MEDPACE

MOLECULAR GENOMICS PROFILING AND THE FUTURE OF CLINICAL TRIALS

A Question & Answer with El Mustapha Bahassi, PhD, Director, Medpace Labs, US.

Dr. Bahassi has over 20 years of clinical laboratory research experience specialized in molecular genomics.

KEY TAKEAWAYS:

Precision medicine is becoming a reality with genomic biomarkers that help answer questions about which therapy to choose, who should be given treatment and what dose of drug to use

Quantitative polymerase chain reaction (qPCR), Next Generation Sequencing and Sanger sequencing are specific techniques to support these genomic biomarkers

Genomic data drives patient enrollment in umbrella and basket trials

There are numerous challenges associated with implementing molecular genomics testing in clinical trials. The chosen lab must have rigorous quality control, costly sequencing platforms, experienced lab technicians, well-established data analysis pipelines and an efficient bioinformatics system that assists in the translation of knowledge from the bench towards molecular targeting and diagnosis

1. What are potential applications of genomic biomarkers in clinical trials?

Genomic biomarkers can be classified into three categories: predictive, prognostic and pharmacogenetic. They provide decision-making support for answering questions about which therapy to choose, who should be given treatment and what dose of drug to use.

Predictive biomarkers: Predictive biomarkers identify patients who are likely or unlikely to benefit from a specific treatment.

For example, HER2 amplification is a predictive classifier for benefit from trastuzumab and perhaps also from doxorubicin and paclitaxel. The presence of a mutation in epidermal growth factor receptor (EGFR) may be a predictive marker for response to EGFR inhibitors. A predictive biomarker may also be used to identify patients who are poor candidates for a particular drug; for example, colorectal cancer patients whose tumors have KRAS mutations may be poor candidates for treatment with EGFR inhibitors.

Prognostic biomarkers: Prognostic biomarkers enable the monitoring of the advances of anticancer therapy, the assessment of the stage of the tumor and its potential malignancy, as well as the prognosis of disease remission in every case individually.

Prognostic biomarkers are assigned to a specific tumor type by determining the occurring polymorphism, mutation, the change in DNA methylation or gene expression, or by detecting the presence of specific microRNA (miRNA) molecules or Circulating Tumor Cells (CTCs) in the peripheral blood. Amongst the most consequential mutations are mutations within the genes whose products participate in DNA repair, such as BRCA1, BRCA2, ATM and P53. Mutations in these genes predispose the patients to an increased risk of developing breast cancer.

Pharmacogenetic biomarkers: Pharmacogenetic biomarkers refer to polymorphisms in genes encoding proteins involved in drug pharmacokinetics and pharmacodynamics or immunological responses that control the inter-individual variability in drug efficacy and risk of adverse reactions.

Pharmacogenetic research has identified a multitude of gene-drug response associations, which have resulted in genetically guided treatment and dosing decisions to yield a higher success rate of pharmacological treatment. Enzymes encoded by the cytochrome P450 (CYP) superfamily of genes are responsible for > 75% of phase 1 drug metabolism and thus constitute major modulators of drug response. Importantly, CYP genes are highly polymorphic between individuals and across populations, which can have important implications for the bioactivation and/or detoxification of medications. It is helpful to distinguish between (i) germline biomarkers, which can influence systemic drug pharmacokinetics and pharmacodynamics and (ii) biomarkers in the somatic cancer genome, which modulate how cancer cells respond to drugs. Besides genetic factors, epigenetic modifications of DNA or histones have been linked to differences in drug response.

2. What genomic techniques are used to support the predictive, prognostic and pharmacogenetic biomarkers in clinical trials?

Molecular laboratories use a diverse array of techniques to support these genomic biomarkers in clinical trials. These include quantitative polymerase chain reaction (qPCR), Next Generation Sequencing and Sanger sequencing.

Quantitative polymerase chain reaction: qPCR is usually used when only one or few single nucleotide polymorphisms (SNPs) or small deletions or duplications are being targeted. Primer oligos and probes specific to these known mutations are used for PCR amplification and detection.

A classic example is to detect the presence of mutations in EGFR gene in non-small cell lung cancer (NSCLC) tumor cells to help determine whether a patient may benefit from targeted therapy with tyrosine kinase inhibitors (TKIs) such as gefitinib or erlotinib.

Additionally, testing may be used after treatment is initiated to determine whether the cancer has acquired new mutations that make it resistant to the current treatment. For example, one of the known resistance mutations, specifically T790M, may be detected by qPCR and can then be targeted with a different drug that has been designed especially for this mutation. The FDA-approved cobas EGFR Mutation Test v2 from Roche, a real-time PCR test that identifies 42 mutations in exons 18, 19, 20 and 21 of EGFR gene, including the T790M resistance mutation.

Other examples include $BRAF^{V600E}$ mutation in melanoma as a predictor of response to vemurafenib, and KRAS mutations in colorectal cancer to predict resistance to anti–EGFR monoclonal antibodies.

Next generation sequencing (NGS): The power of NGS is its ability to detect multiple types of genomic alterations, including nucleotide substitutions, small insertions, and deletions, copy number variations and chromosomal rearrangements.

There are two main types of experimental approaches for genomic DNA sequencing — whole-genome and targeted sequencing.

Whole-genome sequencing, as its name implies, provides a comprehensive characterization of the entire genome. An alternative strategy is targeted sequencing.

Targeted sequencing can refer to either panel sequencing (a small panel of a few genes or a large panel made of hundreds of genes) or exome sequencing also known as whole exome sequencing (WES). WES is a genomic technique for sequencing all the protein-coding regions of genes in a genome. This approach has the advantage of providing increased depth of coverage while generating information quickly and cheaply.

The use of small gene panels is ideal when the mutations that are targeted are known in advance. Targeting few genes or few exons within genes allows greater sequencing read depth which is extremely important especially when dealing with somatic mutations at very low allele frequency. When the mutations are not known, the use of exome sequencing will allow detection of mutations in all genes while achieving a still good level of read depth that will allow identification of mutations that are present at low allele frequency. Both gene panels and exome sequencing will also detect copy number variations.

Whole genome sequencing (WGS) allows better detection of structural variations such as large deletions and insertions as well as variations in the intronic sequences but analyzing the large amounts of produced data represents a real challenge. WGS also does not achieve the read depth needed to detect somatic mutations that are present at a low allele frequency.

Another NGS technique used to interrogate the whole transcriptome is RNA-seq (RNA-sequencing). RNA-seq is a technique that can examine the quantity and sequences of RNA in a sample. It analyzes the transcriptome and allows determination of differential expression of genes. This differential expression can be used as a prognostic biomarker to monitor therapy and follow disease progression.

Sanger sequencing: Sanger sequencing is a quick method (and less expensive than NGS) but has the disadvantage of low throughput and low sensitivity towards low-frequency mutations. This is because Sanger sequencing relies on relative peak heights at a given position when determining a nucleotide base call. A minor allele will likely have a low signal-to-noise ratio that is indistinguishable from the background. This will be particularly problematic for cancer genomic tests since cancer cells are heterogeneous in nature and many clinically relevant changes or mutations may only be present in a fraction of the cells.

GENOMIC TECHNIQUES AT MEDPACE

Our state-of-the-art laboratories in the US and Belgium are designed with engineering and procedural controls to minimize risk of reagent and sample contamination including dedicated suites with private access, directional airflow and pressure control, environmental monitoring systems and the most up-to-date automated instruments.

Both laboratories use the same cross-validated methods based on guidelines from the Clinical and Laboratory Standards Institute (CLSI) and in accordance with CAP and CLIA regulation allowing harmonization of assays with global platforms, calibrators, QC, methodology, SOPs and reference ranges.

Medpace is an Illumina-based NGS laboratory with several sequencing instruments including Mi-Seq and Next-Seq. For Sanger sequencing, fragment analysis and MLPA, Medpace uses the Applied Biosystems SeqStudio and 3500 XL Genetic Analyzers. For qPCR, Medpace laboratories are equipped with several automated “sample-to-report” platforms that allow nucleic acid extraction, mixing of PCR reactions and PCR amplification with minimal operator involvement reducing any chances for errors or contamination.

We use Sanger sequencing to detect mainly germline mutations. Other techniques used at Medpace that rely on capillary electrophoresis (the same technique used for Sanger sequencing) include fragment analysis that is used for detection of small repeats and Multiplex ligation-dependent probe amplification (MLPA) used to detect copy number variations.

Learn more about our capabilities or take a virtual tour of our laboratories, including the molecular and genomics suite.

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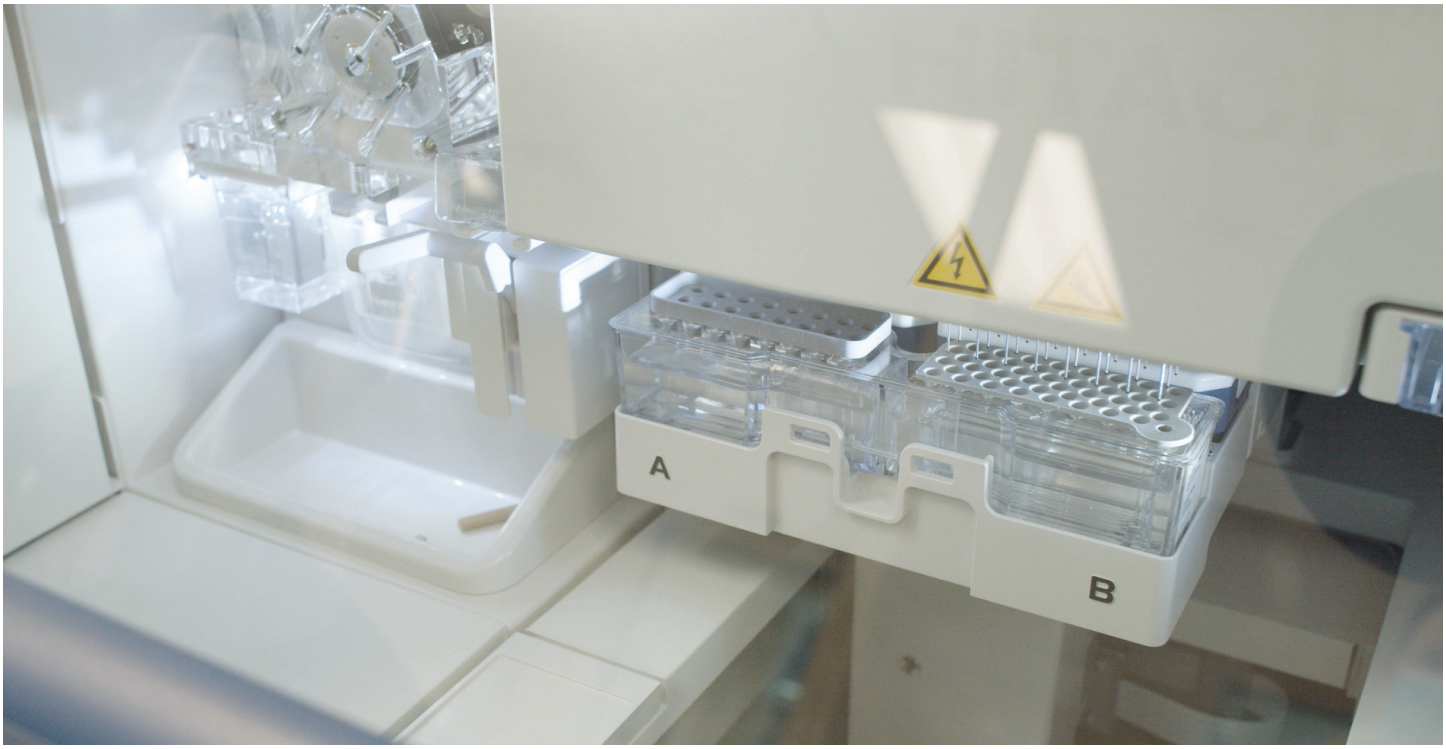

3. What are Umbrella trials and Basket trials and how is genomic data used in patient enrollment?

There are several types of clinical trials. Oncology trials include treatment trials, prevention trials, screening trials, supportive/palliative care trials, and natural history studies. In contrast to traditional trial designs, where a single drug is tested in a single disease population in one clinical trial, the FDA recommends efficient clinical trial design strategies to expedite the development of oncology drugs and biologics. Such modern trial designs include basket (bucket), umbrella and platform trials. They use a single infrastructure, trial design, and protocol to simultaneously evaluate multiple drugs and/or disease populations in multiple sub-studies, allowing for efficient and accelerated drug development.

Umbrella trials: In umbrella trials, patients are enrolled into distinct treatment groups (arms) based on the molecular profile of their tumor; in this way a new drug targeting the particular molecular process within each disease sub-type can be tested.

This type of trial is beneficial for cancer types known to be driven by many molecular abnormalities (e.g. breast cancer that is driven by Estrogen Receptor, Progesterone Receptor and Her-2.) Although, the patients have the same type of cancer (breast cancer), they are put in different subgroupings and treated with different therapies.

Basket trials: In this trial design the focus is on the molecular abnormality/mutation or biomarker that the tumor carries regardless of the tissue where the cancer originated. The trial enrolls patients into a single arm of treatment targeted at the molecular abnormality in question.

This design is beneficial where the molecular abnormality driving the cancer is found in many cancer types, but it is not highly common within one cancer type. For example, the anti-PD1 drug pembrolizumab was found to work in cancers with high microsatellite instability (a predisposition to have a high mutation rate due to defects in DNA mismatch repair) or high tumor mutation burden (TMB) across many indications. At Medpace, defect in Mismatch repair and TMB can be derived from exome sequencing data.

4. What are the challenges of implementing molecular genomics testing in clinical trials?

Although NGS technology is a powerful tool, it is also new and not yet standardized among clinical laboratories. Different sample collection and processing methods, sequencing chemistries, instruments, protocols, and data analysis methods can affect NGS assay results. Therefore, a robust, standardized, and reproducible assay that is compliant with federal regulations must be fully validated for use in clinical trials.

Furthermore, clinical molecular diagnostics laboratories in the US must be accredited through the Clinical Laboratory Improvement Amendments (CLIA) to be able to offer complex testing such as NGS. The analytical performance of a genomic test including analytical sensitivity, specificity, reproducibility, and limit of detection of the assay must be established before it can be used in support of precision medicine trials.

Nucleic acid used in NGS can be extracted from a broad range of sample types, from cell lines to fresh tissue, formalin-fixed paraffin-embedded (FFPE) samples, blood, and other challenging sample types which will require multiple validations for the multiple sample collection and processing methods.

For example, in oncology trials, nucleic acid isolation is done mostly from fixed core biopsy tissue embedded in paraffin blocks. Quality control and quality assurance evaluation is performed by a pathologist using a section cut from the block and stained with hematoxylin and eosin (H&E). The isolated DNA is then processed into libraries. Library preparation allows DNA or RNA to adhere to the sequencing flow cell and allows the sample to be identified.

The core steps in preparing RNA or DNA for NGS analysis are:

I. Fragmenting and/or sizing the target sequences to a desired length
II. Converting target to double-stranded DNA
III. Attaching oligonucleotide adapters to the ends of target fragments
IV. Quantitating the final library product for sequencing

These libraries are then loaded on the sequencing instrument. To ensure the quality of results, thresholds are usually set for library yields, number of DNA reads, number of RNA reads, RNA read length, uniformity, and percentage of amplicons with at least 100× coverage.

Once sequencing is complete, raw data is ready for data analysis by a bioinformatics scientist who uses computational pipelines to identify reliably genomic alterations and mutations from the molecular profiles of each patient. After rigorous quality control, a meaningful report is delivered to the clinicians and biologists for the therapeutic decision.

All these steps and processes require costly sequencing platforms, experienced NGS lab technicians, well-established data analysis pipelines and an efficient bioinformatics system that assists in the translation of knowledge from the bench towards molecular targeting and diagnosis. These resources are usually not accessible to all institutions which makes routine implementation of NGS in clinical trials a challenging task.

SUMMARY OF KEY CHALLENGES & CONSIDERATIONS FOR NEXT GENERATION SEQUENCING

- It is a new technology - ensure your lab can provide a standardized and reproducible assay that is fully compliant with regulations
- US labs must be CLIA accredited
- The analytical performance of a genomic test must be established before it can be used in support of precision medicine trials
- Extracted nucleic acid requires multiple validations for the multiple sample collection and processing methods
- NGS requires rigorous quality control, costly sequencing platforms, experienced lab technicians, well-established data analysis pipelines and an efficient bioinformatics system that assists in the translation of knowledge from the bench towards molecular targeting and diagnosis

Medpace Capabilities in Molecular Genomics Testing	
DNA Extraction	Concentration, purity, yield. Both manual and automated from different sample types
RNA Extraction	Concentration, integrity, yield. Both manual and automated from different sample types
PBMC Processing	Cell count and cell viability. Using CPT tubes or regular anticoagulant tubes
Companion Diagnostics	Using qPCR, Sanger sequencing or NGS
Next-Generation Sequencing	• B cell/T cell receptor repertoire profiling • Small and large gene panels (Familial Hypercholesterolemia, Myeloid panel, DNA Damage Repair, etc) • Pharmacogenomics (PGx) • Exome sequencing • Whole genome sequencing • Germline and somatic mutations for all disease types • Liquid Biopsies (Cell free DNA by NGS or digital PCR) • Micro Satellite Instability (MSI) and Mismatch Repair (MR) • Minimal Residual Disease (MRD) • RNAseq gene signatures • Tumor mutation burden for Immunotherapy studies • Gut Microbiome and Metagenomic Sequencing (Shotgun and 16S ribosomal RNA sequencing) • Bioinformatics data analysis
Sanger Sequencing	• Single gene sequencing or single mutation Genotyping • Fragment Analysis • Germline mutations for all disease types • MLPA for gene copy number determination • NGS validation
Recombination Competent Retrovirus (RCR), Recombination Competent Lentivirus (RCL) and Viral Infectivity in support of:	• CAR-T cell therapy studies • Gene therapy studies • TCR-based therapies
Viral Testing by qPCR	• CAR-T cell therapy studies • Gene therapy studies • Organ and HSC transplant studies • Viral shedding • Viral loads in multiple sample types including serum, plasma, PBMC, stool, CSF, urine

MEDPACE LABS – HOW WE CAN PARTNER

Medpace is rapidly establishing itself as a genomics testing hub across indications and therapeutic areas. We have invested in new NGS technologies (Illumina MiSeq and NextSeq platforms), in building strong bioinformatics teams, scaling up throughput, and automating data processing/interpretation to support panel, exome, and whole genome level studies. Variant calling is based on automated GATK pipelines and variant interpretation is done in accordance with the ACMG and AMP guidelines. These scalable, more efficient, and automated data processing pipelines are incorporated into the Medpace Integrated Genomics System (MIGS) which allows better management, storage, and integration of all the sequencing data.

FULL-SERVICE CLINICAL DEVELOPMENT

Medpace is a scientifically-driven, global, full-service clinical contract research organization (CRO) providing Phase I-IV clinical development services to the biotechnology, pharmaceutical and medical device industries. Medpace's mission is to accelerate the global development of safe and effective medical therapeutics through its high-science and disciplined operating approach that leverages local regulatory and deep therapeutic expertise across all major areas including oncology, cardiology, metabolic disease, endocrinology, central nervous system and anti-viral and anti-infective.