Genomic Testing Simplified

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Summary

One or more genetic abnormalities, either acquired or inborn, combine to cause cancers. Almost all cancers have a distinct set of molecular alterations. Technologies have been created to investigate tumors and identify genomic traits that will eventually affect therapeutic treatment.In fact, the discovery of important genetic abnormalities (molecular drivers) may eventually result in the creation of highly focused treatments that significantly improve patient outcomes. Cancers will soon be routinely characterized due to the increasing availability of newer, more potent, and more affordable technologies like next-generation sequencing, multiplex mutational screening, array-based methods that can determine gene copy numbers, methylation, expression, and others, as well as more advanced interpretation of high-throughput molecular data using bioinformatics tools like signatures and predictive algorithms. Over the past ten years, next-generation sequencing (NGS) has been used more frequently in cancer genomics research due to advancements in modern sequencing technologies. In order to improve individualized cancer treatment, NGS has recently been used in clinical oncology. The molecular justification for suitable targeted therapy is provided by NGS, which is also used to identify novel and uncommon cancer mutations and identify carriers of familial cancer mutations. Clinician has to choose wisely as the testing is available to more number of patients.

Reading, interpretation, and therapeutic application of test results is complex and needs expertise.

Introduction

Cancer treatment journey of a sufferer trails through twists and turns as it goes by systemic anti-cancer treatment by medical oncologist. This happens mainly because of rapid advancement in the field of diagnostics and treatment. The ancient era of broad spectrum chemotherapy is replaced by individualised treatment driven by the tumor's genetic makeup.

With the launch of genetic testing under AB PM JAY, our desire to test for genomics is fulfilled. But, we are yet to understand worth of that. We have started doing NGS on FFPE blocks for selected malignancies.

What and why genomic testing? Is it useful in our patient population?

Molecular methods are classified into two types: chromosomal structural alterations anomalies and other alterations

Cytogenetic methods detect abnormalities in chromosome structure:

I. Karyotyping

- It is a basic test to know about congenital anomalies.
- Used to identify gain and loss of chromosomes in several malignancies
- Example
- In colorectal cancers, gain of chromosomes 7, 8q, 13, 20 and loss of 8p, 17p, 18 is seen
- In breast cancers, gain of chromosomes1q, 8q, 17q, 20 and loss of 8p, 16q, 17p is seen

II. Fluorescence in situ hybridization (FISH)

- Mainly evaluates patients with known /pathogenic translocations and fusions
- Example is commonly found translocation t (9;22) or BCR-ABL fusion gene for patients with Chronic Myeloid Leukaemia (CML)

III. Chromosomal microarrays analysis (CMA)

- Can detect much smaller chromosomal abnormalities than karyotyping and FISH
- Provides First line analysis for developmental delay, intellectual disability, congenital defects.
- Limitation: will not pick up balanced translocations
- Examples - losses of 1p in mantle cell lymphomas and diffused large B cell lymphomas, Gain of 1q or loss of chromosome 13 in multiple myeloma.

IV. Quantitative fluorescence PCR (QF-PCR)

- It Is useful to find the pathogenic variant which is already known like it is present in parents/siblings or children
- Provides Information on quantitative data and not just qualitative aspect
- It does not report structural alteration in chromosomes which is a limiting aspect of the test

Molecular methods

Molecular methods detect abnormalities in DNA sequence.

Molecular testing is basically classified into 3 categories

I. Polymerase chain reaction (PCR)
II. Ligation mediated PCR e.g. MLPA
III. Next Generation Sequencing (NGS)

I. Polymerase chain reaction (PCR)

- Here amplification or multiple copies of known sequence/part of DNA is made.
- That sequence DNA is denatured to form single strands.
- Forward (PF) and reverse (PR) primers anneal.
- Further processing step results into amplified/copies of the original target of interest.
- Examples - Microsatellite instability testing in HNPCC-related genes; BRCA1/2 mutations testing by PCR
- Examples of PCR sequence based tests are: A) Targeted and single gene testing and B) Sequence Chromatogram

II. Multiplex Ligation-dependent probe Amplification (MLPA)

- Can detect small rearrangements
- Limitation - Cannot detect copy neutral loss of heterozygosity
- Example - Used for detecting exon deletions in BRCA1, MSH2, MLH1 in hereditary breast and colon cancer

III. Gene Sequencing Technologies

Types are

a) Sanger sequencing and b) Next-generation sequencing (NGS)

IV. Sanger sequencing

- Gold standard along with MLPA
- High accuracy but labour intensive
- Detects only small DNA changes (point mutations and/or indels)
- Limitations - Longer turnaround time and Limited throughput
- Examples –
- BRAF exon 15 containing the p.V600 hotspot mutation in varieties of CNS tumours like gliomas and glioblastoma multiformes
- TERT promoter region containing the c.-124C and c.-146C hotspots that are frequently mutated in IDH-wildtype glioblastoma in adults, IDH-mutant and 1p/19-codeleted oligodendroglioma, anaplastic (malignant) meningioma

V. Next-Generation Sequencing (NGS)

- Vast improvement with accuracy
- Shorter turnaround time
- High throughput
- Requires complex bioinformatics algorithms
- Has potential to detect LGRs
- Examples –
- NGS can help identify ROS-1 mutations in adenocarcinoma of lung which in turn can be treated by giving drugs like crizotinib.
- AKT3 amplificatons can also be detected by NGS in cases of lung cancer wherein drugs like everolimus can help

How to read Next Generation Sequencing (NGS)

NGS can accurately detect all the 4 types of genetic alterations which drive oncogenesis, namely:

1- Base substitution
2- Insertions and deletions
3- Copy Number Alterations
4- Re-arrangements (gene fusions)

NGS testing broads can be broadly divided into two types:

1- Hot spot panels
2- Comprehensive Genomic Profiling (CGP)

Hot spot approach- sequences only selected regions of a gene CGP- sequences coding regions of selected genes in their entirety.

Components of a CGP report

1- Patient Demographics: patient's name, DOB, Sex, ID number
2- Date of specimen collected, date of receiving, date of reporting results
3- Ordering physician's name
4- Specimen details – type (FFPE, Liquid biopsy), tissue information with diagnosis and tumor cell content.
5- Results:biomarker findings (MSI and TMB) and genomic findings (results along with variant allele frequency (VAF))
6- Range of the genes tested and coverage of testing
7- Sequencing depth
8- Methods and the steps of process involves the presence of target and its details, specimen enrichment method, limitation of detection of particular genetic alteration and other pitfalls of testing
9- Analytic interpretative comment
10- Clinical interpretative comment including approved therapies, level of evidence and clinical trial options based on genomic finding.
11- Pathologist / designee signature.

The classifications and scale used for interpretation of genetic variant detected:
ESCAT grading system for cancer treatment decision-making:

	Readiness for use in clinical practice	**Current examples of genomic alterations**
Tier (-A, -B, -C)	Targets ready for implementation in routine clinical decisions	HER2 in breast cancer BRCA1/2 in ovarian and breast cancer EGFR, ROS/ALK in NSCLC TRK, PD1 in multiple cancers BRAF in metastatic melanoma
Tier (-A, -B)	Investigational targets likely to define patients who benefit from a targeted drug, but additional data needed	PTEN pathway (PIK3CA, AKT1)
Tier (-A, -B)	Clinical benefit previously demonstrated in other tumour type or of similar molecular targets	BRAF in non-melanoma cancers PALB2 and other non-BRCA DNA repair mutations
Tier (A, B)	Preclinical evidence of actionability	Hypothetical targets for future clinical testing
Tier	Evidence supporting co-targeting approaches	P1K3CA in ER+, HER- breast cancer
Tier	Lack of evidence for actionability	

NSCLC = non-small cell lung cancer

ACMP/AMP tier system (American College of Medical Genetics and Genomics / Association for Molecular Pathology):
5- Pathogenic
4- Likely Pathogenic
3- Variant of Uncertain Significance (VUS)
2- Likely Benign
1- Benign

AMP 4 tiered system:
Tier 1- Variants with strong clinical significance
Tier 2- Variants with potential clinical significance
Tier 3- Variants of unknown clinical significance
Tier 4- Variants deemed benign or likely benign

Benign and likely benign variants:
1- population frequency > disease prevalence
2- No impact on amino acid sequence
3- Changes amino acid at poorly conserved position
4- Inheritance not supportive of a disease causing role

VUS: Not enough information available

Likely Pathogenic and pathogenic:
1- Rare
2- Severe protein impact
3- Reported in other individuals with consistent phenotype
4- Segregates with disease in families
5- De novo occurrence
6- Functional studies supportive of an impact

Basics of the NGS testing which needs to be checked before attempting to interpret genomic alterations

1- Sample: tumor cell content of the samples – the results may be falsely interpreted as range of detection for variety of genetic alteration varies with various assays. So, it is true that low tumor content results in false negativity for the genetic alteration in concern.
2- Assay Validation: before interpretation of particular genetic assay, assess for accurateness, preciseness, biological reference range and any restrictions of reportingetc One should also look how sensitive and specific is the analysis.Clinician ordering the test should know the validation of the requested array is done by the performing laboratory or not. The laboratory should also be doing regular and mandatory quality check and Q/A programmes.
3- Sequencing Depth versus Sequencing Coverage: Read depth indicates how many reads detected a specific nucleotide. Low depth means poor representation. Coverage is the percentage of bases covered by sequencing reads eg. 95% coverage means that 95% of the bases in sample have been sequenced (at depth 'n'). Good coverage is required for accurate variant calling.

Optimum depth is not defined but on an average it is taken as > x30.

NGS sequencing data is only reliable when supported by a sufficient number of reads.

As amount of DNA being sequenced increases, coverage will be sacrificed (decreased) .

Molecular insights of NGS/CGP reports
An assessment on following 4 levels is desired:
1- gene
2- specific variant

Databases with genomic data and where to check for relevance of alterations	
Database	**Comments**
Cancer Genome Atlas (TCGA)	Large databases including cancer-associated genomic alterations of >20000 cancer patients
International Cancer Genome Consortium (ICGC)	Global initiative to build a large database of genomic alterations in the most common tumor types
OncoKB	Memorial Sloan Kettering Cancer Centre precision oncology database including link to FDA levels of evidence
MyCancerGenome	Large database including cancer-associated genomic alterations of almost 100000 tumor samples
CIViC	Clinical interpretation of variants in cancer, open access open source, community driven
COSMIC	Large catalogue of somatic cancer mutations including data from >37000 genomes
ClinVar	Freely accessible, public archive of reports of the relationships among human variations and phenotypes, with supporting evidence
Online Mendelian Inheritance in Man (OMIM)	Comprehensive, authoritative compendium of human genes and genetic phenotypes
VarSome	Variant knowledge community, data aggregator and variant data discovery tool
Breast CncerInformatiomn Core (BIC) Database	Large BRCA1 and BRCA2 gene mutation database
ARUP BRCA1 AND BRCA2 mutation databases	Provides information on BRCA1 and BRCA2 gene mutations and their impact on risk of developing breast cancer, ovarian cancer and certain other cancers. Two types of databases are provided. One is a list of mutations curated from critical review of literature and family studies. The other provides in silico prediction of risk to help understand variants of unknown significance

3- sensitivity or resistance to a drug or group of drugs
4- tumor specific context

Detailed description of each genetic variant identified in tumor: type of alteration, exon position, transcript ID and Variant Allele Frequency (VAF)%.

VAF%:

- Gives the % of DNA that has the variant in the tissue. It identifies driver mutations and gives insights into various clones in the tumor.
- A report containing various mutations and amplifications with different VAF% represents multi-clonality of tumor.
- VAF% closer to 50% points towards the variant to be potential germline variant.
- VAF% also gives an idea regarding driver mutation (more VAF- likely to be the driver).
- Cut-off value of VAF% - when is it too high to be pathogenic? It depends on a number of factors : how common is the disease (prevalence), inheritance pattern, penetrance, how many genes or variants cause the disease.

Report also provides us description of studies and clinical trials giving insights to targeted therapy for the specific variant.

It also provides details on prognosis and frequency of the variant in cancer.

Mutations that are not targetable are also important as they can be helpful in finding which therapy might not be useful. (example- CTNNB1 (B-catenin) gene mutation – resistance to immune checkpoint inhibitor).

Important area to consider-

1- prioritization of targeted treatment options in case more than one actionable genomic alteration exists.
2- identification of the most promising targeted anticancer treatment when considering standard of care systemic treatment options as an alternative

Conclusion

Though genetic testing is now available to the patients, physician should consider it for the patients who are eligible for targeted treatment or when useful for diagnosis.

The test reports open up the Pandora's box with plethora of information and interpreting and applying in the right way is very important.

References

1. Genetic Alliance; The New York-Mid-Atlantic Consortium for Genetic and Newborn Screening Services. Understanding Genetics: A New York, Mid-Atlantic Guide for Patients and Health

Professionals. Washington (DC): Genetic Alliance; 2009 Jul 8. APPENDIX I, GENETIC TESTING METHODOLOGIES. Available from: https://www.ncbi.nlm.nih.gov/books/NBK115548/

2. Wan TS. Cancer cytogenetics: methodology revisited. Ann Lab Med. 2014 Nov;34(6):413-25. doi: 10.3343/alm.2014.34.6.413. Epub 2014 Oct 28. PMID: 25368816; PMCID: PMC4215412.
3. Chrzanowska NM, Kowalewski J, Lewandowska MA. Use of Fluorescence In Situ Hybridization (FISH) in Diagnosis and Tailored Therapies in Solid Tumors. Molecules. 2020 Apr 17;25(8):1864. doi: 10.3390/molecules25081864. PMID: 32316657; PMCID: PMC7221545.
4. Homig-Holzel C, Savola S. Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. DiagnMolPathol. 2012 Dec;21(4):189-206. doi: 10.1097/PDM.0b013e3182595516. PMID: 23111197.
5. Guan YF, Li GR, Wang RJ, Yi YT, Yang L, Jiang D, Zhang XP, Peng Y. Application of next-generation sequencing in clinical oncology to advance personalized treatment of cancer. Chin J Cancer. 2012 Oct;31(10):463-70. doi: 10.5732/cjc.012.10216. Epub 2012 Sep 17. PMID: 22980418; PMCID: PMC3777453.
6. Personalized Cancer Therapy (https://pct.mdanderson.org)
7. OncoKB (https://www.oncokb. org).
8. ESMO Open: How to read a next-generation sequencing report: what oncologists need to know y.J Cases ObstetGynecol 2016; 3:50-52