Comprehensive Insights

Combating cancer through comprehensive genomic profiling

Please click the circles to navigate

- Liquid Biopsy Insights into Cancer
- Webinars
- Product Portfolio
- Published Results
- Portfolio Updates
- Educational Resources

Liquid Biopsy Insights into Cancer

The amount of time between the arrival of a new technology and its deployment in the diagnostic laboratory is shrinking. Take immuno-oncology (IO), for example – this powerful new approach harnesses the body's own immune system and has quickly become one of the most promising approaches to cancer treatment. Yet given the growing number of guideline and clinical trial biomarkers that can be explored, tissue sample availability is becoming a limiting factor. Liquid biopsy provides a noninvasive alternative, not subject to tissue limitations, to gain access to the mutational landscape of tumors.

As molecular pathology assays aim to cover a growing number of guideline-recommended and emerging biomarkers (1, 2), existing assays that interrogate a small number of variants can now be consolidated onto a single panel, facilitating comprehensive genomic profiling (CGP) of multiple actionable biomarkers and key investigational IO biomarkers through next-generation sequencing (NGS). Covering these needs with a combination of revolutionary sample collection and consolidated testing is key, especially in current times with infectious threats adding another layer of challenge for oncology patients.

Enabling CGP from liquid biopsy sounds like the perfect answer; the approach is noninvasive and enables repeat sampling. However, it raises questions about the feasibility of analyzing a variety of tumor variants and genomic signatures that are circulating in the blood with with sufficient sensitivity at low variant allele frequencies (VAF). We spoke to two experts working in molecular pathology and translational genomics to explore the merit of liquid biopsy to identify tumor insights at a very low limit of detection and learn about the early success of Illumina's comprehensive research use only assay for liquid biopsy, TruSight™ Oncology 500 (TSO500) ctDNA.

Multiple markers

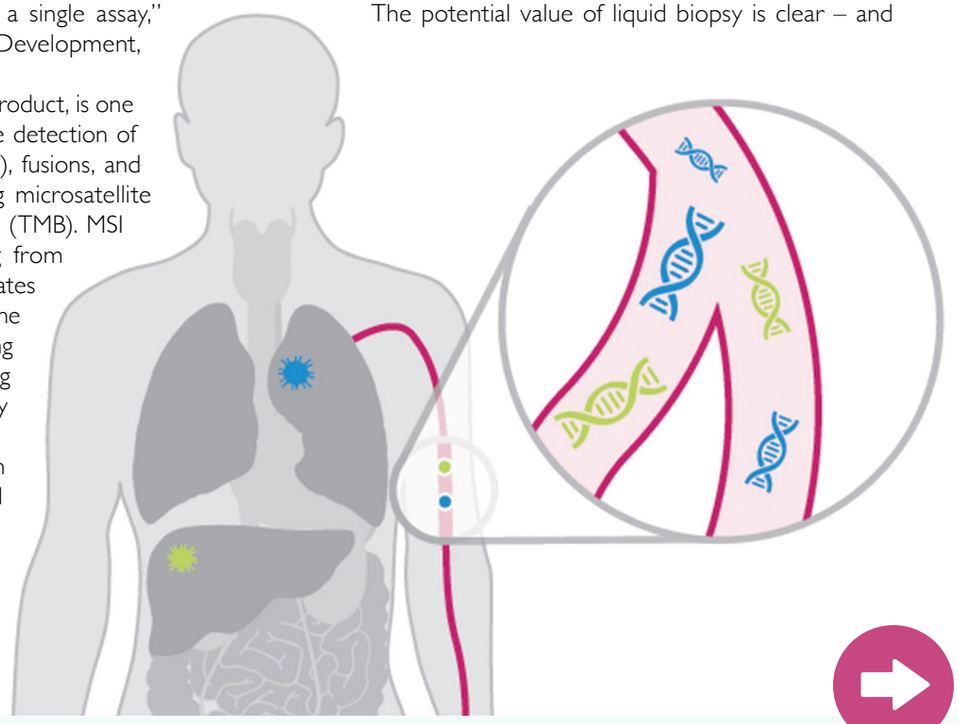
"When it comes to sequencing and beyond, the days of the single-focus assay are numbered. There are now hardly any tumor entities in which we only look for one marker," says Wilko Weichert, Professor of Pathology and Chairman of the Institute of Pathology at the Technical University of Munich, Germany. Larger panels, with a variety of markers enabling the identification of multiple potential driver alterations can provide a more detailed perspective on possible therapy response to support clinicians in their selection of the most appropriate therapy according to individual tumor characteristics. "The choice between targeted panels and comprehensive assays depends on intended use – but for anything from exploratory biomarker discovery to composite biomarker testing, it's important to have multiple markers consolidated into a single assay," says Stephanie Hastings, Manager in Assay Development, Translational Genomics at Q2 Solutions, USA.

TSO500 ctDNA assay, a research use only product, is one such highly multiplexed assay that involves the detection of small variants, copy number variations (CNV), fusions, and key genomic signature biomarkers, including microsatellite instability (MSI) and tumor mutational burden (TMB). MSI is a unique pan-cancer biomarker resulting from defective DNA mismatch repair, which indicates predisposition to mutations (3). TMB measures the number of somatic mutations within the coding sequence of the tumor genome (4). Combining multiple genomic aberrations provides a highly personalized assessment.

However, pathologists routinely work with solid tumor tissue samples – so why would they use liquid biopsy? "There are two main advantages of using liquid samples. Blood is readily available, and you can collect it via a minimally invasive procedure, repeating several times if necessary," explains Weichert. "It's also accessible in almost every patient – even those in whom you can't reach the lesion by traditional biopsy due to an increased risk of side effects, such as lung cancer patients with emphysema."

Another factor to consider is the biology of the two different sample types. Although solid tumor samples might correlate well with histology and cellular phenotypes, they represent only a small, localized primary tumor profile. In contrast, multiple metastatic lesions might all shed DNA into the bloodstream. The subsequent liquid biopsy sample would provide a comprehensive patient tumor profile that could be more predictive of therapy response than information from a biopsy of a single tumor site.

The potential value of liquid biopsy is clear – and



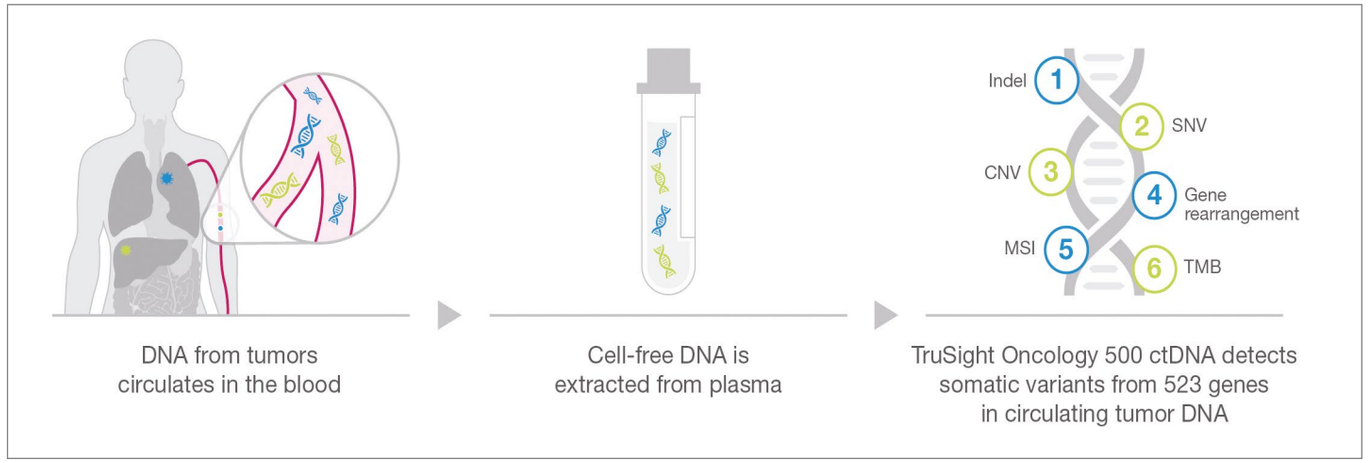
Weichert sees several scenarios where a liquid sample could prove beneficial. "I believe we will see liquid biopsy used to follow patients, particularly because it's easier to obtain blood sequentially than tissue. For example, you can measure ctDNA in the blood to check for cancer recurrence or monitor changes in molecular profiles to detect whether resistance mutations have occurred."

Effective capture

"The real challenge of working with a liquid biopsy sample is its potential for low stability; lysis of white blood cells can cause genomic DNA to spill into the plasma fraction," says Hastings – and such contamination could potentially conceal the targets of interest in the blood sample.

Therefore, appropriate collection tubes, preanalytical considerations, and using the most effective workflow are key to capturing low-frequency molecular alterations present in cell-free DNA (cfDNA). "When it comes to sample quality for liquid biopsy, the way that blood is collected is crucial – for example, the tube type used can affect the level of stability," says Hastings. "Once you've isolated the ctDNA, the way in which you quantify the amount of material you have is also important for assay performance." It is critical to use an electrophoretic quantification method, such as Fragment Analyzer or TapeStation, that can specifically measure the cfDNA fraction and exclude high-molecular-weight DNA contamination. Fluorometric methods are not recommended because they quantify all species of DNA sizes contained in the sample, which could potentially overestimate the amount of cfDNA and fail to create a robust library (5).

From a library preparation perspective, using hybrid capture-based target enrichment chemistry enables users to generate results from liquid biopsy samples with very high sensitivity (6). Hybridization probes have tolerance to capture targets even when mutations exist in the hybridized regions and can cover the span of the entire gene sequence. In comparison, amplicon-based chemistries only amplify a subset of the fragmented DNA due to the possibility of break points between primer binding sites. This also makes amplification of novel fusions challenging. Hybrid capture is more versatile than amplicon-based chemistry and can be used to detect SNVs, indels, CNVs, fusions, and other structural changes with higher accuracy (7). "A hybrid-capture approach is therefore more robust than amplicon because of this feature; you will gain higher sensitivity by having a purer sample for analysis."



Working in collaboration with Illumina since 2018, Hastings has been able to evaluate the assay chemistry of TSO500 ctDNA that has enabled the detection of low allele frequency variants such as *EGFR* L858R, *MYC* indels, and *NTRK2* fusions. "Early access to the pre-released version has allowed us to comprehensively evaluate its performance – and, to date, we have analyzed over 1,000 samples using the TSO500 ctDNA assay."

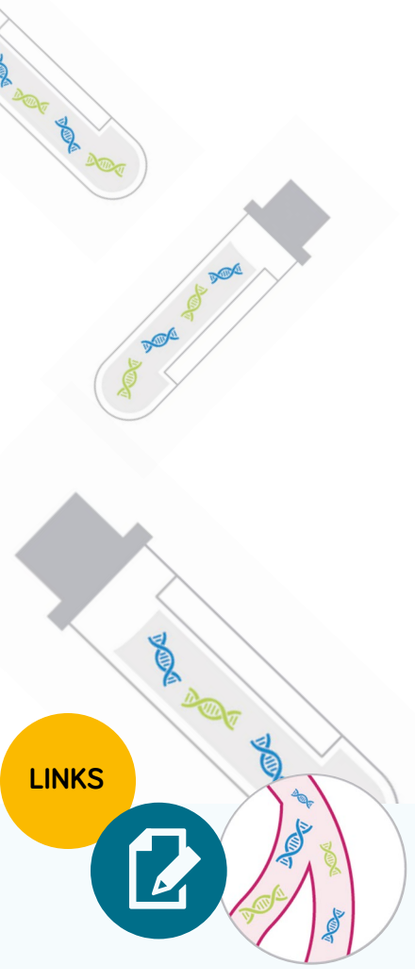
Liquid biopsy's future

Liquid biopsy is still very much an evolving application – and its increased use in clinical trials could accelerate adoption. To that end, Weichert believes that a combination of both tissue and liquid samples should be used wherever possible in these scenarios; "We need even more directly comparable data that indicate whether liquid samples are more predictive than tissue samples, as we envisage they might be."

Whether in the diagnostic laboratory or in clinical trials, liquid biopsy is a fast-moving field that directly feeds into the future of precision medicine. TSO500 ctDNA assay is proving to be an effective research tool delivering reproducible comprehensive information from liquid biopsy samples across 523 cancer-related genes. Hastings certainly believes in the potential of the platform, given that Q^2 recently performed analytical validation of TSO500 ctDNA.

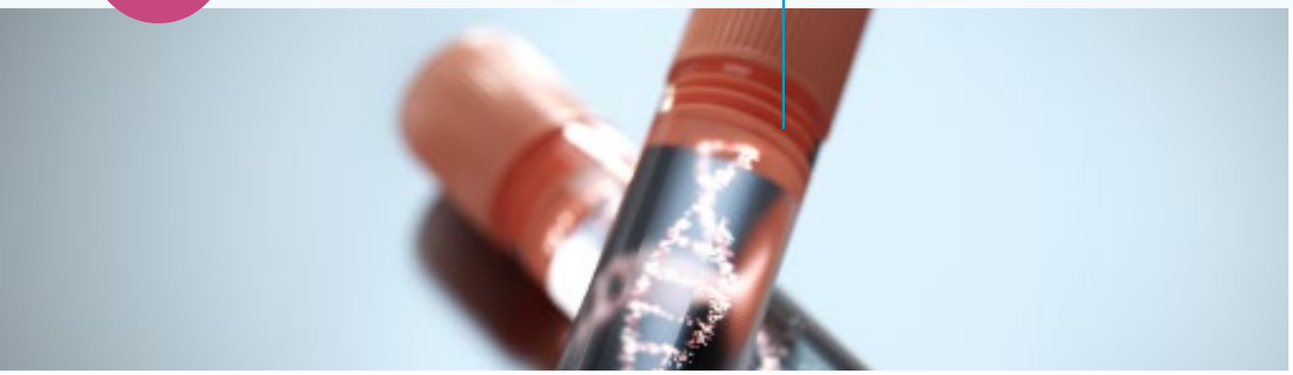
References

1. *https://www.decibio.com/resources-library/white-papers/liquid-biopsy-stakeholder-toolkit/*
2. *Mosele F, Remon J, Mateo J, et al. 2020. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. Annals of Oncology, Vol. 31, issue 11, P1491-1505.*
3. *Yamamoto H, Watanabe Y, Maehata T, Imai K, Itoh F. Microsatellite instability in cancer: a novel landscape for diagnostic and therapeutic approach. Arch Toxicol. 2020 Oct;94(10):3349-3357.*
4. *Fancello L., Gandini, S., Pelicci, P.G. et al. Tumor mutational burden quantification from targeted gene panels: major advancements and challenges. J. immunotherapy cancer 7, 183 (2019).*
5. *Illumina, "Accurate quantification of ctDNA for use in TruSight Oncology 500 ctDNA" (2020). Available at: https://www.illumina.com/content/dam/illumina-marketing/documents/products/appnotes/cfdna-quantification-tech-note-1170-2020-001.pdf*
6. *Illumina, TruSight™ Oncology 500 ctDNA assay data sheet, available at https://www.illumina.com/content/dam/illumina/gcs/assembled-assets/marketing-literature/trusight-oncology-500-ctdna-data-sheet-1170-2019-006/trusight-oncology-500-ctdna-data-sheet-1170-2019-006.pdf*
7. *Samorodnitsky E, Jewell BM, Hagopian R, et al. Evaluation of Hybridization Capture Versus Amplicon-Based Methods for Whole-Exome Sequencing. Human mutation. 2015;36(9):903-914.*



LINKS

Download - TruSight™ Oncology 500 ctDNA Data Sheet



Comprehensive genomic profiling from liquid biopsy samples in clinical trials

Stephanie Hasting, Ph.D
Staff Scientist II, Assay Development,
Translational Genomics
Q² Solutions

Clinical trials are increasingly using liquid biopsy samples to complement or as an alternative to invasive tissue sample collections. With the increasing complexity and diversity of biomarkers to include for clinical trials stratification, liquid biopsy panels that enable comprehensive genomic profiling (CGP) allows consolidation of individual biomarkers into a single NGS assay. In addition, sophisticated bioinformatics solutions allow for the detection of variants at low allelic frequencies, making the assay suitable for biomarker development programs and clinical trial use. In this webinar, we will discuss critical workflow and bioinformatics considerations when consolidating biomarkers testing into a panel that enables CGP from liquid biopsy samples in clinical trials.

Learning Objectives

- Trends contributing towards the use of panels enabling CGP testing from liquid biopsy samples in clinical trials
- Workflow and technical considerations when selecting a liquid biopsy CGP assay
- The requirements and impact of choosing a suitable bioinformatics solution to enable low frequency variants detection and meeting the needs of translational researchers

Watch webinar now

Enabling scalable comprehensive genomic profiling from FFPE samples

Brian Piening, PhD
Technical Director,
Clinical Genomics
Providence Cancer Center

Comprehensive genomic profiling (CGP) is becoming standard of care for cancer and allows pathology labs to consolidate individual biomarkers into a single NGS assay. It enables the assessment of all key biomarkers cited in guidelines and clinical trials using a minimal amount of formalin-fixed, paraffin-embedded (FFPE) tissue while increasing the chance of finding a positive biomarker for every sample.

Illumina's TruSight Oncology 500 High-Throughput (TSO500 HT) assay enables labs to perform in-house CGP with scalability on a NovaSeq™ 6000 platform.

In this CellPress webinar, Brian Piening of Providence Cancer Center will discuss why his team decided to implement in-house CGP and will share analytical performance data from their TSO500_HT runs. He will also provide details of the center's end-to-end workflow, including the data analysis flow and how they build their final reports.

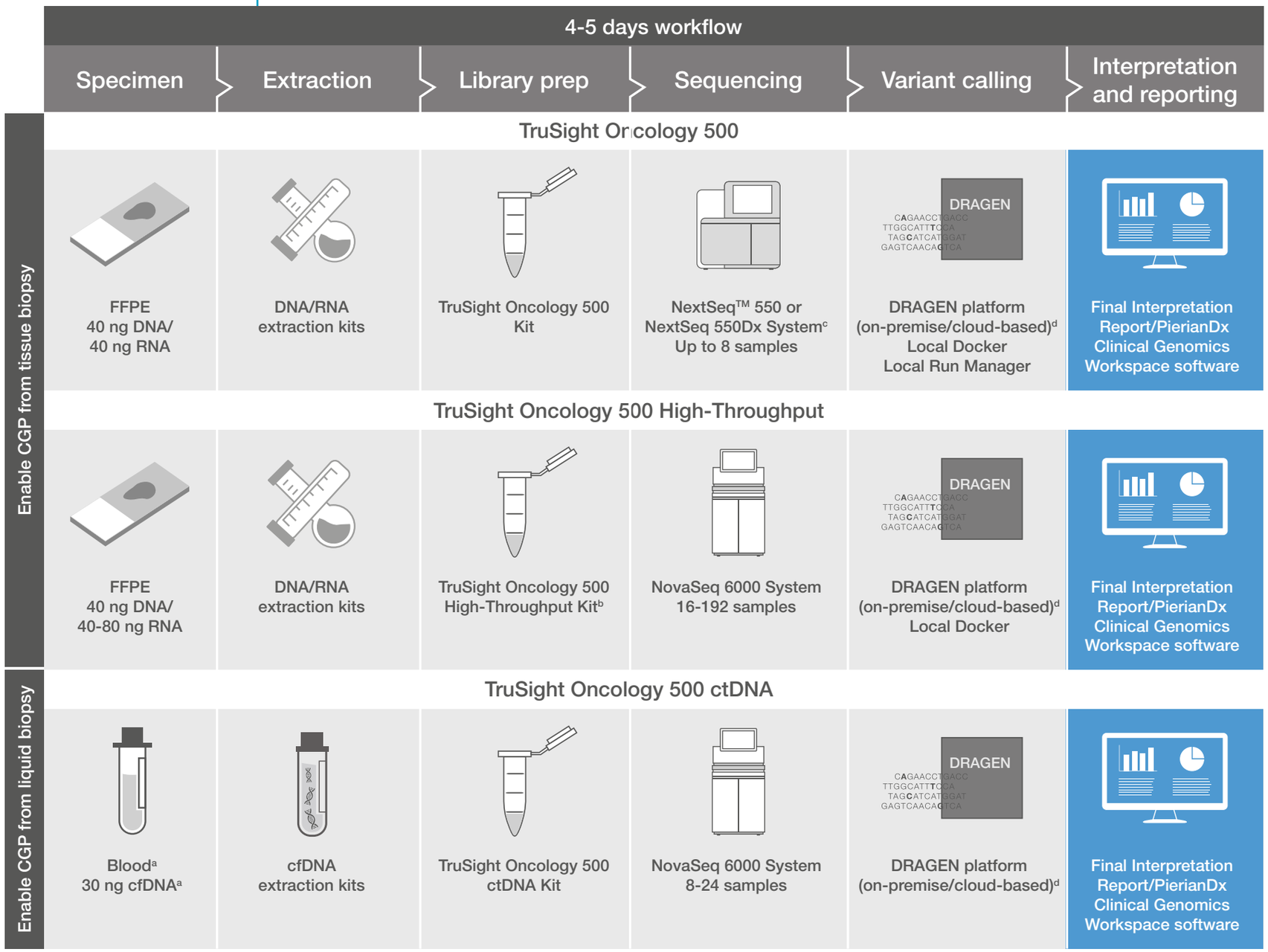
Learning Objectives

- Providence Cancer Center's implementation of in-house CGP
- Illumina's solution enabling in-house CGP

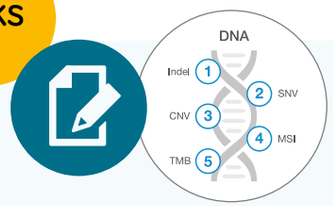
Watch webinar now

TruSight™ Oncology 500 Porfolio Workflows

Comprehensive, streamlined, sample-to-results workflows, starting with tissue or liquid biopsy samples



LINKS



Download - TruSight Oncology 500 High-Throughput Data Sheet

For Research Use Only. Not for use in diagnostic procedures.

Sponsored by illumina®

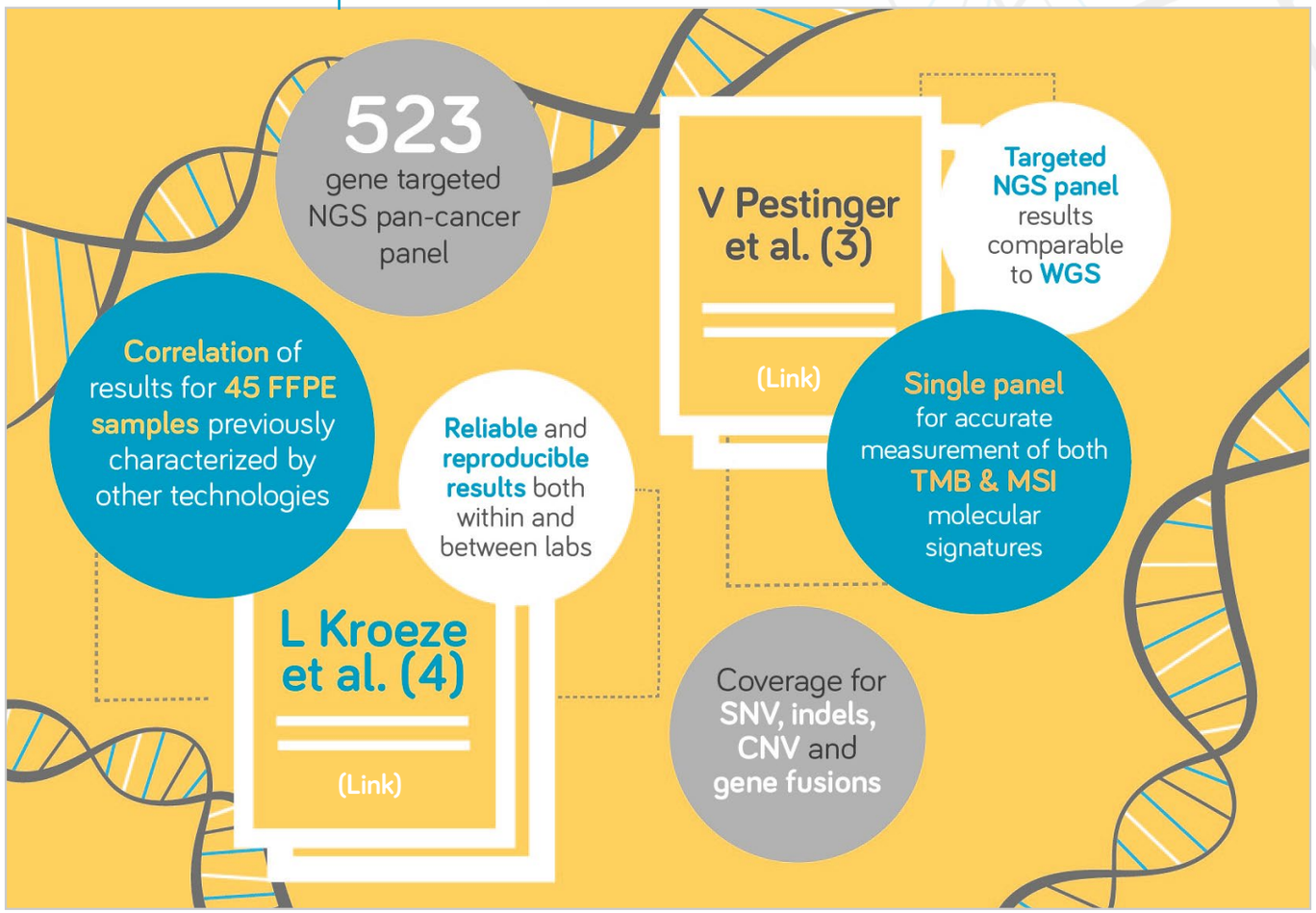
Produced by the Pathologist®

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 as a "one-stop shop" for cancer samples. 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 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

LINKS

Webpage - TruSight Oncology Portfolio

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 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 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.

Andrew Beggs is a Professor of Cancer Genetics and Surgery in the Institute of Cancer and Genomic Sciences, University of Birmingham, UK.

Leonie Kroeze is a clinical scientist in molecular pathology in the laboratory of Marjolijn Ligtenberg at Radboud University Medical Center, Nijmegen, the Netherlands.

References

1. *I Alborelli et al., "Tumor mutational burden assessed by targeted NGS predicts clinical benefit from immune checkpoint inhibitors in non-small cell lung cancer," J Pathol, 250, 19 (2020). PMID: 31471895.*
2. *"TruSight™ Oncology 500: Enabling Comprehensive Genomic Profiling for Every Laboratory," The Pathologist (2019). Available at: https://bit.ly/3juuB6V.*
3. *V Pestinger et al., "Use of an Integrated Pan-Cancer Oncology Enrichment Next-Generation Sequencing Assay to Measure Tumour Mutational Burden and Detect Clinically Actionable Variants," Mol Diagn Ther, 24, 339 (2020). PMID: 32306292.*
4. *LI Kroeze et al., "Evaluation of a Hybrid Capture-Based Pan-Cancer Panel for Analysis of Treatment Stratifying Oncogenic Aberrations and Processes," J Mol Diagn, 22, 757 (2020).PMID: 32205293.*

TruSight™ Oncology 500 Portfolio Updates

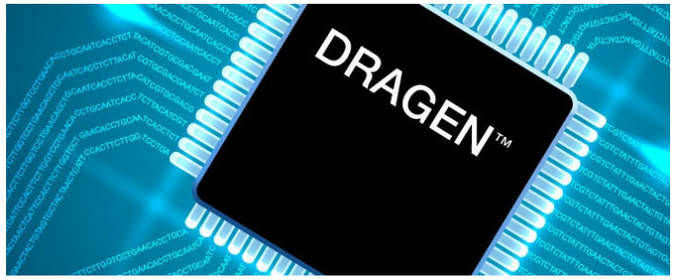
Improvements to enable a simpler, faster, and more scalable sample-to-results workflow for in-house comprehensive genomic profiling (CGP).

Automation-friendly library prep

- ~50% less hands-on time with new kits and methods*
- Efficient scaling with reduced manual errors
- Qualified methods by Illumina and third-party vendors

Increased sequencing efficiency

- TruSight Oncology 500 High-Throughput and ctDNA are now compatible with the new NovaSeq™ 6000 v1.5 Reagent Kits
- Improved inventory management with extended reagent shelf life (six months)
- Reduced sequencing costs



Ultra-rapid variant calling

- On-premise and cloud-based variant calling software solution with the DRAGEN™ Bio-IT Platform† coming soon for the entire TruSight Oncology 500 Portfolio
- 2-10× faster analysis time, reducing overall time from sample to final interpretation report
- Streamlined variant calling workflow featuring simultaneous processing of data from large batches

Liquid biopsy pipeline enhancements

- Includes surrogate measurement for tumor fraction (maximum somatic allele fraction (MSAF))
- Provides improved sensitivity for the immuno-oncology biomarker microsatellite instability (MSI), now > 2,400 sites

* Applies to the TruSight Oncology 500 automation kits/methods available now, as well as TruSight Oncology 500 High-Throughput automation kits/methods available 1H 2021

† On-premise DRAGEN solution currently available for TruSight Oncology 500 ctDNA; on-premise and cloud-based solutions for the entire TruSight Oncology 500 portfolio coming soon

LINKS

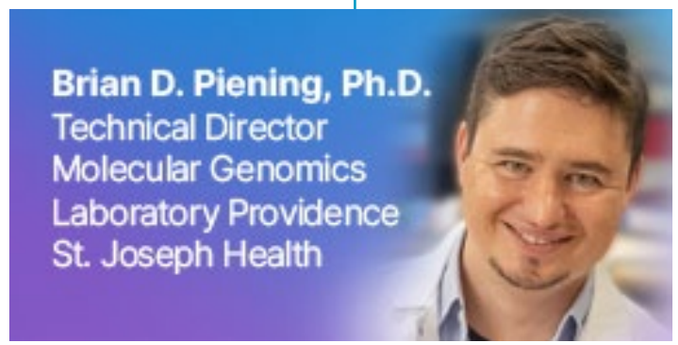
News Center: TSO 500 Updates

Educational Resources

Learn about enabling comprehensive genomic profiling

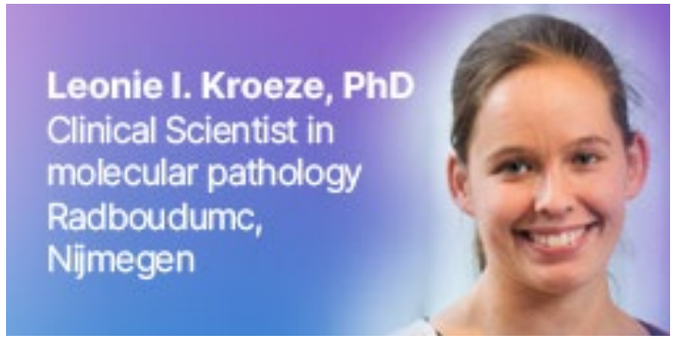
Podcast

Listen to Dr. Rachel Sanborn of the Providence Cancer Institute explain how genomics technologies are empowering precision oncology.



Podcast

Listen to Dr. Brian Piening of the Providence Cancer Institute explain how NGS is changing the standard of care in oncology.



Video On Demand

Comprehensive genomic profiling enabled by TruSight™ Oncology 500 - experience of the Radboudumc Nijmegen Leonie Kroeze

Video On Demand

TruSight™ Oncology 500 ctDNA Liquid Biopsy Panel – Application within Almac Diagnostic Services Laboratory Killian McSorely

Contact an Illumina Representative