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Next Generation Sequencing in Clinical Oncology

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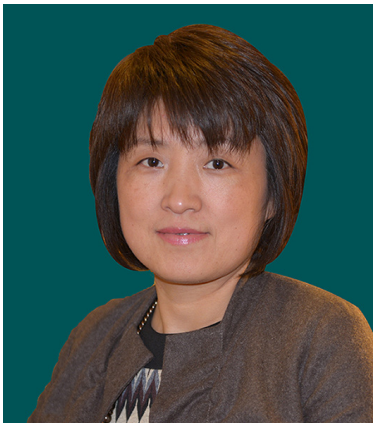

Next generation sequencing (NGS), often referred to as massively parallel sequencing, is a relatively new technique in molecular diagnostics that has truly served as a "disruptive technology" in the area of cancer treatment. Prior to the availability of NGS, one small fragment of DNA could be sequenced per reaction, using a technique called Sanger sequencing, that at the time of its discovery was also quite revolutionary. In Sanger sequencing, terminator nucleotides are used randomly in a PCR (polymerase chain reaction) reaction to create fragments of differing lengths with a known base at the terminal end. The fragments can then be lined up to deduce the sequence of the entire region being targeted. In contrast, NGS can sequence millions of fragments simultaneously, by sequentially adding *reversible* terminator nucleotides to numerous fragments of DNA from different samples, throughout all regions of interest, in one reaction. This is accomplished by adding adapters to the ends of the DNA fragments (used to affix fragments to a fixed surface, to "barcode" individual samples within a larger reaction, and to prime the PCR reactions), and then adding the reversible terminator nucleotides one by one, measuring the addition, and then cleaving the "terminator" portion so that another nucleotide can be added.

In this way, the amount of sequencing data retrieved per reaction is increased by an almost inconceivable amount. This benefits cancer patients in two ways. The first is that we can examine many genes that may be relevant to the patient's tumor simultaneously, with a high level of accuracy, using only a small amount of tissue and for a reasonable cost. The second clear benefit is that we can look at these regions in replicate, typically hundreds to thousands of sequences from each individual targeted area, across all regions of interest. This is particularly important in cancer because tumor DNA is never 100% pure (there are always some stromal cells, inflammatory cells, or normal tissue cells included in the tumor, even after microdissection), and most solid tumors are known to be heterogeneous, meaning that an important mutation may only appear in a small amount of the total tumor DNA. It is much easier to identify a minor population of mutated DNA when there are thousands of sequences to compare, than when there is one tracing representing a single DNA sequence. The net

result is a dramatic increase in the quality and quantity of information obtained.

SYMGENE™ Cancer Panel from CellNetix

The SYMGENE™ 68 gene Cancer Panel assay, developed at CellNetix Pathology and Laboratories, is an affordable, custom-designed targeted NGS assay, which serves effectively for both solid tumors and hematologic malignancies. Genes and the targeted regions within each gene have been carefully selected in collaboration with oncologists from the Swedish Cancer Institute and through an extensive literature review focused on known clinical utilities for 8 major types of cancer. The actionable mutations associated with approved therapeutic agents (such as EGFR, KRAS, BRAF, and ERBB2) are very well covered in the panel, along with many other gene targets that show promise for multiple different tumor types and have many associated clinical trials or experimental therapies (such as PIK3CA, KDR, and SMO). There are also several other genes that could potentially be of benefit in guiding treatment, along with a few pharmacogenomic markers.

One distinguishing feature at CellNetix is the large degree of pathologist involvement in NGS cancer testing. A molecularly trained pathologist evaluates the tissue and directs microdissection, and will then continue to correlate the morphology with the laboratory results at every step along the way, from the evaluation of DNA quality, to the initial analysis of sequencing reads and variants, to the actual clinical report. In this way, problems in sample analysis that are tissue-specific can be identified and rectified at many different levels. The pathologist is also able to serve as a physician colleague to explain any subtleties in results, or challenges in processing, to the treating oncologist. At CellNetix, we also utilize our numerous subspecialty pathologists to aid in delineating any subtleties in morphology that could affect tumor analysis or usefulness of results.

Another distinguishing feature is the reporting format and process. We strive to make our reports succinct, direct, and clinically useful. Mutations are categorized at the beginning of the report according to clinical utility, and are divided into three categories: Actionable (approved drug for a specific tumor type, or a relevant contraindication), Applicable (approved therapy in another tumor type, or a directed drug in a specific tumor type available in a clinical trial setting), and Unknown Significance. Next, each gene and mutation is summarized, along with the most relevant drugs and clinical trials, each accompanied by a symbol to delineate the therapeutic confidence level. Again, the highest level indicates an approved drug for a specific tumor type, and the lowest level is a therapy that may only have a theoretical indication, or only pre-clinical supporting evidence. The electronic version of our report contains live links to clinical trials and literature references. We intend for this report to be useful to most practicing oncologists, regardless of the clinical setting. In the event that the oncologist requires additional information or assistance, our pathologists are always readily available for consultation.

Personalized Oncology Care and Pathology

NGS cancer testing is part of a rapidly growing movement in medicine to provide more personalized, or precise, care to individual patients. Nowhere is the concept more immediately relevant than in oncology treatment. Cytotoxic chemotherapy was dramatically innovative at the time of its discovery, and these methods continue to save lives, and to provide the basis for modern cancer care. However, these therapies impact cell growth and division as a global process within the body. The hope is that the most rapidly growing and dividing cells exist in the tumor, and that a sufficient dose of the right drug, or combination of drugs, can be given to kill the tumor without actually killing the normal cells. The downside of this approach is that often the side effects of these drugs can be significant, and often the desired effect cannot be achieved. Ideally, a therapy targeted towards a specific aberration found only in the tumor would kill the tumor cells selectively, with greater effectiveness and efficiency, and with many fewer side effects. We have already seen this happen, most notably with EGFR inhibitors in lung cancer, but also with BRAF inhibitors, and the combination of BRAF and MEK inhibitors in melanoma. The field of targeted drug therapy development is rapidly expanding, with new drugs and drug combinations emerging frequently. Currently there are a small number of known drug targets with approved therapies, but this number will undoubtedly multiply many times in the coming years. This rapidly changing treatment landscape can be challenging for

both physicians and patients, but the promise it holds for a better cancer treatment paradigm is vast. Next generation sequencing is currently the best testing tool to help this process progress, and pathologists are in the best position to implement the technology in an effective way.

The arrival of this technology has enabled oncologists to obtain a more complete molecular profile of each patient's tumor. The most immediate advantage is that many of the most commonly targeted genes have specifically associated therapies that can be made available to the patient based only on the result of the test. Many of these therapies have molecular targets that are already being tested for individually, by various methods, depending on tumor type. The more subtle benefit is that there will also frequently be mutations that point to therapies that may be less obvious for that particular tumor type, such as a drug that is approved for use in another tumor type, or an experimental drug that is available via a clinical trial that the patient may be able to enroll in. These are areas that often provide challenges to the treating oncologists, because off-label drug use and clinical trial enrollment can be difficult and time-consuming for both physicians and their office staff. However, as this technology becomes more widely available clinically, and as the body of evidence for targeted therapies continues to grow, these activities will become increasingly necessary components of a quality clinical practice. Pathologists and oncologists will need to continue to work cooperatively to assure that there is effective communication and synchronization of effort to increase the availability of therapies indicated by NGS testing.

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