to 37% BRAF alterations in malignant melanomas. Prior Hotspot testing misses up to 38% KRAS alterations in colorectal cancer. In breast cancer, ERBB2 non-amplification alterations are undetectable by IHC and FISH.

All the above genomic alterations are detected by CGP. CGP has revealed clinical associations in response to immune therapy in Head and Neck cancers. CGP testing has demonstrated clinical applications in diffuse gliomas. Clinical utility of CGP testing has also been demonstrated in the management of rare as well as refractory cancers. ESMO now recommends the use of CGP testing in patients with metastatic NSCLC, cholangiocarcinoma, ovarian cancer, prostate cancer etc. It also recommends CGP to test TMB in well and moderately differentiated NETs, cervical, vulval, thyroid and salivary gland cancers. The benefit to the patient needs to be weighed against the cost to the public health care system.[135–139]

Some of the most popular commercially available CGP solutions include Caris Life Sciences, Foundation One CDx, Oncomine Comprehensive Assay Pus (OCA Plus) etc.

ctDNA, Circulating Tumor Cells (CTCs) and Liquid Biopsies

"The tissue is the issue" for performing molecular testing. However, now liquid biopsy is also feasible for this purpose. Nucleic acids extracted from blood, CSF, saliva, urine, semen, pleural and ascitic effusions can be sources of liquid biopsies and can be used for the detection of hyper and hypo DNA methylation, deletions and amplifications, mutations and chromosomal rearrangements by NGS testing. When tissue biopsies are unavailable, CGP can be performed on liquid biopsies which represent a groundbreaking advancement in oncology, offering a non-invasive method for detecting and monitoring cancer by detecting minute quantities of cancer-derived DNA. Unlike conventional biopsies, which require invasive procedures to obtain tumor samples (while metastatic tumors are often inaccessible), liquid biopsies are transforming cancer diagnostics by analyzing promising potential biomarkers like circulating tumor cells (CTCs), cell-free DNA (cfDNA)/circulating tumor DNA (ctDNA), exosomes and extracellular vesicles (EVs), circulating microRNAs (miRNAs) etc. in body fluids including blood.

CTCs are whole (intact) cancer cells that detach from primary or secondary solid tumors and enter the lymphatics or bloodstream and represent potential triggers for cancer metastasis. They may, in fact, be key drivers of cancer metastasis. Used as biomarkers, they can be detected in blood samples even at early stages of cancer and are associated with more

aggressive behavior. Despite their scarcity in body fluids, they correlate with cancer outcomes, underscoring their potential to guide cancer decision-making. CTCs not only reveal the presence of tumors, but they also indicate that cancer is progressing or spreading and may help determine whether a patient may be at a higher risk of relapse. They may help guide therapy selection. In summary, CTCs are prognostic indicators for survival and cancer progression, and for assessing effectiveness of cancer therapies. Tumor cells are not the only cells which release fragments of DNA. cfDNA is DNA shed in blood stream from non-cancerous cells. cfDNA fragments are typically longer strands of DNA while ctDNA fragments are of typically shorter length. cf-DNA is shed into, and circulates freely in the bloodstream through a natural process of cell death, while ct-DNA is a subset of cf-DNA released into the bloodstream specifically from cancer cells by necrosis, apoptosis, secretion etc.

ct-DNA

These are small free-floating DNA fragments released by cancer cells into the bloodstream via necrosis, apoptosis, or active release which carry genetic alterations that are specific to cancer, such as mutations, deletions, or amplifications. Ct-DNA is a type of cell-free DNA. It acts as a non-invasive biomarker carrying cancer specific genetic information and enables timely adjustments to treatment plans and helps in initiating additional treatment strategies based on genetic profile of the cancer to prevent relapses, facilitating early intervention, improving patient outcomes and personalizing treatments. ct-DNA is useful in early detection of cancer, monitoring tumor progression, and identifying resistance mutations. The primary challenge with ct-DNA is very low sensitivity as it constitutes less than 1% of the total cf-DNA in the bloodstream which can make detection challenging, especially in early stages or when the tumor burden is low. This means that ct-DNA may be difficult to detect using current technologies, potentially leading to false-negative results. The sensitivity of ct-DNA can vary depending on factors such as cancer type and stage, and the specific mutations being targeted. Thus, detecting ct-DNA in early-stage cancers or when the cancer has a low frequency of mutation can be problematic. However, use of methods like single-strand DNA libraries for NGS are effective in improving detection. Another significant limitation is the risk of contamination from external sources especially from FFPE tissues. Contaminants can include DNA from the environment, laboratory reagents, or even from the collection and processing of samples. Contamination makes it difficult to accurately distinguish between genuine ct-DNA and background cf-DNA from non-tumor sources. Stringent measures are required to avoid contamination, and results need to be interpreted carefully to ensure accurate diagnostic and prognostic assessments.

EVs are small lipid-bilayer membrane-bound vesicles derived from various types of cells. They play a crucial role in intercellular communication. EVs derived from cancer cells contribute to tumorigenesis by promoting metastasis, initiating stromal support for cancer angiogenesis, suppressing the anti-tumor immune response, and enhancing cancer cell proliferation. EVs are present in higher concentrations in body fluids making them promising biomarkers for cancer detection, prognosis, treatment decisions, and monitoring therapeutic response. Exosomes and EVs are biomarkers for early diagnosis, monitoring therapeutic responses, and understanding mechanisms of drug resistance. CfDNA is used for detecting genetic mutations and methylation patterns, early diagnosis and monitoring treatment response. In addition, cf-RNA and tumor derived RNA (tdRNA) provide information on gene expression changes. They are useful in understanding tumor biology and predicting response to treatment. miRNAs serve as diagnostic and prognostic biomarkers with potential for monitoring treatment response. Liquid biopsies are simple and quick with minimal patient discomfort, a low risk of any complications and can be performed frequently. They can monitor disease progression and treatment response through serial sampling over time. Tumor-educated blood platelets are also emerging as a promising biomarker source for the non-invasive detection of cancer.

ctDNA analysis can help to diagnose certain cancers, especially when tissue biopsies are difficult or impossible to obtain. Changes in ctDNA levels can indicate how well a patient is responding to treatment (treatment monitoring), with decreasing levels suggesting a positive response and increasing levels potentially indicating resistance or recurrence. Its analysis can be used to detect minimal residual disease (MRD) after treatment, potentially signaling the early stages of cancer recurrence (early detection of recurrence). ctDNA analysis can help identify specific mutations in the tumor, guiding the selection of targeted therapies. A lack of ctDNA in the bloodstream in periods with no symptoms following treatment indicates that the cancer has not returned (cancer remission). High ct DNA tumor fraction (TF) is

a characteristic of high shedding tumors, metastatic and more advanced stage tumors and aggressive tumors which are progressing while patient is under active treatment. Low ct DNA TF is a characteristic of low shedding tumors, tumors still limited to tissue of origin or locally advanced cancers, and cancers which are shrinking and responding well to treatment. Thus, low quantity of ctDNA indicates that treatment is successful. ctDNA TF value informs the next steps following a negative liquid biopsy result. It can be used to prioritize confirmatory tissue testing for patients with low ct DNA TF. While promising, it is important to note that sensitivity and specificity can vary, and false positives or negatives can occur. It is not a replacement for and is often used in conjunction with traditional tissue biopsies. Ongoing studies are exploring its full potential in various cancer types and clinical settings.

Thus, knowing more about cancer from a patient's blood sample (liquid biopsy) by examining ctDNA or circulating tumor cells (CTCs) or both in combination is an approach that is now being applied to cancers of breast, colorectum, urinary bladder, prostate, pancreas, and lung as well as malignant melanoma.

Liquid biopsies represent minimally invasive, low risk, repeatable lab tests, most commonly blood, but include other body fluids, that are used to analyze cancer-derived material such as CTCs, ctDNA, RNA etc. They are used in detection and monitoring of cancer. Cancer cells have a constant turnover. They die and make new cells. So, as a tumor grows, the amount of ctDNA may be higher. Through a blood sample, it may be possible to identify genetic mutations that can be targeted with specific drugs or look for markers that make a patient eligible for some therapies. Patients in future could avoid unnecessary drugs, or if they really are at high risk, they can receive therapy which provides a big benefit and change the course of their treatment. For example, presence of PD-L1 protein on a CTC may indicate that immunotherapy may be a better option for a patient with melanoma. Preliminary data also show that certain mutations identified by ctDNA testing can be used to guide treatment choices for lung cancer showing the feasibility of guiding treatment in other cancers based on liquid biopsy. By taking blood samples before and during treatment, it may be possible to track the effectiveness of the therapy or identify new mutations that develop without the need for additional invasive tissue biopsies or imaging studies. We can get answers about what's going on in liver, bones etc. without invasive procedures. The technique is still new, and best use is still undefined. However, the prospect is exciting. Ongoing clinical trials in the next few years may reveal the true value of liquid biopsies and whether they can truly help in providing the best care for cancer patients. Due to the constantly increasing clinical use of ctDNA based CGP for treatment selection, several leading organizations have included recommendations for the use of liquid biopsy in their clinical management guidelines. There are still contradicting suggestions as to whether a "plasma first" or "tissue first" approach should be adopted in a clinical setting. NCCN, ASCO, and ESMO all recommend a "tissue first" approach during the initial diagnosis of NSCLC while the International Association for the Study of Lung Cancer (IASLC) recommends the use of ctDNA CGP as the assay of choice.[140–144]

DNA Methylation Profiling Assays and Transcriptome Studies

These refer to the genome-wide analysis of methyl group additions to DNA, primarily at CgP sites to determine epigenetic (changes especially heritable changes, in the characteristics of cells resulting from altered gene expression that do not involve changes to DNA sequence itself) gene regulation patterns. They are a powerful molecular diagnostic tool for tumor classification, and identifying cancer subtypes. They can aid pathologists in difficult cases, increasing diagnostic accuracy. These are crucial in cancer research, providing insights into cancer development, progression, and potential therapeutic targets. The development of cancer is always associated with genetic and epigenetic changes accumulated within the cell, through which it acquires aberrant biological features specific for cancer cells (such as loss of apoptosis, insensitivity to regulatory factors, uncontrolled growth and cell division). These changes can be considered at three distinct levels: genomic, transcriptomic, and proteomic. The analysis of complex interactions among these three molecular levels forms the basis for the understanding of personalized oncology. The analysis of cancer genome may provide valuable information about the DNA sequence and its structure but could be inadequate to describe the actual phenotype of the cancer cell. Thus, other approaches are needed to find proper molecular diagnostic targets and develop specific therapies for cancer patients. The proteomic approach is closer to the molecular mechanisms determining the cell's phenotype. It focuses on quantitative protein measurements to characterize biological processes and deciphers the protein-dependent mechanisms of gene expression regulation in a living cell. Among proteomics

techniques, microarrays testing which uses monoclonal antibodies for identification of an individual protein is the most common. Protein microarrays have gained extensive applications in molecular diagnostics, especially in cancer biomarker discovery. The pivotal link between cellular phenotype and genetic aspects of tumor biology is the transcriptome. It contains all information encoded in RNA transcribed from DNA. In contrast to the genome which is relatively stable, transcriptome reacts actively to physiological and pathological conditions. Adjustments in the transcriptome reflect the different cell states, developmental stages and regulatory mechanisms. Currently, transcriptomics is at the forefront of cancer research due to the rapid development of RNA sequencing methods. Transcriptome analysis using RNA sequencing studies the complete set of RNA transcripts in a cell. It includes protein-coding RNAs typically referred to as messenger RNAs (mRNAs) and non-coding RNAs. Transcriptome analysis is considered as a useful approach for investigation of constantly changing cancer cells at a molecular level and reveals which genes are actively being expressed by cancer cells. By comparing gene expression profiles between cancer cells and normal cells, researchers can identify genes and pathways that are abnormally expressed and dysregulated in specific cancers, making these genes potential targets for new drugs. Differentially expressed genes identified through transcriptome analysis can serve as biomarkers for early cancer detection, prognosis, and monitoring treatment response. Combining DNA sequencing and transcriptome analysis provides a powerful approach to understand cancer. DNA sequencing can identify a specific mutation in a gene, while transcriptome analysis can reveal whether that mutation is affecting the expression and function of the corresponding protein. This integrated approach helps identify key driver mutations, understand the molecular mechanisms of cancer development, and predict which patients are most likely to respond to specific therapies. By analyzing both DNA and transcriptome (RNA) data, researchers can develop personalized treatment strategies tailored to the specific genetic and molecular characteristics of an individual's cancer. Transcriptome analysis can identify mechanisms of drug resistance in cancer, which can help develop strategies to overcome resistance. Techniques like the whole-genome and transcriptome sequencing (WGTS) offer a comprehensive view of the cancer genome and transcriptome, providing more detailed information for clinical decision making. Thus, DNA and transcriptome studies are emerging as essential tools in cancer and are expected to transform the diagnosis and treatment of cancer. The combination of WGTS and transcriptome sequencing presents an opportunity for a much larger proportion of cancer patients to have access to personalized therapies. The WINTHER precision medicine clinical trial was the first prospective trial in diverse solid cancers that assessed both genomics and transcriptomics to match treatments to specific molecular alterations. WINTHER and other trials increased the number of targetable-omic changes compared to genomic profiling alone. Many issues regarding the complexity of the analysis, its reproducibility and variability, and the interpretation of the results still need to be addressed. The integration of transcriptomics with genomics, proteomics, epigenetics, and tumor immune profiling will improve our understanding of cancer and accelerate the implementation of precision oncology. Transcriptomics are leading to a change in the holistic understanding of cancer, from histopathological and organic to molecular classifications, opening a more personalized perspective for cancer diagnostics and therapy.[145–150]

Molecular Tumor Board (MTB)

Oncologists cannot grasp the full spectrum of actionable molecular events in cancers, hence the concept of MTBs which are a unique form of MDT. MTB comprises of a multidisciplinary group of experts with expertise in molecular diagnostics and targeted therapies who analyze and review molecular profiling data results from a patient's tumor (DNA, RNA and proteins) within the tumor looking for specific mutations, gene fusions etc to recommend patient specific targeted therapies with potential better outcomes. Participants of this forum include medical oncologist, molecular pathologist, histopathologist, geneticist, bioinformatician, clinical trials navigator and pharmacist. It complements traditional tumor boards by focusing on the genetic and molecular characteristics of cancer, rather than just the organ of origin. This approach helps identify targeted therapies and clinical trial opportunities for patients with advanced cancers. Patients' history, imaging, laboratory results, histopathology report and report of CGP are extensively reviewed to facilitate interpretation of complex molecular data. A genomic analyst processes the raw sequencing data by filtering, reviewing, and validating it. She/he then prioritizes and summarizes the functionally significant events in the context of published literature and clinical trials, and a report is generated. The pathologist and oncologist then evaluate the significance of potentially actionable events and incorporate the significant ones into the clinical management of patients.

Thus, all possible therapeutic strategies for patients who are not responding to standard of care systemic anti-cancer therapies are discussed and decisions are taken. Patients with metastatic cancers may not benefit from standard drugs and their cancers may develop drug resistance due to cancer cell heterogeneity and genomic landscape complexity. The aim is to develop a treatment plan by understanding the molecular profile of the cancer and match patients with targeted therapies that are likely to be most effective and recommend specific therapies. MTBs play a crucial role in identifying patients who are eligible for clinical trials based on the cancer's molecular profile thus providing cancer patients with access to cutting-edge therapies. In some cases, germline alterations detected in the patient's normal DNA are also evaluated with the assistance of genetic counselors. Studies have shown that cancer patients who receive MTB-recommended therapies demonstrate improved response and survival rates. By focusing on the most relevant therapies based on molecular data, MTBs help optimize the use of healthcare resources. Access to MTBs is currently variable. Interpreting complex molecular data requires specialized expertise, and the availability of qualified professionals may vary. There is a need for standardized guidelines and reporting for MTB evaluations to ensure consistent and reliable results. Thus, MTBs represent a shift towards precision oncology where treatment decisions are guided by a deep understanding of the molecular characteristics of each patient's cancer.

MTB recommendations should be clearly written documenting parameters such as driver mutations/copy number/structural variations including fusion genes, druggable molecular alterations, MSI, TMB, molecular alterations indicating drug resistance, MTB conclusions/recommendations, and potentially available clinical trials. MTBs can also serve as key educational tools in teaching hospitals where presentation of challenging cases would initiate discussion.

However, MTBs face significant challenges. These include limited availability of and access to targeted agents matching the genomic alterations, limited access to clinical trials, limited currently existing evidence for the therapeutic recommendations, cost of CGP, availability of testing platforms, in-house versus send out testing, tissue versus liquid biopsy for testing, limited availability of expert personnel etc. Access to MTBs may vary depending on the institution. Interpreting complex molecular data requires specialized expertise. The above facilities and human resource may not be available in developing countries and low resource settings.

In summary, MTBs are needed because genomic data is vast, raw and complex and difficult to interpret with many cancers harboring multiple genomic alterations, both somatic and germline. Cancers acquire resistance to anti-cancer drugs. Sometimes, no mutations are detected raising the question of whether this is real or caused by a technical issue. It also needs to be addressed whether all actionable targets were completely assessed or not. Sometimes, critical determinants for care are not detected by NGS but need to be recognized and discussed for optimal management of cancer patients.[151–154]

The Concept of Basket Trials

Basket trials are a groundbreaking approach in clinical cancer research, focusing on patients with specific genetic mutations, regardless of the cancer's location or type. They are a type of clinical trial design which utilizes the same experimental treatment to target a particular mutation, allowing researchers to evaluate the effectiveness of a single therapy across various cancers harboring the same mutation or biomarker, including rare cancers or cancers with rare mutations. Patients are grouped into single cohorts or "baskets" based on their cancer's molecular profile, which means that patients with different types of cancer sharing the same molecular alteration are included. These trials are more efficient, can enroll patients more rapidly and aim to identify potentially effective tumor agnostic therapies more quickly. All patients receive the same targeted therapy. Researchers assess the effectiveness of the treatment across different cancer types within each basket. If a treatment shows promise across different cancer types, it can lead to tumor-agnostic approval, meaning the drug can be used to treat that specific alteration regardless of the cancer's origin. With advances in CGP, new drivers of oncogenesis shared across different tumor types continue to be discovered in virtually all cancers in variable proportions and are becoming available for pharmaceutical inhibition (eg fusions of RET, FGFR 1, 2, 3 and NRC 1, 2, 3 as well as mutations involving BRAF, EGFR, MET, RET, ERBB2, PIK3CA, Ras, AKT and many others). In other words, a mutation common to multiple cancer types can, with the help of basket trials, lead to targeted intervention and ultimately the development of tumor agnostic therapies.

Basket Trials for highly addictive oncogenic drivers, Fusion genes: Fusions arise because of genomic rearrangements which include chromosomal inversions, deletions, duplications, and translocations. The fusion gene leads to the formation of a fusion protein that behaves as a very strong oncogenic driver. Cancers which harbor fusion genes often demonstrate founder fusion alterations (eg Bcr-Ab, ROS1, ALK, PML-RARA etc). Fusions appear to be extremely important tumor targets and optimized therapy may require targeting fusions even in the presence of other molecular alterations. Targeting fusions has shown a much more marked anti-tumor activity than targeting other alteration types (Imatinib targeting Bcr-Abl in CML versus Imatinib targeting cKIT in GIST). More recently, oncogenic fusions in neurotropin tyrosine receptor kinase genes NTRK 1, 2 or 3 (also called TRK-tropomyosin receptor kinase) and the RET gene provide prime examples of histology independent oncogenic alterations which can be present across diverse types of cancers. Keynote-158 study is a prominent example of a non-randomized basket trial.[155–159]

Wayfind

Precision oncology is rapidly evolving to become a pivotal part of cancer management, supported by regulatory approvals of biomarker-matched targeted therapies and immunotherapy. NGS-based technologies have revealed an increasing number of molecular-based cancer subtypes with rare patient populations, leading to difficulties in recruiting for and conducting traditional clinical trials. Therefore, approval of novel therapies based on traditional interventional studies may be difficult and time consuming, with delayed access to innovative therapies. Real-world data (RWD) generated in routine clinical practice can help elucidate the clinical utility of NGS-based genomic profiling, multidisciplinary case discussions, and targeted therapies. WAYFIND- is the first of its kind global cancer registry which aims to advance precision oncology by collecting RWD from patients with solid tumors who have undergone NGS-based genomic profiling. Its objectives include characterizing the treatment and clinical course of solid tumors in patients who have undergone NGS testing, developing a database to better understand health outcome and cancer care processes, and providing a collaboration platform to support the design and conduct of further clinical and epidemiological research in cancer. WAYFIND-R will optimize best practice for NGS-based treatment decisions by clinicians, help develop global collaborations between cancer centers, allow robust conclusions regarding outcome data to be drawn, and improve understanding in disparities in the access of cancer patients to advanced diagnostics and therapies.[160,161]

Suitable Alternatives and Actionable Strategies for Overcoming Obstacles and Challenges in Cancer Care in Resource-Limited Settings

Global cancer incidence is rising and this rise is disproportionately affecting low-and-middle -income countries (LMICs). It is expected that approximately 70% cancer deaths in the next two decades will occur in these countries. This disparity is due to socioeconomic barriers and lack of even conventional cancer care facilities due to poor infrastructure and manpower shortages. There is reduced access to cancer care with delays in diagnosis and treatment, and preventable mortality is a major concern. Cancer drugs are very expensive and unaffordable for the majority of patients. In such a scenario, hoping for a transformation in cancer care based on new molecular techniques and tests seems a distant dream. Equity in cancer diagnosis and management between developed and developed countries is essential and is a moral imperative. WHO has a duty to address inequity in cancer care and help in increasing the capacities of LMICs in essential molecular diagnostics, treatment of cancer, and ensuring accessibility of new cancer therapies. WHO should advocate justice and also keep the WHO blue books for cancer in step with changes in capacity of LMICs in fighting cancer. At the same time, it is essential for LMICs to invest extensively in the prevention, diagnosis, and treatment of cancer. Governments, even if they have the will, cannot do this on their own, and the importance of collaboration between public and private sector cannot be overemphasized. Philanthropy needs to play an important role. Oncologists and pathologists need to convince hospital administrators, government officials, and rich entrepreneurs regarding the urgent need of radical changes and massive investment in cancer care for improvement of infrastructure, training, recruitment and retention of cancer care physicians and staff (to overcome manpower shortages by offering attractive financial incentives, and above all the reduction in the high costs of cancer testing and latest treatment modalities so that the latest facilities are accessible to all cancer patients irrespective of their socioeconomic status. Collaborations with

international health organizations and major oncology centers should be developed. Since even major hospitals in poor countries can find it difficult to perform molecular testing, establishment of regional and national molecular testing centers and modern cancer hospitals, equipped with the latest state of the art techniques and adequately stocked with the latest anti- cancer drugs, by public-private partnerships. By investing in digital/telepathology, remote consultations with internationally renowned experts in difficult and challenging cases will be possible ensuring the best diagnosis. Artificial Intelligence (AI) can help in making skilled opinion accessible for all patients and overcome the shortage of skilled pathologists. At present, there is lack of specialized training for performing and interpreting molecular tests. Low resource settings need to focus on cost-effective, rapid, user-friendly technologies. Need for expensive molecular testing can be obviated in many cases by the use of surrogate immunohistochemistry (IHC) markers. They can be used for early diagnosis and to determine the response of cancer to treatment and whether it will respond to specific targeted therapies such as tyrosine kinase inhibitors. Histopathology labs need to add surrogate IHC markers to their IHC repertoire. Examples include SS18: SSX antibodies in synovial sarcoma, DDIT in myxoid liposarcoma, P16 in HPV-related cancers, IDH1 in gliomas, P53 for TP53 mutations in gliomas and other cancers. Even in countries like India, NGS remains underutilized due to cost constraints and limited availability. Experts believe that cost-effectiveness may be improved through strategic hotspot panel selection. It needs to be emphasized that investment in NGS technologies and integration of molecular biomarkers in clinical flow will be essential in next few years even in developing countries. Targeted hotspot panel testing is preferable over CGP in LMICs due to greater availability and affordability. Multigene assays are recommended as they are more efficient and cost-effective. CRISPR diagnostics are suitable for LMICs as they are accurate, rapid and low-cost and can bypass the high costs and longer turn-around times associated with NGS.

Point-of-care technologies (POCTs) can offer a transformative approach to decentralizing cancer diagnostics by providing rapid, affordable, accurate, portable testing in resource-limited settings. Laboratory technologists and nurses can be trained to perform and interpret these tests to cover the shortage of pathologists. Recent advancements including loop-mediated isothermal amplification and multiplex lateral flow immunoassays enable high sensitivity detection of cancer biomarkers without the need for complex lab infrastructure.

Sustainable financing is required through public-private collaboration so that poor cancer patients can be offered targeted subsidies, and community-based screening programs for different cancers can be started and sustained. It is essential to acquire emerging targeted therapies through international collaborations and public-private partnership and ensure access of poor patients to these therapies. National health insurance systems need to be implemented to cover for expensive personalized targeted therapies. Some major cancer centers in countries like India are developing their own drugs. Local research and drug development should be fostered. Investments need to prioritize local capacities and research. In other words, sustainable local capacity needs to be developed. Biswas et al in a study of molecular biomarker testing on NSCLC from India demonstrated the usefulness of strategic Hotspot panel selection instead of the more expensive CGP. They showed that strategic Hotspot panel testing was more affordable and readily available compared to CGP and required limited lab infrastructure. They developed their own panels to perform molecular testing, an example of developing and building local capacities. However, they emphasized that CGP was essential in future for ensuring the most optimal personalized cancer care. In Pakistan, Qazi et al developed innovative in-house molecular testing for solid cancers and demonstrated good results. These examples highlight the great importance of developing local capacities to perform more cost-effective, affordable molecular testing. They emphasized the need to educate and raise awareness among lab professionals, hospital administrations and government officials about the importance of molecular testing in the early detection and optimal treatment of cancer and the urgent need to invest in new molecular technology, building infrastructure, and training of personnel in these techniques. In Africa, there are access initiatives like Cancer Access Partnership which is a collaboration between African Cancer Coalition, Clinton Health Access, and American Cancer Society can help. Investments from non-profit organizations should be matched with long-term commitments from biotech companies, government agencies, regional and international governing bodies such as Africa Union and World Bank. Regional and national cancer registries must be established. Molecular diagnostics should be integrated with affordable treatment. Another major issue in LMICs is the lack of regulatory oversight and ethical concerns associated with genetic testing. National regulatory bodies need to be established to resolve these issues as FDA stresses the essential importance of regulatory oversight and approval for molecular testing and molecular-based therapy.

Collaborations with major international cancer organizations are also needed to ensure participation of cancer patients from underdeveloped countries in in clinical trials in oncology. Currently, patients from these countries are grossly underrepresented. Ntekin and Ologada discussed innovative strategies for developing biomarker based clinical trials in precision oncology in Sub-Saharan Africa.

Thus, molecular oncology in resource limited settings focuses on developing affordable and portable diagnostic tools. Key strategies include using microfluids and POC devices for rapid on-site testing. These highly cost-effective technologies often target infection-related cancers and help guide treatment with limited infrastructure. They aid in both diagnosis and treatment monitoring. Portable, compact, automated microfluid-based POC molecular testing is revolutionizing testing by allowing non-specialists to perform complex tests with minimal, low-cost reagents and robust small instruments. However, training of personnel (nurses and lab technologists) and sustainable funding are still required. Fostering public-private partnerships is extremely important to develop and sustain these services.[162–168]

Conclusion

There is a paradigm shift in the diagnosis and management of cancer in the new era of precision oncology. This is the age of biomarkers in which cancers of different histological types across multiple organs will increasingly be treated based on shared molecular characteristics, identified through advanced molecular testing, by tumour agnostic targeted therapies which target the molecular changes common to different cancer types. These therapies are the cornerstone of precision oncology. Many of the major changes in cancer care have been discussed above. Pathologists need to be aware of these changes, become familiar with the ever-expanding field of new biomarkers and their crucial role in cancer care and adapt to new technologies such as NGS and CGP, liquid biopsies. DNA transcriptome analysis etc. They need to familiarise themselves with tumour agnostic and immunotherapies, concepts such as tumour heterogeneity and resistance to therapy. They must understand that they can no longer be morphologists alone and will be required to adapt their practices to become collaborative clinicians with major roles in the prevention, diagnosis, prognostication, and treatment of cancer. We hope the above discussion will raise awareness about these fundamental alterations in cancer care among pathologists, especially pathologists in developing countries working in resource limited settings.

Disclosure

The authors report no conflicts of interest in this work.

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