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CURRENT TOPICS IN BREAST PATHOLOGY

Comprehensive genomic sequencing and the molecular profiles of clinically advanced breast cancer

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Summary

Targeting specific mutations that have arisen within a tumour is a promising means of increasing the efficacy of treatments, and breast cancer is no exception to this new paradigm of personalised medicine. Traditional DNA sequencing methods used to characterise clinical cancer specimens and impact treatment decisions are highly sensitive, but are often limited in their scope to known mutational hot spots. Next-generation sequencing (NGS) technologies can also test for these well-known hot spots, as well as identifying insertions and deletions, copy number changes such as *ERBB2* (HER2) gene amplification, and a wide array of fusion or rearrangement events. By rapidly analysing many genes in parallel, NGS technologies can make efficient use of precious biopsy material. Comprehensive genomic profiling (CGP) by NGS can reveal targetable, clinically relevant genomic alterations that can stratify tumours by predicted sensitivity to a variety of therapies, including HER2- or MTOR-targeted therapies, immunotherapies, and other kinase inhibitors. Many clinically relevant genomic alterations would not be identified by IHC or hotspot testing, but can be detected by NGS. In addition to the most common breast carcinoma subtypes, rare subtypes analysed with CGP also harbour clinically relevant genomic alterations that can potentially direct therapy selection, illustrating that CGP is a powerful tool for guiding treatment across all breast cancer subtypes.

Key words: Next-generation sequencing; comprehensive genomic profiling; breast carcinoma; *ERBB2*; HER2; triple-negative breast cancer; tumour mutational burden; clinically relevant genomic alterations.

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INTRODUCTION

The accumulation of mutations underlies all cancer development. For many years the association of carcinogenesis with alterations in the cancer cell genome, including base substitutions, short insertions and deletions, homozygous deletions, amplifications and rearrangements (e.g., translocations), has been widely accepted.[1] As genetic mechanisms that initiate or drive cancer progression have been elucidated, the stage has been set for the development of personalised cancer treatment.[2–5] Although the total set of critical alterations known to drive a given cancer type may be large, the number of driver alterations in any given patient's tumour is typically low and unpredictable.[2,6,7] Directly sequencing a large panel of genes across numerous pathways is necessary to identify which alterations drive an individual patient's disease. Targeting specific mutations that have arisen within a tumour is a promising means of increasing the efficacy of treatments.[8–12]

Breast cancer is no exception to this new paradigm of personalised medicine. The process of comprehensive genomic profiling (CGP) through next-generation sequencing (NGS), also termed massively parallel sequencing, has been applied to clinical breast cancer samples, typically in patients with advanced stage, clinically relapsed disease that is refractory to treatment with endocrine and/or cytotoxic chemotherapy. As is commonly seen in the context of lung cancer, CGP can distinguish between several distinct, targetable pathways and direct treatment toward appropriate agents.[13] Building on the four molecularly defined subtypes for primary breast cancers (basal, luminal A, luminal B, and HER2 enriched),[14,15] genomic profiling of relapsed and metastatic breast cancers can further refine treatment strategies and provide valuable information for clinical management of rare histotypes.[16]

THE EVOLUTION OF DNA SEQUENCING TECHNOLOGIES

Traditional DNA sequencing methods used to characterise clinical cancer specimens and impact treatment decisions are highly sensitive, but are often limited in their scope to known mutational hot spots, for example *BRCA1/2* testing in the context of hereditary breast and ovarian cancer[17] or evaluation of *ESR1* following disease progression.[18] Although targeted and quick, the rate of false negatives, limitations in the type of alterations that can be identified, and the missed opportunities for identifying other potential drivers are significant disadvantages to low-throughput molecular tests.[19,20] In addition, the low throughput and narrow range of detection for methods such as allele-specific real-time PCR (polymerase chain reaction),[3] analysis of melting curve quantitative PCR (qPCR),[21] or the PCR clamp method,[22] limit the comprehensive analysis that is essential to categorise tumours into one of several molecular groupings.

Next-generation methods have the capability to sequence a much larger set of alleles simultaneously, providing scale and breadth of analysis that was not previously possible.[23–25] Two NGS technologies are widely used for clinical applications, those that rely on the incorporation of fluorescent nucleotides and subsequent imaging and those using

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semiconductor-based detection. Illumina has developed the most widely used NGS platform, utilising dye terminator methodologies, and offers three major clinical instruments: the HiSeq 2500, the HiSeq 3000/4000, and the MiSeq. HiSeq platforms can sequence up to 1 trillion bases in about 3 days or approximately 10 billion bases in a rapid run mode that takes as few as 7 hours. The MiSeq is a much cheaper, lower capacity instrument used for rapid turnaround (it can sequence 500 million bases in 4 hours). A number of technical issues, particularly those involving aberrant nucleotide incorporation rates, place major responsibility on the bioinformatics systems and computational biologists to correctly interpret the raw sequencing data produced by the Illumina systems.

Semiconductor sequencing relies on the detection of hydrogen ions that are released during the polymerisation of DNA, and is the basis for the Ion Ampliseq (Ion Torrent) system.[26] It has been widely adapted for use in clinical molecular diagnostics laboratories. This approach is now owned by Thermo Fisher, which claims that PostLight sequencing technology has the major strength of being the first of its kind to eliminate the cost and complexity associated with the extended optical detection currently used in all other sequencing platforms. The uses of this system appear to be focused on rapid and affordable short sequence determination of exons containing hotspot mutations.

COMPARISON OF TRADITIONAL AND NGS STRATEGIES FOR CANCER GENOMIC SEQUENCING

Prior to NGS testing platforms, traditional hotspot DNA sequencing had reached the bedside for the treatment of a variety of tumours including non-small cell lung cancer, colorectal cancer, haematological malignancies, and melanoma.[5,27,28] NGS technologies can also test for these well-known driver mutations,[29] as well as identifying copy number changes, such as *ERBB2* (HER2) gene amplification in the context of breast or upper gastrointestinal tumours,[29] and a wide array of fusion or rearrangement events.[30,31]

Tables 1 and 2 compare traditional DNA sequencing approaches with NGS methodologies. The relative cost of the two approaches is of great importance to test providers, consumers and payers. Although the cost per base sequenced for the traditional approaches is high, these narrow approaches focused on one gene or a few hotspots are often less expensive overall than the cost of an NGS assay that evaluates hundreds of genes with more expensive reagents and equipment. Without question, the expertise required to perform clinical NGS testing for cancer patients, especially in computational methodologies, is significantly higher than for traditional sequencing. In daily clinical pathology practice, both traditional and NGS sequencing approaches are challenged by several concerns: which sample should be tested (e.g., primary versus metastatic tumour tissue or tumour tissue versus circulating DNA); small sample size, a feature of fine needle aspiration biopsies (FNAs); heterogeneity of genetic abnormalities within individual tumours; and extensive necrosis or samples with very low tumoural DNA compared with non-cancerous tissue.

The restriction of traditional sequencing to analysis of one gene at a time, and generally to hotspots within that gene, is a significant drawback. NGS platforms allow for large-scale gene sequencing known as comprehensive genomic profiling (CGP) that can both determine the status of mutational hotpots 'expected' in a given clinical situation and discover 'unexpected' sequence abnormalities that could significantly alter the treatment plan.[32] Novel mutations with clinical impact continue to be discovered, even for established cancer genes, but are undetectable using narrow panels or hotspot assays. In addition, unlike traditional PCR-based sequencing, CGP can provide information on gene copy number, identifying homozygous or heterozygous deletions and gene amplifications.[29] CGP can also detect the wide variety of translocations and fusions that drive therapy selection in cancer,[30] including rearrangements affecting *ESR1* that confer resistance to endocrine therapies in breast cancer.[33–35] Furthermore, the sensitivity of CGP can match or exceed traditional approaches when the mutation is present in only a small percentage of the total DNA extracted from the specimen.[32]

Rapidly analysing many genes in parallel, as is possible with CGP using the Illumina HiSeq NGS technology, facilitates the identification of clinical trials with potential relevance for a given patient.[9,25,36] Knowing a patient's comprehensive genomic profile can indicate either a selective trial investigating therapeutic strategies in the limited context of one biomarker and/or disease,[9,36] or suggest that patients could benefit from enrollment into an umbrella or basket trial with potentially fewer restrictions on tumour type or molecular profile.[9]

The turn-around time for a CGP assay performed on a multiplex (>100 gene) panel is currently longer (approximately 7 days) than traditional single-gene hotspot sequencing, but this difference is expected to narrow rapidly as knowledge and bioinformatic analysis capabilities expand to more quickly interpret the tumour cell sequence and distinguish rare, harmless germline polymorphisms from possibly significant somatic mutations.[37,38] With the growing demand for more personalisation in oncology practice, the development of a unified laboratory report is likely, combining the results from high-throughput sequencing as well as other diagnostic tests such as slide-based assays [immunohistochemistry (IHC) and fluorescence *in situ* hybridistion (FISH)], mRNA profiling, and epigenome analysis.

CHALLENGES TO CLINICAL NGS TESTING

Several challenges must be overcome to deliver CGP results in the course of clinical management,[27] from limiting cost and defining specimen requirements to reducing turn-around time and ensuring high-quality analysis and data interpretation.

Adequate sample

Clinical CGP can be successfully performed for solid tumour samples (generally formalin fixed, paraffin embedded material),[29,39] bone marrow,[40] and blood, although many other tissue samples such as FNAs can be analysed.[41] In general, a sample approximately 15 mm^2 with a minimal depth of 40 μm is adequate for CGP.[29] Major resection specimens nearly always provide an adequate amount of nucleic acids, but small needle biopsies, FNA biopsies and fluid cell block