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Introducing Liquid Biopsy: The Revolutionary Aspect of Molecular Oncology

Dr Mirette Saad



Cancer, a leading cause of mortality, is associated with mutated genes. Analysis of tumour associated genetic alterations is increasingly used for diagnostic, prognostic and treatment purposes. Precision or personalised medicine harnesses genomic knowledge banks to tailor individualised treatments based on patients' or their tumours' genetic signatures.

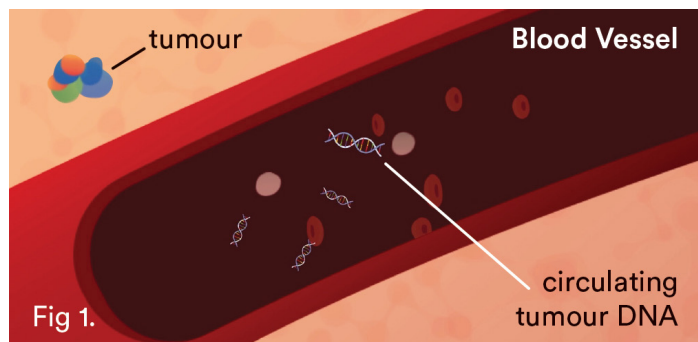
Somatic Mutations in Cancer

A high-quality genomic analysis is critical for personalised pharmacotherapy in patients with cancer. Identifying activated therapeutic gene targets (e.g., *BRAF* in melanoma, *EGFR* in lung cancer and *KRAS* in colorectal cancer) with established cancer associations, using focused panels for targeted cancer sequencing, allows for deeper coverage of those genes and higher sensitivity to confidently call rare variants (e.g., *PTEN* and *KIT*) in rare tumour subclones, including FFPE tissue. The advent of therapies targeting genomic alterations has improved the care of patients with certain types of cancer dramatically. Thus, elucidating the genetic profile of a given tumour is potentially useful in designing tailored treatment regimens that avoid unnecessary toxic therapy.

Liquid Biopsy

The genetic profile of solid tumours is currently obtained from surgical or biopsy specimens; however, performing biopsies, particularly in lung cancer, is not always possible in advanced disease stages and is associated with potential complications. Also, information acquired from a single biopsy provides a limited snap-shot of a tumour and might fail to reflect its true genetic and cellular heterogeneity.

While molecular targets were initially detected in nucleic acid samples extracted from tumour solid tissues, detection of circulating nucleic acids in blood has enabled the development of what has become known as “liquid biopsy”.



Certain fragments of DNA, cell-free DNA (cfDNA), have been shown to be elevated in the plasma of patients with cancer. This increase is the result of the rapid turnover of cells within the tumour, releasing DNA into the circulation¹ (see Fig. 1). With new highly sensitive technologies, cell-free circulating DNA can now be isolated and analysed. cfDNA analysis can complement and in many instances correlate to solid tissue biopsies^{3,4} for real-time molecular monitoring of treatment, detection of recurrence, and tracking resistance. Circulating tumour DNA (ctDNA) reflects the overall tumour information and is not biased by analysing only a small fraction of the tumour. It is always accessible, in contrast to the lung cancer tissue.

Various studies in breast, lung and colorectal cancers have demonstrated the potential application of ctDNA analysis at each stage of clinical management: early diagnosis^{12,13}, molecular

profiling¹⁴, prognostication^{12,15,16}, detection of residual disease^{7,17}, monitoring response and clonal evolution^{18,19,20}. Lastly, there have been recent approvals by the Federal Drug Administration (FDA) and the European Medicines Agency (EMA) for detection of the tyrosine kinase resistant clone *EGFR* p.T790M mutation in plasma as a companion diagnostic for second-line treatment of metastatic *EGFR*-mutant non-small cell lung cancer (NSCLC)^{21,25}.

Prospective research cohort studies are ongoing to explore the possible clinical applications of ctDNA in cancer. The inclusion of ctDNA analysis into multiple clinical trials (ClinicalTrials.gov), signals the recent integration of this form of biomarker into routine clinical oncology.

Implementation of Aspect Liquid Biopsy

Clinical Labs has analytically validated an integrated test process for a comprehensive mutation profiling assay for clinical oncology patients using circulating tumour derived DNA extracted from blood.

Our technology, including (NGS), MassArray Agena Biosciences ULTRAseek and Droplet Digital PCR (ddPCR), is able to identify clinically relevant variants at a sensitivity down to 0.5% or less.

Aspect Liquid Biopsy is Less Invasive

Surgical tissue biopsies have some inherent shortcomings and risks, and do not guarantee enough material will be collected for accurate analysis in clinical practice. Liquid biopsy provides a non-invasive alternative sample source, allowing the identification of genomic alterations that can be addressed by targeted therapy. This non-invasive type of liquid biopsy can be taken easily and repeatedly over the course of a patient's treatment. ctDNA provides new insight into diagnosis, prognosis and patient follow-up compared to traditional tissue biopsy.

The Quality Choice for Cancer Treatment Response Monitoring

Early detection is the holy grail of cancer management. The biggest advantage of liquid biopsy is the ability to detect the cancer biomarkers in blood earlier than conventional methods^{22,23}. While correlating with imaging scans, it has been demonstrated 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^{22,23}.

In the future, instead of extensive imaging and invasive tissue biopsies, employing ctDNA as liquid biopsies could be used to guide cancer treatment decisions and perhaps even screen for tumours that are not yet visible on imaging.

Over the past several years there have been multiple studies demonstrating the clinical utility of liquid biopsy ctDNA analysis following surgical resection of colorectal cancers (CRCs)⁷. Most studies demonstrate better outcomes when no tumour-derived DNA is found in patients following surgery, or chemotherapy in colorectal cancer patients⁷, whereas those where tumour DNA

is still present do better with the addition of more aggressive targeted treatment or chemotherapy.

It is important to note that for melanoma patients who harbour a *BRAF* mutation, targeted therapy remains the first-line treatment, particularly in Australia¹¹. Tracking of *BRAF*V600 ctDNA levels as patients undergo targeted treatment can potentially help identify the period when resistance to targeted therapy emerges. Gray et al. and Lee et al.^{22,24} showed that baseline ctDNA levels predict response to immunotherapy in melanoma patients, and low basal ctDNA levels were significantly associated with long-term clinical benefit. Nevertheless, prospective clinical studies of survival in a larger cohort of patients are underway to validate the predictive value of ctDNA in melanoma patients treated with immunotherapy.

Aspect Liquid Biopsy Can Guide Treatment Decisions

Liquid biopsy can offer valuable insights into how best to treat cancer. ctDNA can be used to monitor the effects of cancer treatment and give an early warning about possible recurrence. The detection of resistant clones can offer clues to the reasons for treatment resistance^{5,6} (see Fig.2). Recently, a third-generation *EGFR* Tyrosine Kinase Inhibitor (*TKI*), which is effective in tumours harbouring the T790M *EGFR* mutation (~50-60% of lung cancer patients^{5,6,10}), was approved in Australia for patients with NSCLC harbouring the *EGFR* T790M mutation following progression on an *EGFR TKI*⁹.

Serial analysis of ctDNA from the time of diagnosis throughout treatment can provide a dynamic picture of molecular disease changes, providing evidence that this non-invasive approach could also be used to monitor the development of secondary resistance and identify heterogeneous sub-clonal populations of tumour cells developing during the course of treatment. NCCN guidelines⁸ recommend the use of liquid biopsy for lung cancer patients as an alternative for tissue in initial T790M *EGFR* testing^{2,6}. However, if the plasma is negative, then a tissue biopsy is recommended, if feasible.

Tumour Progression

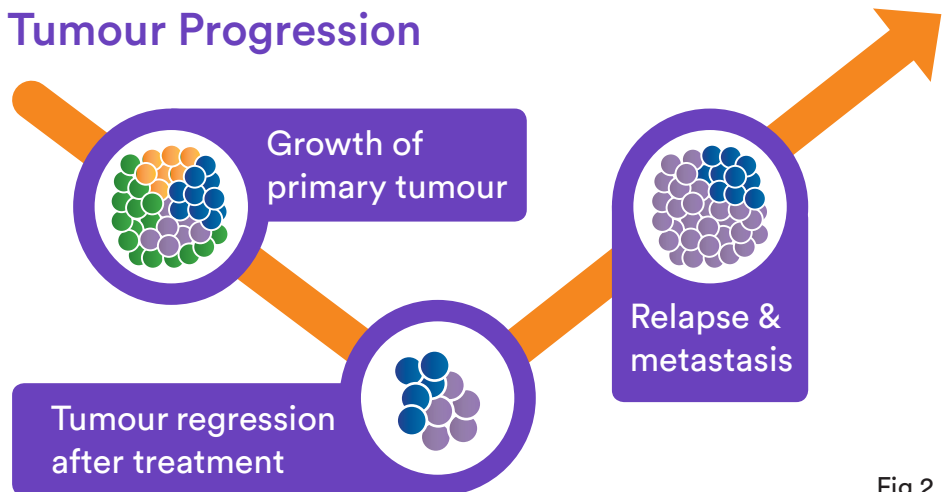


Fig 2.

How to Order?

Health Practitioners can order Aspect Liquid Biopsy for cancer patients using the Aspect request form. Some required information is listed on the form, including type of cancer and whether it is a new diagnosis and if the patient is on anti-tumour therapy (if known). Health Practitioners can download the Aspect Liquid Biopsy form on the Clinical Labs website at clinicallylabs.com.au/aspect

What is the cost?

Out-of-pocket fee of \$550. (No Medicare rebate available)

When will results be available?

Results will be available after 5-7 business days from the sample receipt date.

How is it collected?

This test requires two 10ml blood samples which can be taken at any of our collection centres.

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Dr Mirette Saad is a Consultant Chemical Pathologist and the National Clinical Director of Molecular Genetic Pathology at Australian Clinical Labs. At Clinical Labs, Dr Mirette Saad leads the Molecular Genetic testing for Non-Invasive Prenatal Testing (NIPT), carrier screening, personalised drug therapy and cancer. She is a member of the RCPA Chemical Pathology Advisory Committee and a Chair of precision medicine services at Australian Clinical Labs.

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1. Norton et al. N Engl J Med. 2015 Apr 23;372(17):1589-97.
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6. Data on file, Roche

**Patients are asking — and
clinicians need to be equipped
with the right knowledge.**

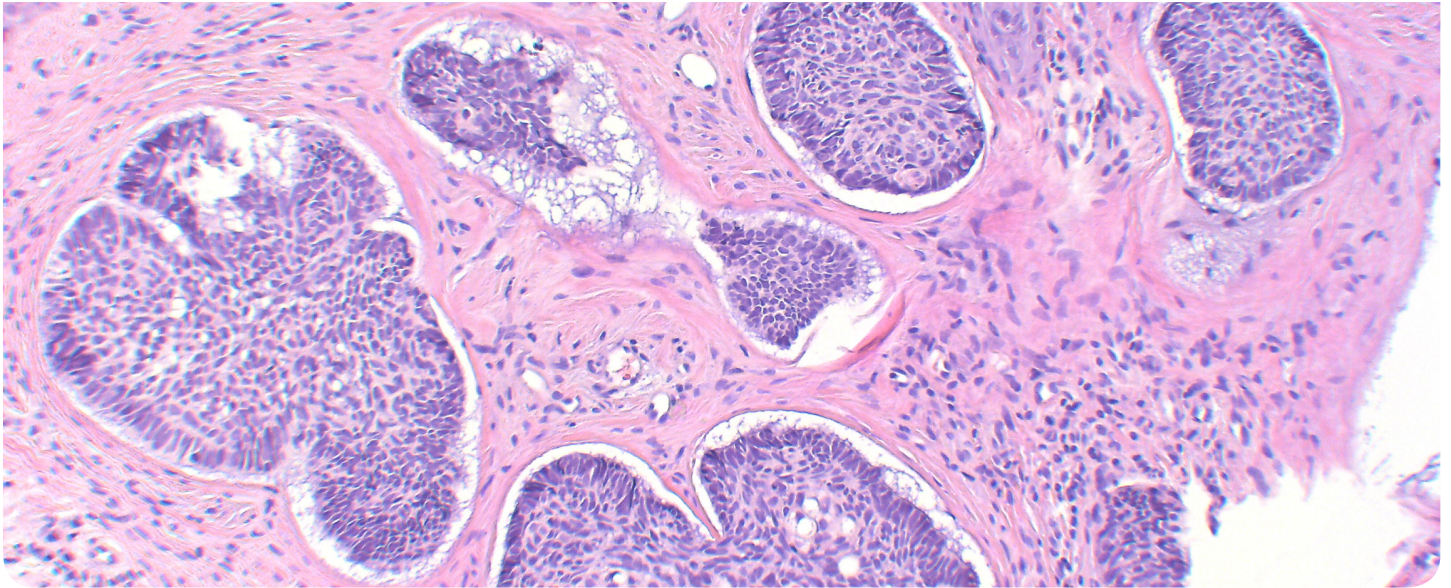
Harmony[®] is the most broadly studied non-invasive prenatal test (NIPT) for Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), and Patau syndrome (trisomy 13).¹⁻⁵ The Harmony Prenatal Test uses a proprietary, targeted DNA-based technology to provide you and your patients a greater level of assurance—simply requiring a maternal blood sample.

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Skin Biopsy: Lesions and Inflammation

Dr Jenny Grew



Biopsies of the skin are generally used to diagnose skin pathologies, but occasionally are used in treatment. This information sheet serves to outline the techniques of biopsy, including: excision (elliptical, saucerisation/shave, punch), incision, superficial shave, punch and wedge. The type of biopsy a clinician uses is dependent on the pathology of the individual patient.

complete removal of in situ/thin lesions. The margins of these samples must remain intact and flat, enabling assessment of the entire sample (achieved by flattening the sample on a card). If atypical/pigmented areas are seen at the base following saucerisation, punch or elliptical excision of the base area may be required to remove further pathology.

Punch biopsy is preferable to shave biopsies, which are prone to sample errors. Many clinicians prefer to use a punch biopsy technique for thick, keratotic lesions. Large, deep lesions require an incisional or wedge biopsy, which includes tumor/fat margins, for accurate diagnosis.

Non-Pigmented Discrete Lesions



- A biopsy should be performed when a diagnosis is uncertain.
- Complete excision is recommended for suspected malignancy.
- Small and/or non-suspicious lesions can be removed using punch or shave biopsy.

Excision Biopsy: A well-differentiated squamous cell carcinoma or keratoacanthomas is diagnosed by excising the entire lesion. Partial biopsy often does not present enough information to make an accurate diagnosis.

For a patient with a broad lesion who is prone to keloid/hypertrophic scar formation, saucerisation could aid in negating surgical and/or cosmetic difficulties, and can be used for the

Pigmented Lesions



Suspected melanoma:

- Excision margins 1-3 mm
- Small or superficial biopsies may not be representative - subsequent tumor staging may be difficult.

Partial biopsies can be used:

- If melanoma is unlikely
- For benign, pigmented melanotic macules on the genitals
- Cosmetic concerns
- Broad lesions
- Surgically difficult sites

Saucerisation (including a 0.5-1.0 mm margin) can be performed for 'ugly duckling' dysplastic naevi.

If partial biopsy of suspected melanoma is unavoidable:

- The thickest area/s should be biopsied at greater depth.
- Multiple small shaves across different areas of lentigo-maligna-type lesions are recommended.

Small suspicious acral lesions can be removed using saucerisation which include a margin of healthy skin.

Annular lesions (e.g. porokeratosis): Incisional biopsies should be taken from the center of the lesion in an outwards direction and include a 1 mm margin of normal skin.

Lupus erythematosus, dermatomyositis and vasculitis: The biopsy site is crucial. For lupus and dermatomyositis, biopsy an established lesion (>6 months old) that is active for routine histology and DIF. For vasculitis, biopsy an established, purpuric lesion (>72 hours old) for routine histology and an acute lesion (<24 hours old) for DIF.

Panniculitis: A deep or incisional biopsy, including subcutaneous fat, is recommended. If necrosis is present, a sample should be sent for culture.

Rashes/Inflammatory Conditions



- 4 mm punch biopsy
- Incisional biopsy for larger, deeper lesions
- Saucerisation to remove entire blisters

Many rashes have similar histological patterns. A clinical description of the rash should accompany the biopsy sample and include: distribution, duration and course, other history (e.g. medication), clinical photographs (sent via email or text message) and clinical diagnosis to ensure accurate categorization of the sample.

Stating the biopsy type and reason for the biopsy will ensure that the sample is handled appropriately.

Lesions: A small rim of perilesional skin should be included in a biopsy of tissue that has not been excoriated or ulcerated. Frictional sites and lower limbs may have secondary features resulting in false negative results, and biopsy of these areas should be avoided. Multiple biopsies of the rash and a concurrent biopsy of normal skin may be helpful in disorders that are of polymorphous and pigmented appearance, respectively.

Blisters: Biopsies maintaining the attachment of the blister roof are important. A second biopsy of a non-blistered lesion or normal skin immediately adjacent to the lesion should be taken for direct immunofluorescence microscopy (DIF) and preserved in immunofluorescence transport medium or phosphate buffer saline.

Alopecia



- Two punch biopsies
- 4 mm diameter
- Parallel to the direction of hair growth/emergence.

Alopecia: Two biopsies should include the entire follicular unit, subcutaneous fat and be at least 5-6 mm in depth (no hairs emerging from the deep aspect). Such biopsies will allow the pathological examination of both vertical and horizontal sections of the biopsy. Clinical information including area of scalp involved, race, duration and clinical differential diagnosis are important.

Biopsy site

- **Non-cicatricial alopecia:** Most prominent area of hair loss.
- **Cicatricial alopecia:** Area of hair loss but not completely bald (active area where at least 6 months duration). Dermoscopy help identify suitable biopsy sites, e.g. areas of scaling or abnormal pigmentation. A second biopsy from the same area can be sent for DIF.

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Updates From The Lab

Northern Beaches Hospital

Clinical Labs will anchor a new laboratory in the Northern Beaches Hospital (NBH), New South Wales. Opening in late 2018, the new state-of-the-art, 488-bed hospital will cater for both public and private patients. We have met with pathology staff currently working at the Mona Vale and Manly Hospitals, many of whom have seen the site and are excited at the prospect of being part of the new hospital. We look forward to welcoming them to the Clinical Labs team this year and being part of one of the best hospitals in Australia.

Hawkesbury District Health Service

Clinical Labs is also proud to offer our laboratory and pathology service to the Hawkesbury District Health Service (HDHS), a 130-bed, privately-run public hospital in North West Sydney. Our Clinical Labs team in NSW will keep HDHS staff and our referring doctors updated with any further news, with the aim of getting the onsite lab and collection centre in place by mid-May.

Skin Excision Evaluation Program

Thank you to those NSW referrers who have signed up to our Skin Excision Evaluation Program. The response so far has been very positive, and as the program grows we look forward to helping more doctors deliver better diagnostic outcomes for their patients.

As we head into the winter months, now would be a fantastic time to encourage your patients to have a post-summer skin check-up.

For those who may not be aware of the program, Clinical Labs offers GPs the ability to earn CPD points by submitting their provisional diagnosis on cases where histological evaluation is needed. A monthly Skin Excision Evaluation Report is then generated for participants showing their performance. If you would like to sign up, you can register online at ehealth.clinicallabs.com.au/harp or email us at skinaudit@clinicallabs.com.au



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