The role of NGS in stratified cancer medicine

Clinical Scientists Dr Matthew Smith and Dr George Burghel reveal insights into the present and the future of next generation sequencing within stratified cancer medicine.

Introduction

Cancer is a leading cause of morbidity and mortality worldwide with approximately 14 million new cases and 8.2 million cancer-related deaths per year.¹ One challenge to overcome in the treatment of cancer is its high level of inherent genetic complexity and heterogeneity. Characterising the genetic profile of each individual tumour can therefore help guide the delivery of effective treatment strategies, and this approach of stratified or personalised medicine is driven by the latest advances in genetic technologies.

Due to the success of research efforts over recent years, the application of these technologies in cancer research is shifting, particularly with regards to the rising popularity of next generation sequencing (NGS). As this technique evolves, falling costs and increasing accessibility have led to its increased uptake. To better understand where we are in its current integration into the clinical laboratory, NHS Clinical Scientists Dr Matthew Smith and Dr George Burghel discuss the strengths of this approach and factors to consider for its application, as well as the challenges faced.

How is stratified cancer medicine being realised, and what are the advantages of NGS to this approach?

“Mutations, some inherited and others acquired during life, within certain driver genes are central to tumour development,” says Dr Burghel. “We are learning about more and more specific mutations which have diagnostic, prognostic or predictive implications (mutations that are termed “actionable”). Some of these mutations identify cancers which can be treated with specific “targeted” therapies (“druggable” mutations). Targeted therapies can act directly on the protein product of the mutant gene or act indirectly on the molecular pathways which have been disturbed by the mutation. This exciting field is developing very rapidly: for example, until this year inherited variants within the BRCA1 and BRCA2 genes indicated an increased risk of breast, ovarian and prostate cancer, but were actionable only in the sense that the disease risk could be managed (e.g. through mastectomy and oophorectomy) but we now regard these mutations as “predictive” in the sense that ovarian cancers containing the mutations respond to a new class of drugs called PARP inhibitors. Since the early and dramatic success of directly targeting mutations such as the BCR-ABL fusion protein in chronic myelogenous leukaemia, the list of druggable or “predictive” mutations is growing year on year.

Identifying such actionable or druggable mutations in tumours is the key to stratified cancer therapy, informing clinicians and helping to guide treatments. “By profiling tumours at a molecular level we can identify the cancer prognostic markers and mutations that can be targeted for treatment. This has huge implications for cancer therapy as they only receive the most appropriate treatment dependent on the underlying molecular profile of the tumour,” adds Dr Smith. Personalised therapy for cancer based on this premise is proving to be safer and more effective than traditional approaches, and therefore forms the rationale behind Cancer Research UK’s Stratified Medicine Programme.²

A variety of genetic testing technologies are available for profiling these targets, both well-established and emerging. Dr Smith comments: “Since the breadth of testing is currently limited to a handful of targets, the majority of routine diagnostic testing in the UK utilises well-established techniques such as Sanger sequencing, pyrosequencing and qRT-PCR. This enables us to turn around tests in a clinically actionable timeframe, and provides a cost-effective strategy — but only as long as the number of tests per individual or sample are limited.”

In light of their restricted capacity for multiplexing applications, these techniques are not wholly compatible with ongoing trends. “With the list of actionable genetic markers and targeted therapies expanding with the latest research, these types of tests are becoming less feasible,” Dr Smith goes on to explain. For example, in the last few years half a dozen actionable mutations in Non-Small Cell Lung Cancer (NSCLC) have been identified, requiring a combination of sequencing, fluorescence in situ hybridisation (FISH) and immunohistochemistry to characterise from very limited amounts of tumour material. “With a reduction in cost and improvements in library preparation and sequencing, it’s NGS that has the capability for testing larger, multi-gene panels.”
NGS can provide a wealth of data, which can sometimes be overwhelming. How are the latest targeted NGS approaches overcoming this challenge?

The data load generated by NGS is well-known as a bottleneck, requiring time and expert knowledge in extracting meaningful results. This is particularly true within the clinical genetics workflow, where turnaround times are a major priority.

As a highly efficient alternative to whole genome sequencing, targeted sequencing is well suited to the clinical laboratory. By capturing specific genomic regions of interest from DNA samples prior to sequencing, only the regions of interest are sequenced. Focusing in on relevant areas of the genome, targeted sequencing panels significantly reduce the sequencing and data load, in turn reducing both time and cost. This approach also enables an increased depth of coverage, providing the sensitivity needed for heterogeneous samples and overcoming many of the challenges typically faced in NGS. In addition, numerous options exist for further streamlining the process of data analysis, from third party software to in-house solutions (Figure 1). “Cancer-specific gene panels and enrichment methods are becoming increasingly popular, and a number of laboratories and commercial companies have recently developed and validated these for clinical use,” explains Dr Burghel (see Box 2).

Dr Burghel expands on this: “The introduction of high-throughput NGS is revolutionising cancer genomics research and diagnostics, which are rapidly moving from single-gene mutation analysis to cancer genomic profiling.” This is also driven by initiatives such as the ‘The 100,000 Genomes Project’ (see Box 1).

Raising an important point regarding NGS technology, he adds: “Nevertheless, routine sequencing of the entire cancer genome by diagnostic laboratories remains unfeasible as it is still relatively expensive, and time- and labour-intensive.”

**Box 1**

**The 100,000 Genomes Project**

Run by Genomics England, this project is underway and will sequence 100,000 whole genomes from National Health Service (NHS) patients by 2017, leading to new scientific discoveries and medical insights.

Cancer is one of the three main areas of focus, with a number of cancer genomes having been included in the initial pilots. They will sequence both cancerous and non-cancerous cells of the patient, comparing them in order to pinpoint the precise changes that contribute to the disease.

The sequencing of so many cancer genomes is sure to drive the discovery of actionable genes for more cost-effective targeted NGS studies in the future.

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**Figure 1:** The SureSeq™ Interpret Software is provided as standard with all SureSeq NGS panels and services from Oxford Gene Technology (OGT). The report provides researchers with simple and rapid identification of meaningful results, without the requirement for in-house bioinformatics resources.

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Box 2

Which capture method for targeted NGS?

The type of capture method is of utmost importance for targeted NGS, and the two main approaches fall into either the hybridisation or amplicon-based categories — each with its own set of advantages and drawbacks. Utilising PCR, amplicon strategies tend to be quick and easily integrated into existing laboratory workflows. However, data quality tends to be less robust when compared to a hybridisation-based approach, as it is very hard to determine and remove bias introduced by PCR (e.g. polymerase errors, formation of secondary structures and preferential amplification of some fragments due to differences in GC content). Dr Smith explains the role of the hybridisation approach in more detail: “With the ability to easily capture larger target regions, this is the method of choice for larger panels. Traditionally they have required considerably more DNA input, and the library preparation tends to be longer when compared with PCR based methods.” The technology has been improving, however: “Both of these factors have been recognised by OGT in the development of the SureSeq Solid Tumour Panel, with extensive research validation of lower input DNA and a focus on making the whole process more streamlined having really advanced hybridisation-based technologies.”

With the utility of targeted NGS in stratified cancer medicine dependent on the genomic regions analysed, how is the content chosen for targeted sequencing panels?

When choosing content for a new panel, the current focus of molecular pathology labs is on delivering results that can be translated into meaningful clinical action. However, this can be complex, as Dr Smith explains: “The content of any panel is a balancing act between trying to maximise the utility of the panel with expected sample numbers and desired throughput.” In general, for a diagnostic panel the focus is often very narrow, maximising cost-efficiency and sample throughput, while limiting the amount of surplus sequencing data that, as yet, has no recognised clinically actionable relevance. Without any known effect on treatment, variants of unknown significance (VOUS) therefore tend not to be covered.

The breadth of content can range from mutational hotspots through to full exons, and Dr Burghel describes how for each laboratory this will depend on the target genes and the clinical literature. “For example, KRAS and BRAF carry mutational hotspots with well-characterised effects on drug response, and can therefore be specifically targeted. For other mutations, such as those in KIT and PDGFRA genes, which have implications for the aetiology of gastrointestinal stromal tumours, diagnostic labs need to look for mutations spread over specific exons.” Sometimes known as ‘hot exons’, exhibiting high levels of actionable mutations throughout the entire exon, these can provide a wealth of information.

Another point to consider is that investigations evolve with new discoveries, and when mutations within certain genes, for example the tumour suppressor TP53, become more clinically actionable, it will then become important to look for variants spread over the whole gene. However, there is a lag between discoveries in research and their clinical application. “Interpreting novel variants provides a significant challenge, and requires bringing together in silico analysis, literature review, current drug trials etc.,” says Dr Smith. The panel must then be re-evaluated following the addition of any new content. Additional content must therefore be carefully considered, and provide very strong evidence for a tangible difference in patient treatment. Moving forward, Dr Smith proposes: “One model would be to review the content after set time periods and add additional content, if required, in batches.”

Moreover, a particularly interesting way targeted NGS technology has adapted in response to this challenge is with the emergence of custom panels, which enable the user to select a chosen pool of relevant hybridisation probes. The flexibility of such systems facilitates researchers in investigating variants relevant to their specific study, increasing the speed at which new content can be validated and decreasing the time lag from the laboratory to the clinic.
For NGS to be routinely applied for stratified cancer treatment, how must the technology adapt for compatibility with tumour samples?

Solid tumour samples present two primary challenges. Firstly, due to tumour heterogeneity and the presence of DNA derived from non-tumour cells, a variant of interest may only occur at a relatively low allele frequency in the sample. Since this is facilitated through deep sequencing, researchers are particularly interested in NGS platforms that allow considerable depth (i.e. targeted panels).

In addition, the use of DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tissue can present many technical challenges, impacting on both DNA integrity and yield. Dr Smith explains: “Due to the fixative process, the DNA can be degraded and we often have to work with very small amounts of DNA.” Because of this, a number of quality control matrices are analysed, including the accurate measurement of low level DNA. Targeted capture methods are also carefully considered to ensure the uniform representation of all regions of interest.

When adopting a new technology for clinical application, quality control is a key consideration. How is this being addressed?

Quality control procedures are vital to ensure accurate NGS data: “All new tests undergo extensive validation initially with well-characterised samples, including a variety of positive, no-mutation and no-template controls, which also includes validating the laboratory-based work and the bioinformatics pipelines. The validation estimates the sensitivity, specificity, reproducibility and repeatability of the tests,” Dr Burghel says.

Following the initial validation, positive and negative controls are included in each assay. The sequencing quality, coverage, depth and mutant allele frequency are all determined, and data is analysed and validated by two scientists. The report summarises the interpreted results clearly, and is once again checked and authorised by a second experienced scientist. “Not only do we have to report the variants found but we must also be able to verify that the reason a variant was not detected in a region of interest was because it was generally not there or below the reported sensitivity of the test, and not because of lack of sequencing depth,” says Dr Smith. In addition, the challenge of tumour heterogeneity is also considered. “If the test’s detection sensitivity has not reached the level required to detect low allele frequencies, then this needs to be fed back to the clinician so additional testing can be performed if desired.”

Considering the latest research discoveries is also important, and published literature and known databases (such as COSMIC) are frequently used in interpretation and reporting.

What do you see for the future of NGS in molecular pathology?

The fundamental premise of stratified cancer therapy is to ensure the right treatment for the right person at the right time, and with the area of genomic medicine growing at an unprecedented rate, it is becoming clear that targeted next generation sequencing is the key to this.

As clinical genetics experts Dr Matthew Smith and Dr George Burghel indicate, this technology is becoming increasingly embedded within the clinical laboratory, with new panels emerging and evolving in response to the latest genetic discoveries. These panels provide the capability to detect low-level mutations from the ever-increasing catalogue of clinically actionable aberrations and markers for directing cancer therapy, and in fact, Dr Burghel comments that many of these genetic markers are already in use today. However, it is also clear that a certain level of consideration is necessary in order to accommodate the particular needs of the clinical laboratory, including the requirement for accuracy and sensitivity.

As Dr Smith affirms, “Along with existing and emerging testing strategies, NGS has an extremely important role to play in future cancer characterisation and treatment.”
About OGT

Oxford Gene Technology (OGT) provides world-class genetics research solutions to leading clinical and academic research institutions. OGT’s SureSeq NGS products and Genefficiency™ Sequencing Service offer an accessible approach to highly sensitive and specific sequencing of cancer samples. Targeted sequencing services also include whole exome or custom panel designs. All results are delivered in an interactive report, which can be run on a PC with no requirement for additional software or local bioinformatics support, helping researchers get straight to the variant of interest.

For more information, please visit www.ogt.com or email contact@ogt.com

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References