Circulating Tumour DNA in Oncology

Subtitle: Liquid Biopsy - Detection of somatic gene variants in plasma from cancer patients

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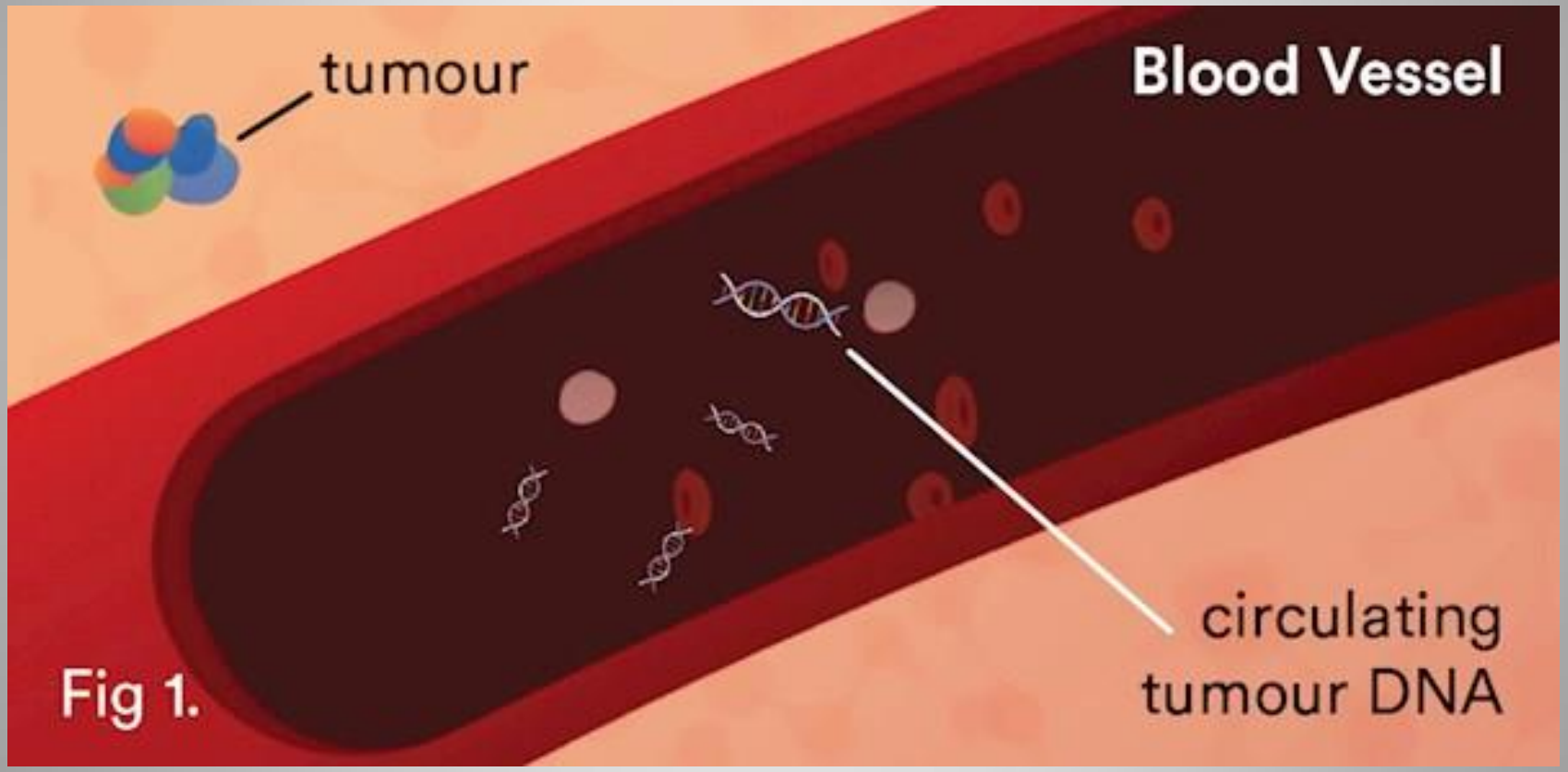
OUTLINE

- What is liquid biopsy?
- What are the immediate considerations?
- When to use liquid biopsy?
- How is liquid biopsy performed?
- What is the laboratory process?
- Interpretation of the result.
- What are the pros and cons?

Simply put…



What is Liquid Biopsy

- In simple terms, Liquid Biopsy is a minimally-invasive method to analyse the presence of circulating tumour cells or tumour-derived DNA (circulating tumour DNA [ctDNA]) in blood;
- More specifically, the detection in blood (or other sources) of gene variants in DNA of tumour origins that can be used for treatment decisions by clinicians;
- Can potentially be used to monitor patients in remission, detect tumour relapse or development of treatment resistance through variant evolution.

The Upfront Considerations

- Tumours often have a diverse heterogeneity;
  - A single tissue biopsy may not provide the best overview of tumour status;
- Factors influencing sample quality –
  - Blood collection, transport, temperature and sample processing;
- Patient individuality and impact on sample suitability –
  - High BMI, tumour type, location and stage;
- What gene/variant coverage will get the best result given the clinical situation (tumour type);
- Relationship of variant frequency in plasma versus that measured in a tissue sample.

When to use Liquid Biopsy

Most obvious situation to use liquid biopsy is when solid tissue biopsy is not an option:

- Compromised patients where surgery or other procedures pose inherent risks to patients;
- Metastatic tumours where a representative tissue sample is unlikely;
- Tumours (often metastasies) in locations that are difficult to access for biopsy;
- Patients in remission, monitoring treatment or for progression of tumour;
  - Solid tumours not practical for longitudinal studies .

How is the test performed?

- Not necessarily novel - NIPT testing uses similar principles to analyse cfDNA;
- cfDNA is short in length (120 to 200bp) and short lived;
- Blood collection and appropriate DNA isolation methods are critical for a reliable result;
- Isolate DNA from plasma – serum is unsuitable for Liquid Biopsy purposes;
- Need an analysis instrument platform that has high sensitivity and specificity;
- The circumstance of the patient case can determine the workflow for the platform used;
- Provide a profile of gene alterations (variants) and their variant allelic frequency (VAF).

Blood collection

- Preferably in special preservative blood tubes;
- Roche or Streck cfDNA tubes are most suitable;
- The more plasma extracted, the better the cfDNA yield;
- Depending on test platform, require between 10 to 40ng of DNA minimum to get the most reliable result.

- Preservative prevents further leucocyte lysis thereby reducing contamination with WT genomic DNA;
- This can be important in maintaining test sensitivity;
- The more endogenous (wild type) DNA present, the more the impact on variant detection and VAF reliability.

Plasma Extraction

- Separation of plasma from blood cells should be done as soon after collection as practical;
- Most routine extraction methods are optimised for large genomic DNA fragments, not short cfDNA (up to 200bp);
- Manual and automated commercial kits are available for cfDNA;
- Usually extract between 2 and 8 mLs of plasma;
- Routine yields of 0.2 to 1ηg/µL (up to 100 µL extraction volume) are usually achieved but can be higher;
- Low yields or poor quality can compromise reliability and sensitivity;
- It has been reported that there is a potential correlation between DNA yield and tumour status so consistent extraction is important;
- DNA yields can be higher after treatment, increased activity or unrelated health issues.

Qiagen Manual Extraction kit

Qiagen QIAsymphony

Promega Maxwell

Analysis Platforms

- Several platforms are currently used to investigate genetic variants for ctDNA;
- Although all are appropriate, they do vary in sensitivity, specificity and variant coverage;
- The choice of platform could potentially be determined by the investigation situation (for example new cases should be tested on a platform with a broad gene/variant coverage);
- However, the platform used would usually be determined by the testing laboratory.

1. Next Generation Sequencing

- Probably the most popular platform available;
- Provides the ability to test a broad range of genes / variants in a single test;
- For most cases, a panel of genes would be tested incorporating whole genes or selected exomes of interest;
- In particular, ‘hot spot’ regions of mutation can be comprehensively covered capturing all alterations in those areas;
- Whole genomic or exomic coverage is also available but clinical utility for these options needs to be considered;
- Can be expensive but prices are reducing - best value if samples can be batched;
- Good sensitivity (down to 0.5%) but can be impacted by starting quality / quantity of DNA;
- Generally a 3 day workflow.

Illumina MiSeq

Thermo Fisher Ion Torrent

2. Mass Array

- Analysis based on molecular weight of amplified probes that determine variant sequence;
- Is target based investigating a set of specific variants in a multiplex style assay;
- Is readily expandable by adding primer/probe sets within an assay;
- Good specificity but sensitivity for liquid biopsy application may be less than other platforms (>1%);
- Is able to handle lower DNA concentration and lesser quality starting DNA;
- Cost effective and scalable;
- A two to three day workflow.

Agena Mass Array CPM

3. Digital PCR

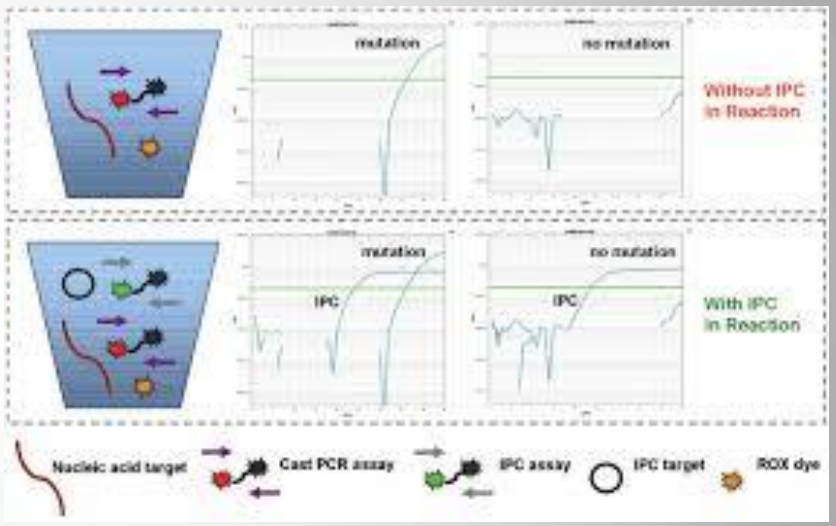
- Perform individual reactions to detect specific variants;
- The greater the range of variants to be detected, the more reactions that need to be performed;
- Has a high sensitivity (<0.5%) so would be the platform of choice in studies for early detection of a specific mutation (eg EGFR T790M);
- Also applicable for monitoring patient's response to treatment by investigating variant frequency status;
- Can be completed within 24 hours of sample receipt;
- Cost effective but can become expensive if multiple variants being investigated.

Bio-Rad QX200™ ddPCR System

Life Technologies Next-Gen dPCR

4. Real Time PCR (qPCR)

- Several commercial options available and would be available to any molecular lab as don't necessarily need dedicated instrumentation;
- Like dPCR, limited multiplexing available so the more variants to be investigated, the more it costs;
- However, each reaction is low cost so can be appropriate;
- Has lower sensitivity (>2%) and can be prone to false positive results if appropriate performance and analysis measures are not maintained;
- Can be performed within 24 hours of sample receipt and is a very straight forward work flow.



5. Others

Other options available but not in widespread use;

- Microarrays:
  - Provides good gene coverage;
  - Good sensitivity and can be quantitative;
  - Can be costly.
- Biocartis Idylla :
  - Fully automated qPCR;
  - Being used for FFPE but introducing plasma option;
  - Test can be done as samples receipted in the lab;
  - Sensitive and semi-quantitative;
  - Rapid but expensive.

Illumina iScan Microarray Reader

Biocartis Idylla

Result Reporting

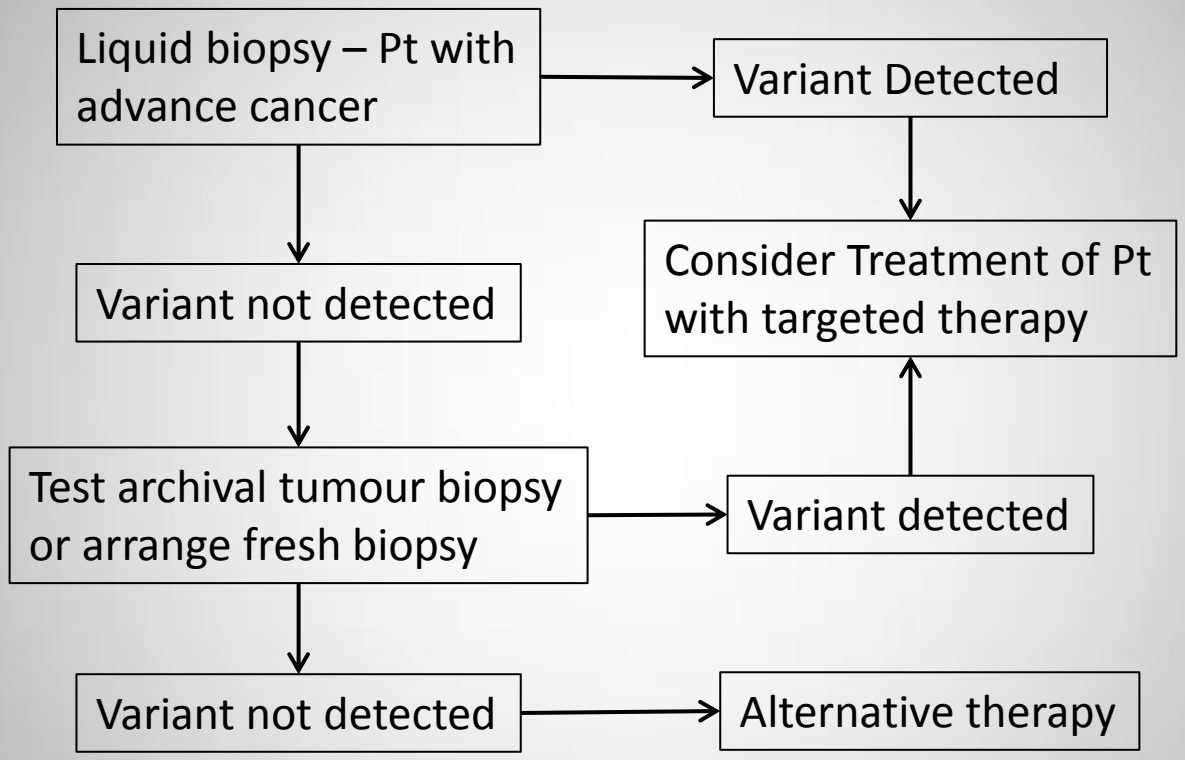
- Reports will include relevant gene variants detected and the allelic frequencies of those variants;
- Should display results from previous ctDNA tests for the patient being tested;
- Total DNA yield may also be reported in nanograms DNA per millilitre (ηg/mL) of plasma extracted;
- Total cfDNA may indicate active tumour progression but will be influenced by other factors such as any medical treatments, exercise, tumour type and site;
- Report may not include the detection of variants of unknown significance but these may still be an indicator of relapse;
- Report may not include any earlier or concurrent solid tumour testing or other relevant patient status of disease.

Result Analysis & Interpretation

In most cases, liquid biopsy will be performed to follow previous testing; as such results should be analysed with reference to those earlier results.

- The result reflects the variant status for the whole tumour burden of the patient, not a single site (intra-patient tumour heterogeneity);
- Variant frequency is determined from total cfDNA analysed (WT & ctDNA) and thus will be impacted by other factors outside tumour activity;
- Interpretation will be influenced by reason for test (for example monitoring patient in relapse or determining effectiveness of treatment).

The Liquid Biopsy Workflow



1. Monitoring

- Comparing the variants detected with those known to be associated with the tumour (if any) from previous analysis, usually on FFPE;
- Recommend that a ctDNA baseline profile (including variant frequencies) is determined soon after diagnosis or post initial treatment;
- An increase in frequency of known variant may indicate tumour relapse;
- Presence of previously unidentified variants may help direct future treatments.

2. Disease Relapse

- May be indicated by raised total cfDNA levels;
- Prompt investigation of solid tumour status - regrowth at original site or metastatic spread;
- Determine whether the ‘current’ tumour has the same or different variant profile;
- Determine if development of treatment resistance is due to variant evolution;
- Presence of new variant that could be targeted with next level drugs (e.g. Osimertinib and EGFR T790M in NSCLC)?

Application in NSCLC

Clinical Utility of ctDNAs in Lung Cancer

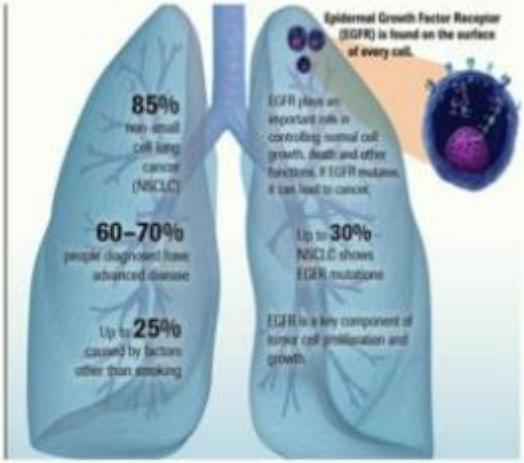
< Previous Article | January 2018 Volume 13, Issue 1, Pages 112–123 | Next Article >

Longitudinal Cell-Free DNA Analysis in Patients with Small Cell Lung Cancer Reveals Dynamic Insights into Treatment Efficacy and Disease Relapse

Presented as a poster presentation at the International Association for the Study of Lung Cancer Annual Meeting. February 22–25, 2017; Santa Monica, CA. Presented as an oral abstract at the American Association for Cancer Research Annual Meeting. April 1–5, 2017; Washington, D.C. Presented as a poster presentation at the American Society of Clinical Oncology Annual Meeting. June 2-6, 2017; Chicago, IL. Presented as an oral abstract at the Yale Lung SPORE Annual Meeting. June 21-22, 2017; New Haven, CT.

Karinna Almodovar, PhD, Wade T. Iams, MD, Catherine B. Meador, MD, PhD, Zhiguo Zhao, MS, Sally York, MD, PhD, Leora Horn, MD, Yingjun Yan, MS, Jennifer Hernandez, BS, Heidi Chen, PhD, Yu Shyr, PhD, Lee P. Lim, PhD, Christopher K. Raymond, PhD, Christine M. Lovly, MD, PhD

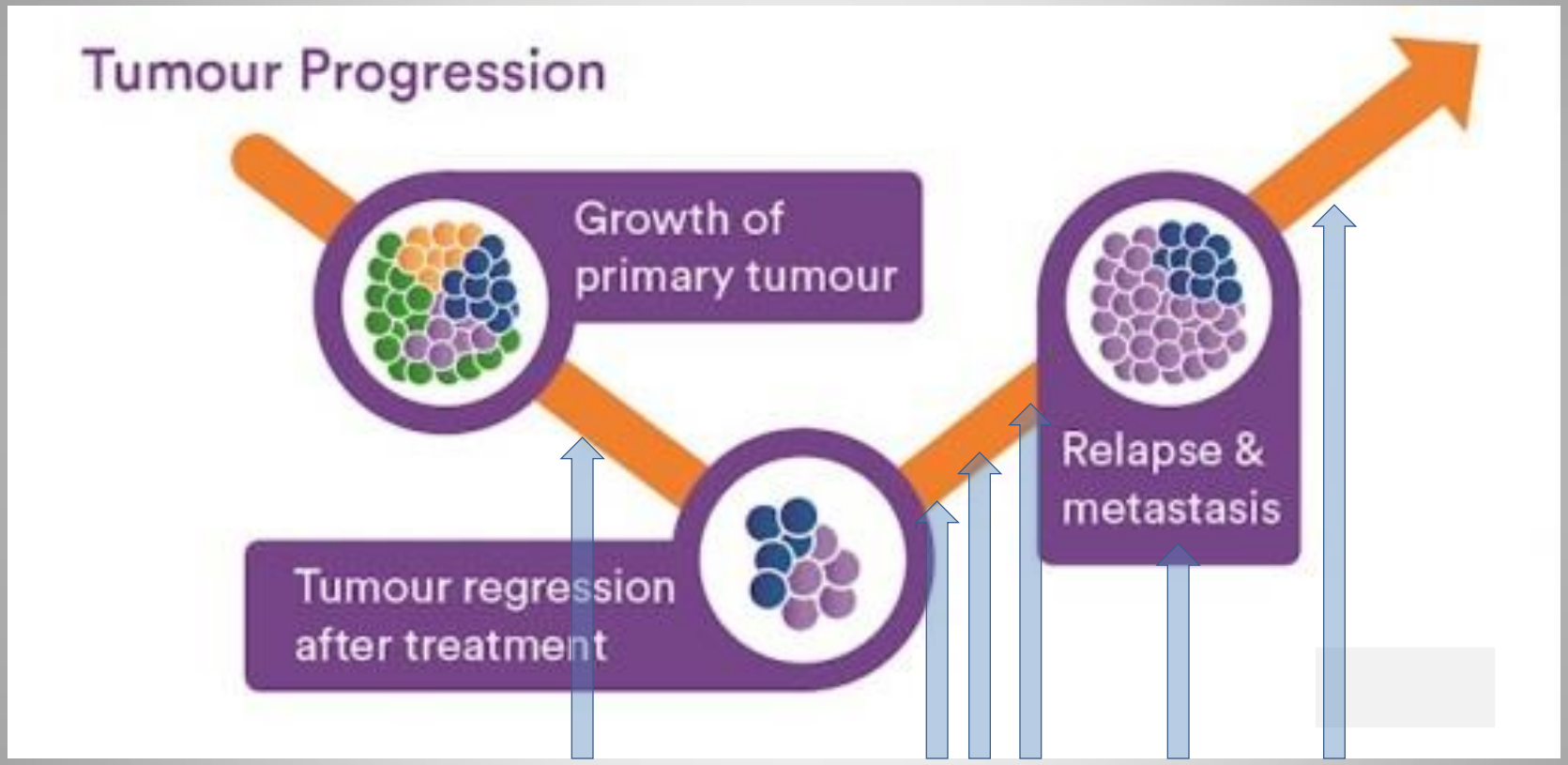
Open Access PlumX Metrics



- **Approximately 50-60% of NSCLC patients become resistant to TKI therapy through an epidermal growth factor receptor (*EGFR*) T790M mutation**
- **Only <5% of NSCLC patient have this mutation detectable in the primary biopsy**
- **Most studies showed that monitoring for tumour-derived DNA in the plasma can identify relapse or drug resistance well before clinical signs and symptoms appear**
- **Enabling earlier intervention and better outcomes**

AUSTRALIAN Clinicalabs

When to perform ctDNA analysis
(future application of Liquid Biopsy)



After initial diagnosis…

… and then to monitor response or detect relapse

Challenges

Although there is huge potential in Liquid Biopsy technologies there are still some questions:

- How frequently should testing be performed?
- Currently no Medicare rebate for ctDNA analysis;
- At what variant frequency does a detected variant have clinical importance (consideration of variant detected and tumour type and status)?
- Is there a use for Liquid Biopsy as a screening test or in early stage cancer ?
- So what is the clinical utility of ctDNA analysis?

Some Reading…

- Narod A. & Schramm J: Liquid Biopsy Tests in People with Cancer: An Expert Review. Published on *ASCO* (https://www.asco.org), March, 2018.
  - *Summary: More evidence needed to establish effective and appropriate use in the clinic.*
- Merker JD *et al.* Circulating Tumor DNA Analysis in Patients with Cancer; American Society of Clinical Oncology and College of American Pathologists Joint Review. Arch Pathol Lab Med **142** (Oct 2018): 1242 – 1253.
  - *Conclusions: … Some ctDNA assays have demonstrated clinical validity and utility with certain types of advanced cancer; however, there is insufficient evidence of clinical validity and utility for the majority of ctDNA assays… There is no evidence of clinical utility and little evidence for clinical validity of ctDNA assays fro early-stage cancer, treatment monitoring, or residual disease detection.*
- Genomic Assessment of Blood-Derived Circulating Tumor DNA in Patients with Colorectal Cancers: Correlation with Tissue Sequencing, Therapeutic Response, and Survival. JCO Precision Oncology, published on https://ascopubs.org/journal/po, September 2019.
  - *Conclusion: Patients with colorectal cancers have heterogeneous ctDNA profiles, and most harbor potentially actionable ctDNA alterations. Matched therapy yielded higher rates of stable disease for 6 months or more, partial response, or complete response. CtDNA assessment may have clinical utility and merits further investigations.*

Thanks for listening…

Open for discussion!