A New Dawn for Comprehensive Genomic Profiling

Published results from early access program demonstrate the reproducibility and reliability of TruSight™ Oncology 500

The more we learn about the complex molecular pathology of different cancers, the more powerful comprehensive genomic profiling (CGP) becomes. Using next-generation sequencing (NGS) to identify genetic alterations that drive cancer, CGP simultaneously examines multiple biomarkers that are included in guidelines and clinical trials, reducing both tissue and time requirements compared to sequential testing methods. An important genomic signature covered by the panel is microsatellite instability (MSI) – an inactivation of mismatch repair genes that prevents the correction of DNA replication errors – which was the first pan-cancer signature approved by the US Food and Drug Administration (FDA). Additionally, coverage for tumor mutational burden (TMB), the recently FDA approved immuno-oncology genomic signature, can be used to estimate the effectiveness of immune checkpoint inhibitor therapy (1).

Both of these genomic signatures, in addition to DNA and RNA variants reveal important information about tumor heterogeneity. TruSight™ Oncology 500 (TSO500), research use only (RUO) assay, analyzes hundreds of these cancer-related genes across 1.94 MB of genomic content using sophisticated software algorithms. Launched in 2019, TSO500 was tested by 13 leading European cancer centers in an early access program (2). Data recently published by the University of Birmingham and Radboud University Medical Center Nijmegen returned particularly promising results (3, 4). We spoke to Andrew Beggs from Birmingham and Leonie Kroeze from Radboud Nijmegen to learn more.

What were the main findings of your recent publication?

Andrew Beggs: We used the TSO500 panel to carry out comprehensive molecular profiling of cancers and compared the results with those from whole-genome sequencing (WGS). The panel was as accurate as WGS and orthogonal techniques at measuring TMB, MSI, single-nucleotide variants, indels, copy number/structural variation, and gene fusions. One of the main benefits of the TSO500 is that it is less expensive than WGS. This lower cost makes it more feasible to complete mass genomic profiling and means that you could theoretically use it for every patient who presents with cancer as a "one-stop shop" for cancer profiling. We also found that the deep sequencing on a targeted panel facilitated a better understanding of tumor heterogeneity and detected rare variants that might otherwise have been missed.

Leonie Kroeze: By using a large sequencing panel, such as the TSO500, we can analyze many biomarkers which will be important for diagnosis and therapy decisions using a limited amount of material. One of the major advantages of the TSO500 is that it includes unique molecular identifiers, which show how many unique DNA molecules have been sequenced. This feature is particularly important to judge the reliability of the detected DNA variants when the DNA quantity is low.

We especially focused on the reproducibility of TMB and MSI values, because both are relatively new NGS-based biomarkers important for predicting response to immunotherapy. After repeating a sample in 10 different sequencing runs, we obtained highly reproducible values. More importantly, the results from 11 different labs across several countries were comparable; interlaboratory reproducibility is crucial if we are to use the same cutoff values for MSI and TMB across the world. We found it is particularly important to define minimum acceptance criteria for DNA quality and quantity when evaluating TMB.

How does a large panel such as the TSO500 affect laboratory efficiency?

AB: It's highly automatable, which means it can be built into a workflow that is mostly hands-off and left unattended to run overnight. The level of automatization also makes it extremely reproducible and allows for consistent results, and we have found it allows a 50 percent reduction in hands on time and a subsequent increase in efficiency.

LK: The complete workflow – from DNA isolation to final clinical report – takes us approximately six days. Although more expensive than small NGS panels, the larger panel provides results for many biomarkers at once. There is no need for sequential testing or multiple parallel tests, thereby decreasing the total turnaround time.

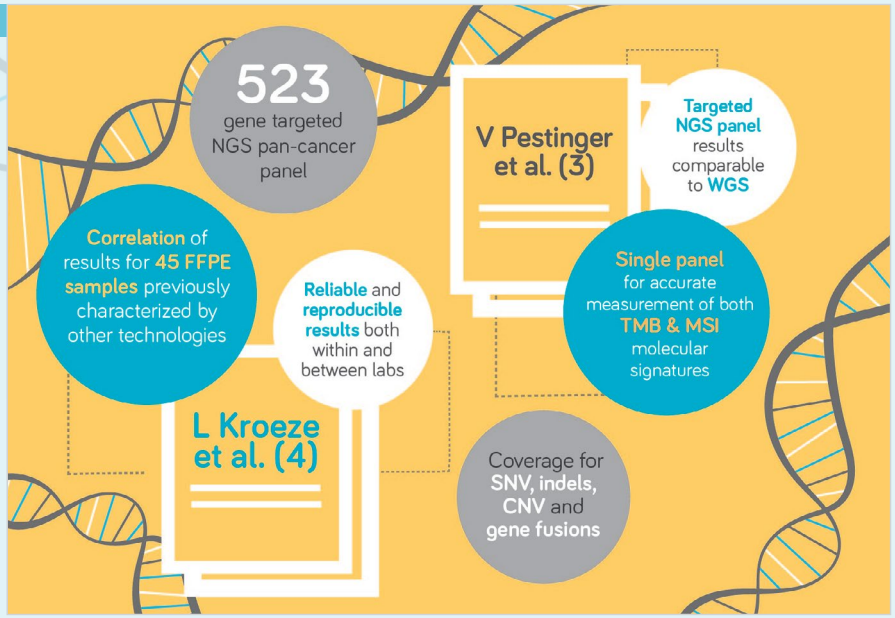
How did the TSO500 perform when analyzing multiple biomarkers and variant types simultaneously?

AB: Using a "TMB-high" threshold of 10 mut/Mb, the TSO500 classified samples with 100 percent accuracy. The panel was reproducible across multiple samples and tumor types and shows that a panel of this type would be suitable for the clinical determination of TMB status across different sample types and DNA inputs. The same can be said for MSI, which we detected in all samples that had over 10 percent unstable MSI sites.

The targeted RNA-seq assay component of TSO500 offers a unique advantage to detect known and unknown fusions events – and we reliably detected *NTRK*, *ALK*, and *RET* fusions. We think the hybrid-capture enrichment used in TruSight technology is superior to conventional pathology techniques for detecting fusions because you don't need to know the other end of the fusion breakpoint. As long as one of the partners is on the fusion panel, you can

work out novel fusions and find potentially pathogenic fusions that couldn't otherwise be detected.

LK: We compared the TSO500 results with our current NGS approach and were able to detect all previously determined mutations, amplifications, and MSI present in the samples. One of the main benefits of a larger panel is that less material is needed overall than for separate assays. For example, a lung cancer brush biopsy produces only a small amount of material – but the TSO500 maximizes the information obtained from that limited sample.



What advice would you give to anyone implementing the TSO500 into their workflow?

AB: I think a basic knowledge of molecular biology is helpful. You also need to have the correct equipment, which requires a small initial capital investment. In terms of workflow, the most important aspect is to work out how many samples you're going to process each week; it's not worth stepping up to an automated workflow if you're only doing a handful. If you process hundreds each week, then an automated workflow is the favored option.

LK: It's possible to manually analyze the list of variants produced by the TSO500 – but we built an additional bioinformatic workflow that annotates the variants and makes filtering easier. For that reason, the assistance of a bioinformatician was very helpful during implementation. I would also advise to optimize the DNA shearing which is especially important for reliable MSI calling, because the sequencing reads should be long enough to span the complete microsatellite regions.

What are the main advantages of performing CGP in-house?

AB: I think the primary benefits are speed and breadth of assay. Comprehensive panels would also support consideration of multiple novel therapy options. I would argue that, in many solid tumors, CGP will replace testing methods that use smaller gene panels. For example, colorectal cancer patients should be tested for *KRAS* and *BRAF* mutations – but limited panel sizes mean that doesn't always happen.

Although some pathologists question the standardization of assays that enable local CGP testing, we demonstrated that the TSO500 minimizes interlaboratory variability. Consistent results both within and between labs are obviously critical to devolve testing down to the local level. This kind of in-house testing provides quicker turnaround times, greater confidence in results, and easier communication with molecular pathologists.

LK: The main advantages of CGP are that less material is required, turnaround times are shorter without sequential testing, and there is a higher chance of finding actionable targets. We anticipate that this latter advantage will also result in more patients who are eligible for clinical trials, which ultimately leads to better knowledge of new therapies.

As molecular biologists, we prefer to analyze sequencing results ourselves so that we have a better feeling of the quality and reliability of results. This confidence is crucial when it comes to communicating with clinicians about the consequences of our molecular findings for therapy – and we can easily respond to additional questions that would ordinarily make life more difficult when liaising with an external organization. The highly reproducible TSO500 provides this reliability and unlocks the benefits of local CGP testing.

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References

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