Review Article

Emerging Molecular Technology in Cancer Testing

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Article Info

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Keywords

genomics, cancer, laboratory medicine and molecular technology

Abstract

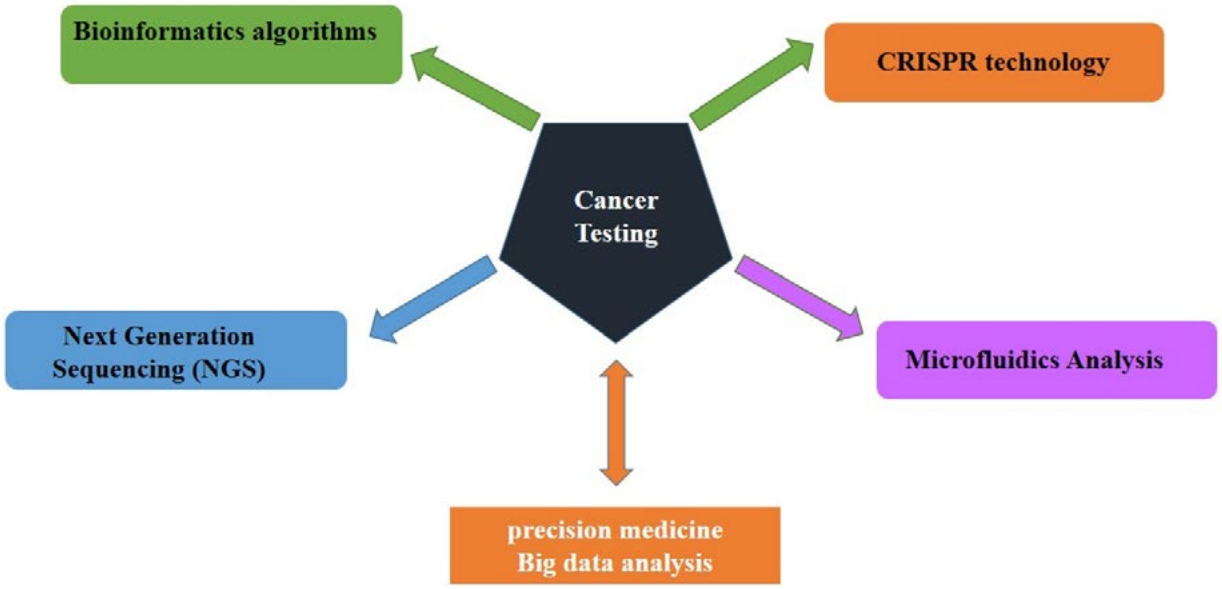
Background

One of the areas that have most likely profited from technological and methodological advancements in genomics is cancer research. Genomic technologies are reaching the point where genetic variation in patients can be identified with high precision and at a lower cost, offering the promise of profoundly changing medicine. The next task at hand is to apply the amazing tools and resources created in genomics to improve our knowledge of health and illness. Personalized medicine offers tailored treatment that targets the appropriate drugs for the right person at the right time based on that person's unique profile. This improved understanding should revolutionize the medical sector to leverage genomics in this transformation. Content: Clinical biomarker discovery can be advanced by high-throughput technologies like genomic sequencing and gene expression microarrays. Commercially and at particular academic cancer centers, targeted cancer gene panels (50–250 genes) are offered by Clinical Laboratory Improvement Change-certified laboratories. Precision cancer medicine is becoming a reality thanks to the abundance of data that genome sequencing and other high-throughput technologies provide, both in terms of cost and efficiency. Summary: This review sheds light on the newly emerging molecular technology of diagnostic applications in the clinical laboratory for cancer diagnosis using genomics.

Introduction

The potential of genetic information to ameliorate disease is a great deal of excitement. In the last ten years, genetic technology has become more widespread with tests that help manage drug usage, help prevent and treat cancer, and identify other health hazards (Figure 1) [1]. These advancements show that genomic information will likely be used in clinical care in a large way going forward, going beyond the assessment of single-gene abnormalities to include overall disease vulnerability. Clinical utility, however, becomes a significant problem when the focus moves from highly penetrant genetic diseases to less penetrant genotypes and genetic risk profiles [2]. Precision medicine, which offers individualized medical care based on a patient's specific information and unique genetic profile, is a result of technological advancements. When opposed to the more conventional indiscriminate radio/chemotherapy strategy, this notion has been demonstrated to be effective in improving clinical results [3]. The FDA has approved a rapidly expanding list of medications to treat

Figure 1: Emerging cancer testing methods.



advanced solid tumors that specifically target specific genetic changes. Therefore, it is important to understand and evaluate current and emerging molecular methods for cancer diagnosis and the various molecular techniques available to map the molecular heterogeneity of tumor for effective treatment strategies. Due to the scarcity of nucleic acids in the heterogeneity of tissue and samples, the identification of nucleic acid and Biomarkers was difficult before the invention of Polymerase Chain Reaction (PCR). Using more sensitive quantitative PCR (qPCR) tumor-specific DNA can be amplified to detectable levels [4]. RNA-based biomarkers such as miRNAs can be detected using quantitative reverse transcription-PCR (RT-qPCR) [5]. As technology develops, techniques like qPCR, digital PCR, and NGS can be used to identify exceedingly rare biomarkers like tumor-specific cfDNA. When using qPCR to detect highly rare events or gene variants in a patient sample proves to be challenging, the most recent PCR option to hit the diagnostic market is digital PCR. Conversely, NGS denotes high-throughput nucleic acid sequencing systems that employ PCR-amplified and fragmented DNA at a rate of similar efficiency to digital PCR [6]. This review sheds light on uses of the emerging technologies such as CRISPR, Microfluidic chip-on devices, precision medicine, and NGS big data challenges for cancer.

Novel Cancer Diagnostic Technologies

Recent studies to develop the diagnosis of cancer have broken multiple records for diagnostic test speed and accuracy. Although these molecular diagnostic techniques were first employed as research instruments [7], they have now been demonstrated to be helpful in a therapeutic context [8]. The availability of multiple high-throughput and high-resolution approaches to identify abnormalities in these novel biomarkers has made the use of these biomarkers in cancer diagnosis easier, as Table 1 illustrates.

Table 1: Current high throughput tests for cancer diagnosis.

Analysis	Methods	References
MicroRNA and RNA	Microarray technology	[8]
Methylation analysis	Quantitative sequenom and pyrosequencing	[9]
Single nucleotide polymorphism (SNP) arrays, gene arrangements	Capillary electrophoresis	[11]
Single nucleotide polymorphism genotyping	Matrix-associated laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) sequenome	[12]
70 Gene microarray panel analysis in breast cancer	MammaPrint	[13]
Hotspot cancer mutations	Ampliseq	[14]
Hotspot cancer mutations	Afirma gene profiling	[15]

New platforms, including targeted gene panel sequencing, microarrays, FISH, capillary electrophoresis, real-time PCR-TaqMan assays, nested PCR, sequencing/pyrosequencing, sequencing, and qualitative PCR-ARMS and RFLP, are available for clinical use in cancer diagnosis [9]. These platforms are based on basic, translational, and clinical research [8]. Single nucleotide polymorphism (SNP) detection, gene expression profiling, viral load quantification in cancer, and monitoring, and tracking the outcomes of a patient's treatment are just a few of the many uses for quantitative polymerase chain reaction (PCR), which is widely used in the detection of DNA, RNA, and miRNA abnormalities in the primary diagnosis of cancer. There are several drawbacks of gel electrophoresis, including low resolution, inaccurate results, and non-measurable outcomes. In order to identify gene rearrangements, single nucleotide polymorphisms (SNPs), and loss of heterozygosity (LOH), capillary electrophoresis was created and is now commonly utilized [10]. A panel of more than 46 genes may now be sequenced for a cancer diagnosis in 48 hours for about $1000, thanks to rapid technical advancements and sequencing. As most tests are more accessible and useful than PCR and NGS, qPCR is currently the molecular method of choice for biomarker discovery. Also, test results can be obtained within a day. However, performance is limited to multiple targets and requires prior knowledge of the target DNA. On the other hand, an NGS study can provide valuable information, including mutations, chromosomal rearrangements, and genetic changes, without prior knowledge of the target. Results last about 7 days [11]. Setting up a central and private cancer center as a model for a major city or provincial health center has many challenges. Sensitivity testing is required, which involves sampling from the device during transport, resulting in a longer TAT. Therefore, ill patients may have to wait a day or two weeks for appropriate care [12].

Selection of Molecular platform

The number of biomarkers evaluated in molecular medicine is increasing in clinical practice [13] and advances in precision medicine require caution. Well-behaved cases are rare. The introduction of large-scale sequencing technology (better known as NGS) in molecular diagnostics is an important step towards helping meet these new needs. NGS can be performed using arrays of various sizes that can identify hundreds of genes. The availability of drug studies in clinical research centers requires knowledge of genomic profiling (CGP) of selected patients. CGP may play an important role in future revisions, but it is not a viable method for current revisions [14]. Precision, specificity, and a fast response time are critical when it comes to cancer diagnosis and screening [15]. The qPCR is more widely available than digital PCR and NGS and has a large number of approved assays; it is now the preferred molecular approach for biomarker identification. For instance, hematologic malignancies can be driven by single genetic aberrations that are PCR targets, qPCR is an excellent method for quantifying minimal residual disease. Furthermore, test results may be made available in a single day. However, the activity is restricted to many targets and requires prior knowledge of the target DNA. On the other hand, without prior knowledge of the targets, a single run of NGS can yield significant information, such as mutations, chromosomal rearrangements, and copy number alterations [16], although results can take up to 7 days [6]. Establishing a molecular platform should not be based solely on its superior sensitivity, specificity, or performance. Since advanced molecular laboratories cannot be used for all medical applications, consideration must instead be given to the type of clinical application, cost-effectiveness, and the requirement for greater efficiency. There are a number of drawbacks to setting up a major city or state's central, highly specialized cancer diagnosis laboratory to handle samples from nearby clinical sites. A lengthier TAT is caused by the requirement to handle delicate clinical samples during travel and process samples in batches. Because of this, some really ill patients would have to wait days or even weeks to get the care they need [17].

The Analytical technology selection: NGS versus standard techniques

In the framework of clinical practice, the number of biomarkers evaluated in molecular targeted therapy is constantly raised [18]. Advances in point-of-care diagnostics, where careful standardization of the protocol and assure the procedure selection to optimal execution within clinical needs and frequently with limited biological material available. Molecular diagnostics' advent of massively parallel sequencing techniques, or NGS, is a significant technological advancement to address these emerging clinical requirements. Several sizes of panels that can analyze the tens to hundreds of genes can be used for NGS. A complete genome profile (CGP) in specific patient populations is becoming more and more necessary for clinical research facilities to offer drug-targeted investigations. Although CGP is not a practical strategy in modern clinical practice, it is expected to play a significant role in therapy adaptation in the future [19]. Depending on the number of detectable molecular targets, their complexity, and the proportion of patients with biomarkers approved by regulatory bodies and national and international recommendations, NGS approaches should be used in clinical practice for a subset of advanced malignancies. Thus, typical diagnostic protocols include NGS testing for the identification of biomarkers recognized in clinical practice [20]. The cancers that need to be examined using NGS analysis, comprise ovarian, prostate, lung, and cholangiocarcinoma was much examined. Using NGS technology for these cancers enables the best possible utilization of tissue samples and/or the discovery of recently identified alterations that are not detectable through conventional methods of analysis. All molecular genetic alterations by clinical indication, including point mutations, insertions/deletions (indels), gene copy number variations (CNVs), and structural rearrangements such as fusions, must be covered by the NGS panels that are used. RNA sequencing offers greater

diagnostic reliability for fusions and NGS panels available in a range of sizes. In clinical practice, panels of just ten biomarkers that are approved are adequate. While entire exome sequencing (WES) is currently less suitable for clinical application, the use of large CGP panels covering hundreds of genes should be permitted within the framework of a clinical trial protocol. When tests could only find one biomarker per study, genomic profiling was first restricted to identifying a small number of identifiable alterations [19]. For a long time, this method has been enough to give details on a drug's sensitivity or resistance to a particular tumor site. Many molecular targets and related medications are now available for a variety of malignancies, and developments of understanding and technology, increase the possibilities of precise and customized treatment. Traditional genetic change analysis techniques do not enable the identification of several biomarkers based on the quantity and time range of biological material used in clinical practice. With a general molecular genetic profile of the tumor, NGS technologies enable even more accurate patient selection that is responsive to targeted therapy. It is presently critical to guarantee that NGS tumor genomic profiling tests, whose significance and proof are acknowledged, are equally accessible to cancer patients across the nation. In fact, based on current knowledge and guidelines created at the national and international levels, the application of these technologies must satisfy appropriateness requirements in regard to tumor type, molecular targets, and accessible medications. The ability to deliver tailored medication, identify a molecularly defined subgroup, and assess the epidemiological impact for each patient are all important factors to take into account. While PCR remains the gold standard for the majority of diagnostics based on nucleic acids [19]. However PCR chemicals are expensive, and the method calls for sophisticated lab equipment and skilled workers [21]. Nonspecific amplification can lead to a reduction in detection specificity, even if isothermal nucleic acid amplification eliminates the necessity for thermocycling species. Additional readouts, such as fluorescence probes, oligo chain displacement probes, or molecular markers, can increase specificity [22].

CRISPR: A Novel Approach to Molecular Diagnosis

Numerous biological applications have made use of Clustered Interspaced Short Palindromic Repeats CRISPR-based diagnostics, most notably the identification of nucleic acid-based biomarkers for infectious and non-infectious diseases as well as the identification of mutations and deletions suggestive of genetic disorders [23]. Furthermore, the method has been modified to identify proteins and other tiny compounds. These unfulfilled demands might be satisfied by diagnostics based on Clustered Interspaced Short Palindromic Repeats (CRISPR) [24]. In order to discover mutations, laboratory procedures such as genotyping single nucleotide polymorphisms (SNPs), heteroduplex analysis (HA), and genome sequencing must be conducted using bench-based methods that do not rely solely on hardware. The SHERLOCK system's demonstration of SNP detection sheds light on the use of CRISPR-based detection in genetic point-of-care mutation screening [25]. It can be coupled and incorporated with current testing technologies to enhance current designs, or with new, free, portable devices to allow for on-site treatment [26]. CRISPR-Cas systems have been adapted for a number of uses to date, including targeted editing of genomes [27], epigenomes [28], and transcripts [29], nucleic acid bioimaging [30], cellular event recording [31], and nucleic acid detection. In general, the quickly expanding field of CRISPR-based diagnostics depends on the programmability, specificity, and user-friendliness of CRISPR technology and seeks to produce point-of-care (POC) assays based on nucleic acids that may be used in standard clinical settings. Managing CRISPR-based diagnostics can help track genetic markers that show response to treatment, like BRAF gene alterations, which are frequently utilized to treat melanoma skin cancer [32]. Furthermore, by identifying cell-free mRNA, CRISPR-based diagnostics can be utilized to track gene expression in real-time across different tissues [33]. Naturally, newly created CRISPR assays need to be validated in clinical trials [34], and the assay's validity needs to be confirmed and upheld during clinical use. Nonetheless, we think that the field of nucleic acid-based detection technology will change due to the quick advancements in CRISPR-based diagnostics. The specificity of CRISPR-based diagnostics may alter the existing requirement for benchtop equipment or genome q sequencing for the detection of genetic alterations such as single nucleotide polymorphisms (SNPs). For instance, scientists swiftly created a SHERLOCK-based test during the Zika virus outbreak to find an SNP linked to prenatal microcephaly in Zika virus patients [35].

Furthermore, findings were obtained using the CRISPR-Chip platform in a few fifteen minutes of use. can determine the removal of two exons linked to Duchenne muscular dystrophy in just fifteen minutes [36]. Due to their extremely small concentrations in serum, cell-free DNA and circulating tumor cells would be difficult to detect without a highly sensitive assay. This is where CRISPR-based assays come into play. While PCR-based diagnostics remains the gold standard today, CRISPR-based diagnostics has advanced quickly since its 2017 launch and offers a number of benefits, including simplicity, speed, and reduced cost. They are perfect for care environments where prompt outcomes can expedite therapy and aid in the containment of infection. There are many more drawbacks to PCR-based diagnostics, including dependable access to personal protective equipment, sample reagents, and nucleic acid extraction. CRISPR-based diagnostics, on the other hand, allow for simple readouts and do not require sophisticated laboratory facilities with benchtop thermocyclers. The development of a single-step diagnostic test that complies with the Clinical Laboratory Modification Act (CLIA) must overcome these obstacles, and portable CRISPR-based diagnostics are expected to transform the clinical diagnosis area in the near future [37]. Modern management systems use artificial intelligence to enhance information retrieval; the classification of this

intelligence modifies the feasibility of sharing [38]. When making judgments through the cloud, some researchers, like Ibrahim and his colleagues, merged their expertise in MI machine learning CRISPR detection based on signals transmitted by radio networks with the Internet of Things (IoT) [39].

Personalized medicine is revolutionizing health care in all therapeutic domains

In recent years, there have been breakthrough technological advancements in cancer medicine. For many years, direct biopsy of the tumor tissue for histological and pathological examination has been the primary method of cancer diagnosis. Recent advances in next-generation DNA sequencing and bioinformatic genomics analysis have brought to light a paradigm change in the field, moving from microscopic histological diagnosis levels to molecular genome levels for the diagnosis of cancer [40]. Variations in patient variability and disease heterogeneity result in variations in drug safety and efficacy. This unpredictability sets off a process of trial and error that doesn't stop until each patient receives a safe and efficient prescription. By using predictive biomarkers to inform therapeutic decisions, personalized medicine aims to do away with trial and error. Many of these tests are currently approved by the US Food and Drug Administration (FDA) and can be categorized as complementary or adjunctive diagnostics [41]. These prognostic tests can be useful in other areas of care, even though they are typically utilized in conjunction with oncology treatment. Oncology has been at the forefront of the creation and expansion of the companion and complementary diagnostics sector [42]. The Dako PD-L1 IHC 28-8 pharmDx was approved by the FDA in 2015 as the first supplementary diagnostic tool for patients with non-small cell lung cancer using the anticancer medication nivolumab [43]. Atezolizumab and nivolumab, two cancer medications, now have expanded indications that include melanoma and urothelial carcinoma, making them complementary diagnoses. About 87% of the companion diagnostics market in North America and 95% in Europe is accounted for by oncology. Oncology is anticipated to continue down the path of personalized therapy, with roughly 60% of medications at the conclusion of clinical development based on biomarker data [44]. Complementary diagnostics for various diseases are also being developed by biopharmaceutical businesses sourced from the oncology field. According to some estimates, non-oncology biomarker analysis is used in about half of the medicines that are presently undergoing phase 3 clinical trials [45]. The use of artificial intelligence among the AACC semi-finalists will not only bring efficient, scalable, and effective solutions to a wide range of health problems but also advance clinical precision medicine. These new technologies use and interpret large volumes of patient data that dramatically increase our understanding of the human at the molecular level. In particular, the Numares AXINON® system uses massive metabolomic datasets to obtain molecular information about different organ systems. In addition to its machine learning and metabolomics platform, Numares has identified constellations of patient metabolites that identify acute kidney transplant rejection. This AI-based approach can facilitate faster medical intervention and better outcomes for those suffering from transplant failure. OncoHost's PROphet also analyzes immunotherapy patients' proteomic signatures to inform individual cancer treatment strategies [46]. Instead of relying on standardized immunotherapy protocols, PROphet informs each patient of a more individualized clinical strategy based on molecular profiles that ideally advance treatment. These exciting new platforms have the potential to change the way we approach clinical testing. They use new technology that can ease the burden on healthcare workers. In addition, these systems facilitate fast, accurate, and personalized testing that can provide better treatment plans for various health problems and thus significantly improve cancer patient outcomes [47].

Utilizing molecular Signature for prediction

Gene expression assays such as Oncotype DX (Genomic Health, Redwood City, CA), MamaPrint (Agedia, Inc., Irvine, CA), and Genomic Grade Index (GGI; Affymetrix, Santa Clara, CA) have recently been made commercially available. These technologies identify a prognostic gene signature to predict response to treatment using real-time PCR or microarray technology. The Amsterdam 70 gene profile or signature, which is based on gene expression profiling, is the basis for the development of the MamaPrint test, which has been authorized by the US Food and Drug Administration [48]. Using a microarray platform, this group of researchers found a predictive signature of 70 genes in patients with node-negative breast cancer who were under 55 years old. Genes related to the cell cycle, invasion, metastasis, angiogenesis, and signaling were included in this signature. The 70-gene prognostic signature was proven to be a robust predictor of distant metastasis-free survival, regardless of adjuvant therapy, tumor size, histological grade, age, and node-positive and node-negative tumors as well as treated and untreated individuals. Another validation was carried out with adjuvant chemotherapy-free node-negative T1-2 breast cancers and compared with standard clinical parameters. An enhanced prognosis for distant metastases and overall survival was demonstrated by a 70-gene profile [49]. In node-negative patients receiving tamoxifen treatment, Oncotype DX, a 21-gene recurrence score (RS) prognostic indicator, forecasts the chance of distant recurrence. Patients with ERþ breast cancer patients [50]. 21 genes out of the 250 clinical genes in the National Surgical Assistance Breast and Bowel Project have their expression levels detected by the study. Researches Formalin-fixed, paraffin-embedded tissues were subjected to real-time RT-PCR, which quantified the expression of 21 genes and computed RS. The patients were categorized into three risk groups: high, medium, and low risk. The National Surgical Adjuvant Breast and Bowel Project validated the 21-gene recurrence score in 675 ERÍ node-negative patients receiving tamoxifen. The results demonstrated that RS corresponded with distant recurrence, time between relapses, and overall survival, regardless of age or tumor

size. Additionally, Oncotype DX testing contains indicators including ER, PR, and HER2 that are frequently employed in diagnosis. In order to reclassify patients with histological grade 2 tumors, the GGI signature was created. This information is useful for making clinical decisions. After examining microarray data from 189 invasive breast cancer cases, Sotiriou et al. found 97 genes that were linked to histological grade; the majority of these genes are involved in the control and proliferation of the cell cycle. There was a difference in the expression of these genes between breast cancers of different grades. The expression pattern of intermediate-grade tumors was comparable to that of low- or high-grade patients. GGI can improve therapy choices and increase the accuracy of tumor classification [51]. Tests for Oncotype DX and GGI have restricted coverage, even if MamaPrint is appropriate for patients who are either hormone receptor-positive or hormone receptor-negative and have either positive or negative lymph node depth. The 21 genes and #40, Oncotype DX and #41 profile, were designed to predict chemotherapy response in cancers that were hormone-positive and lymph node-negative, as well as distant recurrence within ten years. After ten years, 15% of patients with ER-positive, lymph node-negative cancer who were treated with tamoxifen had a distant relapse [52]. Although the introduction of these molecular signatures can improve the clinical management of breast cancer patients, the cost of these tests is relatively high, approximately $4,000 for MamaPrint and $3,500 for Oncotype DX compared to conventional pathology tests such as IHC [53]. An important issue for IHC is the accuracy of pre-analytical factors (ie, fixation duration, type of processing, and type and intensity of antigen used) and post-analytical factors (ie, slide scoring) and the cut-offs used to define positive results. and negative results. Therefore, typical pathological testing was compared with these types of molecular signatures. Knauer et al. found that 80% of tumors classified as grade 1 by traditional methods were classified as having a low-risk prognosis by the MamaPrint test, while 20% showed a high-risk prognosis by the MamaPrint test [54]. Class 3 patients had an index of score of molecular profile by compound diagnostics that varied with patients with other comorbid conditions; 88% of these patients were classified as high risk and 12% as low risk based on the 70-gene profile; the average showed approximately 55% low risk and 45% high risk in MamaPrint [55].

Microfluidics Lab-on-Chip Platform

The only detection system currently approved by the US Food and Drug Administration for the enrichment, detection, and enumeration of CTCs is CellSearch (Menarini Silicon Biosystems Inc., San Diego, CA). This system is based on the expression of epithelium-specific cell adhesion molecule (EpCAM) on the surfaces of epithelial-derived CTCs. An average recovery sensitivity of 85% or higher was observed by Allard et al. In addition to the list, whose underlying biological knowledge and applications are limited to achieve widespread clinical adoption, new approaches have been developed to capture CTCs, including microfluidic platforms such as the CTC-Chip, where CTCs interact with an EpCAM coating, micro columns, Under laminar flow conditions [56, 57]. This positive capture platform option and other such platforms still rely on EpCAM detection, which might not be the most accurate way to define CTCs, even with a more straightforward approach [58]. The application of EpCAM-independent enrichment techniques was pioneered by various research in order to address the problem of CTCs exhibiting an EMT phenotype and perhaps as a result of low negative EpCAM expression. This method was described by Sollier et al. as being used to separate and count CDTCs from the blood of patients with breast (25–51 CTCs/7.5 mL) and lung (23–317 CTCs/7.5 mL) [59]. By thoroughly examining CTCs using these novel techniques, either genomically or molecularly, the molecular diagnostic platform's promise for several clinical applications can be further realized. Fan et al. used CTCs to diagnose hepatocellular carcinoma with high sensitivity and specificity. They were also able to use CTCs as a real-time parameter for risk prediction and therapy monitoring, which allowed for the early selection of tumor-tailored and effective treatment plans [60]. A sensitive and effective method for assessing prostate CTCs was developed by Miyamoto et al. using microfluidic cell enrichment, and they claimed that this strategy may be useful in guiding treatment decisions for both localized and metastatic prostate cancer [61]. Ilie et al. investigated the expression of the MET biomarker and enriched stage III/IV NSCLC patients using CellSearch and ISET technologies (Rarecells, Paris, France). CellSearch revealed that 83 out of 256 patients (32%), had CTCs. CTCs were detected in 80 out of 106 patients, or 75%, using ISET. 72% of ISET CTCs had MET expression, whereas 65% of patients had positive MET expression in the matching patient tissue (93% concordance). Tissue and CTC MET expression exhibited a substantial positive connection, according to quantitative MET expression analysis using H-score [62]. A worm-based (WB) microfluidic system was created by Zhang et al. to quickly monitor biochemical signals connected to metastasis in a controlled setting. The rate of epithelial-mesenchymal transition is correlated with the risk of cancer metastasis. An effective method for assessing the possibility of metastasis is the creation of an EMT index using extracellular vesicles (EV) produced from tumors. Every epithelial cell and EV formed from mesenchymal cells has to be extracted independently in order to produce an EV-based EMT index [63]. In 2021, Hogyeong Gwak and associates created a special microfluidic instrument to divide two kinds of electric cars. In just 6.7 minutes, they discovered that over 90% of EVs expressing both a mesenchymal marker (CD49f) and an epithelial marker (EpCAM) could be eclectically distributed per 100 µl sample volume [64]. Microfluidic systems are novel approaches to cancer diagnosis and treatment that hold great promise for enhancing therapeutic outcomes. Furthermore, compared to other popular procedures, these techniques are better suited for the diagnosis of cancer [65]. These benefits include lower medication and biological sample consumption, more accurate

spatiotemporal parameters and fluid control in the TME, real-time cell invasion and interaction monitoring, accurate tumor and TME mimicking, and improved environmental control [66]. Additionally, various tumor populations respond to therapy in different ways, which presents a difficulty for medical professionals treating cancer. By preserving cancer cell heterogeneity and serving as the appropriate in vivo TME, in vivo microfluidic technologies help to overcome this obstacle [67]. Furthermore, 3D microfluidic tumor models can impose chemokine gradients and alter cytokine transport for adoptive cell-mediated cancer immunotherapies. Additionally, customized immunotherapy approaches to fight cancer can be found since patient-derived cells can be seeded into microfluidics [68].

Despite these significant benefits, microfluidic devices have certain drawbacks that could influence the study of cancer treatments. A few drawbacks of PDMS, which is frequently utilized in the production of microfluidic devices, include toxicity brought on by the slow release of oligomers and the absorption of molecules. It also takes cell types and matrix compositions that are medically appropriate to mimic the natural TME. To further better capture the physiological complexity of in vivo systems, it is necessary to enhance the current microfluidic devices [69]. Advanced technology and sophisticated production procedures are needed to manufacture micrometric structures. Each microfluidic system must use the right materials depending on its intended use. Mass production and commercialization of microfluidic devices also require high levels of experimental knowledge to enable these systems to be widely used in most nations. It will take time to find solutions to the major problems associated with using laboratories and encouraging the general use of this technology for cancer diagnosis and treatment [70]. Currently, the majority of microfluidic devices are restricted to almost two-dimensional planar forms, and the possibility of creating microfluidic devices using 3D printers is being considered.

Big data in the field of cancer

Even though the big data revolution in biomedicine is still in its infancy, oncology in particular has benefited greatly from it. The quantity of data uncovered by cutting-edge technology has already surpassed Moore's Law, which is the benchmark for the exponential rise in computer processor capacity over the previous 50 years. Just 1% of the digital data collected in all fields up to this point has been evaluated, with over 90% of the data produced in the last two years [71]. This trend is expected to continue in the near future due to the growing need for computer processing power and cloud data storage from the billions of smart devices. Oncology is rapidly becoming digital, just like many other fields, and it already faces comparable difficulties with data integration, quality, sharing, and analysis [72]. "Omics" approaches are typically used to describe large-scale investigations that seek to objectively characterize the full range of biological molecules in a particular tissue or individual. They comprise transcriptomics (spectrum and variants of expressed RNAs), proteomics (expressed proteins and their patterns), genomics (point mutations, copy number variations, single nucleotide polymorphisms), and epigenomics (genome-wide investigation of DNA modifications, e.g., cytosine methylation). isoforms), metabolomics (study of various metabolites), and so forth. Occasionally, these methods result in the identification of a single marker that is medically meaningful, like a causal gene or potential therapeutic target [73]. Nonetheless, a more typical outcome of "omics" research is the creation of intricate molecular profiles. Numerous classifiers based on omics offer the semi-automated capability for differentiating between states, such as cancer and health. The majority of high-throughput research works with datasets in which the quantity of observed features greatly outweighs the number of instances that are examined. For instance, even though expression microarrays may assess over 20,000 genes at once, the number of individuals with various illness features is typically only a few hundred observations at most. In any instance, human intellect is primarily responsible for creating workable hypotheses and analyzing the data because this volume of data cannot be handled in a meaningful way by hand. Many studies are being conducted with the goal of incorporating high-throughput technology into clinical trials [74]. Artificial intelligence is developing at a very rapid pace. Meeting the needs of individual cancer patients requires predictive and repeatable treatment strategies based on models with statistical power to advance knowledge about cancer types, patient characteristics, and clinical experience. The flow of "big data" presents a significant challenge for translational research. Molecular profiles of individual patients can be determined by oncologists with the use of powerful techniques like next-generation sequencing. Precision medicine's advantages must be demonstrated for specific tumors as well as for different cancer kinds and subtypes. Similar to this, cancer immunotherapy can have significant advantages for certain individuals, but finding these people is a key obstacle to its broad use. Information sharing is hampered by a variety of issues, such as the technological difficulties in developing systems that are compatible and the simple pricing structures that encourage data security. Research is needed to understand the relationship between disease and phenotype with strata representing more homogeneous populations. Schadt et al. found that associations between various physiological phenotypes (such as physiological traits) and molecular phenotypes (such as DNA variants, RNA transcript level variations, RNA transcript variants, protein abundance, or metabolite levels) together form a functional unit [75]. All of this could hasten the identification of illness subgroups that may have therapeutic or prognostic implications and aid in the creation of more effective treatment plans. Consequently, phenotypic analysis plays a crucial role in clarifying the molecular and cellular physiology and pathophysiology of networks by offering insights into gene, RNA, or protein groups that form pathways or modules, the failure of which can result in phenotypic consequences. The usefulness of linking phenotypes to characteristics of genetic or cellular networks at the genome

size has been demonstrated by a number of recent research [76]. The new discipline of "Health Knowledge Engineering" aims to use deep phenotypic data to bridge the gap between research and clinical practice, enabling results-based research that informs decision-making in a PM and stratified setting.

Future Directions and Challenges

While the field of complementary and adjunctive diagnostics is now dominated by oncology, advancements in other areas of care are being made possible by the identification of predictive biomarkers and technology advancements. The development of companion and supplementary diagnostics for these polygenic disorders is hindered by gaps in our understanding of the disease's progression and the absence of prognostic biomarkers. The difficulty of collecting samples and the lack of blood biomarkers are additional obstacles. The companion and complementary diagnostics industry is projected to grow at a rate exceeding $7 billion by 2024, despite these obstacles. There is a change in the industry about the "one drug, one test" approach. a paradigm where businesses are currently developing multi biomarker panels and high-throughput devices to evaluate several medications at once. In order to guarantee patient safety, this modification necessitates that regulatory bodies create new guidelines for laboratory testing and diagnosis. Whole transcriptome analysis is a useful technique for examining several genes implicated in the development of breast cancer and finding novel prognostic and prognostic indicators, more sophisticated technologies including epigenetics, proteomics, metabolomics, and next-generation sequencing still need to be developed as follows: i) provide a better understanding of breast tumorigenesis, ii) identify new genetic and epigenetic genes, iii) characterize intratumoral heterogeneity, iv) identify mechanisms of therapy resistance, and v) identify new biomarkers for prognosis and prognosis, resulting in better and more accurate breast cancer monitoring [55]. Quick advancements in novel molecular methods yielded fresh insights into the tumor's biological properties and resulted in a molecular reclassification of breast cancer. New biomarkers for neoplastic invasion, survival, and development are found by these genomic approaches and can be progressively added to clinical trials. Patients with breast cancer are receiving more customized care thanks to the combined advancements in genetics and imaging [77]. The identification of an increasing number of biomarkers is necessary to make treatment decisions due to precision medicine's rapid development. Numerous diagnostic labs are using NGS technology in their clinical practice and research because of this demand, particularly in the public health and academic sectors. Access to NGS test networks plays an increasingly vital role in the application of precision medicine in clinical practice in an era where molecular genetic profiling of cancer acquires an increasingly essential role in treatment decision-making [19]. NGS makes it possible to analyze somatic mutations and RNA profiles of spontaneously occurring malignancies, characterize germline DNA in great detail, analyze microbiomes methodically, and more. New hereditary disorders and molecular targets are being found as a result of the ongoing accumulation of data. cancer therapy, diagnostic markers unique to individual tumors, etc. It is important to realize that it took years for the clinical integration of tests that were comparatively easy to interpret and straightforward, such as EGFR mutation testing or BRCA1/2 analysis, and that many issues are still unresolved to this day. Given that each of the several new potential markers is made up of a variety of uncommon and unique molecular events, it is impossible to foresee how practical medicine will handle such a huge number of markers without individual clinical validation. These developments might need to be taken into account concurrently with clinical trial guidelines, information sharing, and collaboration between laboratory and clinical specialists [78]. In the coming decades, as the incidence of cancer increases in LMICs, improving cancer care will become a growing public health priority. To close the current global cancer effectiveness gap, efforts to increase access to cancer care should be combined with strengthening health infrastructure, including capacity for cancer diagnosis and monitoring. Although innovations in cancer molecular engineering can facilitate this process, they have been underutilized. Going forward, comprehensive applied research, deployment of context-appropriate technology, and continued multidisciplinary investment in molecular cancer diagnosis are key to achieving universal health care and equity in cancer care [79]. This will not be a sudden revolution, not least because the quality and affordability of the new genomic technologies are sufficiently high quality and affordable to be available to the majority of the world's population without quality health care to take care of It is almost certain that the technical problems with the accuracy of the sequence data will soon be resolved; however, this does not apply to problems of interpretation. Although the detailed discussion of interpretive paradigms deserves detailed scientific investigation and thorough discussion among basic scientists, clinicians, and policymakers, it is important to emphasize a few key points.

Conclusion

Molecular changes usually precede the clinical manifestations of the disease, longitudinal measurements combined with clinical phenotyping can identify new diagnostic and therapeutic targets for the disease. Technological advances and cost savings now allow us to obtain much deeper personal multi-omics profiles. Finding connections between molecular markers and disease, as well as which self-layer is disrupted and more informative for each disease, can be accomplished by collecting this data from the same person at different times. Assessing the molecular consequences of specific mutations in genes that encode transcription factors, signaling molecules, and other genes might disclose the regulatory networks and pathways that underlie the disease under investigation and provide potential targets for treatment. These methods may work especially well when applied to fields like cancer, which are still poorly understood.

Declaration of Conflict of interests

The authors of this article declare that there is no conflict of interest with regard to the content of this manuscript.

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