



# Liquid Biopsy

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In the note below, we shall be discussing in brief about liquid biopsy in cancer under the following headings.

- Definition
- Need/advantages of liquid biopsy
- Types
- Methodology
- Limitations
- Uses in oncology
  - Approved
  - In research
- Future implication
- References

## Definition

It is sampling and analysis of nonsolid biological tissue, primarily blood, to inform management.<sup>1</sup>

## Need of Liquid Biopsy

There is a need of a technique that can complement or replace the traditional tissue biopsy overcoming the disadvantages and/or limitations associated with it.

Liquid biopsy:

- Offers the same simple, noninvasive, and faster than traditional biopsy.
- Allows assessment of intratumor heterogeneity, monitor tumor dynamics, and study tumor dormancy.
- Problems of tissue inadequacy are avoided as a few copies of mutant DNA are sufficient.
- Allows for serial evaluation (► Fig. 1).<sup>2</sup>

## Types

Cancer cells shed intact cells as well as their cellular components (nucleic acids, exosomes) and with advancement in

the laboratory techniques these can be isolated and analyzed (► Fig. 2).<sup>2</sup>

## Circulating Tumor Cells

Cancer cells shed tumor cells into peripheral circulation mainly because of physical forces such as tumor growth, invasion of vessels rather than through epithelial mesenchymal transition that is an important mechanism in the development of metastasis.<sup>3,4</sup>

## Circulating Tumor DNA

- Cancer cells undergo necrosis or apoptosis, releasing their DNA into the peripheral circulation.
- The circulating tumor DNA (ctDNA) has a short half-life and is present as small double-stranded DNA fragments of ~150 base pairs of nuclear or mitochondrial origin in body fluids.
- The ctDNA is stable when plasma is stored at –80°C.<sup>2</sup>

## Exosomes

- They are membrane-bound vesicles containing various proteins, RNA among other materials. They are released into a variety of body fluids.
- Exosomes derived from malignant cells have a higher concentration of proteins and a different RNA profile.<sup>3,5</sup>

## Tumor-Educated Platelets

Several normal cells form a part of the tumor microenvironment. The interaction of these cells with tumor affects not only the gene expression in the tumor but also RNA expression in the platelets. Such platelets are called as tumor-educated platelets.<sup>6</sup>

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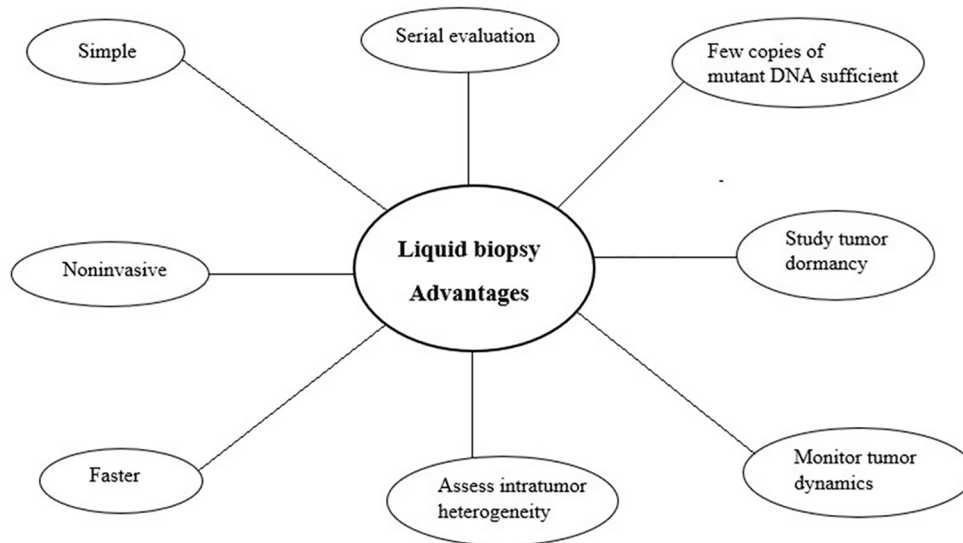


Fig. 1 Advantages of liquid biopsy.

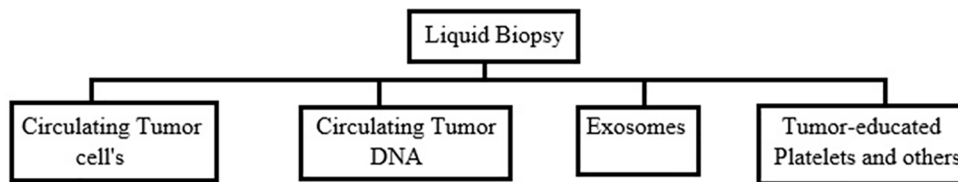


Fig. 2 Types of liquid biopsy.

## Methodology

It involves

Sample collection



Isolation of circulating tumor cells (CTCs) and/or cellular components (nucleic acids, exosomes) by various enrichment techniques\*



Isolated CTCs and/or ctDNA are analyzed further through wide variety of applications such as NGS, polymerase chain reaction, or fluorescence in situ hybridization to look for copy number, mutations, translocations, amplification, deletions, chromosomal abnormalities, RNA expression, fusion transcripts, protein expression, and phosphorylation and culture and data obtained from it is analyzed and interpreted.

\*Several enrichment techniques used are positive selection using antibodies against epithelial markers, negative selection using anti-CD45 antibodies that will remove the white blood cells, and other mechanical methods like filtration are used.<sup>3,4</sup>

## Limitations

- It cannot be used to determine histology (adenocarcinoma, squamous cell carcinoma), establish programmed death ligand 1 status.

- A negative test may not rule out true negativity example: Epidermal growth factor receptor (EGFR) negative on CTCs may still be positive on biopsy, nonshedders.

## Uses in Oncology

### Approved

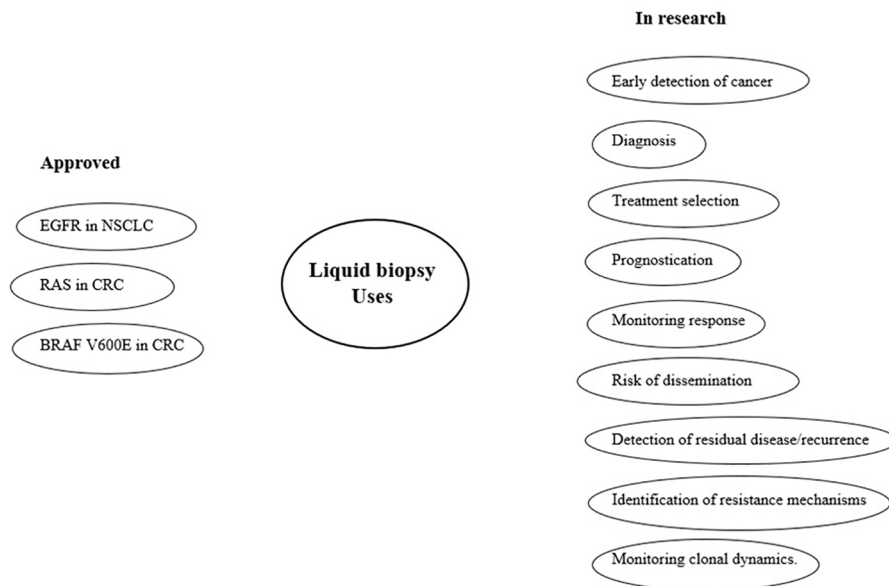
- To detect actionable EGFR mutation in nonsmall cell lung cancer and RAS (KRAS and NRAS) and BRAF V600E mutations in colorectal carcinomas (→ Fig. 3).

### In Research

- Role in early detection of cancer is postulated.<sup>7</sup>
- Diagnosis (especially in difficult biopsy access site/ patient related problems for biopsy).
- Treatment selection (molecular profiling), prognostication (number of CTCs), and monitoring response (serial evaluation).<sup>8</sup>
- Risk of dissemination and detection of residual disease and recurrence.
- Identification of resistance mechanisms and monitoring clonal dynamics (→ Fig. 3).

## Future Implication

Liquid biopsy is emerging as an important noninvasive technique that has both diagnostic and prognostic value. It is currently limited in routine use by the lack of convincing data



**Fig. 3** Uses of liquid biopsy. CRC, colorectal cancer; EGFR, epidermal growth factor receptor; NSCLC, nonsmall cell lung cancer; RAS, .....; BRAF, .....

to replace the traditional tissue biopsy. However, with rapid advancements in the enrichment and isolation procedures, liquid biopsy represents an important prospect because of its many advantages.

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Nil.

#### Conflicts of Interest

There are no conflicts of interest.

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