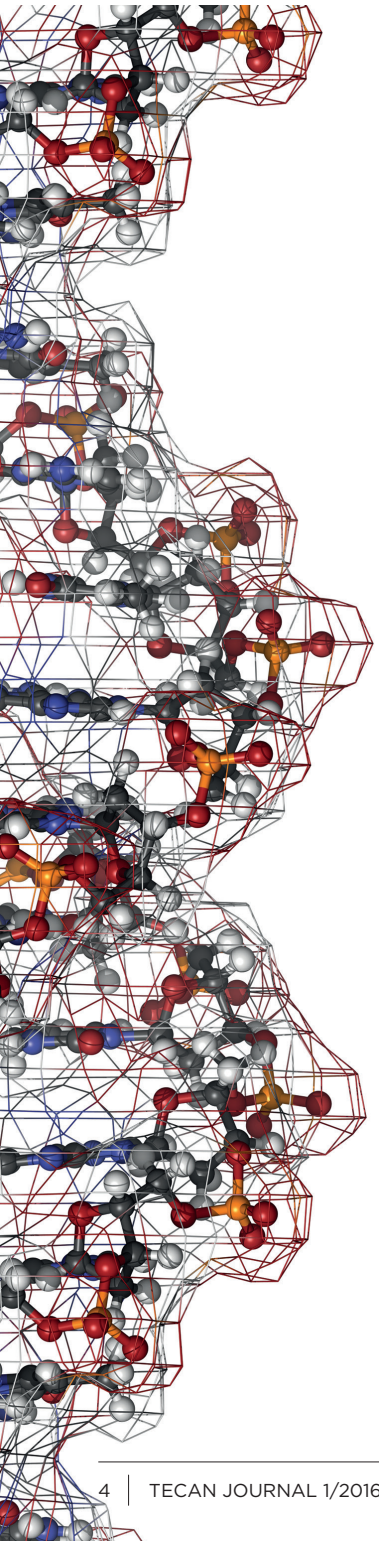
Ramping up NGS in oncology: is sequencing tumor DNA enough?



Massively parallel sequencing has rapidly become a must-have tool of the trade in molecular biology and drug discovery research. In recent years, the cost of next generation sequencing (NGS) has declined exponentially, while throughput, accuracy and read lengths have soared, and multiple regulatory-compliant sequencing technologies have now achieved commercial success. Advances in NGS – combined with global efforts to identify and catalog genetic mutations in a range of cancer types and tumors, as well as to implement these discoveries in diagnostic, therapeutic and prognostic applications – are driving the adoption and, in the not-too-distant future, even routine use of sequencing and related techniques in clinical laboratories.

With the emergence of NGS in clinical oncology have come abundant examples in the literature of the value of tumor-derived DNA sequencing. These include identifying hotspots in cancer-predisposing genes, or specific cancer-associated mutations in one or more genes that might contribute valuable diagnostic or prognostic insights. This information could also help to guide clinicians in therapeutic decision making and drug selection to maximize the efficacy of treatment, limit patient exposure to toxic chemotherapeutic agents not likely to have a beneficial effect on disease progression or patient survival, and minimize the risk of developing drug resistance.

Moreover, clinical oncologists are recognizing the value of additional information to be gained from RNA sequencing (RNAseq) to define the tumor transcriptome. Perhaps a bit farther off in the future will be direct clinical applications of sequencing data derived from non-coding RNA species – such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) – to detect aberrations that may affect gene expression, and overarching gene regulatory networks that control biochemical pathways essential for tumorigenesis, malignant disease progression and metastasis. Furthermore, characterizing the epigenetic changes in tumor DNA and determining how these might relate to cancer diagnostics, the monitoring of disease progression and drug response, and the prediction of drug sensitivity and resistance, is an area still in its infancy.

Immunotherapeutic strategies designed to stimulate the body's immune system to recognize and destroy tumor cells are increasingly being developed to complement chemotherapeutic regimens. The results of NGS can be used to predict patient response to immunotherapy, as well as to inform the design of therapeutic cancer vaccines. Exome sequencing can reveal whether a neoantigen – an antigen created by a somatic mutation in a tumor – is presented by the major histocompatibility complex for recognition by sensitized T cells.[1] The growing interest in clinical applications of NGS in oncology has also

recently spurred discussion and debate on what and how much sequence data is needed to ensure accurate interpretation and appropriate utilization of genomic information in patients with cancer.

Too little information is not an option

One issue swirling around at present is whether sequencing the DNA from a tumor biopsy is sufficient for diagnostic and therapeutic purposes, or if sequence data generated from a healthy sample from the same patient should serve as a matched control for comparison purposes. As noted in the article *Cancer Sequencing Controls*, sequencing a patient's normal DNA is not common practice in clinical labs and would certainly add to the cost compared to analyzing only tumor DNA.[2] However, the extra workload and cost must be weighed against the risk of basing treatment decisions on inaccurate information and an incomplete diagnosis.

The results of an analysis of 815 paired tumor-normal samples from patients with 15 different tumor types illustrated the potential to misinterpret somatic alterations identified in the tumor genome using NGS as tumor-specific mutations.[3] Many of the same changes were shown to be present as germline variations in NGS analysis of the normal sample, and only about a third of the mutations found on sequencing of the tumor exome were tumor-specific. The other two thirds were germline alterations, and would have led to false positive findings – including in cancer-predisposing (potentially actionable) genes – if only the tumor DNA had been sequenced, and that information alone used to inform therapeutic decisions.

Sequencing overload – preparing for the future

An emerging trend towards sequencing matched tumor and normal DNA samples would clearly increase the NGS volume and related sample extraction and library preparation workflows in diagnostics labs. The discovery of new tumor-specific genetic alterations that may be relevant for predicting cancer progression, metastatic potential and drug sensitivity or resistance – and the eventual translation of these molecular tools to the bedside – would have an even greater impact on the demands placed on clinical labs.

The envisioned transition in healthcare to a more efficient and cost-effective strategy based on personalized medicine will require access to an abundance of genetic information for each and every patient, at various stages of health and disease. Thus, rapid, high throughput, massively parallel sequencing performed in a regulated environment will be the new norm for clinical labs. Clinical NGS will become a commodity, essentially as it has in the research setting. This extensive application of NGS will be a reality across a broad range of diseases, but perhaps most acutely in cancer.

> "NGS-based diagnosis is specially promising for diseases that have a highly complex and heterogeneous genetic composition [such as oncology, which is] very well positioned to benefit greatly from such an approach."
>
> *according to Pant and colleagues.*[4]

> "It is easy to imagine that soon every patient will have both their constitutional and cancer genomes sequenced, the latter perhaps multiple times in order to monitor disease progression, thus enabling an accurate molecular subtyping of disease and the rational use of molecularly guided therapies."
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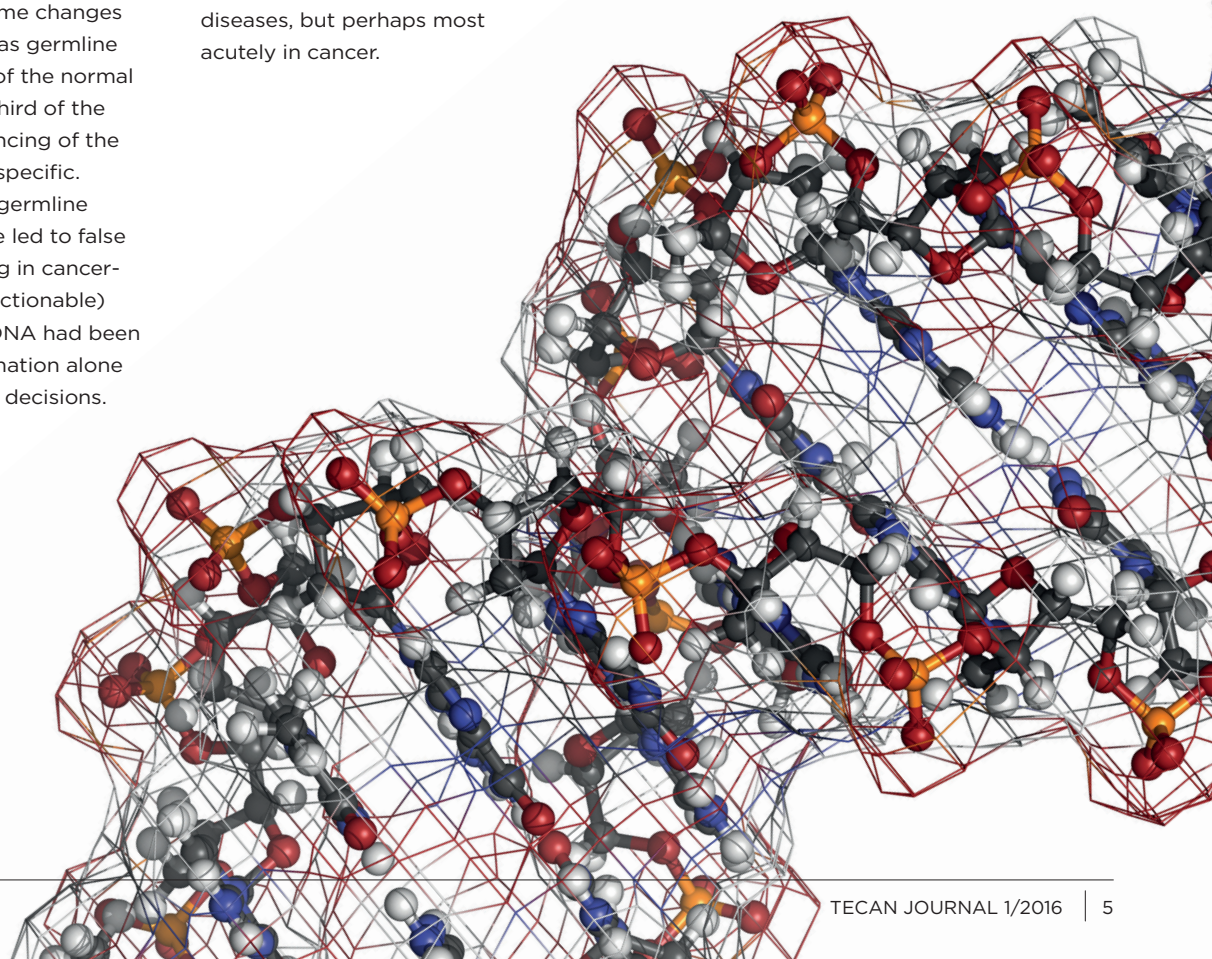
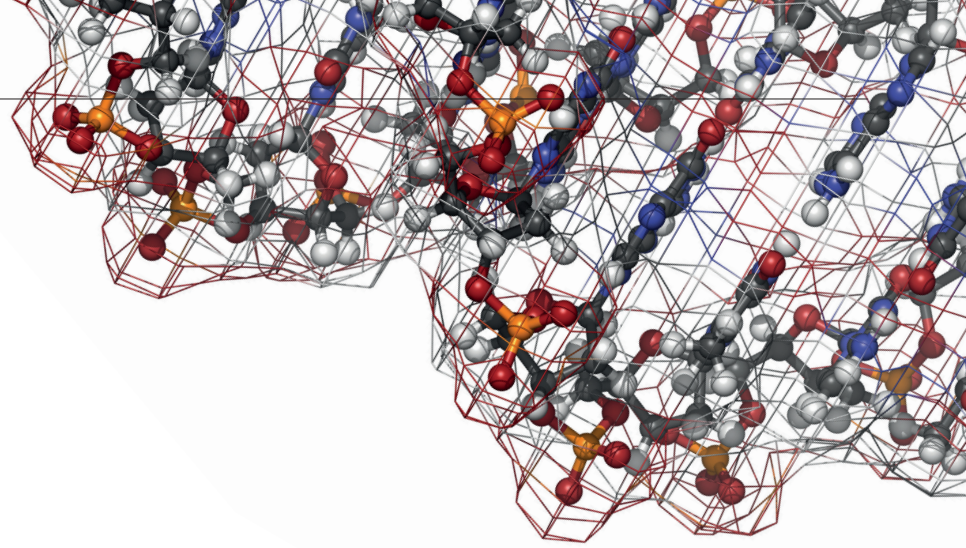
> *state Meldrum* et al.[5]





Regardless of the sequencing technology used, the biggest bottleneck and challenges lie in sample preparation. This involves the laborious and often difficult process of DNA extraction, especially when working with formalin-fixed paraffin-embedded specimens. Pant *et al*. contend that the current testing paradigm for precision medicine is unsustainable.

> “Recent results from clinical studies support the emerging concept of the ‘mutation signature’ or spectrum of correlated mutations in cancer.”

In other words, combinations of mutations will be more predictive of treatment response than individual gene mutations. Therefore, physicians will want to examine the tumor’s whole genome, both somatic mutations and transcriptional changes, to identify the most effective personalized therapy. Thus, the use of RNAseq to analyze the transcriptome of tumor cells and assess the relative expression of a mutated gene will likely become a much more common application. Rizzo and Buck note that NGS-based RNAseq studies continue “to identify and implicate key somatic mutations in oncogenesis.”[6] They point out that certain oncogenic mutations identified in tumor samples using RNAseq have also been shown to alter gene function *in vivo* in a way that agrees with the tumor’s clinical behavior.

The various trends described here – including advances in NGS technology and other factors driving personalized medicine – all point to a future on the horizon in which massively parallel sequencing will be routinely used for cancer diagnosis and to guide therapeutic decision making. As clinical labs begin to realize a dramatic increase in demand for NGS, rapid adoption of high throughput solutions for upstream sample handling and library preparation in NGS workflows will be critical.

Robotic systems that automate sample prep enable faster, more efficient and more secure sample processing, with better accuracy and consistency than manual techniques. They can also provide the flexibility to accommodate both commercial and customized, laboratory-developed diagnostic tests and sample prep protocols. In addition to improved speed and productivity, automation and computer-driven systems bring several crucial advantages to the clinical laboratory. Not least of these is an emphasis on sample tracking and a secure chain-of-custody, as well as ensuring the quality controls, validation and documentation required in a regulated environment. Furthermore, robotic liquid handling and sample processing minimize the risk of contamination and maximize accuracy and reproducibility compared to manual techniques.[7]

With increased throughput, efficiency and productivity typically comes cost savings. Thus, as labs ramp up their sequencing activities in response to increasing demand for massively parallel sequencing capabilities – and transition to automated systems to handle the added volume of NGS and workload related sample prep functions – they are likely to realize quite quickly that cost need not be a barrier to meeting the evolving needs of clinical oncology.

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