Molecular diagnostics: parallels between infectious disease and emerging oncology testing

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Evolving molecular techniques used in the clinical laboratory are becoming increasingly important across nearly all fields of medicine. An increased understanding of carcinogenesis and the use of targeted cancer therapies has resulted in a demand for new types of molecular oncology test to help in cancer diagnosis and as tools to predict response to targeted therapeutics for cancer patients. Understanding the need for and the function of these emerging molecular oncology tests by both clinicians and laboratorians is often problematic. Although many of these molecular testing techniques and strategies are relatively new to oncology, similar testing has been performed in the field of infectious diseases for many years and is now widely accepted and understood. Recognizing the parallels between the molecular testing that is now standard for infectious diseases and testing being introduced to aid in the care of cancer patients will accelerate the acceptance, implementation and correct utilization of molecular assays for oncology.

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1. Introduction

The past 20 years have seen an incredible growth in the diagnostics industry, particularly in the molecular diagnostics industry. Laboratory medicine has experienced the full breadth of clinical, molecular applications and is now witnessing the automation of this field. The conversion of polymerase chain reaction (PCR)-based gel electrophoresis assays to real-time PCR and capillary electrophoresis for DNA sequencing and fragment size analysis has allowed clinical laboratories to keep up with the growing demand for extra test requests and growing test volumes. The introduction of these more advanced molecular technologies to the clinical laboratory, as well as their subsequent automation, was driven by the need for faster and higher throughput testing in the field of infectious diseases. Although these technical advances are now used for a wide range of clinical molecular applications, a particularly interesting parallel can be observed between the fields of infectious disease and oncology with respect to the use of molecular techniques for diagnosis, disease monitoring, personalized medicine and drug resistance.

2. Molecular testing in infectious diseases

Whereas the use of molecular techniques in the clinical setting began with hereditary genetic disease applications, molecular infectious disease testing required more advanced molecular technologies. Initially, qualitative detection of bacterial targets, including Chlamydia trachomatis and Neisseria gonnorrhoeae, was the leading
clinical use of molecular techniques. The introduction of these applications to the clinical setting brought attention to the need for various controls, including those for sensitivity, specificity and inhibition. In addition, when DNA and RNA extraction methods became automated, it became apparent that control materials would be needed that could be taken through the entire testing process, including extraction.

Qualitative assays for many different pathogens grew rapidly, especially for those organisms that were difficult to culture by traditional microbiologic techniques. By the early 1990s AIDS had become a major global health issue and the pharmaceutical industry, fueled with insight from the research community, responded with new therapeutics that would target specific proteins or pathways needed for viral replication. Treating HIV-1-infected patients with targeted therapies against the viral protease and reverse transcriptase resulted in the need to monitor viral levels as a mechanism of determining the efficacy of the therapies being administered. Hence the ‘viral load’ testing to monitor therapy was born [1,2].

The use of these new targeted therapies, at first as single agents and then in various combinations, often achieved viral load values below the limits of detection of the most sensitive molecular assays. Unfortunately, in many patients the viral load levels would subsequently increase after prolonged treatment. This typically indicated non-compliance or the development of viral resistance. This acquired resistance to antiretroviral therapies resulted from the selective pressure of targeted drugs along with the high rates of replication and mutation observed in HIV that resulted in the appearance of new mutations in the targeted genes. As these resistance mutations became recognized, DNA sequencing to determine the viral mutation spectra and to predict which therapeutics would be effective was introduced in the clinical laboratory as a new tool in the care of HIV patients [3]. By sequencing various regions of the HIV-1 genome, laboratory could predict which therapeutics would be most effective against the current virus infecting the patient. Genotyping to predict resistance or to detect resistance mutations quickly became the standard of practice for the management of HIV-1-infected patients.

3. Molecular testing in oncology

The concept of performing qualitative, quantitative and genotypic assays for distinct purposes in patient management became fully ingrained through these types of infectious disease application. Two decades later, we are returning to this same clinical testing paradigm as newer molecular assays are being introduced to the field of oncology. Once again molecular laboratories and diagnostic manufacturers are working hard to implement new qualitative, quantitative and genotyping or mutation detection assays, this time as diagnostic, prognostic and predictive tools to aid oncologists and pathologists in the care of cancer patients. The evolution of BCR-ABL testing in leukemia is one of the best examples of this molecular testing paradigm in oncology.

Following the discovery of the t(9;22) ‘Philadelphia chromosome’ translocation detected in nearly all cases of chronic myelogenous leukemia (CML), cytogenetic testing for this chromosomal rearrangement has become standard practice to aid in CML diagnosis confirmation. In addition to being used as a diagnostic tool, it soon became evident that this translocation could also be used as a marker to monitor disease progression by calculating the percentage of cells with the Philadelphia chromosome and the change in this percentage from diagnosis, through treatment, remission and relapse. This progression from using the Philadelphia chromosome as a qualitative diagnostic tool to using it as a marker in quantitative testing became more useful as newer techniques, including fluorescence in situ hybridization (FISH), PCR and real-time PCR, were applied [4]. The use of these newer technologies was made possible by research identifying the two genes on chromosomes 9 (ABL1) and 22 (BCR) that contain the breakpoints involved in the translocation. Sequences on either side of the translocation could then be used to design FISH probes that could be used to detect the resulting BCR-ABL fusion gene in any cell, not just in metaphase cells required for traditional cytogenetic analysis. Similarly, primers flanking the breakpoint could be designed to amplify BCR-ABL mRNA by RT-PCR for increased analytical sensitivity. When combined with real-time PCR technology, this allowed for quantitative detection of the fusion transcript down to minute levels undetectable by either traditional cytogenetic techniques or FISH. With increased quantitative techniques available, clinicians are now able to monitor levels of BCR-ABL in patients as a marker for the degree of tumor involvement, response to treatment and as an early indicator of relapse [5].

In addition to identifying the genes involved in the translocation, researchers also revealed that the resulting BCR-ABL fusion gene produces a protein with a constitutively activated tyrosine kinase domain from ABL1 that results in unregulated cell cycle progression. The finding that the translocation was not only an indicator of CML but also a leading cause of the disease led to the use of BCR-ABL as a pharmaceutical target. Tyrosine kinase inhibitors (TKIs) such as imatinib (Novartis), able to block the activity of the fusion gene product, are now widely used and highly successful in the treatment of CML. Unfortunately, in the same way that drug resistance was found to occur in HIV patients being treated with antiretroviral therapies targeting essential HIV proteins, CML patients being treated with TKIs also develop resistance owing to the appearance of new secondary mutations in the BCR-ABL gene such as the T315I mutation. DNA sequencing or other genotyping methods to detect this mutation are now needed to help identify the source of TKI resistance and to direct oncologists to more appropriate therapy options [6].
4. Expert opinion

Although the use of BCR-ABL as a qualitative and quantitative marker along with the need for genotyping of this fusion oncogene may be the most direct parallel to the use of similar molecular testing strategies for infectious diseases, a continually expanding list of other targets for molecular testing exists for other forms of cancer.

Qualitative assays for the detection of mutations or other somatic variations in the cancer genome are becoming more and more common. This type of testing is not often used for diagnostic purposes because there are few examples such as CML where a particular form of cancer and mutation are so closely linked. More commonly, however, qualitative molecular testing in oncology is used for prognostic or predictive purposes. In the case of acute myeloid leukemia (AML), the presence of internal tandem duplication (ITD) mutations in the FLT3 gene indicates a poor prognosis, whereas c-KIT or NPM1 mutations carry a more favorable prognosis. In addition to its prognostic significance, testing for AML-associated mutations will probably play a role in predicting which therapy will be most effective in a specific patient once drugs targeting some of these mutated proteins, now in development or clinical trial, are introduced to the market [7].

In the case of some solid tumors, including non-small-cell lung cancer (NSCLC) and colorectal cancers, TKI- and monoclonal antibody-based therapies, respectively, targeting the epidermal growth factor receptor (EGFR), are in use at present. In colorectal cancer the detection of a KRAS mutation is a negative predictor for anti-EGFR antibody therapies because the antibodies only inhibit EGFR activity that acts upstream of the constitutively active KRAS oncogene. TKIs acting on EGFR in the setting of NSCLC are likely to be ineffective in the presence of a KRAS mutation, but testing for EGFR mutations in these patients is more commonly used because the small subset of patients originally found to respond well to these TKIs were later found retrospectively to harbor specific mutations in exons 19 or 21 of the EGFR gene [8]. With pharmaceutical companies shifting more of their resources towards the development of these types of targeted therapy, the use of molecular testing for alterations in BRAF, PI3K, mTOR, MET and other genes will become increasingly common. This type of testing is used at present in clinical trials and as proven relationships between mutation status and drug response are revealed, these predictive tests will be quickly translated into clinical practice [9,10].

Quantitative clinical assays using PCR, real-time PCR or other target/signal amplification technologies for cancer patients are not well established beyond the BCR-ABL testing described previously. FISH testing, however, has been used extensively to quantify the degree of amplification in the ERBB2 (also known as HER2/NEU) gene in predicting response of breast cancer patients to the anti-ERBB2 monoclonal antibody trastuzumab [11]. As the use of qualitative prognostic and predictive molecular testing for bloodborne malignancies becomes more pervasive, many of these assays will probably be modified for quantitative testing, resembling the use of BCR-ABL to evaluate patients’ responses to therapy and to monitor minimal residual disease levels.

Following treatment with targeted drugs, many cancer patients develop drug resistance, often caused by the appearance of and selection for acquired mutations in the targeted gene. The T315I BCR-ABL mutation and the T790M mutation in EGFR are both detected in neoplastic cells from patients showing secondary or acquired resistance to the TKIs imatinib (for CML) and gefitinib or erlotinib (for NSCLC), respectively. DNA sequencing and other mutation detection strategies are often used to determine the cause of drug resistance and to help determine future treatment options [5,8]. The discovery of more secondary resistance mutations will probably continue as more targeted therapies are introduced to treat patients with diverse forms of neoplastic disease.

The clinical laboratory has been and will continue to be an essential source of support for physicians treating patients suffering from various ailments. When these patients are being assaulted with unwanted, fast growing and quickly evolving parasitic invaders, in the form of bacteria, viruses, or cancer cells, success is often dependent on treatment decisions and options made possible to physicians by researchers, pharmaceutical companies and clinical laboratory tests. Clinical molecular testing, in particular, has played a key role in providing critical information in the treatment of infectious diseases through qualitative, quantitative and acquired mutation testing for diagnosis, prognosis, drug selection, and for elucidating the causes of drug resistance. Similar testing needs and solutions are evident in oncology testing in the clinical molecular laboratory. Recognizing and understanding the parallels between infectious disease and oncology testing strategies will help ease the development, implementation and acceptance of new cancer-related tests that can be expected to expand the field of influence of molecular testing in the coming years.

Declaration of interest

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Bibliography


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