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REVIEW ARTICLE

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Oncology Breakthroughs to A New Pace of Precision Diagnostics and Therapeutics

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ABSTRACT

Background: When it comes to developing new cancer diagnostics (Dx) and therapeutics (Rx), time is of the essence-for the patients that need treatments most, every day counts. Traditional benchmarks of Dx and Rx have delivered favorable clinical outcomes on a slower timeline than patients can afford.

Methods: Transformative Dx tools such as liquid biopsy in combination with next-generation sequencing (NGS) have emerged as a standard of care to inform clinical decision, treatment response and prognosis much sooner.

Results: As the cutting-edge Dx technology continues to advance, it will complement Rx innovation like antibody-drug conjugates (ADCs), chimeric antigen receptor T-cell (CAR-T) therapy and messenger RNA (mRNA) medicine, and help lifesaving treatments reach patients earlier than ever.

Conclusions: Highly personalized Dx and Rx breakthroughs can empower providers and researchers alike with precise and real-world data on each patients' cancer, helping them to develop and deliver real-time precision oncology care.

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Introduction

According to the National Cancer Institute, close to 2 million people were diagnosed with cancer in the United States in 2022, with over 600,000 people dying from the disease last year. While much has been done, much still needs to be done. Due to cancers' inherent heterogeneity, there is a continuous effort to determine the cause of various cancers to develop effective diagnostics (Dx) and therapeutics (Rx). Indeed, the complexity of tumor clonal evolution, immuno-oncological networks and variable therapeutic responses among different cancers make developing suitable solutions a daunting task. Today, precision Dx and Rx is the name of the game, as scientists seek and have access to multi-layered information on how different facets of tumor cells interact with one another and with adjacent microenvironment (TME).

Today's precision Dx and Rx is driven by the diverse approaches enabled by next-generation sequencing (NGS). At the beginning, the task was about simply sequencing DNA. After that, we started sequencing RNA. Then, methylation patterns, the microbiome, the transcriptome, and so on. The early 2010s brought single-cell resolution to the fore, and all of these methods developed during the first five or ten years of NGS are now being applied at the single-cell level, which is really a stepping stone for establishing spatial and functional genomics. Cancer is probably the clearest example of why precision Dx and Rx approaches are important (Figure 1). Not only scientists want to see whether you have certain mutations, but they also need to understand tumor heterogeneity at the multiple levels—which means looking at tumor and non-tumor TME in their spatial context, e.g., tumor-infiltrating lymphocytes. This insight will guide us on how we can develop novel and effective Dx and Rx against cancer.

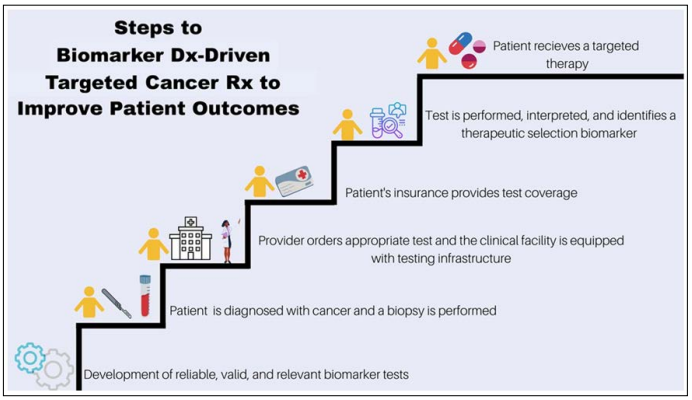

Figure 1: As illustrated here, several steps are involved before a cancer patient can receive a targeted cancer therapy. Many therapies require that a patient is first tested to identify and evaluate specific biomarkers to determine if they are eligible for therapy. However, barriers to patient access for biomarker

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testing (Dx) can arise beginning at test development and can persist through the interpretation of test results in the clinic and can prevent cancer patients from receiving therapies (Rx) that improve survival and quality of life.

Next-Generation Sequencing

Next-generation sequencing (NGS) has been used in precision cancer Dx to detect multiple actionable biomarkers simultaneously. Being able to test for hundreds of targets in a high-throughput format, and possibly discover novel genetic alterations, NGS can potentially speeds up the development of Dx and Rx solutions [1, 2]. As using NGS typically requires weeks to generate results, any improvement in processing times is a definite benefit. Ultimately, shorter sample-to-report time (while still maintaining efficient and high-throughput workflows) lead to results being generated more quickly, which allows for faster clinical decision-making and improving patient outcome. There's been a major shift in the focus of NGS over the past couple of years. First it was all about new genomes, new techniques, and discovery. Now it's all about clinical genomics and genomic medicine. We are entering a new era in advanced sequencing, one in which NGS technologies will not only be used for discovery and translation, but will be integrated into standard clinical care.

The unprecedented throughput of NGS and the nature of the sequencing technology brought a whole host of challenges too, notably: highly sophisticated workflow, bioinformatics, sequencing accuracy, costly with limited access, and operational competency [3-5]. Getting access to large sample cohorts is another challenge. Given the widespread availability of exome and genome sequencing, samples are the new commodity. High-quality DNA samples from informative sources—matched tumor tissue and blood samples of the same patients pre-, during and post-therapy, are increasingly valuable. Nevertheless, we all know that NGS is destined for the clinic. Targeted sequencing panels are already in routine use at many cancer centers; in time, this will likely become exome/genome sequencing [6, 7]. Possibly transcriptome (RNA-Seq) and methylome (Methyl-Seq) as well. Now that NGS has made considerable progress, we must deliver actionable information to the clinic to benefit as many real-world patients as possible.

NGS is certainly a successful story of technological revolution, but the simple fact is that most targeted therapies didn't emerge from large-scale genomics studies, but from a deep understanding of specific pathways involved in defined tumor types. Further, the successful identification of (and targeted therapy against) a driver mutation in one tumor type does not guarantee it will work in another type. Other factors—tissue specificity, genetic environment, and tumor microenvironment—must be considered as well. In many current clinical trials, gene expression and mutation data are being concomitantly assessed for insight into patient stratification and therapeutic response. These sorts of trials are necessary to close the gap between new knowledge from large-scale cancer genomics and its application in the clinic. The feedback loop needs to work both ways: clinical trial results should inform future NGS oncogenomics studies as well. It's clear that we will need both creativity and cross-discipline expertise to carry the mission forward from here. Beating cancer is an important but incredibly difficult mission, and it won't be solved by one technology breakthrough alone. Collaborative efforts by cross-discipline Dx and Rx teams are going to be necessary.

Liquid Biopsy

Liquid biopsy detects and characterizes various tumor-derived biomarkers present in the blood of a cancer patient. These biomarkers are almost undetectable in healthy cancer-free individuals, and they can be analyzed by liquid biopsy assays throughout development, tumorigenesis and progression [8]. This presents the opportunity to perform detailed profiling of analytes for early detection, tailor treatments based on specific molecular characteristics of a particular cancer, and monitor patient responses, drug resistance, and recurrence. Genomics-based methods are providing access, to more molecular data than ever before, giving cancer researchers the tools needed to fulfill that promise. Furthermore, these technologies enable hypothesis-free interrogation of biofluid analytes, providing discovery power to assay genes and pathways that were not considered prior to experimental design.

Most tumors release cell-free tumor DNA (ctDNA) into the bloodstream through various cellular mechanisms, including apoptosis, necrosis, phagocytosis, and active secretion [9]. Even so, ctDNA only represents 1-10% of total cell-free DNA (cfDNA) in the blood, the majority of which is released by erythrocytes, leukocytes, and endothelial cells [9-11]. The rarity of ctDNA combined with the fact that different cancer types at different stages shed ctDNA at different rates complicates analysis. Even so, genomics-based liquid biopsy analysis of ctDNA can provide information of cancer type, stage, and vascularization [12]. Furthermore, ctDNA sequencing identifies variants in the tumor from which it originates, including both driver and passenger mutations, those that do and do not contribute to tumor initiation or progression, respectively [9]. Given the rapid turnover of cfDNA and ctDNA in the bloodstream (half-life of 16.3 minutes), liquid biopsy can be used to assay tumor burden in real time and monitor response to therapy [13]. Changes in DNA methylation occur early during tumorigenesis and result in a state where most of the genome becomes hypomethylated and CpG islands become hypermethylated [14, 15]. This switch in global methylation patterns leads to genomic instability and silencing of tumor suppressor genes, driving tumor progression and metastasis [16, 17]. Importantly, multiple studies have demonstrated that the methylation pattern of ctDNA recapitulates the pattern present in the cell/tissue of origin [18, 19]. This indicates that analysis of ctDNA methylation can be used for early detection of cancer, analysis of tissue of origin, surveillance of minimal residual disease (MRD), monitoring therapy response, and more. Circulating ctRNAs are also potential biomarkers that can be assayed by liquid biopsy to identify specific cancers, detect cancer initiation, reveal tissue-of-origin, elucidate molecular mechanisms of disease, monitor therapeutic response, and more [20, 21]. Unlike DNA, which is identical in every cell/tissue (except for genetic variants), RNA is dynamically and differentially expressed between cell types and tissues. This enables ctRNAs to be used to detect cancer and potentially

localize it in the body. The diverse nature of RNA expression may also enable use of ctRNAs to determine and classify cancer subtypes early in disease, which is important given the wide range in progression, treatment options, and prognosis between cancers, even those associated with the same organ or tissue [22, 23].

Circulating tumor cells (CTCs) are cells shed from tumors into the bloodstream. Multiple studies have demonstrated that CTCs have metastatic potential and are associated with aggressive or advanced disease and poor prognosis in various cancer types [24, 25]. While CTCs can serve as biomarkers for liquid biopsy–based cancer characterization, their rarity and isolation in the bloodstream (< 1 to < 50 CTCs in 7.5 ml blood on average) remains a significant challenge for detection and characterization [26]. Historically, CTCs have been analyzed in bulk due to the low numbers that can be successfully isolated. However, bulk analysis of CTCs limits potential insights into tumor heterogeneity. Technological advances in single-cell isolation and single-cell sequencing methodologies have enabled detailed analysis of single CTCs at the genomic, transcriptomic, and epigenomic levels. Combining liquid biopsy with single-cell sequencing of CTCs can elucidate the cellular heterogeneity that contributes to tumor biology [27, 28].

Liquid biopsy can provide more comprehensive information about a tumor's heterogeneity, mutational complexity, tissue of origin, and more. As technology continues to advance, there is the potential to apply multiomic approaches that integrate analyses of the genome, transcriptome, epigenome, and proteome to liquid biopsy [29]. The ability to combine multiomics with liquid biopsy will provide unique discovery power for deeper insights and comprehensive answers to the mechanisms of cancer. Beyond molecular characterization of cancer, in the future, screening by liquid biopsy could be incorporated into standard preventive care as part of an annual physical exam, like cholesterol and blood glucose tests to detect tumors at their earliest stages. For cancer patients, the noninvasive nature of liquid biopsy will enable longitudinal studies to track cancer over time, monitoring response to therapy and recurrence of disease. Ultimately, when combined with targeted therapies, immunotherapies, and other emerging approaches, liquid biopsy will help provide cures for many cancer patients.

Antibody-Drug Conjugates

Developing therapeutic antibodies or antibody-drug conjugates (ADCs) are among popular strategies for cancer Rx. ADCs are a type of targeted cancer therapy that combines the specificity of monoclonal antibodies with the potency of chemotherapy drugs. Essentially, the antibody component of the ADC specifically targets the cancer cell, allowing the ADC to enter the cancer cell and release the ADC's chemotherapy drug component once inside the cancer cell. The idea is to use an antibody to deliver a toxic payload directly to cancer cells, while sparing healthy cells. There are currently several FDA-approved ADCs on the market, and many more are in development. However, there are also some challenges associated with ADCs, including the complexity of the manufacturing processing and quality control [30, 31]. Overcoming these challenges requires in-depth analytical characterization of the produced ADCs as well as in vivo pharmacological studies to ensure drug safety. Characterizing biological drugs is a critical step throughout the drug development process, starting from development to clinical evaluation. However, several characterization methods are unique to ADCs due to their manufacturing complexity and nature as both synthetic (cytotoxic payload) and biological (antibody).

Pharmacokinetics (PK) and pharmacodynamics (PD) studies are critical to influencing decision-making in dosing, dosing schedules, and maximum tolerated dose for clinical trials [32]. Due to the complexity of ADCs and its reliance on different cytotoxic payload release methods, understanding the pharmacokinetics of both antibody and cytotoxic payload is critical. The quantification of the drug-to-antibody ratio (DAR) of an ADC is another important aspect of ADC analysis, as it can provide insight into the homogeneity and potency of the ADC [33, 34]. DAR analysis is vital for quality control during ADC manufacturing. The DAR can vary depending on the conjugation method and other manufacturing parameters, and it is important to ensure consistency and reproducibility to produce a safe and effective product.

ADCs are a complex biomolecule for analysis, with each component having different gold standards for characterization. With the addition of matrix complexity and species, DAR and PK/PD studies need to be comprehensively evaluated for each component.

Chimeric Antigen Receptor T-cell Therapy

Chimeric antigen receptor (CAR) T-cell therapy is another cell therapy of interest. T cells are collected and isolated from the peripheral blood of a cancer patient or that of a donor, CAR is added to the T cells, and the T cells are then expanded in vitro and infused into the cancer patient. When returned to the cancer patient, the T cells attack cancer cells without damaging healthy ones.

The past decade has seen significant advancements in CAR-T cell therapy. As evidenced by a growing number of successful clinical trials and FDA approval, CAR-T therapy has emerged to revolutionize cancer treatment. Since 2017, the FDA has approved six CAR-T cell therapies used to treat blood cancers such as lymphomas, leukemia, and multiple myeloma. A 2022 report revealed approximately 2,754 active cell therapy agents in the global immunotherapy pipeline, a 36% increase from the number reported in 2021. CAR-T has the most significant number of pipelines and ongoing trials of all immunotherapies, with 1,432 pipelines and 857 trials worldwide, indicating that it remains the most active and promising immunotherapy [35].

The sequential production stages include the collection and expansion of patients' T cells; CAR molecule design, screening, and transfection; target selection; and quality control. The first step in CAR-T production is collecting T cells from a patient. As the number of cells is limited, it requires expansion to a certain amount (billions to even tens of billions of CAR-T cells) before infusion back to the patient, making CAR-T cell expansion an essential step during development. Notably, cytokines can stimulate CAR-T cell activation and expansion.

For example, IL-2, IL-4, IL-15, IL-7, and IL-21 are widely used in CAR-T cell preparation and culturing [36].

The CAR molecule guides the CAR-T cells to the target on the cancer cell surface and activates them into cancer-killing agents. Therefore, the CAR molecule must recognize the cancer antigen specifically and precisely to target cancer cells while avoiding healthy cells effectively. In addition, to provide a better recognition ability for CAR-T cells, the CAR molecule should be effectively designed, as it affects the durability and persistence of the CAR-T cells in the body. A potent CAR molecule should have several key features: high specificity for the target antigen, efficient activation of CAR-T cells, and reduced off-target effects [37]. The fact that four of the six FDA-approved CAR-T therapies use CD19 as a target and the other two use BCMA indicates that these targets are significant and feasible. Unlike hematological malignancies, solid tumors have a more complicated microenvironment, which limits the ability of CAR-T cells to reach and penetrate tumors. Therefore, CAR targets for solid tumors require careful selection and screening.

Owing to several FDA approvals, CAR-T-cell therapy remains the most promising cell therapy for patients with hematological malignancies in comparison to other CAR-engineered cell therapies, such as CAR-NK and CAR-M. However, some challenges must be addressed to guarantee the real success of CAR-T therapy in cancer treatment.

Messenger RNA Therapeutics

The success of mRNA-based COVID-19 vaccines accelerated the market for RNA-based medicines and enabled manufacturers to gain traction. Messenger RNA-based therapies gained popularity for several reasons: (i) mRNA is a versatile molecule that can be easily synthesized and modified; (ii) mRNA-based therapies are considered safe and are effective in COVID-19 pandemic; (iii) mRNA-based medicine has the potential to treat a wide range of diseases. Messenger RNA can target specific cells, encode proteins, and trigger immune responses, enabling its use in vaccines and cell and gene therapies. However, the development of mRNA-based vaccines and therapies can still face challenges, such as targeted delivery, efficacy, stability, and scalability. Nevertheless, the potential of mRNA therapeutics is significant. Their rise has been exponential, and the number of therapies in clinical trials continues to increase.

Messenger RNA vaccines, which deliver genetic instructions to cells to make a specific protein to trigger an immune response, are highly adaptable and can be quickly designed and manufactured to target new cancer biomarkers. An exciting branch of RNA therapeutics is mRNA-based immunotherapy. It involves the delivery of CAR-encoding mRNA to T cells. Essentially, the approach uses mRNA to reprogram T cells so that they target and kill cancer cells. It has the potential to treat a wide range of cancers, including leukemia and lymphoma. Conventional CAR T-cell therapies have high manufacturing costs due to the infrastructure and viral reagents needed. Lipid nanoparticle–based mRNA delivery system negates the need to expand T cells outside the body and can be a more cost-effective method. Combinatorial therapies by CAR T-cell and mRNA vaccine are also promising [38].

The success of the mRNA-based COVID-19 vaccines owes much to the research that was originally focused on cancer vaccines. There are many clinical trials in progress for mRNA vaccine treatment of melanoma, colorectal and pancreatic cancers [39]. Messenger RNA cancer vaccines can work with a one-vaccine-to-many-people approach, or as a personalized therapy. Dendritic cells take up mRNA from the vaccine and present it to T cells, teaching them to search out and destroy cancer cells. Personalized vaccines are manufactured based on molecular features of tumors from individuals to identify genetic mutations that could give rise to neoantigens. Algorithms are used to predict which neoantigens will bind to T-cell receptors and create an immune response. Speed of manufacturing is particularly important in this case, which is one of the reasons that mRNA is a suitable modality.

Protein replacement therapies address protein deficiencies, raising protein levels that are too low, or substituting functional for nonfunctional proteins. Messenger RNA can be used to produce therapeutic proteins within the body—for example, by instructing cells to produce a specific protein that corrects a disease-causing genetic effect. For example, in cystic fibrosis, mRNA can encode a functional copy of the cystic fibrosis transmembrane conductance regulator protein, which is deficient in patients [40].

Messenger RNA can also deliver gene editing tools such as CRISPR-Cas9 to cells and tissues, making it possible to correct deleterious mutations that cause genetic disorders. For example, mRNA-delivered gene editing tools can eliminate aberrant splicing sites that cause beta-thalassemia [41]. It can also deliver gene editing tools to cancer cells, enabling precise genomic modifications that make the cells more vulnerable to immune attack. When gene therapies are delivered by mRNA, “one dose and done” treatments are possible. In contrast, mRNA therapies that rely on mRNA to express therapeutic proteins typically involve repeat doses.

Conclusion

Cancer treatment is transforming rapidly. What was once a wasteland, entered a long period of focus on drugs targeted to specific mutations. But only about 20% to 30% of patients benefit so greatly from these treatments, and researchers have been unable to expand their reach. But the horizon has shifted. For one thing, we have seen the rise of tumor-agnostic therapies, several of which have been approved by the FDA. Also, Rx-guiding Dx testing is becoming much more widespread, with more than a dozen companies offering extensive services, and many cancer centers doing their own. Finally, completely new biomarkers are coming to light. So, the future focus will turn to new generation Dx biomarkers for the corresponding breakthrough Rx. Life-extending targeted treatment options are emerging at an accelerated pace, but cancer will continue to significantly impact the lives of many.

Author Contributions

CY: conceptualization and writing—review and editing. HCL and CY: writing—original draft preparation. WHK: literature review, documentation, writing and supervision. All authors have read and agreed to the published version of the manuscript.

Ethical Approval

Aggregate data were extracted from published studies, no patients were involved in the conduct of this review, thus ethical approval and informed consent were not required.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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