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Genomic profiling for cancer therapy

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About the Reviewer

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Logan is a Rutherford Discovery Fellow at the University of Otago Christchurch. He obtained his PhD in molecular genetics from the University of Otago in 2007, and subsequently joined the QIMR Berghofer Medical Research Institute (Brisbane) to undertake research in the field of familial cancer genetics. He currently leads the NZ Familial Breast Cancer Study which links genomic information with clinical decision making to facilitate management of cancer patients and their family members. He is an active member of the international consortia, ENIGMA (Evidence-based Network for Interpretation of Germline Mutant Alleles), CIMBA (Consortium of Investigators of Modifiers of BRCA1/2), and the Global Alliance for Genomic Health (GA4GH) BRCA Interpretation Group. His research focuses on advancing molecular tools used to identify individuals who are most vulnerable to developing cancer.

This publication is intended as an educational resource for health care professionals with an interest in genomic profiling. The review discusses the topic of genomic profiling in cancer patients and its impact on cancer therapy. New techniques such as hybrid capture-based next-generation sequencing (NGS), also referred to as massive parallel sequencing (MPS), provide insights into the genomic profile of a particular cancer and engender the best possible treatment for each patient. Such comprehensive profiling allows for the planning of future treatments should the disease progress. Genomic profiling is not only helping to improve cancer patient outcomes by matching known alterations driving cancer to currently available therapies, thus enabling personalised care, but is also providing new insights and guiding the development of new targeted therapy.

Introduction

In 1977, Professor Frederick Sanger and colleagues were the first to sequence a full genome in a reliable and reproducible manner.[1] In 2003, the Human Genome Project sequenced the whole human genome.[2] Advances in the field of DNA sequencing and the ability to process large amounts of data quickly, have led to major advances in our understanding of cancer as a collection of hundreds of diseases each with its own genetic makeup (genomic profile). It is now known that four major classes of genome changes can lead to cancer: base substitution, insertions and deletions (indels), copy number alterations and rearrangements.

It is now possible to analyse an individual's full genetic sequence of three billion DNA base pairs (the genome) in a few hours via NGS, a process of DNA sequencing on a massive scale using minimal amounts of tissue. This type of comprehensive interrogation of clinically actionable genomic aberrations can be used to gain valuable individualised biomarker information and determine the genetic drivers of a specific cancer.[3]

Emerging clinical complexity

Increasing numbers of potential genomic targets are being identified.[4-8] In 2015, 96 genomic alterations were identified in lung adenocarcinoma alone.[4] As the number of known cancer genes increases, so does the number of targeted therapies.[6,9-11] In 2016, there were 836 new drugs and vaccines listed under development in clinical trials, with approximately 73% targeting specific genomic aspects of tumours.[11]

Other biomarkers

In addition to genomic targets, other biomarkers such as tumour mutational burden (TMB; total number of somatic mutations per coding area of a tumour genome[12]) and microsatellite instability (MSI; a result of defects in DNA mismatch repair[13]) have been described that help us understand more about tumour profiles, even when driver mutations are unknown.[10,12,14-17] High TMB levels may help to predict response to cancer immunotherapies, while MSI may help to predict response to immunotherapy in patients who have failed conventional therapy.[12,14,15-18]

Limitations of standard genomic testing

Traditional genomic tests for tumour characterisation, such as fluorescence *in situ* hybridisation (FISH), immunohistochemistry (IHC), real-time or reverse transcriptase polymerase chain reaction (RT-PCR), Sanger sequencing and gene signature microarrays, are highly specific and require the genomic target and class of alteration to be pre-determined.[19-25] These diagnostic approaches work well when you know what you're looking for, but only detect some classes of alterations and miss others.[20-22]

Studies have revealed that 17% of epidermal growth factor receptor (*EGFR*) -activating alterations were missed by current diagnostic approaches;[25-28]

- 35% of patients with non-small cell lung cancer (NSCLC) harbouring anaplastic lymphoma kinase (*ALK*) rearrangements were missed by FISH
- 15% of ERBB2-activating alterations in breast cancer were missed by FISH and IHC
- 15% of patients with advanced lung cancer carry an *EGFR/ALK* alteration after false-negative results by RT-PCR/FISH (53% carried an *EGFR/ALK* alteration that RT-PCR/FISH cannot detect).

Furthermore, current diagnostic approaches only test for a handful of possibilities.[28] For example, *HER2/ERBB2* amplifications and overexpression occur in a wide range of tumours, but they are only routinely tested for in a handful of tumour types using FISH and IHC.[28] The significant number of genomic alterations not routinely tested for, or not detected by standard techniques, leaves many actionable alterations unidentified and this impacts on treatment possibilities for patients.[28,29]

Another limitation with current diagnostic testing is that sequential tests are often required, resulting in the depletion of available tissue, potentially precluding future tests for additional markers.[4] Among a group of patients with lung adenocarcinoma, two-thirds required multiple biopsies to complete standard diagnostic testing.[4] Some clinicians believe there is a need for methodologies that can detect all genomic alterations in a single, tissue-sparing test.[10] The US National Comprehensive Cancer Network (NCCN) NSCLC Guidelines strongly advise broader molecular profiling to identify rare driver mutations to ensure patients receive the most appropriate treatment.[30]

Next-generation sequencing

NGS can overcome the shortcomings of standard genomic techniques.[10,31] Unlike FISH, IHC and RT-PCR, NGS can detect anomalies without the need for pre-determination, can detect unknown genomic alterations, and can detect all anomalies in a single, tissue-sparing assay.[4,10,21,22,31] The utility of NGS, however, depends on how it is applied and not all NGS platforms look broadly across the genome.[4,10] Multi-gene hotspot analysis for example sequences only selected regions of a gene, leaving many gene alterations missed. PCR-based NGS often employs a selective approach to analyse the gene sequence.[19] The use of primers with pre-determined start and stop locations means reads are stacked in vertical columns at the same region of the gene, missing alterations in other regions (**Figure 1**).[19]

Comprehensive genomic profiling

In contrast to PCR-based NGS, hybrid capture-based NGS employs a tiled approach which can recreate the entire gene sequence and provide a comprehensive genomic profile, identifying all four classes of genome alterations across hundreds of genes know to drive cancer.[10,19] The use of an overlapping probe means reads are stacked in a staggered or step-wise manner (**Figure 2**). This creates continuous alignment, ensuring no alterations are missed.[19]

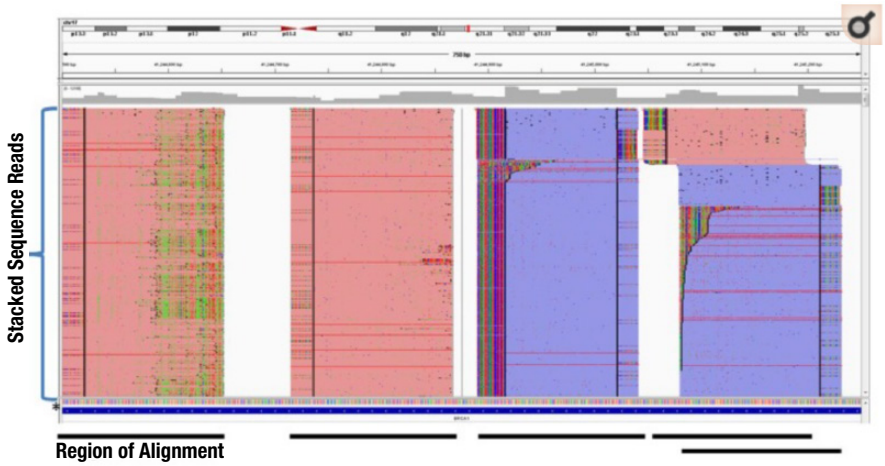

Figure 1. PCR-based next-generation sequencing (NGS). Sequence read alignment from an alignment-based enrichment method. Individual sequence reads (red or blue horizontal lines) are stacked vertically and aligned to a reference sequence at the base of the stack. The region of alignment is depicted by black bars across the bottom; all sequence reads within a stack have the same start and stop positions.[19]

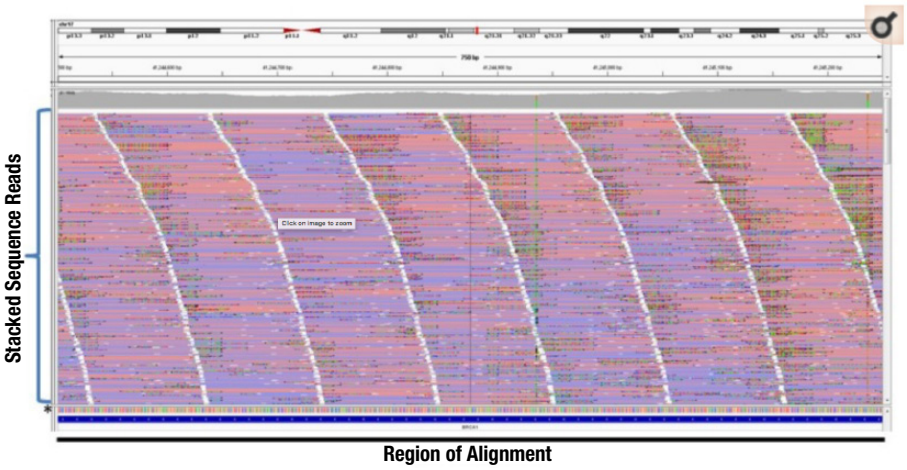

Figure 2. Hybrid capture-based next-generation sequencing (NGS). Sequence read alignment from a probe-based enrichment method. Individual sequence reads (red or blue horizontal lines) are stacked vertically and aligned to a reference sequence at the base of the stack. The black bar depicts the region of alignment across the bottom, showing a continuous region of alignment with reads stacked in a staggered or step-wise manner. Reads within a stack will have unique start and stop coordinates.[19]

Commercial NGS services

A number of commercial services are available. One such service is FoundationOne® (for solid tumours) developed by Foundation Medicine. It is a single assay that uses a comprehensive genomic profiling approach utilising hybrid capture-based NGS to identify a patient's individual molecular alterations and match them with relevant targeted therapies and clinical trials.[10,32] FoundationOne® interrogates the entire coding region of relevant cancer genes and selected introns and finds genomic alterations missed by current diagnostic approaches.[4,10,25-27,32,33]

FoundationOne® is validated to detect (with high sensitivity and specificity) over 300 cancer-related genes, including introns from over 25 genes often rearranged or altered in solid tumours.[10,34] The assay also identifies TMB and MSI status.[10,12,18,32,33]

Other established providers of comprehensive genomic profiling include Caris Life Sciences, with their Caris Molecular Intelligence® Comprehensive Genomic Profiling Plus (CGP+) and OncoDEEP™ by OncoDNA. Caris Molecular Intelligence® CGP+ analyses DNA, RNA and proteins, revealing a high quality molecular blueprint to provide reliable, high-quality information to guide more precise and individualised treatment options.[35] OncoDEEP™ combines DNA analyses of solid tumour samples sequencing 75 genes linked to approved targeted therapies and protein analysis using IHC testing, combined with MSI and TMB analysis.[36]

Real world experience of comprehensive genomic profiling

In 2016, Lim et al. demonstrated that maximally identifying actionable genomic alterations in advanced lung cancer patients is an important factor in improving clinical outcomes.[37] In their study, a total of 51 patients with lung adenocarcinoma who had previously tested negative for *EGFR/KRAS/ALK* by conventional methods subsequently underwent NGS-based comprehensive genomic profiling using FoundationOne®. The frequency of genomic alterations identified can be seen in **Figure 3**. Comprehensive genomic profiling revealed that 31% of patients harboured clinically relevant genomic alterations that were not previously identified; a genomic alteration with a corresponding targeted therapeutic (according to NCCN guidelines) was identified in 39%. In a further 27% of patients, genomic alterations for which clinical trials of targeted therapies could be considered were identified and objective responses were seen in an encouraging number of these patients. All patients who received matched targeted therapy derived clinical benefit (evidence of tumour shrinkage or objective radiologic responses).

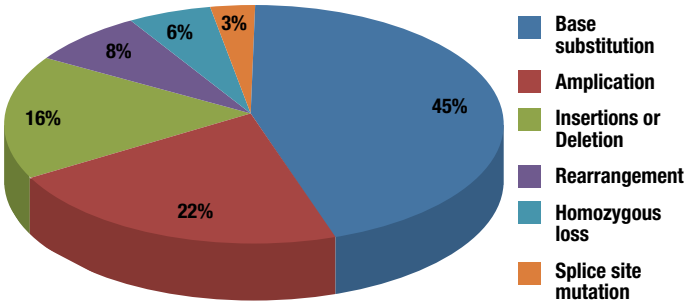

Figure 3. Frequency of genomic alterations identified in a study of 51 patients with lung adenocarcinoma who had previously tested negative for *EGFR/KRAS/ALK* by conventional methods who subsequently underwent NGS-based comprehensive genomic profiling using FoundationOne®.[37]

Supporting the above findings, Drilon et al., using FoundationOne®, identified actionable genomic alterations in 65% of tumours deemed without targetable genomic alterations by earlier extensive non-NGS testing in 31 patients with lung adenocarcinomas.[4] Meanwhile in a study by Schrock et al. involving patients with NSCLC assayed with FoundationOne®, and found to be harbouring classic *EGFR* Δex19 deletions, 12 of 71 cases (17%) had previously tested negative for *EGFR* mutations.[26] Given the clinical benefit in progression-free survival conferred by *EGFR* tyrosine kinase inhibitors in patients with these alterations, these authors recommended that comprehensive genomic profiling be considered in the initial presentation of advanced NSCLC and also in those patients who have previously tested negative for *EGFR* mutations.

A recent large scale, prospective clinical sequencing initiative using a comprehensive assay, the Memorial Sloan Kettering Cancer Center's hybrid capture-based NGS panel (MSK-IMPACT), in a cohort of over 10,000 patients with metastatic cancer also demonstrated the advantages of using such a technique. It was found that 81% of mutations detected by MSK-IMPACT were missed by commercially available PCR-based hotspot panels.[38]

The feasibility and utility of comprehensive genomic profiling was investigated in a study by Suh et al. in 6832 consecutive cases of NSCLC between 2012 and 2015.[32] They concluded that comprehensive genomic profiling was practical and by enabling simultaneous detection of genomic alterations (point mutations, small indels, copy number changes and rearrangements) involving all seven driver oncogenes (*ALK*, *EGFR, BRAF, ERBB2*, *MET*, *ROS1*, *RET*) and *KRAS*, facilitates implementation of the NCCN guidelines for NSCLC. In addition, they found that comprehensive genomic profiling identifies patients with pan-negative lung adenocarcinoma who may benefit from enrolment in mechanism-driven clinical trials, without additional tissue use or profiling cost.

The clinical impact of hybrid capture-based NGS (FoundationOne®) on treatment decisions was recently investigated by Rozenblum et al., in their retrospective study involving 101 patients with advanced lung cancer.[25] In 51.5% of patients this testing was performed before first-line therapy and in 48.5% it was performed after treatment failure.[25] Hybrid capture-based NGS identified clinically actionable genomic alterations in 50% of patients and in 15 patients it identified *EGFR/ALK* aberrations after negative results with prior standard testing. After hybrid capture-based NGS, the treatment strategy was changed for 42.6% of patients and the overall response rate was 65%, with a complete response of 14.7% and a partial response of 50%.

Expert's comments on real world studies

A significant minority of patients derive direct clinical benefit from cancer care that has been guided by genomic sequencing. As shown above, such advances in targeted therapy have been well demonstrated in patients presenting with an oncogene driven advanced stage NSCLC. Several genes are also being used to guide therapies in other cancers, such as melanoma (*BRAF*) and colorectal cancer (*KRAS, NRAS, BRAF*), and this list continues to expand as many more promising genomic targets are discovered.

The future of genomic profiling in personalised cancer care

Genomic profiling offers great potential to transform clinical care, however implementing this approach also presents key challenges for patients and their doctors. Currently, the power of NGS to detect genetic changes is far greater than our ability to interpret and fully understand the genetic findings. As we move into an era of matching targeted therapies to potentially actionable mutations, it is essential for genetic changes to be identified and evaluated using validated approaches. Until bioinformatic analysis of NGS results leads to accurate classification of new genetic changes, manual curation will be required to assess these changes for clinical utility.

EXPERT'S CONCLUDING REMARKS

The value of genomic profiling is not only in its ability to affect diagnosis and treatment, but also in its capacity to provide opportunities for researchers to advance this area. To address the many analytical challenges faced by genomic profiling, laboratory scientists (basic and translational) and clinicians will need to collaborate, providing access to genomic data and accompanying clinical information. Such integrated services are essential if patients are to truly benefit from the potential of genome-driven targeted therapies.

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