Currently available molecular analyses for personalized tumor therapy (Review)

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Abstract. Targeted therapies are becoming more common and genetic tumor profiling is becoming more precise and affordable. The aim of the present review was to demonstrate the importance of molecular analyses in tumors, summarize the current situation, provide an outlook on how to improve diagnosis to facilitate individualized therapy, including the use of specific methodologies for tumor marker analysis to improve patient treatment. Most predicted metabolomic and proteomic biomarkers have not progressed from the laboratory to clinical trials, as most of the trials were stopped at the initial stage of biomarker identification. The use of liquid biopsies as a clinical tool improves cancer screening, diagnosis and prognosis; furthermore, is able to improve the classification of more diverse disease entities, assess therapy response and identify treatment-resistant clones, allowing for more stringent patient monitoring. Based on specific clinical populations and the unique molecular features of a cancer, the identification of a suitable targeted therapy may be accomplished. The present review provides insight into cancer genomic testing in the clinical setting and the available methods, supporting the prioritization of molecular therapeutic tumor targeting.

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1. Introduction

The combined use of genetic tumor testing and genetically matched targeted medicines has resulted in significant advances in cancer treatment outcomes over the last few decades (1). Imatinib has been used for KIT-mutated gastrointestinal stromal tumor, vemurafenib for BRAF V600-mutated melanoma (2), trastuzumab for HER2-amplified breast and gastric cancer (3) and crizotinib for ALK-activated lung malignancies (4). Modern molecular biology tools have enhanced the understanding of complex changes during carcinogenesis in basic research and their parallel use in clinical practice has improved the identification of malignancies. To make an accurate diagnosis of a tumor, estimate the prognosis of a patient and plan tumor therapy, molecular markers and genetic analyses are essential today (5).

In the era of molecular medicine, with 60 different molecular analyses, the majority of approaches (57.4%) are using targeted multigene next-generation sequencing (NGS), accompanied by whole-exome sequencing (16.4%) and RNA sequencing (13.1%), array-based comparative genome-wide hybridization (4.9%), whole-genome sequencing and Sanger sequencing (both 3.3%) and mRNA sequencing (1.6%). Targeted NGS of the most essential cancer-associated genes, ideally combined with analysis of clinically important gene fusions, is the most suited analysis for detecting actionable changes (e.g., kinase fusion genes) (6). This analysis is readily available and combines quick sequencing with manageable bioinformatics.

Since most clinicians obtain minimal training in genetics (7), the abundance of genetic tests and information poses a serious challenge: In a recent survey of physicians in a tertiary cancer center, 22% reported low confidence in their genetic knowledge and 18% expected to test their patients infrequently (8). The current genetic 'under-testing' may be due to a lack of knowledge; for instance, in the Netherlands, during the years 2008 to 2014, after crizotinib and EGFR inhibitors were approved, ~50% of patients with NSCLC had their ALK rearrangements tested and ~70% had their EGFR mutations tested (9). It took an additional two years to bring ALK testing coverage to 80% after it was included in NSCLC guidelines (10). The literature search was performed in the PubMed database using the following key words: Precision therapy and cancer, personalized medicine and cancer,

molecular analysis and cancer therapy and precision medicine and clinical trials. The last 20 years of publication were considered. The inclusion criteria were that precision medicine and cancer were included.

2. Translating complex biomarkers into molecular diagnostics

The incorporation of mutational profiles and/or gene expression signatures into a biomarker development strategy is accompanied by the translation of the clinical outcome-related biomarker into a robust assay adaptable to widely used analysis platforms. A review of the scientific literature revealed that most published biomarkers are insufficient to replace the existing clinical tests or are only useful for diagnosing advanced disease phases with low survival rates. Numerous molecular or genetic biomarkers have indeed been proposed for the diagnosis of various diseases; however, the majority of these lack the essential sensitivity and specificity. Although several molecular and -genetic biomarkers have indeed been proposed for diagnosing various diseases, they usually lack the essential sensitivity and specificity. In addition, most predicted metabolomic and proteomic biomarker results have not yet progressed or proceeded from the laboratory to clinical trials, since they were stopped at the initial stage of biomarker identification (11).

Multiple cancer genome databases are currently available for the interpretation of profiling results, including canSAR (https://cansar.icr.ac.uk), cBioPortal (https://www.cbioportal.org/), My Cancer Genome (https://www.mycancergenome.org/), COSMIC (https://cancer.sanger.ac.uk/cosmic), ICGC (https://dcc.icgc.org/) and The Cancer Genome Atlas (TCGA; https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga), and various institutes host their own online variant interpretation tools (such as www.mycancergenome.org, Vanderbilt-Ingram Cancer Center; pct.mdanderson.org). However, to translate genomic profiles into treatment recommendations, searches across numerous databases are usually required, as none of these repositories appears to be sufficiently useful on their own (12). Overall, molecular tumor research is likely to become increasingly significant in the division of molecular oncology.

3. Molecular markers for the selection of cancer therapy

Several medicines have one thing in common: They all target different parts of cancer hallmarks or features that are required for successful tumor proliferation and dissemination. The advancement of molecular-scale technology has been critical in the discovery of new cancer targets and it is not a coincidence that better medicines have appeared at the same time that our understanding of cancer genetics has increased thanks to gene expression arrays and DNA sequencing. Advanced tumor pathology is now studied at the molecular level, with immunohistochemical biomarkers, gene signature classifiers and gene mutations all providing important information about whether patients may react to targeted therapy regimes. The broad categories of targeted medicines utilized in clinical settings are briefly discussed in the present review, as well as a brief history of immunohistochemistry, gene expression and DNA sequencing technologies, before looking at the following three tumor types: Breast, lung and colorectal malignancies. To review the current standard therapy for each of these cancer types, prior to focusing on the pertinent targeted therapies and the pathways they impede, molecular analyses provide information on the subclinical manifestation of the disease. Finally, certain strategies that are critical to the growth of targeted anti-cancer medications may be planned. Clearly, a deeper knowledge of the mechanisms of action of drugs and associated biological effects is required, particularly given that magnetic resonance imaging is also unable to distinguish tumor cells from edema. Hence, the necessity of being able to assess a clinical trial drug's effect should be emphasized as a goal of modern analysis methodologies, such as genomics, proteomics and even functional imaging analysis (13).

The most significant category is the heterogeneous population of uncommon tumors, with sarcomas being the most prevalent, followed by breast, brain, gynecological, lung and colorectal cancers (14). The use of NGS technology has proven that there are commonly mutant genes that are aberrant across various cancer types, which may thus respond similarly to a specific targeted therapy (15). NGS-based methods that rapidly generate the mutational profile of a cancer genome in the clinical setting are now being combined with baseline information about frequent genomic alterations in cancer generated in the research setting by sequencing the DNA of thousands of tumors to inform genome-guided cancer medicine (16).

4. Liquid biopsy and personalized therapy

In recent years, oncology research has focused on liquid biopsies, which rely on the detection of cancer-derived components in patients' biofluids, such as circulating tumor cells (CTCs) (17,18), circulating tumor DNA (ctDNA), ctRNA (19) and extracellular vesicles (EVs) (20), and may reveal disseminated aggressive clones. Liquid biopsies are not confined to blood; however, this is what much of the liquid biopsy studies focus on. Urine, saliva or cervical fluid may be employed, as genetic information is present in these fluids (21). ctDNAs have emerged as promising biomarkers, particularly in cancer, and is being widely examined in translational and clinical research (22). Several efforts are being made to evaluate the potential of ctDNAs for early cancer screening, and both qualitative and quantitative cell-free DNA (cfDNA) alterations have been investigated (23). Despite extensive study, only a small number of cfDNA-based assays have been implemented in clinical practice. Conflicting data on total nuclear cfDNA concentration, for example, make it difficult to develop and use cfDNA-based tests in the clinic: Plasma cfDNA concentrations in patients with cancer range from a few ng/ml to many thousand ng/ml, which overlaps with the range of concentrations in healthy subjects (24). The finding of cells discharged into the bloodstream or migrating from tumors is critical and has resulted from 20 years of intense investigation. CTCs are difficult to separate and frequently do not represent genetically malignant cells. Several studies have evaluated the utility of CTCs in identifying various types of cancer (25,26).

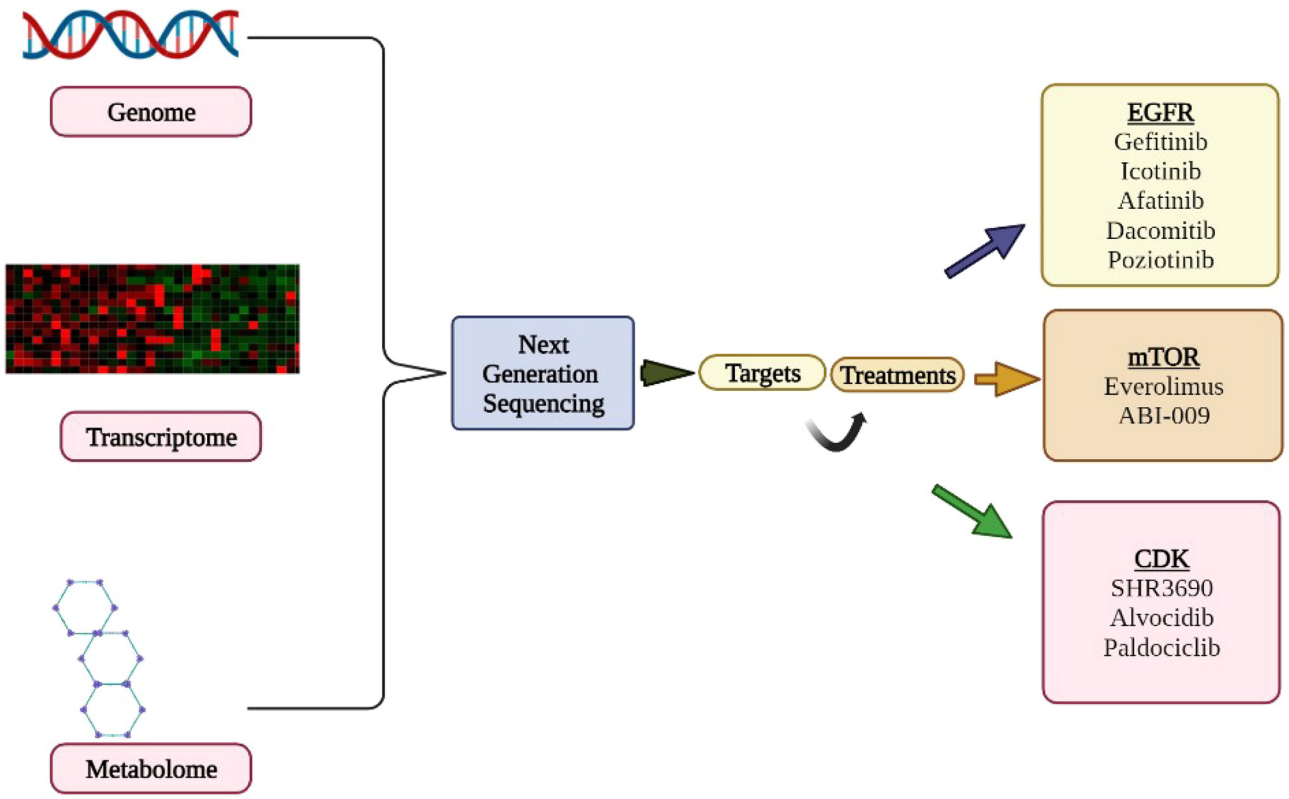

Figure 1. Molecular analyses for targeted cancer therapy.

Tanaka *et al* (27) demonstrated that CTC enumerations exhibited an unsatisfactory discriminating capacity between individuals with lung cancer and those with non-malignant lung lesions [area under the receiver operating characteristic curve=0.598 (P=0.122)]. Standardization of pre-analytical parameters and an improved understanding of the precise origin and structure of cfDNA are additional steps in implementing its analysis (27). Advances in sophisticated quantitative PCR technologies or NGS (Fig. 1) will certainly increase the analytical performance and dependability of future tests (28).

5. Precision medicine (PM) trials and treatment algorithm

PM is a method of improving patient outcomes by combining clinical and molecular patient data to analyze the genetic causes of a disease (29). The goal of this technique is to improve patient outcomes while offering better safety profiles than standard population-based cancer treatments (30).

The goal of progressive PM clinical studies is to determine whether tumor molecular profiling has therapeutic utility and whether treatment selection based on molecular changes delivers better outcomes than unselective treatment. Treatment algorithms are used in these trials to allocate patients to specific targeted medicines based on tumor genetic changes (31).

PM in oncology was developed nearly two decades ago with the introduction of molecular targeted agents (MTAs) and is now mostly based on the molecular-genetic characteristics of the patients' malignancies. MTAs affect the function of specific molecular targets in cell signaling, proliferation, apoptosis, angiogenesis, metabolism, migration or invasion, whereas cytotoxic drugs destroy rapidly dividing cells by stimulating the DNA and cell division machinery. It is now known that the bulk of harmful genetic modifications are shared by different tumor types (32). MTAs have been indicated to be beneficial in a variety of tumor types that have a common molecular abnormality, e.g., trastuzumab and lapatinib for HER2, which is amplified and overexpressed in 10-15% of breast and gastric cancers. Molecular changes are frequently observed in a relatively small fraction of patients with solid tumors. Due to the division of all classic human cancers into comparatively small subgroups, it is critical to reach an agreement on the best methods for establishing the action of experimental therapies in rare molecular subsets (31).

Clinical trials provide a scientific evaluation of investigational drugs, technologies or biologics, such as chemotherapeutic agents, blood products or gene therapies, in human volunteers. Prior to being granted Food and Drug Administration (FDA) approval, proposed medicines frequently go through a rigorously regulated and time-consuming multi-phase clinical trial process. Significant changes to present clinical trial designs will be required in the future to move toward a more individualized approach. An example of a novel accelerated attempt to assess targeted medicines is an adaptive trial design. Researchers may use this design to assess study data collected during anticipated intermediate time periods and change the direction of an individual's research project or the trial ultimately (33).

Mazo *et al* (34) provided an *in silico* analysis, according to which four well-recognized numerical risk scores (OncotypeDX, OncoMasTR, EndoPredict and tumor-infiltrating leucocytes) were significantly associated with neoadjuvant chemotherapy complete remission to neo-adjuvant chemotherapy in patients with estrogen receptor-positive and HER-2-negative breast cancers. The outlook for recurrent acute myeloid leukemia (AML) remains dismal, but a small number of effective treatments are available. According to the findings of Uckun *et al* (35) phase IB study, older adults with relapsed AML generally tolerate the integration of the cytotoxic chemotherapeutic drugs combrestatin A1 diphosphatase (OXi4503) and cytarabine well. A microvesicles-based study examined the therapeutic response of glioblastomas to temozolomide and concurrent radiation therapy (36). Melo *et al* (37) indicated that the serum levels of glypican-1-positive EVs are highly sensitive and specific for detecting early- and late-stage pancreatic cancer and correlate with the tumor burden. Uckun *et al* (38) described a method to overcome the blood-brain barrier, as it may restrict the intra-tumoral availability of therapeutically effective compounds. They demonstrated that intra-tumoral administration of the RNA therapeutic OT101 through convection-enhanced delivery inhibits the immunosuppressive effects of transforming growth factor β2, which then leads to clinically significant single-agent activity. Castration-resistant prostate cancer (CRPC) is treated with docetaxel as the first-line chemotherapy, but resistance is common due to acquired induction of P-glycoprotein, which is expressed by the multidrug resistance protein 1 gene. A new taxane is being used in resistant patients due to its low affinity for P-glycoprotein. It has been indicated that docetaxel-resistant patients had significantly higher levels of P-glycoprotein in their serum EVs than docetaxel-susceptible patients. Therefore, this test has the prospect to be utilized as a guide for choosing the right taxoid in patients with CRPC (39). It is well known that genetic and epigenetic events build up gradually to form carcinomas. As a result, the current standard of care is guided by prognostic and predictive biomarkers such as KRAS and microsatellite instability. Emerging biomarkers and cutting-edge liquid biopsy platforms, as outlined by Koulis *et al* (40), may open the door to novel combination treatments that target both the tumor microenvironment and tumor cells. Approximately 5% of NSCLCs have an anaplastic lymphoma kinase (ALK) mutation. As a result of this discovery, FDA-approved ALK blockers, such as crizotinib and ceritinib, which are given to patients who test positive for the ALK mutation, were created. Another promising application is that of the drug olaparib, an inhibitor of poly ADP ribose polymerase, for BRCA-mutant ovarian cancer. The HER2 proto-oncogene, a frequent target for drugs used in personalized therapy, is overexpressed in ~25% of human breast cancers (41). However, a sizeable percentage of patients receiving trastuzumab, a monoclonal antibody against HER2, eventually relapse or experience progressive disease (42). According to a recent study, EVs with the specific markers EGFR, p-EGFR and genomic DNA were produced in large quantities after treatment with the EGFR inhibitor cetuximab (43). This study further supported the idea that 'targeted agents may induce cancer cells to change the EV emission profiles reflective of drug-related therapeutic stress'. Thus, it may be assumed that such EV emission profiles may be defined and used to evaluate the efficacy of various treatments in different patients. The concept of acclimation is not entirely new. In radiation oncology, it has been successfully used and implemented in recent years (44).

As the knowledge of biomarkers and that particular EGFR mutations lead to superior outcomes with EGFR tyrosine kinase inhibitors (45), bio-markers have emerged as an essential aspect in planning treatment for NSCLC. Erlotinib, KRAS/BRAF (sorafinib), retinoid-EGFR signaling (bexarotene and erlotinib) and VEGFR (vantetanib) are among the targets. The primary objective of the study was the 8-week disease control rate (DCR), which was defined by Response Evaluation Criteria in Solid Tumors as a full or partial response or stable disease. Treatment efficacy was characterized as >80% probability of obtaining a DCR of >30% in similar patients, with efficacy of treatment defined as >80% possibility of reaching a DCR of >30% (46).

6. Conclusions

In conclusion, none of the studies that were chosen employed a standard scale to classify molecular changes based on their therapeutic value. The need for genetic testing will grow along with the rapid advancement of the genetic understanding and development of medical science. Whole-genome and whole-exome sequencing technologies are already widely employed for research objectives, also including providing prognostic or predictive profiles or screening patients for early clinical trials. Whole-genome and whole-exome sequencing technologies are projected to become the standard of care in the near future. Clinicians will be challenged with increasingly complex genomic information and a growing number of platforms from which to choose. While large-scale sequencing is far more instructive in most circumstances, tailored in-depth sequencing may be better in others.

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Ethics approval and consent to participate

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Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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