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INVESTIGATING SOLID TUMOURS IN THE ERA OF PRECISION ONCOLOGY

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INTRODUCTION

Cancer is a leading cause of death worldwide, with the World Health Organization (WHO) estimating that malignancies accounted for nearly 10 million deaths in 2020.

As a genomic disease, malignancies develop from the accumulation of mutations that drive unregulated cell growth. Rapid advancements in high-throughput technology and the advent of next-generation sequencing (NGS) technology have enabled scientists to improve their understanding of underlying genomic alterations and biological pathways that lead to cancer development and progression. This has revolutionised the field of oncology by allowing for precision-based approaches in the management of cancer. By understanding the effect of mutations in key regions of the genome, new possibilities in cancer therapy are being unlocked.

Ampath is one of the leading providers of comprehensive genetic testing in South Africa. The Oncomine™ Precision Assay (OPANGS) is the latest in a string of state-of-the-art technologies being offered that allows for the simultaneous investigation of several solid tumour malignancies using a singular approach.

THE BENEFITS OF NEXT-GENERATION SEQUENCING (NGS)

NGS is the laboratory cornerstone of precision medicine. It is a high-throughput technology that allows for the concurrent detection of multiple molecular variants across a range of classes, including the following:

- Single nucleotide variants (i.e. point mutations – SNVs);
- Small insertions or deletions of bases (indels);
- Copy number variants (amplifications or deletions of genes); and
- Gene rearrangements and fusions.

NGS offers several advantages over other older diagnostic modalities listed below. While polymerase chain reaction (PCR), real-time PCR (RT-PCR) and fluorescence *in situ* hybridisation (FISH) are robust and comparably inexpensive tools for detecting genomic alterations, they are limited by requirements for specific primers or probes for a limited number of targets.

By providing a more comprehensive tumour profile for each individual patient, NGS allows clinicians to refine disease prognosis and select appropriate therapies based on specific tumour molecular characteristics.

Next-generation sequencing has the following advantages:

- NGS can analyse many biomarkers simultaneously with a single test.
- NGS can be time saving, so individualised treatment decisions can be made sooner.
- NGS requires less DNA (as little as 10 ng) and therefore saves tissue for further tests.
- High specificity and sensitivity allows for the detection of genomic variants, even if they are present at a very low fraction within the cell.
- Cost and resource saving (it is cheaper to run one NGS test than multiple single PCR assays).
- An NGS panel can identify additional mutations that may offer further treatment options.
- NGS is sensitive enough to be used on liquid biopsies.
- A single comprehensive test will allow for reduced laboratory sample-handling errors.

THE VALUE OF OPANGS IN PRECISION MEDICINE

Precision oncology focuses on the molecular characteristics of tumours rather than traditional histopathology to match treatment strategies. This strategy has been applied in the management of many malignancies with the most focus given to the following:

- Epidermal growth factor receptor (*EGFR*) mutation, anaplastic lymphoma kinase (*ALK*) fusion, B-type Raf kinase (*BRAF*) mutation, c-Ros oncogene 1 (*ROS1*), rearranged during transfection (*RET*) alterations, Kirsten rat sarcoma viral oncogene homolog (*KRAS*) G12C and *MET* in non-small-cell lung cancer (NSCLC);
- Human epidermal growth factor receptor 2 (*ERBB2*) in breast and gastric cancer;
- Kirsten rat sarcoma viral oncogene homolog (*KRAS*), neuroblastoma ras viral oncogene homolog (*NRAS*) and *BRAF* mutation in colon cancer;
- *BRAF* mutation in melanoma; and
- Fibroblast growth factor receptor 2 (*FGFR2*) alterations in cholangiocarcinoma.

The identification of specific molecular signatures via NGS aids in the effective matching of therapy to the tumour and the patient.

In this way, targeted therapy has the potential to reduce trial-and-error inefficiencies, reduce adverse drug events, enhance adherence to treatment and avoid unnecessary delays in treatment.

FUSION DETECTION AND POTENTIAL FOR TUMOUR-AGNOSTIC THERAPY

RNA sequencing techniques allow for the detection of fusions, including *ALK, ROS1, RET, MET, FGFR2* and *NUTM1*. RNA sequencing is superior to other molecular techniques in its capacity to detect gene fusions. NGS can assist in identifying specific fusion variants that have potential impact on treatment decisions (e.g. by identifying specific treatment resistant variants).

Tumour-agnostic therapy treats malignancies based on genetic features, regardless of the tumour type and tumour site (Table 1). All *NTRK* gene-fusion isoforms can be detected with the OPANGS. Regardless of the tumour, patients with NTRK fusion-positive solid tumours can now potentially benefit from tumour-agnostic therapy.

NTRK gene fusion isoforms that can be detected using OPANGS include *TPM3 – NTRK1, LMNA – NTRK1, IRF2BP2 – NTRK1, SQSTM1 – NTRK1, TFG – NTRK1, AFAP1 – NTRK2, NACC2 – NTRK2, QKI – NTRK2, TRIM24 – NTRK2, PAN3 – NTRK2, ETV6 – NTRK3, BTBD1 – NTRK3*.

TABLE 1: TARGETS WITH APPROVED TUMOUR-AGNOSTIC THERAPIES

Tumour type	Target/biomarker	Approved therapy
Unresectable or metastatic colorectal carcinoma or solid tumours	MSI-H (microsatellite instability high) / dMMR (deficient mismatch repair)*	Keytruda® (Pembrolizumab)
Solid tumours	*NTRK* gene fusion	Vitrakvi® (larotrectinib), Rozlytrek® (entrectinib)
Unresectable or metastatic TMB-H solid tumours	TMB-H (tumour mutational burden high)*	Keytruda® (Pembrolizumab)

* *MSI-H/dMMR testing is not included in the OPANGS. The dMMR testing can be performed by immunohistochemistry on specific request.*

* *TMB not included in the OPANGS.*

LIQUID BIOPSY AND OPANGS

The approach to tumour biopsies is evolving with the introduction of liquid biopsy as an alternative and less-invasive method to detect oncogenic mutations. Liquid biopsies detect molecular aberrations in circulating tumour DNA, shed by malignancies or released when cancer cells die. In addition to other advantages listed below, liquid biopsy allows for real-time feedback regarding the molecular landscape.

The advantages of liquid biopsy are as follows:

- It is minimally invasive.
- It can predict the risk for metastatic relapse or metastatic progression.
- It allows for the stratification and real-time monitoring of therapies.
- It has the potential to assess tumour evolution and the development of resistance mutations (e.g. *EGFR* T790M mutation).
- The test can easily be repeated if needed; and can be used as often as necessary to monitor the patient's progress.
- It is potentially much less costly to perform than a formal tissue biopsy (in the setting of relapse and metastatic disease – particularly when the cost of surgery, theatre time, ICU, hospitalisation and histologic work-up are taken into consideration).
- It gives a more accurate representation of intra-tumoural genetic heterogeneity.

Note: *Liquid biopsy has a complementary role in the management of advanced carcinomas and is not recommended in lieu of histopathological diagnosis. Tissue remains the gold standard for diagnosis.*

CONCLUSION

Testing strategies are evolving to account for the increase of new targeted biomarkers. Identifying specific molecular signatures by NGS aids in the effective matching of the therapy to the patient and offers an unprecedented opportunity to make precision, personalised patient care a reality.

For more information, contact the NGS laboratory at ngs@ampath.co.za.

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REFERENCES AVAILABLE ON REQUEST