The importance of NGS panel customization for your assays: Good practices and key takeaways

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Precision medicine is the present and future of oncology research, opening doors to more effective and less toxic treatments. Accountable for the death of almost 10 million people in 2020 alone, cancer is a leading cause of death worldwide.[1] With limitations in the efficacy of traditional chemotherapy and radiotherapy treatments and their negative, often substantial side effects, the dawn of precision medicine is promising for incrementally improving patient outcomes in oncology.

From identifying biomarkers to detecting actionable mutations, molecular profiling is the backbone of precision medicine. An improved understanding of the molecular mechanisms behind these cancerous tumors is also essential when it comes to facilitating the development of precision medicines that target these specific mechanisms for more effective treatments.

For instance, over the past decade, the treatment of patients with advanced non-small cell lung cancer (NSCLC) has become reliant on tissue specimens and biomarkers to help guide targeted treatment options. Moreover, thanks to the early detection of those biomarkers, medical professionals are avoiding treatments that are unlikely to benefit patients. There are now numerous biomarker-defined patient subgroups, with evidence showing that treatment with targeted and immuno-oncology therapies has superior clinical outcomes when compared to cytotoxic agents. The National Comprehensive Cancer Network® (NCCN) is currently recommending molecular testing in NSCLC practice guidelines, which includes *EGFR* mutations, *ALK* rearrangements, *KRAS, ROS1, BRAF, NTRK1/2/3, METex14* skipping, *RET* and *ERBB2* (HER2). They are also recommending PD-L1 testing through immunohistochemistry (IHC).[2] Several of those biomarker-driven subgroups currently have a large number of approved targeted therapies, except for high-level *MET* amplifications, which is currently an emerging NSCLC biomarker. [2]

The revelation of next-generation sequencing in oncology

The current form of precision oncology relies on next-generation sequencing (NGS) for the most part, which can analyze multiple genetic aberrations, such as, but not limited to, single nucleotide variants (SNVs), small insertions/deletions (indels), and copy number variants (CNVs), simultaneously. Before the incidence of NGS, information on tumor mutational status was limited, as traditional Sanger sequencing and PCR methodologies can only analyze one gene at a time. Although useful when conducting an assay to isolate a specific, known target gene, these methods become incredibly time-consuming when working to discover new target genes. Rebiopsies are also sometimes required to investigate other single genes, making Sanger sequencing and PCR methodologies quite impractical for identifying new gene alterations. On the other hand, NGS can analyze millions of genetic segments in parallel, allowing multiple cancer-driving genes to be sequenced in a single assay with increased sensitivity and limited cancerous tissue availability. See Table 1 for an outline of the benefits and disadvantages of these methods and how, when used together, they can compliment each other for an optimized clinical outcome.

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Table 1: Summary of the benefits, uses, and disadvantages of NGS and Sanger sequencing in oncology precision medicine.

	Advantages	Disadvantages
NGS	• Account for locus heterogeneity • Accurately and sensitively analyze multiple genes in one assay with limited tissue • Fast and less labor-intensive method	• Increased chance of missing relevant mutations • Prone to sequencing artifacts • Increased false positive rate
Sanger sequencing	• Offers precise confirmation of genetic variants of clinical significance • Low false positives rate • Useful to fill in regions that have failed to amplify in complex sequences	• Does not account for locus heterogeneity • Can only analyze one gene in an assay, therefore tissue continues to be the issue • Time consuming and labor intensive

Since its clinical potential was recognized several years ago, NGS has been utilized in many oncology clinical trials and is currently being relatively successfully implemented in clinical practice. NGS is also the most commonly deployed technique, with broad molecular profiling being a key component of improved patient care.[2] Thanks to NGS, the genome of individual cancer patients have now been sequenced, from which genetic changes relating to cancer can be identified. However, processing the vast amount of data provided by whole genome sequencing is highly challenging, impractical, and too expensive for patient diagnosis.

The recent development of high-throughput assays of customized NGS-based panels that examine clinically relevant genes offers a palpable solution to these challenges, enabling faster and more cost-effective investigation of the genomic abnormalities relating to a particular cancer and disease type. With the ability to sequence at a deeper level for more precise detections at a reduced cost, NGS-customized broad molecular profiling is facilitating improved clinical applications. Broad-based genomic testing is essential to providing more personal and precise targeted therapies through more efficient and accurate identification of potentially actionable mutations. Incorporating clinically relevant target sequences into these customized panels is key to individualized oncology treatment.

This whitepaper discusses the importance of panel customization assays with regards to the assessment of the therapeutic value of a treatment and the ability of a patient to respond to a potential therapy, with a focus on NGS of formalin-fixed paraffin-embedded (FFPE) samples and the more recent usage of circulating tumor DNA (ctDNA).

Next-generation sequencing broad panel assays

Many current precision oncology clinical trials depend on customized, targeted NGS-panel assays to identify actionable targets. With customized NGS panels that range from 20 to over 500 genes, users can rapidly and reliably identify the most common genetic aberrations associated with a particular cancer type.[5] This customized panel design gives NGS-panel assays a higher depth of coverage, allowing for a lower threshold value for detecting intratumoral heterogeneity and low frequency of variant allele changes. As these are inherent to most cancer types, their detection is vital to precision oncology treatments.[6]

At Cebra Research, we are continuously adapting our oncopanels to meet the solid and liquid tumors international guidelines to ensure that we can provide our customers with the most relevant, up-to-date panels for more efficient assays and faster administration of more effective treatments.Our genomic experts are here to assist you throughout your assays and will accurately customize your panel for and/or make it more visible for you to assay.

Using our customized panels in your NGS assays, you can focus on the genes pertinent to your research and enhance the detection of actionable variants for the identification of potential druggable targets. Cebra Research provides you with the tools and expertise to examine the number of somatic mutations in your DNA sequence and to determine the number of alterations in one or more nucleotides. Having an extensive history in genomics and state-of-the-art capabilities and expertise, including robust multiplex panel assays, Cerba Research is adept at providing broad-panel assays that optimize the analysis of patient FFPE samples. Our NGS panels can detect a great variety of cancerous hallmarks, such as Indels, SNVs, and CNVs, as mentioned above. Our broad panel NGS assays can also determine the homologous recombination deficiency status (HDR), tumor mutational burden (TMB), and microsatellite instability (MSI) status from your precious, and often limited, patient samples.

As customized panels provide more efficient and sensitive data collection, the bioinformatic processes required to analyze the collected data are thus simplified and, therefore, less time-consuming, further contributing to our fast turnaround times. We have a large variety of oncopanels available across the globe, and our specialists are always on hand to help you choose which is best suited to your oncology clinical trial. If our available NGS panels meet your trial requirements, we can offer you a rapid turnaround time of 10-15 days, depen–ding on the geographic location of our laboratories. If required, we can also create a totally new NGS panel for your research and add additional genes to an existing panel. Such customization normally requires a three-month fit-for-purpose validation turnaround time. Thanks to this high level of efficiency, patients can receive faster-targeted treatment, which is essential when studying such an advanced disease with dismally low survival rates.

Customized panel assays are not only important for NGS-based oncology molecular profiling but are also key to improving the efficiency of immunohistochemistry (IHC) assays, which sometimes assist NGS assays in a reflex fashion, such as reflex PD-L1 stains. IHC assays also complement NGS assays by verifying co-expression and spatial organization in multiple targets and characterizing tumors to identify biomarkers to predict/examine a patient's response to immunotherapy. Multiplex and simplex IHC is also necessary to facilitate the move from preclinical to clinical trials as it validates therapeutic targets and helps to characterize treatment efficacy and patient selection to an appropriate treatment or clinical trial option. Together, our large range of customized NGS and IHC panels can help you provide more effective and tolerable treatment options to your patients.

Table 2 provides a deeper insight into what Cerba Research can offer in the NSCLC biomarker space, aligned with various international guidelines. As mentioned by the NCCN guidelines, "broad-based genomic testing approaches that efficiently utilize limited biopsy tissue while maximizing diagnostic genomic information are most commonly NGS-based".[2]

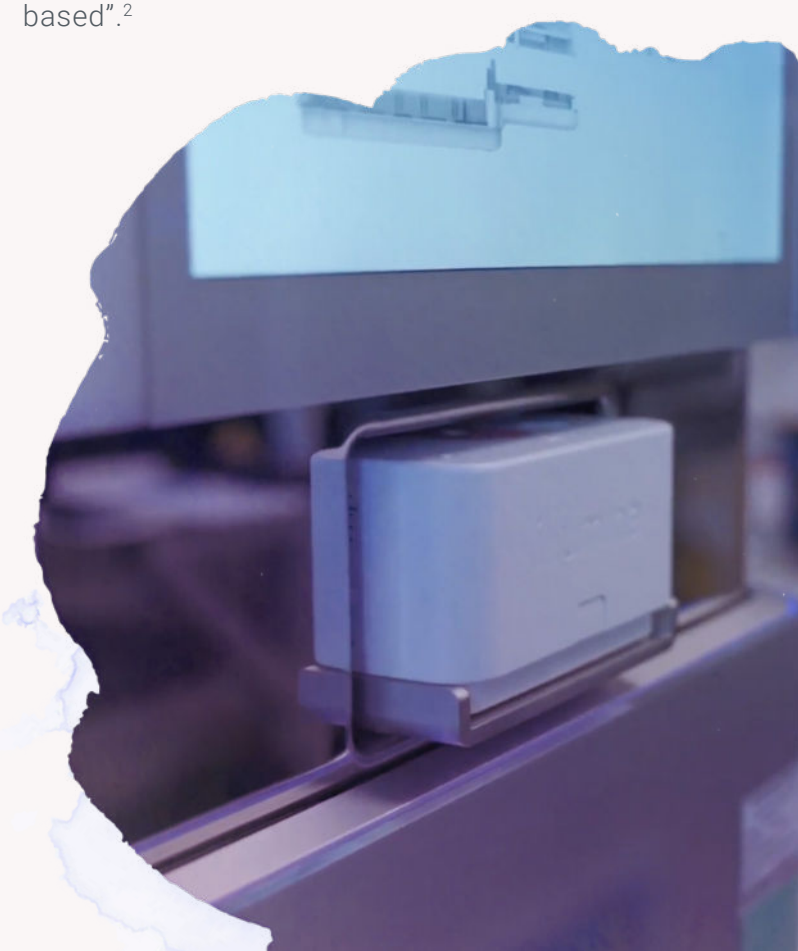

Table 2: What are NSCLC guidelines proposing? Aligned with Cerba Research NGS, IHC, and FISH capabilities

Lung Cancer Biomarker	Most commonly deployed[1-4]	Additional Assay(s)[1]	Cerba Research NGS[+]	Cerba Research IHC[+*]	Cerba Research FISH[+]
EGFR	NGS, RT-PCR	Sanger sequencing, single gene	X	X	
ALK	NGS, IHC, Liquid Biopsy	FISH (reflex), RT-PCR	X	X	X
ROS1	NGS	FISH (reflex), IHC, RT-PCR	X	X	X
BRAF	NGS, RT-PCR, Sanger sequencing	IHC	X	X	
KRAS	NGS, RT-PCR, Sanger sequencing		X	MEK1	
MET	NGS, RNA-based NGS		X	X	X
RET	NGS, RNA-based NGS	FISH, RT-PCR	X	X	X
NTRK1/2/3	NGS, RNA-based NGS	FISH, IHC, PCR	X	X	X
EGFR T790M	NGS, Liquid Biopsy		X		
PD-L1	IHC			X	
HER2	NGS	Sanger sequencing, targeted PCR	X	X	X

1.NCCN guidelines 2023; 2.Bebb et al. Curr Oncol 2021; 3.Cabillic et al. ESMO Open 2018;3(6):e419; 4.Li et al. J Nat Cancer Center 2021; †Cerba Research Data In-house mostly available through the ACTOnco®/ Cerba Paris (NGS) or Cerba Montpellier/NY (IHC) or Cerba Paris (FISH); *Validation level may vary; IHC=Immunohistochemistry; NGS=Next-generation sequencing; FISH=Fluorescent *in situ* hybridization.

Selection of NGS broad panel assays

To take full advantage of the power of NGS panels to screen numerous genetic markers simultaneously, the genes selected to be included on a panel are of paramount importance, and our experts are here to guide you with this choice. When conducting routine diagnostic assays of samples, predesigned panels with different genes that have an established predictive and/or prognostic significance which enable flexible examination of tumors from different genetic origins, are advised.[7] The customization of NGS panels is also necessary for extracting the correct information on the molecular profile of cancerous tumors to accurately assess the therapeutic value of a compound and a patient's ability to respond to treatment. Thanks to our large bioinformatic database, Cerba Research can offer a wide variety of already existing panels on a global scale that have been validated in diagnostic laboratories and then implemented in a clinical and routine setting, thus providing you with data of high clinical value.

The NSCLC precision treatment trials are a pertinent example of the importance of creating customized panels. According to guidelines, a minimum of 20 genes should be examined when conducting molecular screening for therapeutic trials in NSCLC treatment, including *EGFR, BRAF, HER2, KRAS, PI3KCA, NTKR, ALK, MET, AKT1, BRCA1/ BRCA2, HRAS, NRAS, ROSI, RET, MET, FGFR1/2/3,* and *NOTCH1/NOTCH2*.[2,8] NCCN also acknowledges "that many currently available NGS-based assays used to fully genotype NSCLC are larger than the 50-gene limit threshold."[2] Cebra Research can offer you customized OncoSign panels for these valuable NSCLC genes and our experts will advise you on the best panel customization for efficient and precise NSCLC tissue assays, to ensure that the most appropriate treatment can be provided as fast as possible.

You can now also benefit from our extra-large Cerba OncoSign 600+, which is capable of detecting 638 DNA-based genes, including 20 fusion genes, that cover known mutations with established, emerging and exploratory value in lung, ovarian, breast, colon, melanoma, bladder, GIST, rare tumors and more. Along with this, we can also determine the MSI, TMB, and HRD status that are often requested for various tumors such as breast (TMB, MSI), ovarian (HRD, MSI, TMB), and prostate cancer (TMB).[2] With our customized broad-based panels, we provide efficient, fast, and affordable NGS assays for state-of-the-art precision oncology technologies. See below some of our available comprehensive oncopanels.

Examples of our available oncopanels

Cerba Paris:

Cerba OncoSign 600+

- 658 genes
- 20 RNA (fusions)
- TMB, MSI, and HRD
- Analysis of significant cancer hallmarks on FFPE tissue

Cerba OncoSign

- 59 genes
- 42 DNA
- 17 RNA (fusions)
- 15 microsatellites
- HRD status (CE-IVD marked available in a separate panel)
- Analysis of significant cancer hallmarks in FFPE tissue

Cerba OncoSign ctDNA

- 42 DNA
- 15 microsatellites
- Analysis of significant cancer hallmarks on ctDNA (liquid biopsies)

Alternatively, we can create tailor-made panels customized explicitly to your oncology research and/or add relevant genes to an already existing broad panel assay to fulfill any study requirements. The most practical approach in creating these panels is to use traditional assays, such as Sanger sequencing or pyrosequencing, to screen for single genes to develop these novel markers.[7] These novel markers are then added to the panel and revalidated for implementation with minimized redesigning and revalidation of the panel.

Primer or probe multiplexing has been found to increase as new genetic markers are added to panels because target capture techniques are either multiplexed primer- or probe-based, possibly reducing the overall performance of conventional panel assays.[7] Cebra Research assays account for these issues, and the formation of primer dimers is reduced by masking regions in which primers cannot bind when making the primer design. We have the tools, data, and expertise to successfully create customized panels with your gene(s) of interest with a real-world validation turnaround time of about 3 months, allowing for more rapid development and implementation within clinical trial operations.

The future is here with circulating tumor DNA (ctDNA)

Biopsy tissue from solid tumors is often limited, requiring an invasive procedure, and on occasion, it cannot even be garnered, with a fifth of our patients usually not having enough tumor tissue to perform NGS.[9] Circulating cell-free tumor DNA (ctDNA), also referred to as 'liquid biopsies,' offers a much less invasive, lower risk, less painful and easier method of sampling for NGS. ctDNAs are released into the bloodstream during the apoptosis or necrosis of tumor cells and so can be easily extracted by taking patient blood samples.[10] With a vast array of expertise in genomic assays, Cerba Research can offer a range of liquid biopsy assays from our already existing ctDNA-based panels, customize a new panel for you, or add a new gene to an existing panel that is specific to your trial.

Below are just some of the ctDNA panels that we currently offer, alongside their instruments and gene numbers (where applicable).

- Cerba OncoSign ctDNA (Illumina, 59 genes)
- Cobas® EGFR mutation test (Roche Cobas)
- EGFR exons 18-21 (Illumina)
- Rapid EGFR (42 variants/PCR)
- EGFR, KRAS, BRAF, HER2, MET, STK11 & KEP1 panel (Illumina)
- TSO500 ctDNA (Illumina)
- ACTMonitor® + (Ion Torrent, 50 genes)
- ACTMonitor® Lung (Ion Torrent, 11 genes)
- ACTMonitor® Colon (Ion Torrent, 13 genes)
- ACTMonitor® Breast (Ion Torrent, 8 genes)

Conclusion

With an extensive portfolio of broad-panel assays produced from both FFPE samples and ctDNA, and the option to customize your own panel, Cerba Research offers state-of-the-art, efficient, cost-effective assays, along with medical-grade reports for clinical trials, hopefully guiding your precision oncology trials towards more effective and more tolerable treatment options. Data generated from our customized panels are advantageous to clinical trials compared to 'off-the-shelf' panels because they allow users to focus on genes pertinent to their research, providing more clinically valuable data that is likely to improve patient care. Thanks to our NGS capabilities and expertise in genomics, our large array of oncopanels, and our access to large bioinformatic databases worldwide, Cerba Research has you covered when it comes to im–proving patient outcomes with precision on–cology and our reliable experts are always on hand to assist you through your clinical trials. Also, always remember that, if we do not currently have it, we can customize it.

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