



Cancer Biomarkers in Australia



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The biomarker image on the front cover of the report forms part of an ambitious research program led by Professor Doug Brooks from the University of South Australia to develop, validate and implement a new biomarker in prostate cancer.

Research to date has revealed that the entire cellular pathway is altered in prostate cancer, with changes in more than 20 genes and proteins. This has provided a large panel of biomarkers which have the potential to support the diagnosis of prostate cancer, predict disease severity and guide treatment timing.

Foreword

A cancer biomarker is a molecule either produced by the cancer or by the body in response to a cancer that can be measured in blood, body fluid or tissues and indicates the presence or status of the cancer. Biomarkers associated with altered (mutated) genes can be used to screen for the risk of developing cancer. They can be used to help diagnose cancers. They may indicate targets for treatment and then give information on dosing by indicating how easily the body will eliminate the drug. They can be used to monitor treatment response and subsequently recurrence of a cancer.

What has made the use of biomarkers essential is the advent of personalised medicine. Advances in genomics, which describe the mutations in genes which are responsible for the growth of cancers, has allowed targeted therapies to prevent that growth. Moreover, at the time of diagnosis, cancers which share the same mutations may respond to the same targeted drugs. Determining treatment by the genetic make-up of a cancer may be more important than by using the organ of origin, which has been the traditional practice.

An optimal set of biomarkers is required to accurately detect the cancer, to predict its severity and to determine which treatment strategy is most appropriate. Further, the presence of biomarkers will enable drugs to be prescribed specifically to patients whose tumours have the targets, allowing those without to avoid the side effects and cost of being treated with drugs which are unlikely to be effective. In trials, response to treatment as measured by a decrease in the biomarker, will occur much earlier than waiting for a survival advantage, so that biomarkers may be earlier surrogate markers of the success of a new drug. This will enable drugs to be provisionally approved for widespread use until a survival advantage can be demonstrated.

To take advantage of the optimal use of biomarkers, methods for standardising the timely assessment of their efficacy need to be developed. Regulatory frameworks need to be developed to approve and fund the testing for biomarkers. This is especially the case when biomarkers are companion diagnostic tests for targeted drugs to ensure the most cost-effective use of targeted drugs. Patent law must not become a barrier for global access to biomarkers.

This report reviews the evidence for the efficacy of biomarkers and examines overseas precedents for their regulation and the Australian context. It also reports on the opinions of the end-users of biomarkers who are ultimately responsible for their measurement being translated into improved patient care and outcomes.

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Executive summary

BACKGROUND

In cancer therapy, there has been a major shift from non-specific cytotoxic drugs that indiscriminately kill cells to targeted small molecules, monoclonal antibodies and immune regulators. At the same time, cancer biomarkers are increasingly being used for screening and diagnosis, prognosis and as surrogate endpoints in cancer therapy trials. In particular, they are being used co-dependently with cancer drugs to stratify the patient population into those for whom the treatment is most likely to be successful, and those for whom the side effects will be fewer. We are also now seeing more flexible, but more complex study designs that are beginning to replace standard trials of new cancer drugs.

In response to these and other innovations such as orphan drugs, regulatory authorities in many countries are modifying or changing the ways in which drugs or drug-biomarker combinations can be licensed and funded. Australia's regulatory authorities have recognised the need for change, but still lag behind many OECD countries in this regard.

METHODS

To address these issues, we have undertaken:

1. An overview of current international and Australian policy on cancer biomarkers;
2. A review of the published literature for selected biomarkers;
3. A survey of Australian experts in cancer biomarkers;
4. Interviews with key players in the cancer biomarker arena.

FINDINGS

The survey of experts found a high use of biomarkers, but only just under half of the respondents thought they were reliable. Respondents called for better trials to demonstrate the efficacy of biomarkers, and agreed that biomarker development and validation would be more successful if the biomarker can be shown to be part of the underlying pathological process of tumour development.

More detailed interviews with stakeholders focussed on the funding issues of pairing a drug to its biomarker. Stakeholders commented on the potential for risk sharing amongst those who would benefit from this pairing, such as those who developed the testing technology. They were interested in regulatory models that would mandate pairing a targeted drug with its biomarker as a condition of funding the drug. They also recognised the difficulty of designing and interpreting biomarker trials.

RECOMMENDATIONS

1. Allow approvals and reimbursement of targeted drugs to be based on the genomic similarities of cancers expressing the target, rather than approving drugs only on histopathology.
2. Align the approval and funding of a targeted drug with that of its co-dependent biomarker, preferably by the same agency where end-user benefit can be a part of decision-making.
3. Allow provisional drug approval based on surrogate biomarker endpoints.
4. Develop standards for evaluation of biomarkers as predictive tools.
5. Develop bioinformatics capabilities to analyse large genomic datasets.
6. Develop electronic health records and laboratory systems to allow for capturing and linking biomarker tests and data.
7. Develop guidelines for the use of biomarkers.
8. Ensure that patent law does not restrict biomarker development.

OVERVIEW OF THE LITERATURE

Cancer biomarker policy

BACKGROUND

In 1998, the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.”¹ Clinical biomarkers can be broadly classified into those used for diagnosis, prognosis, prediction, a surrogate endpoint, and those that can identify a potential target against which a therapeutic agent can be produced and to which it can be directed. Some biomarkers can fit into more than one category.^{2,3}

Diagnostic biomarkers are used to detect or confirm the presence of a disease or condition of interest, or to identify individuals with a subtype of the disease. For example, gene expression profiling may be used as a diagnostic biomarker to segregate patients with diffuse large B-cell lymphoma into subgroups with different tumour cell of origin signatures.⁴

A prognostic biomarker provides information about the patients’ overall cancer outcome, regardless of therapy. A clinically useful prognostic marker must be a proven independent, significant factor that is easy to determine and interpret and has therapeutic consequences.⁵ For example, oestrogen receptor-positive (ER-positive), progesterone receptor-positive (PR-positive) and HER-2 expression are prognostic biomarkers in breast cancer.

Predictive biomarkers allow clinicians to target patients who are likely to respond positively to a treatment. This has the potential to reduce the cost of drug development by reducing the size of the study population required to demonstrate safety and efficacy. Further, by demonstrating that a drug will only be effective for a particular subset of patients, this can reduce the number of patients having adverse side effects, and the cost attached to providing the drug to patients in whom it will be ineffective.

Biomarkers can also be used as surrogate endpoints.⁶ These can be measured sooner than the classical clinical endpoints they substitute for, and thus have the potential to reduce the length and cost of clinical trials. To date, few biomarkers have met necessary regulatory standards to be used in formal drug or clinical trials.⁷

Some “classic” examples of cancer biomarkers include the protein PSA for prostate cancer (screening/diagnosis/

prognosis), the BRCA 1/2 genes for breast and other cancers (prognosis), the FMC7 cell surface antigen CD20 on B-cells (differential diagnosis of lymphoma and leukaemia), HER2 for breast cancer (prognostic/predictive), CA 19-9 for pancreatic and other cancers (diagnostic/predictive), and CA-125/MUC16 antigen for ovarian and other cancers (diagnostic). While these biomarkers are used in clinical practice, their performance is not always optimal. This is the case with PSA, which was originally developed as a prognostic marker only, and then adapted for screening and diagnosis; unfortunately, PSA has major problems with specificity that question its usefulness in diagnosis. This highlights a significant problem in the biomarker field, with a need for strict guidelines for biomarker development, validation and implementation and use - so that governments and medical institutions can rationalize and justify the use and funding of biomarkers in clinical practice.

To date there has been a lack of progress in biomarker development due to the difficulties in discovering suitable candidates, verifying that the biomarker is genuine, proper clinical evaluation and commercialisation. The US Food and Drug Administration (FDA) has recognized the potential for biomarkers and the emerging field of pharmacogenomics to transform drug development. The FDA is committed to advancing the development and use of biomarkers by modifying its regulatory review processes.

Biomarkers have been used in a diagnostic capacity in medicine for decades, with biomarkers having a wide variety of analytical targets, including metabolites, nucleic acids, proteins, lipids and unusual entities such as exhaled gases. This usage is further encouraged by the range of laboratory-based and ‘near patient’ point of care (PoC) platforms and devices available commercially.

There are several areas in which diagnostics for cancer need to be improved including: primary disease diagnosis, prediction of disease course, and for monitoring treatment effect and disease recurrence. It is currently a common requirement for many treatment efficacy trials to have a paired diagnostic test to monitor outcomes.

Another potential pathway for biomarkers is for follow-on care, especially in patients thought to be at elevated risk. Until recently, a barrier to this was that the utility of biomarker clinical studies were mainly confined to single or dual use, and the landscape was multifaceted. One example of this is the diagnostic and predictive dual use of beta HCG and alpha fetoprotein for testicular cancer.

Biomarkers in future might be delivered by “omics” technology in disciplines such as genomics, proteomics or metabolomics.⁸ Further, since single biomarkers often lack specificity or sensitivity, a multi-biomarker approach might be necessary to achieve diagnostic accuracy.⁹

To continue the progression of biomarker use in a diagnostic capacity, there is a requirement to detect disease pathology early in clinical progression. This can be achieved through tests that have high specificities and positive predictive values. Presently the majority of biomarkers that are available have high negative predictive values. The degree of specificity is an area of debate, thus larger complex panels could prove to be superior to single or low-complexity panels in this capacity.¹⁰ This objective is more likely to be achieved by a combination of biomarkers in a score-like test.¹⁰ Cancer is one of the areas in which there has been continual progress for biomarkers,¹¹ with it being one of the first fields with commercially available diagnostic and stratification tests.

REAPING THE BENEFITS OF BIOMARKERS

The current reactive ‘one-size-fits-all’ healthcare model approach could be revolutionised by novel molecular biomarkers transforming to an increasingly proactive and personalised approach. A personalised approach or “personal medicine”, as it has been termed (also called “precision medicine” or “stratified medicine”) would be effective through treatments directed by the information contained within a patient’s genetic profile; where cancer may be diagnosed, controlled or possibly prevented when the disease is initially detected. Novel molecular information and biomarker-based tests will enable practitioners to optimise treatment strategies as a supplement to their current treatment approach. We are now able to tailor treatments to the molecular characteristics of patient sub-groups. This enables us to minimise side effects and to improve the efficacy of treatments. Incorporating biomarker-based technologies into cancer diagnosis and treatment has many potential benefits:

- Earlier detection can improve health outcomes and minimise treatment costs.
- The use of pharmacogenetics can increase the safety and efficacy of treatments and reduce side effects.
- The use of biomarkers in pharmacogenetics will allow an increased number of safe and effective treatments to become available as drug development costs and timelines are reduced.
- Cytotoxic side effects can be reduced if the biomarker is associated with a process that occurs in cancers but not the surrounding tissues. However, current immunotherapeutic treatments have their own side effects.

These changes may also have positive economic outcomes:

- Regulators and third-party payers may have a reduced risk of accepting cost-ineffective drugs; there will be a smaller variation in patient response and fewer side effects.
- The cost of drug development will likely decrease. At the same time, biomarkers should speed drug delivery and improve safety and efficacy.

There are many challenges before biomarkers are widely adopted into personalised medicine. In particular, the existing regulatory framework lacks sufficient adaption to these diagnostic and prognostic tests. New evidentiary standards are required to introduce these new tools into the healthcare system. Current reimbursement mechanisms do not reflect the value of these new technologies and new business models are required to develop this new industry. Nonetheless, we are seeing rapid progress in the research and discovery sectors.

THE GENOMICS REVOLUTION

Genomics has allowed us to study and better understand individuals’ different responses to disease and treatment, and is allowing us to tailor diagnostic tests, treatment and monitoring to the individual. Further, our response to disease and drugs can be linked to biomarkers. The sequencing of the human genome in 2001 has heralded new insights into patterns of DNA sequence variation. Advances in technology and bioinformatics has allowed us to examine genome differences between individuals and individual susceptibility to cancer and response to drugs. This new technology gives us greater insight into the disease process in different cancers based on biomarkers. Pharmacogenomics will assist a more rapid development of new drugs and targeted therapies.

APPLICATION OF BIOMARKERS IN PERSONALISED MEDICINE

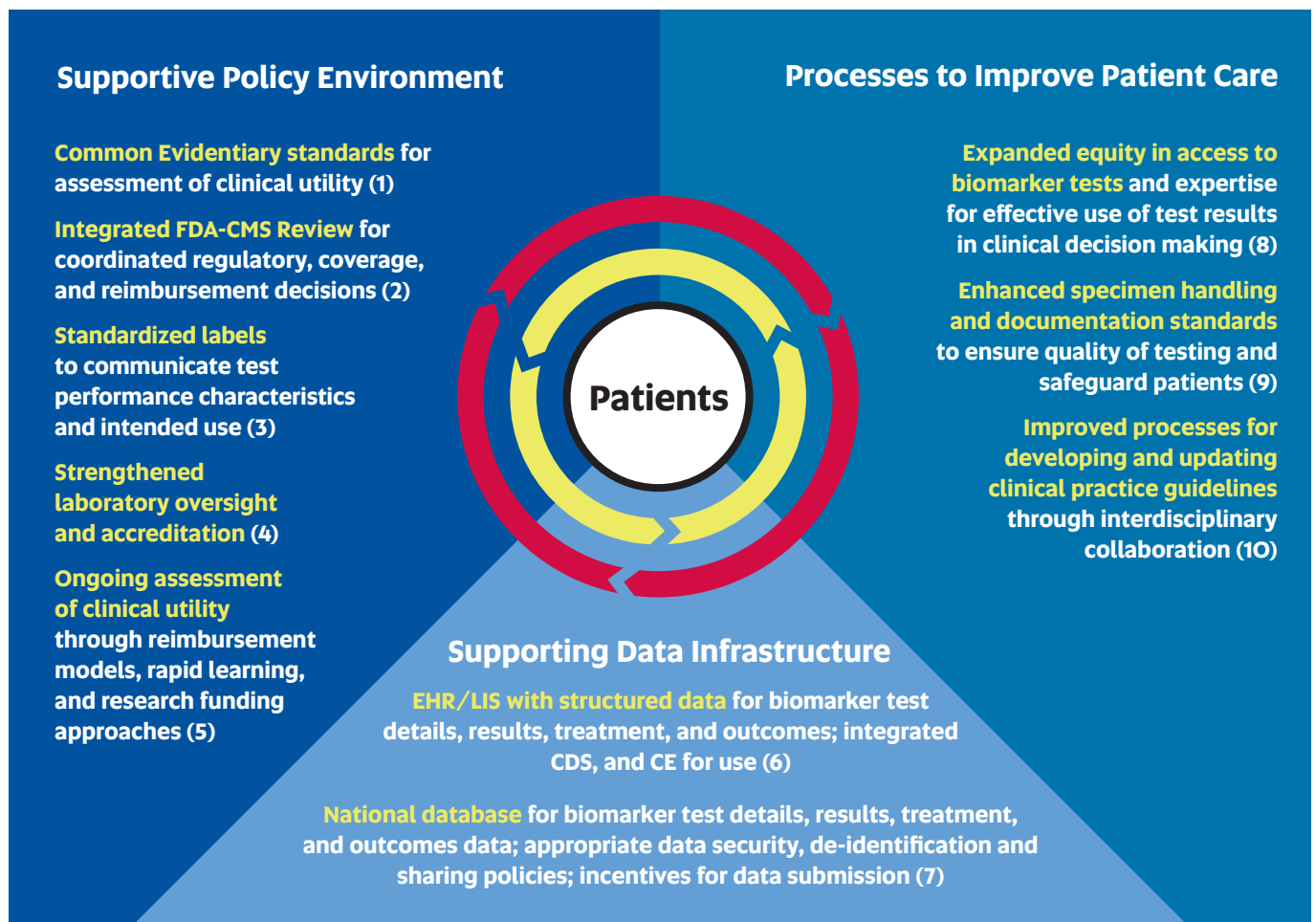
Advances in cell and molecular biology are increasingly being used to develop new diagnostic, prognostic and therapeutic tools. Biomarkers can be used in their own right as a diagnostic test or as companion diagnostics, i.e., tests directly associated with a therapy. Biomarkers are also increasingly being used in a number of pharmacogenetic applications including drug development, the characterisation of diseases and progression pathways. Several types of biomarkers can be identified (see Figure 1).

OPPORTUNITIES AND CHALLENGES

In OECD countries, cancer is a leading cause of death. Importantly, one third of these deaths could have been prevented, and another third cured if detected in time. This has placed a substantial cost on these countries due to the care required.¹² The World Health Organization (WHO)

suggests that in 2030, cancers will overtake ischemic heart disease as the leading cause of death.¹⁵ In 2008, close to 72% of cancer deaths occurred in low and middle-income countries which have lower incidence rates, but poorer survival. The global economic cost due to cancer not including patient care is approximately 900 billion US dollars per year – higher than that of heart disease.¹⁴ All governments are faced with increased healthcare costs, a major component of which is the cost of pharmaceuticals, and the prevention and treatment of chronic disease. Biomarkers may offer a potential way to reduce these costs.

FIGURE 1 - RAPID LEARNING SYSTEM FOR BIOMARKER TESTS FOR MOLECULARLY TARGETED THERAPIES¹⁵



PHARMACEUTICAL CONTEXT

Pharmaceuticals are currently an essential component of the prevention and management of cancers. However, drug discovery and development is taking longer, and is increasingly more expensive. Much of the cost is linked to poor target identification and validation, and to late failure of promising therapies. The inclusion of pharmacogenomics into the process has the potential to speed up drug discovery and delivery, and reduce costs. In particular, biomarkers can enhance the identification of drug targets, thus allowing the identification of patients likely to respond to a drug. This will reduce the likelihood of attrition of new compounds, reduce the size, time and cost of drug trials by using biomarkers as surrogate endpoints, and reduce the risk of side effects in patients.

CLINICAL CONTEXT

There are already a number of diagnostic and pharmacogenetics-based tests currently available. They can assist in the diagnosis of subclinical disease, help identify likely responders and non-responders, help in establishing the appropriate dose for responders, and flag those patients likely to suffer from adverse reactions or side effects.

BIOMARKER IDENTIFICATION AND VALIDATION

To date there have been too few clinical trials allowing biomarker selection; those that have been undertaken have often been underpowered. Studies undertaken have used genomic and gene expression-type platforms,¹⁶ and metabolomics and protein analyses. Running sufficiently powered biomarker studies is complicated by regulatory, ethical and clinical concerns. Further, the generation of vast amounts of data on a relatively small number of patients has required the development of new bioinformatics tools. Other issues include the need: to stratify patients to ensure clean datasets, for secondary confirmation of results, for multiple interrogation pathways, for robust meta-analyses. With respect to the latter, there is now a trend for integrative cross-comparative analyses across published datasets,¹⁷ which are available from publically accessible databanks.

Another barrier to development, especially for nucleic acid-based markers, is the slow development of platforms and technologies. More progress is required to improve platform and assay development and sample preparation before these devices will be at the required technology stage for clinical implementation.¹⁷ In addition, the intellectual property and patent areas are awash with submissions for biomarker tests, often with little inventiveness, from groups having no clear intention of commercial test development.

EVALUATION AND CLINICAL IMPLEMENTATION

To date, the clinical evaluation of biomarkers rarely occurs.¹⁸ Most of published studies have been underpowered and

single-centre, with inherent bias. Only a few biomarkers are regularly used in clinical practice, and some of these have been problematical.¹⁹ Future biomarker studies will have to be sufficiently powered, multi-institutional, and with prospective validation.

CRITICAL ISSUES IN BIOMARKER DEVELOPMENT FOR CLINICAL TRIAL ENRICHMENT

Biomarkers should first be selected based on biological plausibility, followed by validation. This begins by demonstration of an association between the biomarker and the clinical endpoint of interest, followed by independent statistical validation of this association.²⁰ For prognostic biomarkers, statistical validation is relatively easy and can be undertaken through retrospective studies.²¹ However, since predictive biomarkers are used to identify patients likely to have a favourable clinical outcome, validation may require comparing outcomes between biomarker-positive and biomarker-negative patients.²² Randomised controlled trials (RCTs) are best suited for this purpose.²³ New types of RCT are required to allow for the dynamic selection of patient subgroups, using biomarker-based therapies.^{24,25,26} These adaptive clinical trials can speed up the drug development process by collecting data from both biomarker positive and biomarker-negative patients.²⁷ In particular, if the results suggest that the benefits of a treatment are limited to the biomarker-positive subpopulation, an enrichment design strategy in which only biomarker-positive patients are enrolled may be appropriate.²⁷ These new trial designs are still being developed, however it should be noted that these designs do not provide information on the effects of treatment in biomarker-negative patients.

If there is some evidence to suggest that a biomarker can predict that a therapy will be more effective in biomarker-positive patients, but the evidence is not sufficient enough to rule out clinical efficacy in biomarker-negative patients, a biomarker-stratified trial design may be more appropriate.^{28,29} Here, biomarkers are used to guide analysis but not treatment assignment. Biomarker-positive and negative patients are randomly assigned to both treatment groups, providing better evidence for the clinical utility of the biomarker. The FDA has published two draft guidance documents for industry on: (1) enrichment strategies; and (2) adaptive design clinical trials.³⁰

APPROACHES TO COLLABORATIVE CO-DEVELOPMENT

A 'companion diagnostic' is a diagnostic test used as a companion to a therapeutic drug to determine its applicability. Several operational and logistical challenges remain in their co-development with the therapeutic drug.³¹ Ideally, they should be developed at the same time as the drug so that clinical validation of the diagnostic can use data from the development of the therapeutic. However, this is often problematic due to the different developmental

models for diagnostics and therapeutics.³² Other challenges include uncertainty about the regulatory issues, weak financial incentives for investment, and clinical, logistical, and resource-related constraints.³³ The FDA has published guidance documents and a concept paper for industry to address regulatory concerns.³⁴ Co-development is increasing, yet the FDA has traditionally regulated these medical products separately.³⁵ Because of growth in this area, the FDA has taken a number of steps to coordinate and clarify the review process.

In Australia, there is a longer mean time to PBS listing for oncology drugs that require a co-dependent application; approximately twice as long compared to oncology drugs that do not require a co-dependent application.³⁶ Very few co-dependent applications are given first time recommendation by both MSAC and PBAC. The average number of submissions required for PBS listing of co-dependent oncology applications is well over two.

There are examples where co-dependent applications have been delayed or not recommended in Australia but are available in other jurisdictions with a comparable health technology assessment (HTA) process. For example, pembrolizumab was rejected in Australia for use in first and second line non-small cell lung cancer, but recommended as having a clinical benefit in at least one of these patient groups by HTA agencies in Germany, France, Canada and the UK. For co-dependent submissions, the average period from registration to reimbursement in Australia is, on average, much longer than in countries with similar HTA requirements (e.g., Canada, England and France).

The key reasons for the delayed PBS listing of medicines with a co-dependent technology in Australia have been highlighted in a case study submitted by Roche to the Senate Inquiry into Funding for Research into Cancers with Low Survival Rates.³⁷ The case study on HER2 testing for use of trastuzumab in gastric cancer highlighted that *'delays and unpredictability are particularly common for targeted cancer therapies that use companion diagnostic tests'*. Lengthy delays to PBS listing of trastuzumab were attributed to HTA rejections, negative feedback from evaluators and challenges of allocating company resources to a complex submission with a low likelihood of success, requiring six HTA evaluations (three for the medicine and three for the test) to gain approval. However, it should be noted that Australia, unlike some other jurisdictions, requires evidence of both drug efficacy and cost-effectiveness before approval.

The Europa-Bio report provided the following recommendations for health economic policy:³⁸

- Health economic evaluations need to become more flexible and adapt to early launches based on high confidence of therapeutic mechanism and early promising data.
- Relevant diagnosis of patients suitable for treatment with personalised medicines needs to become the norm and embedded in routine healthcare pathways and this should not be viewed as an additional separate step and cost, brought about by having a new medicine available.
- More creative funding strategies, such as coverage with evidence development.
- Health decision-makers should set up systematic evaluations of personalised medicines based on their long-term cost-effectiveness.

ACCELERATING THE USE OF BIOMARKERS AS SURROGATE ENDPOINTS

In oncology, a common endpoint is survival; clinical trials seeking to evaluate the benefits of a drug on survival may require many years before conclusions can be drawn.³⁹ Surrogate endpoints are biomarkers that are intended to substitute for the clinical endpoint, but can be measured sooner or more conveniently and have the potential to reduce the length and cost of clinical trials. These could include earlier endpoints such as progression-free survival. The FDA has developed guidance intended to expedite the approval of therapeutics (based on surrogate endpoints) that treat serious conditions where there is unmet need. A drug that demonstrates an effect on a surrogate endpoint reasonably likely to predict clinical benefit may qualify for accelerated approval or breakthrough therapy designation.⁴⁰

For a biomarker to be considered as a surrogate, it must clearly be associated with the clinical outcome of interest and should capture the full effect of treatment.⁴¹ The latter is much more difficult to demonstrate than the former. For example, the cancer might have multiple pathways, whereas the therapeutic agent only affects one pathway mediated through the surrogate.⁴² Meta-analytic approaches have been developed that attempt to provide the evidence required for both association and treatment effect. Nevertheless, the appropriate use of surrogate endpoints remains difficult, as a particular biomarker's status as a surrogate is context-specific.⁴³ Notably, therapeutics approved through expedited programs are required to meet post-market commitments, including post-approval studies that demonstrate the clinical benefits on relevant outcomes. Yet data suggest that a significant proportion of pharmaceutical sponsors are failing to meet their post-market commitments.⁴⁴

To rationalise coverage and use, a clinical and economic comparison of existing and new treatments must be established. One way to do this would be through early engagement; to align the values and expectations of relevant stakeholders during the drug development process and in the post-market setting.

MAJOR POLICY REPORTS

Two major policy reports for biomarker development have been released, the first by the US National Academy of Medicine in 2015,⁴⁵ and the second by the OECD in 2016.⁴⁶ They are summarised here.

NATIONAL ACADEMY OF MEDICINE

The National Academy of Medicine Committee on Policy Issues in the clinical development and use of biomarkers for molecularly targeted therapies has provided the following insights and recommendations⁴⁹.

Overall Policy Implications⁴⁹

1. Policies, systems and funding mechanisms exist in most OECD countries that allow data of biomarker-disease association to be generated. Such evidence is usually carried out by the scientific community.
2. Policies, systems and funding mechanisms do not exist for the large-scale generation of data to inform the assessment of test performance of diagnostics. This is to be contrasted with therapeutic agents where clinical trials are mandatory. Such evidence is needed to determine the clinical validity of a biomarker.
3. Governments should be aware of this gap and the relevant parties need to discuss their relative roles and responsibilities for funding and establishing such mechanisms.
4. The assessment of predictive or susceptibility (as distinct from diagnostic) tests is in its infancy and will require a reorientation of research effort to focus on (a) the establishment of risk prediction algorithms and (b) determination of the threshold at which preventive interventions should be undertaken.

Biomarker Tests for Molecularly Targeted Therapies: Key to Unlocking Precision Medicine

How can healthcare providers, regulators, payers, and test developers ensure that patients have timely access to tests that can accurately direct targeted treatments, while at the same time protect them from potential harm caused by the use of poorly validated or inappropriate tests? The Institute of Medicine of the National Academies of Sciences, Engineering, and Medicine appointed a committee of experts to examine this question. In its report, the committee recommends

an integrated set of actions aimed at addressing clinical practice, regulatory and reimbursement policy, and data challenges through the framework of a rapid learning system. The committee identified ten goals to further advance the development and appropriate clinical use of biomarker tests for molecularly targeted therapies. The committee's recommended approaches to achieving those ten goals are found below.⁴⁷

1. Development of common clinical utility evidentiary standards. Goal 1: Establish common evidentiary standards of clinical utility—using evidence generated both within and outside the context of clinical trials—across all stakeholders.
2. Secretary of the US Department of Health and Human Services (HHS) should facilitate the development of a new integrated federal review process. Goal 2: Establish a more coordinated and transparent federal process for regulatory and reimbursement decisions for biomarker tests for molecularly targeted therapies.
3. FDA should develop standardized label for IVD and LDT biomarker tests. Goal 3: Enhance communication to patients and providers about the performance characteristics and evidence for use of specific biomarker tests for molecularly targeted therapies.
4. HHS should establish and enforce up-to-date laboratory accreditation standards. Goal 4: Update and strengthen the oversight and accreditation of laboratories providing biomarker tests for molecularly targeted therapies.
5. Centres for Medicare and Medicaid Services (CMS) and other payers should develop reimbursement models that support the ongoing collection of data within a rapid learning system. Goal 5: Ensure ongoing assessment of the clinical utility of biomarker tests for molecularly targeted therapies.
6. Electronic Health Records (HER) and Laboratory Information Systems (LIS) vendors and relevant software developers should enable the capture and linkage of biomarker tests and data. Goal 6: Ensure development and use of Electronic Health Records and related biomedical informatics tools and assessments that support the effective clinical use of biomarker tests for molecularly targeted therapies.
7. Convene a Task Force to develop a sustainable national repository of biomarker tests and data. Goal 7: Develop and maintain a sustainable national database for biomarker tests for molecularly targeted therapies through biomedical informatics technology to promote rapid learning for the improvement of patient care.

8. Conduct demonstration projects to evaluate the collaboration between community healthcare providers and other centres to be part of a rapid learning system. Goal 8: Promote equity in access to biomarker tests for molecularly targeted therapies and the expertise for effective use of the results in clinical decision-making. Agencies that fund the development or evaluation of biomarkers should include funding to identify and overcome barriers to promote equity, access, and public understanding of precision medicine.
 9. Standards for specimen requirements, handling, and documentation. Goal 9: Enhance specimen handling and documentation to ensure patient safety and the accuracy of biomarker test results.
 10. Expand interdisciplinary collaborations to develop integrated guidelines on the appropriate use of biomarker tests. Goal 10: Improve the processes for developing and updating clinical practice guidelines for the effective use of biomarker tests for molecularly targeted therapies.
3. Regulatory processes and reimbursement procedures must be adapted to the specificities of novel biomarker-based clinical tests, and harmonised across jurisdictions.
 4. Business models are being developed within the private sector to support research, development and commercialisation of biomarker-based medical tests. However, in some instances market conditions may not be conducive to development of some biomarker-based products, and situations may arise in which policy intervention may be required to enable the development of biomarkers with a clear clinical value and proven clinical validity in the healthcare setting.
 5. Integration of bioinformatics and genomic tools and other technologies, such as nanotechnology, will be needed in order to create new tools for the development of new biomarker-based diagnostics. Infrastructure, networks and other mechanisms that foster technology convergence should be supported and strengthened.
 6. Networks and other mechanisms that facilitate communication of knowledge about biomarkers, advances in biomarker research, or evidence of their clinical utility should be supported and strengthened. Knowledge networks to improve communication between the medical community and patients are particularly important.

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT (OECD)

This report describes the scientific, industrial, regulatory, and healthcare management system context in which biomarkers are being developed.⁴⁸ It identifies some of the barriers that may impede biomarker research, discovery, development, commercialisation and ultimately uptake in clinics. It also focuses on the use of biomarkers in the healthcare system, as diagnostics and in medical tests, and explores the use of biomarkers for the development of improved medicines. It does not address the role of biomarkers upstream, as tools for basic research.

As governments develop the framework necessary to deliver on the promise of biomarkers and personalised medicine, they may wish to consider six key messages that emerge from this report:⁵⁴

1. Long-term investments in the development of sustainable initiatives and infrastructures, including public-private partnerships, are necessary to facilitate biomarker discovery and development.
 2. There is a need for multi-stakeholder discussions about how to develop and populate an evidence base for molecular biomarker medical test evaluations. Regulatory agencies, healthcare payers, test manufacturers, physicians, and patients require evidence regarding the safety, efficacy, utility, and cost-effectiveness of novel tests to inform their decisions. However, generating, collecting, analysing and protecting such data and information, and making it available to different users is not straightforward.
- Stimulating and supporting the organisation of large-scale infrastructures – gathering the knowledge and data both from the research and industry communities – to foster the discovery and validation of novel molecular biomarkers.
 - Stimulating the development of infrastructures capable of developing an evidence base for biomarker evaluation, particularly in determining if there is clinical evidence of their use.
 - Monitoring the evolution of the industry help to ensure the benefits of biomarker-based products and services achieve clinical application.
 - Adapting regulatory and reimbursement procedures to encourage the translation and penetration of biomarkers with proven clinical utility and healthcare value in the clinical setting.
 - Enabling technology convergence by encouraging the integration of nanotechnology, bioinformatics and genomics and biomarker information in development of new diagnostics to encourage uptake and diffusion of biomarkers in the healthcare setting.

The ways in which policy may help accelerate the use of biomarkers in evidence-based medicine are summarised here:

- Promoting education and communication within the healthcare system to familiarise healthcare providers and patients with the benefits of biomarker-based tests, and to facilitate uptake of these tests within the healthcare system.

THE STATE OF PLAY IN AUSTRALIA

Presentations by John Skerritt, General Manager, TGA, provide insights into the current and future trends at the TGA.^{49,50} Some extracts from Skerritt's presentations are provided below.

THE NATURE OF MEDICINES ALONG WITH REGULATIONS HAS CHANGED

There has been a move from small molecules to protein drugs and biologicals, and there is now the targeting of niche therapies rather than “blockbuster” products. There are now more medicine-device and medicine-IVD combinations. This is the era of personalised medicine.

CANCER THERAPY IS RAPIDLY EVOLVING

Cytotoxic drugs are toxic to tissues with a high cell turnover but are not specific to cancer cells. They kill all cells dividing at the time of administration relying on the normal cells, but not cancer cells, recovering. Monoclonal antibodies are an example, targeting cancer cell antigens such as HER2 (trastuzumab), or destroying overactive B cells via CD20 (rituximab). They also include antibody-drug conjugates, bispecific antibodies. Blocking negative immune regulators (“checkpoints”) can give the immune system the ability to fight cancers by blocking molecules which are preventing an immune response to be mounted against the cancer; for example, T-cell activation by blockade of CTLA-4 by ipilimumab or PD-1 by pembrolizumab and nivolumab.

CHANGES IN THE COMMERCIAL ENVIRONMENT

Drug development costs have been increasing, with less local manufacture. Orphan drugs have become a mainstream business and there has been a shift from short-term use of therapies (e.g. for infections) to management of chronic disease. There have been more near-simultaneous submissions through electronic common technical documents (eCTD) and reimbursement rather than regulatory approval is more often determining market entry. We therefore need greater simultaneous regulatory and HTA dialogue.

ADAPTIVE OR “PROVISIONAL” LICENSING

This is the licensing of medicines prior to full phase 3 trials subject to obtaining ‘real-world’ effectiveness and safety data through an iterative process. This is also where regulatory requirements for expansion from a restricted indication to broader population can be fulfilled. To obtain provisional licensing, a development plan is agreed to provide information on risk versus benefit, to enable subsequent authorisation in a defined group of patients and/or treatments. It may be best suited when early data suggests a positive risk-benefit profile and there is an unmet clinical need, or regulatory data exists on safety, and the proposal is for extension of indications.

REGULATORY IMPACTS

There is current debate on the use of surrogate endpoints/ biomarkers for determining efficacy. This has driven much of the impetus for priority review and provisional approval pathways. The move from organ-based to molecular definitions of cancer has driven companion diagnostics and many submissions for extension of indications. The evaluation of results from new and different trial designs, especially adaptive designs, is challenging.

ORPHAN DRUGS

These are now a mainstream business model with molecular targeting and smaller clinical trials. They account for 19% of all medicines sales, and are growing at 12% pa. Cancers are being divided into smaller subsets by their genetic profile, with more therapies such as monoclonal antibodies and small molecules falling under the definition of orphan drugs for small patient populations. The TGA is reviewing its policies regarding population/prevalence threshold, definition of a serious condition, access to satisfactory alternatives, or new treatment that has a significant benefit over these. European Medicines Agency (EMA) is also updating its policies with regards the meaning of “significant benefit”, application to emerging diseases, simultaneous assessment of products, and extension of indications.

CLINICAL TRIAL DESIGN AND ITS CHALLENGES

We need a better understanding of surrogate endpoints versus clinical outcomes or survival. It is now common to provide patient-reported outcomes – however, their use needs further discussion. There is a need to ensure that populations in trials are representative; disease prevention therapies require long-term trials with large numbers. Benefit/ risk tolerance differs for different populations and individuals. We need to ensure that trials of personalised medicine are adequately powered. We are still working on adaptive trial designs and methods of analysing them. Can regulators provide greater clarity on trial design requirements?

SOME EMERGING CHANGES TO CLINICAL TRIALS FOR CANCER MEDICINES

Trials are now being organised according to genomics of the tumour rather than the organ hosting the tumour. This provides the potential for “basket trials”, i.e., trials of drugs for cancers with genetic similarities. We are now seeing the rolling out or extension of indications of new cancer drugs that appear to be effective against an increasing number of cancer types. This will be beneficial for identifying rare cancers that are often neglected in organ-specific clinical trials.

HOW CAN REGULATORS RESPOND TO THESE CHALLENGES?

- Sometimes new regulatory frameworks are required, for example provisional/adaptive licensing of medicines. Earlier availability of drugs may be highly desirable but also carries the risk of not having identified all of the toxicities. There must also be the ability for drugs approved and funded on surrogate endpoints to have their status altered if they subsequently do not meet the primary endpoint, such as an overall survival improvement. However, the current TGA regulations are flexible enough to meet many of the challenges.
- The TGA could take a more proactive approach. This might involve ensuring frameworks are in place to assess new technologies, ability to evaluate new study designs, ensuring recruitment of staff with the required technical skills, supporting SMEs through the regulatory maze, stronger international regulatory collaboration, and closer relationships with industry.
- Through process improvement, including increased use of electronic submissions, better guidelines and information, a centralised data depository, and a new client self-service portal.

PRIORITY REVIEW AND PROVISIONAL LICENSING

There are now several US pathways including breakthrough designation, priority review, and the EMA PRIME (PRiority Medicines) system. Japan has introduced a regulatory framework for innovative products (SAKIGAKE). Australia will introduce priority review in 2017. EMA now has conditional licensing; Sweden has adaptive approval; and Japan has provisional licensing for cell and tissue therapies. Australia will introduce provisional/adaptive licensing in 2018.

CONCLUSIONS OF POLICY REVIEW

In cancer therapy, there has been a major shift from non-specific cytotoxics to targeted small molecules, monoclonal antibodies and immune regulators. At the same time, biomarkers are increasingly being used for diagnosis, prognosis and as surrogate endpoints. In particular, they are being used co-dependently with cancer drugs to stratify the patient population into those for whom the treatment is most likely to be successful, and those for whom the side effects will be fewer. We are also now seeing more flexible, but more complex adaptive study designs that are beginning to replace standard Phase 3 trials.

In response to these and other innovations such as orphan drugs, regulatory authorities in many countries are modifying or changing the ways in which drugs or drug biomarker combinations can be licensed and funded. The processes must ensure that development and funding of the biomarkers tests occurs simultaneously with therapy development so that the therapy can be specifically targeted at the appropriate population. This will make the process more cost-effective and increase the likelihood of a satisfactory response. Bringing drugs to market earlier based on surrogate endpoints, particularly where there are few existing treatment options, is desirable, but must be balanced with the increased risk of late toxicities not identified prior to making a drug widely available.

Australia is behind some other OECD countries in response to these challenges. In the future, it appears likely that the TGA will have an increased focus on the integration of drug and biomarker development for cancers.

Review of individual cancer biomarkers

INTRODUCTION

The first mention of biomarkers in human cancer was in a publication about the discovery of alkaline phosphatase in seminomas, and dates back to 1954.⁵¹ Since then, there have been over 300,000 papers published about biomarkers in cancer. Initially, much of the research was on the use of biomarkers for cancer diagnosis. Subsequently it was found that biomarkers could also be used for cancer prognosis. This was followed by the use of biomarkers as surrogate endpoints for judging the success of cancer treatment. The ability to genotype individuals and the finding that successful cancer treatments and reduction in treatment side effects can be dependent on genotype, have now led to a new era of precision cancer treatment. Importantly, the move from organ-based to molecular definitions of cancer has driven companion diagnostics and many submissions for extension of indications.

Since new potential biomarkers are discovered every day, it is feasible only to provide a detailed review of a small number. We have therefore selected biomarkers that are either: (1) suggested by the sponsors of this study; (2) suggested by the respondents of a national survey; or (3) are related to priority cancers as judged by survey responses.

The objective of this review is to identify the effectiveness of selected biomarkers on the outcomes of selected cancers.

HISTORICAL PERSPECTIVE

LUNG CANCER

Lung cancer can be categorised generally as small-cell lung cancer (SCLC) or non-small cell lung cancer (NSCLC), which account for 15% and 85% of lung cancers, respectively.⁵² Early biomarkers for lung cancer included Carcinoembryonic antigen (CEA), hormonal peptides and some neurogenic enzymes in small cell carcinoma. Initial examinations of serum for lung cancer biomarkers showed elevated levels of Calcitonin, Adrenocorticotrophic hormone (ACTH), Adrenocorticotrophic hormone (ADH), enolase and Creatine kinase / BB isoenzyme (CK-BB) in a significant proportion of small cell carcinoma patients. Bombesin and neuron-specific enolase were also identified as potential diagnostic markers.⁵³ The expression of these biomarkers was also shown to be directly related to disease progression, displaying

their potential as diagnostic and prognostic markers. The consensus has now shifted toward a multi-biomarker approach, using specific sets of biomarkers for each type of lung cancer, as a single diagnostic/prognostic marker for a broad range of lung cancers appears unattainable. Many of these early protein biomarkers for lung cancer are still used to date, including Programmed Cell Death Ligand 1 (PD-L1), cancer antigen-125 (CA-125), carcinoembryonic antigens (CEA), Cytokeratin-19 fragments (CYFRA 21-1) and neuron-specific enolase (NSE).⁵⁴ Other viable biomarkers include serum single or combinations of tumor-associated autoantibodies (TAABs), which can be used as a tool for the diagnosis of lung cancer in patients at all stages.⁵⁵

PANCREATIC CANCER

Secretin tests performed by Dreiling in 1950 were initially used to measure pancreatic function and as a diagnostic aid in differentiating inflammatory, malignant, and normal tissue. His experience included over 6000 patient examinations, detecting pancreatic cancer with high accuracy.^{56,57,58,59} Unfortunately, this method required an extensive knowledge base and was also costly with respect to time and effort, hence biomarkers such as cancer embryonic antigen (CEA) became the focus. In the early 1970's screening for pancreatic cancer was performed using radioimmunoassay for circulating CEA and tumour-associated antigen (TAA). Elevated levels of CEA were detected in 85% of patients with pancreatic cancer (most of whom were in an advanced stage). More promising evidence for the use of CEA as a pancreatic cancer biomarker was illuminated by Zamcheck et al in 1974 and 1975,⁶⁰ reviewing CEA as a biomarker with a high level of accuracy in predicting pancreatic cancer.⁶¹ In 1980, Mackie et al investigated a number of candidate markers and identified that the serum levels of pancreatic oncofetal antigen, fasting plasma glucose and serum alkaline phosphatase were the most promising for the diagnosis or exclusion of pancreatic cancer.⁶²

Over the next few decades, overexpressed proteins/epitopes such as CA 19-9, CA-50, CEA, and many others held the focus as pancreatic cancer tumour biomarkers.⁶³ These biomarkers were primarily used for the diagnosis of pancreatic cancer, monitoring chemotherapy and determining prognosis. However, these markers displayed limitations like poor sensitivity, false-negative results, as well as false-positive elevation in the presence of other conditions. A preferred marker for pancreatic cancer is still yet to be identified.⁶⁴

BREAST CANCER

An initial screening for breast cancer biomarkers identified seven potential markers including: serum ferritin, C-reactive protein, carcinoembryonic antigen, acid glycoprotein, total alkaline phosphatase, sialyl transferase, and the ratio of urinary hydroxyproline to creatinine.⁶⁵ CEA and human chorionic gonadotropin (HCG) proved useful as early indicators for predicting response to combination chemotherapy in metastatic breast cancer.⁶⁶ Hendrick and Franchimont were among the first to investigate casein as a more specific biomarker for breast cancer, where 81% of patients displayed elevated levels.⁶⁷ A 1980 review highlighted CEA, gamma-glutamyl transpeptidase (γ -GT), and alkaline phosphatase as the most suitable markers. The glycoprotein epithelial membrane antigen (EMA) was also identified as another potential candidate for a breast cancer biomarker.⁶⁸ Markers such as CEA, ferritin, immune complexes, and specially oestrogen receptors all displayed exciting potential as prognostic indicators.⁶⁹ Many of these markers still provide insights into the diagnosis and prognosis of breast cancer to date.

COLORECTAL CANCER

The guaiac faecal occult blood test (FOBT) was the first biomarker for colorectal cancer (CRC) and enabled population screening from the outset of its use.⁷⁰ This simple test was complimented by new colonoscopy procedures that enabled RCTs to be instigated, and proved that screening by FOBT was capable of reducing mortality (e.g. Mandel et. al. 1993⁷¹). These guaiac-based tests may produce false-positive results from the presence of factors such as plant peroxidases, whilst peroxidase inhibitors such as vitamin C may produce false-negative results. Thus, modifications to screening procedures have been made to improve detection sensitivity and specificity by using immunochemical testing.⁷²

PROSTATE CANCER

It was first observed in the 1930's by the respective laboratories of Kutscher and Gutman that the amount of prostatic acid phosphatase in tissue and serum in prostate cancer patients was elevated compared to healthy individuals. They also found that this protein was also highly expressed in metastatic sites on bone.^{73,74} Acid phosphatase was used in prostate cancer diagnosis through the late 1980's,⁷⁵ where it was ultimately determined to be insufficient at predicting disease recurrence due to significantly fluctuating serum levels, compared to prostate specific antigen (PSA),⁷⁶ which became the de facto biomarker for prostate cancer. PSA was first proposed in 1981 by Wang et al.⁷⁷ Despite the shortcomings of the lack of specificity to clinically relevant prostate cancer, PSA blood serum levels are used in diagnosing the disease, with improvements made through use of 'free PSA' in ratio to total PSA in multivariate analyses.⁷⁸

Additional FDA-approved diagnostic tests include testing for PCA3 and TMPRSS2:ERG to improve decision making post-biopsy.⁷⁹

OVARIAN CANCER

Initial biochemical analysis indicated that lactate dehydrogenase and glutamic oxaloacetic transaminase were elevated in patients of ovarian cancer.⁸⁰ Similarly, 'fibrin degradation products'⁸¹ were found in over 70% of patients with malignant cancer versus those with benign changes, who had less than 5%. These were never used as a diagnostic biomarker, and carcinoembryonic antigen (CEA) and human chorionic gonadotropin (β -hCG) could likewise only be used to monitor, but not diagnose, disease status as a result of their lack of specificity.⁸² As of October 2017, there are currently no FDA approved tools for screening populations for early onset of ovarian cancer.

MULTIPLE CANCERS

BIOMARKERS FOR MULTIPLE CANCERS

- PD-1/PD-L1 (Programmed death)
- TRK fusion
- HMGB1
- Immunological metagene signatures
- Long Noncoding RNA MALAT-1
- Acquired resistance to gemcitabine
- Novel biomarkers

Genomic scar signatures

PD-1/PD-L1 (PROGRAMMED DEATH)

Immune checkpoint inhibitors (ICI) are drugs that promote an immune system attack on cancer cells. Checkpoint inhibitors seek to overcome one of cancer's main defences against an immune system attack. Immune system T cells patrol the body constantly for signs of disease or infection. When T cells encounter another cell, they probe on its surface, which serve as a sign of the cell's identity. If the proteins suggest that the cell is infected or cancerous, the T cell will attack. Once T cells initiate an attack, the immune system increases a series of additional molecules to prevent the attack from damaging normal tissues. These molecules are immune checkpoints. Cancer cells often use immune checkpoint molecules to suppress and evade an immune system attack. Checkpoint inhibitors block these proteins on cancer cells, or the proteins on T cells that respond to them.

Programmed cell death (PD-1), and its ligands (PD-L1 and PD-L2) are ICIs. Expression levels are associated with an increase in treatment effect to immunotherapies working on the PD-1/PD-L1 target. These drugs are currently in clinical development

in large numbers of trials in a variety of cancers. Nivolumab, Pembrolizumab and Atezolizumab have been FDA approved and are increasingly used in multiple cancer types.^{83,84,85} Nivolumab, an IgG4 subclass PD-1 inhibitor, is FDA approved for metastatic melanoma, advanced NSCLC (NSCLC),⁸⁶ RCC⁸⁷ and Hodgkin's disease.⁸⁸ Pembrolizumab is FDA approved for metastatic melanoma and advanced NSCLC and Atezolizumab has been recently FDA approved for the treatment of urothelial bladder cancer. In addition, these agents and other antibodies targeting PD-1 or its ligands are under investigation in a broad spectrum of malignancies, such as mismatch-repair deficient colorectal carcinoma, non-Hodgkin's lymphoma and cancers of the head and neck. In addition, multiple clinical trials investigating combination regimens of checkpoint inhibitors are underway.

In Australia, Pembrolizumab is registered by the TGA for first-line treatment of NSCLC, and unresectable or metastatic melanoma and a number of other indications. The PD-L1 test kit is available and used in a number of countries overseas.

TRK FUSION

TRK (*tropomyosine receptor kinase*) fusions occur when one of the TRK genes becomes abnormally connected to another gene. This fusion event causes the TRK gene to be turned on and the cancer to grow. The function of TRK is to monitor cell differentiation and play a role in specifying sensory neuron subtypes. TRK fusion genes have so far been associated with cancers of the following types:⁸⁹ colorectal, soft tissue sarcoma, spitzoid melanoma, AYA sarcoma, congenital infantile fibrosarcoma, papillary thyroid carcinoma, glioblastoma, NSCLC, large cell neuroendocrine tumour, lung adenocarcinoma, appendiceal adenocarcinoma, low grade glioma, pilocytic astrocytoma, head and neck squamous cell carcinoma, intrahepatic cholangiocarcinoma, mammary secretory breast carcinomas, ductal carcinoma, fibrosarcomas, congenital mesoblastic nephroma, radiation-associated thyroid cancer, acute myeloid leukaemia, and gastrointestinal stroma tumour.

A pan-tumour biomarker for TRK fusion currently under expedited review by the FDA,⁹⁰ is used to assess larotrectinib. Larotrectinib is an inhibitor TRK receptors TrkA, TrkB, and TrkC. It was awarded orphan drug status by the FDA in 2015 for soft tissue sarcomas and it was awarded breakthrough therapy status in 2016 for metastatic solid tumours. TRK fusions are thought to be oncogenic drivers, but their clinical significance remains unclear.

HMGB1

A systematic review and meta-analysis was undertaken to assess the association of HMGB1 expression with prognosis in cancer patients.⁹¹ HMGB1 overexpression was significantly associated with poorer overall survival irrespective of cancer types. HMGB1 overexpression had a consistent association with poorer survival when detected by immunohistochemistry in tissues and enzyme-linked immunosorbent assay in serum. HMGB1 has the potential to be a prognostic factor and potential biomarker for survival in cancer.

IMMUNOLOGICAL METAGENE SIGNATURES

A meta-analysis was undertaken to evaluate the association between immunological metagene signatures derived from immunogenic cancer cell death (ICD) with improved survival of patients with various types of cancer.⁹² The authors analysed the prognostic impact of differential gene-expression of 33 pre-clinically-validated ICD-parameters through a large-scale meta-analysis. The ICD-associated parameters exhibited a highly clustered and largely cancer type-specific prognostic impact. They found that the cancer type-independent consensus-metagene acted as an 'attractor' for cancer-specific convergent-metagenes. They concluded that ICD can serve as a platform for discovery of novel prognostic metagenes.

LONG NONCODING RNA MALAT-1

MALAT-1 is significantly overexpressed in various cancers, suggesting that it might be a potential biomarker of cancer. Findings from a large meta-analysis were that MALAT-1 can serve as a molecular marker in different types of cancers.⁹³

ACQUIRED RESISTANCE TO GEMCITABINE

Acquired resistance to the chemotherapy agent gemcitabine is a problem. To date, no genetic factors have been identified that are completely responsible for the resistance process. A meta-analysis⁹⁴ of available microarray datasets for cancer cell lines with acquired gemcitabine resistance was undertaken. By systemic combinational analysis of the three molecular networks, the researchers condensed the total number of differentially expressed genes (DEG) to only seven. GJA1, LEF1, and CCND2 were contained within the lists of the top 20 up- or down-regulated DEGs.

NOVEL BIOMARKERS

A meta-analysis using human meiotic genes identified a new cohort of highly restricted cancer-specific marker genes.⁹⁵ Cancer/testis (CT) genes are an important gene family with expression tightly restricted to the testis in normal individuals but which can also be activated in cancers. The researchers analysed and validated expression profiles of human meiotic genes in normal and cancerous tissue followed by meta-analyses of clinical data sets from a range of tumour types resulting in the identification of a large cohort of highly specific cancer biomarker genes, including the recombination hot spot activator PRDM9 and the meiotic cohesin genes SMC1beta and RAD21L.

GENOMIC SCAR SIGNATURES

Pan-cancer analysis of genomic scar signatures that are associated with homologous recombination deficiency suggests new indications for existing cancer drugs. Ovarian and triple-negative breast cancers with BRCA1 or BRCA2 loss are highly sensitive to treatment with PARP inhibitors and platinum-based cytotoxic agents, and show an accumulation of genomic scars in the form of gross DNA copy number aberrations. Cancers without BRCA1 or BRCA2 loss, but with accumulation of similar genomic scars, also show increased sensitivity to platinum-based chemotherapy. Therefore, reliable biomarkers to identify DNA repair-deficient cancers prior to treatment may be a useful method for directing patients to platinum chemotherapy and possibly PARP inhibitors. A study⁹⁶ explored the pan-cancer distribution of scores of three gene signatures and found a good correlation between scores of the three signatures. They found that cancer types ordinarily receiving platinum as therapy had higher median scores on all three signatures. They also found that smaller subpopulations of high-scoring tumours exist in most cancer types, including those for which platinum chemotherapy is not standard therapy.

SOLID TUMOURS

PREDICTIVE BIOMARKERS FOR SOLID TUMOURS

- Bevacizumab
- Genomic classifiers of solid tumours

PREDICTIVE BIOMARKERS

BEVACIZUMAB

Bevacizumab is a humanised monoclonal antibody that blocks the binding of circulating vascular endothelial growth factor to its receptors. The FDA has approved bevacizumab for the

treatment of several solid tumours. Roviello et al⁹⁷ performed a meta-analysis of all randomised trials where bevacizumab was tested in the first line setting compared with a control arm, including chemotherapy, placebo or other anti-neoplastic agents. They confirmed that bevacizumab-based regimens result in a significant improvement in survival and response in advanced colorectal, lung, ovarian and kidney cancer.

GENOMIC CLASSIFIERS OF SOLID TUMOURS

Recent advances in the understanding of the genetic underpinnings of cancer offer the promise to customize cancer treatments to the individual through the use of genomic classifiers (GCs). At present, routine clinical utilisation of GCs is uncommon and their current scope and status, in a broad sense, are unknown. As part of a review, Prodromidou et. al.,⁹⁸ systematically examined the literature evaluating the utility of commercially available GCs. Most studies were specific to hormone-receptor positive breast cancer, whereas only four studies evaluated GCs in non-breast cancer (prostate, colon, and lung cancers). While there are several GCs that have been validated, the general quality of the data are weak. Further research, including prospective validation is needed, particularly in the non-breast cancer GCs.

TESTICULAR CANCER

BIOMARKERS FOR TESTICULAR CANCER

- Novel cohort of cancer-testis biomarker

NOVEL COHORT OF CANCER-TESTIS BIOMARKER

Some antigenic proteins are only normally present in male gametogenic tissues in the testis and not in normal somatic cells. Aberrant proteins are referred to as cancer/testis (CT) antigens (CTAs). Some CTA genes have been shown to encode immunogenic proteins that have been used as successful immunotherapy targets for various forms of cancer and have been implicated as drug targets. A meta-analysis demonstrated a novel cohort of CT biomarker genes.⁹⁹ The expression profiles of these genes were validated in a range of normal and cancerous cell types. Subsequent meta-analysis of microarray data demonstrated that these genes are clinically relevant as cancer-specific biomarkers. This could pave the way for the discovery of new therapies and/or diagnostic/prognostic monitoring technologies.

COLORECTAL CANCER

BIOMARKER FOR COLORECTAL CANCER

DIAGNOSTIC BIOMARKERS

- BRAF
- EGFR
- PIK3CA exon 20 mutations
- Dysregulated lncRNAs profiling

PREDICTIVE BIOMARKERS

- CEA
- Dysregulated lncRNAs profiling
- PIK3CA exon 20 mutations

Colorectal cancer (CRC) is one of the leading causes of cancer-related death worldwide, but the pathogenesis of CRC remains not well known.

DIAGNOSTIC BIOMARKERS

BRAF

A meta-analysis of BRAF mutation as a predictive biomarker of benefit from anti-EGFR monoclonal antibody therapy for RAS wild-type metastatic CRC was undertaken. Metastatic CRC that has a BRAF V600E mutation was found to have poorer outcomes.¹⁰⁰ However, whether this mutation is predictive of treatment benefit from EGFR monoclonal antibody therapy is uncertain. The meta-analysis found that there is insufficient evidence to definitively state that RAS WT/BRAF MT individuals attain a different treatment benefit from anti-EGFR mAbs for metastatic CRC compared with RAS WT/BRAF WT individuals. They concluded that there are insufficient data to justify the exclusion of anti-EGFR mAb therapy for patients with RAS WT/BRAF MT metastatic CRC.

EGFR

EGFR inhibitors monoclonal antibodies have shown therapeutic effectiveness in patients with metastatic CRC. However, many patients show resistance to treatment. EGFR gene copy number (GCN) is a potential biomarker for predicting treatment resistance in these patients. A systematic review of EGFR gene copy number as a predictive biomarker for resistance was undertaken.¹⁰¹ The results suggest that EGFR GCN represents a predictive biomarker for tumour response in these patients regardless of KRAS mutation. Patients with increased EGFR GCN were more likely to have a better response when treated with cetuximab or panitumumab.

In another study, a meta-analysis of EGFR GCN as a predictive biomarker for the treatment of metastatic colorectal cancer with anti-EGFR monoclonal antibodies was undertaken.¹⁰² The authors found that although increased EGFR GCN is

generally associated with a better outcome of anti-EGFR MAb treatment, especially among patients with wild-type KRAS, the clinical use of this biomarker for selecting recipients of anti-EGFR MAb is severely limited by the heterogeneous scoring system and the poor reproducibility of EGFR GCN enumeration due to technical reasons.

PREDICTIVE BIOMARKERS

CEA

Testing for carcino-embryonic antigen (CEA) in the blood is a recommended part of follow-up to detect recurrence of CRC following primary treatment. A Cochrane review¹⁰³ found substantial clinical variation in the cut-off level applied to trigger further investigation.

PIK3CA EXON 20 MUTATIONS

A systematic review and meta-analysis of PIK3CA exon 20 mutations as a potential biomarker for resistance to anti-EGFR monoclonal antibodies in KRAS wild-type metastatic CRC was undertaken.¹⁰⁴ Clinical outcomes of interest included objective response rate (ORR), progression-free survival (PFS), and overall survival (OS). They concluded that PIK3CA exon 20 mutations may be a potential biomarker for resistance to anti-EGFR MoAbs in KRAS wild-type metastatic CRC.

DYSREGULATED LONG NON-CODING RNA PROFILING

A meta-analysis was undertaken exploring the identification of dysregulated long non-coding RNA (lncRNA) profiling and metastasis-associated lncRNAs in colorectal cancer by genome-wide analysis.¹⁰⁵ RNA sequencing and microarray data obtained from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) were analyzed to find differentially expressed lncRNAs in CRC. They found hundreds of lncRNAs expressions are dysregulated in CRC tissues when compared with normal tissues. By genomic variation analyses, they identified that some of this lncRNAs dysregulation is associated with the copy number amplification or deletion. Many lncRNAs expression levels were significantly associated with overall and recurrence-free survivals. They concluded that taken together, aberrantly expressed lncRNAs may play critical roles in the development of liver metastasis of CRC.

RECTAL CANCER

BIOMARKER FOR RECTAL CANCER

- Predictive factors of the response of rectal cancer to neoadjuvant radiochemotherapy

PREDICTIVE BIOMARKERS

PREDICTIVE FACTORS OF THE RESPONSE OF RECTAL CANCER TO NEOADJUVANT RADIOCHEMOTHERAPY

Locally advanced rectal cancer is currently treated with pre-operative radiochemotherapy, but the response is variable. Identification of patients with higher likelihood of responding is important, as patients with resistant tumours could be spared exposure to radiation or DNA-damaging drugs. A systematic review of predictive biomarkers of response to pre-operative radiochemotherapy was conducted.¹⁰⁶ They found that the majority of the studies did not support the predictive value of p53, while the values of Ki-67, TS and p21 was still controversial.

PANCREATIC CANCER

BIOMARKERS FOR PANCREATIC CANCER

DIAGNOSTIC BIOMARKERS

- Related Biomarkers for PC
- Early detection biomarkers
- Related Serum Biomarkers for PC
- survivin gene
- CA19-9
- IGFBP2 and IGFBP3
- Muc-1
- Circulating microRNA profiling studies
- Diagnostic accuracy of MRI, PET scan
- Molecular imaging technology

PREDICTIVE BIOMARKERS

- Current and Emerging Therapies in Metastatic Pancreatic Cancer
- Vandetanib with or without gemcitabine
- Peptide cocktail therapy

Pancreatic cancer (PC) is a serious threat to human health, due to malignant tumours with concealed onset, rapid development, and poor prognosis. PC is the fourth leading cause of death among all cancers in the USA, with a 5-year survival rate of less than 5%.¹⁰⁷ These outcomes can be attributed to the lack of early diagnoses and the inability to detect precancerous lesions.¹⁰⁸ Therefore, the detection and diagnosis of PC in the early stage are extremely urgent.

DIAGNOSTIC BIOMARKERS

At present, the methods used to diagnose PC include tumour marker detection and imaging. The traditional tumour markers that have been used for the early diagnosis of PC have high sensitivity in clinical use, but low specificity and

these markers are thus prone to false positives.¹⁰⁹ CT and MRI have been used to diagnose and stage the majority of PCs with tumour detection limits of 5–8 mm, when the earliest precursor lesions are in the microscopic range.^{110,111}

RELATED BIOMARKERS FOR PC

The increasing study of PC has established that it is a highly heterogeneous disease involving extremely complex tumour microenvironments that express a variety of antigens and receptors within the tumour cells and surrounding stroma. These related proteins and highly expressed genes in malignant tumours are the foundation of designing functionally targeted nanoparticles. Currently, many differentially expressed genes related to signal transduction are known to play roles in the development of PC that include the stimulation of proto-oncogenes, such as K-ras,^{112,113} HER-2/neu^{114,115} and BRCA^{116,117} and the inactivation of tumour suppressor genes, such as SMAD4¹¹⁸, APC¹¹⁹, P53¹²⁰, and CDKN2A¹²¹. The associated genes that have been identified as being involved in these processes have potential as imaging markers for PC.

Although a wide variety of tumour markers have been associated with PC, these markers cannot fully meet the requirements of imaging targets, primarily because the sensitivity, specificity, and expression quantities are not homogenous. Nonetheless, additional exploration and in-depth study are needed to select the appropriate molecular imaging targets for PC.

EARLY DETECTION BIOMARKERS

Pancreatic ductal adenocarcinoma (PDAC) is largely incurable due to late diagnosis. Better early detection biomarkers are critical to improving PDAC survival and risk stratification. A meta-analysis of PDAC transcriptome datasets identified and validated key PDAC biomarkers.¹²² This study identified and validated a highly accurate 5-gene PDAC classifier for discriminating PDAC and early precursor lesions from non-malignant tissue. This may facilitate early diagnosis and risk stratification upon validation in prospective clinical trials. They found that cell-based experiments of two overexpressed proteins encoded by the panel, TMPRSS4 and ECT2, suggest a causal link to PDAC development and progression, confirming them as potential therapeutic targets.

RELATED SERUM BIOMARKERS FOR PC

Serum autoantibodies against tumour-associated antigens have recently emerged as early stage biomarkers for different types of cancers. A systematic review¹²³ of early biomarkers for pancreatic cancer found that serum autoantibodies might present an option, but more work is needed to identify and validate autoantibody signatures.

SURVIVIN GENE

More recently, the survivin gene, which is a potential marker of PC, has been regarded as a targeting gene, and chitosan-coated magnetic iron oxide particles (MNPs) have been regarded as imaging probes for the detection of PC.¹²⁴ The research not only demonstrated that the survivin gene of PC was detectable by Sur-MNPs but also indicated that Sur-MNPs may become good negative molecular contrast agents in the diagnosis of PC. Further studies evaluating the selective uptake of Sur-MNPs in PC xenografts in vivo are extremely urgent.

CA19-9

Detection of serum tumour markers CA19-9, CEA, CA125 and CA242 may aid in the early diagnosis of PC.¹²⁵ It appears that a reduced rate of apoptosis plays a crucial role in carcinogenesis, and it is one of the most important characteristics acquired by PC cells, which protects them from attack by the immune system and reduces the effectiveness of pharmacological treatment. However, there are some concerns that CA19-9 lacks the sensitivity needed to detect early-stage PC¹²⁶ and to monitor responses to therapy, because of its poor sensitivity and specificity.¹²⁷

IGFBP2 AND IGFBP3

A study by Yoneyama et al.¹²⁸ aimed to identify plasma biomarkers for early detection of invasive ductal adenocarcinoma of the pancreas (IDACP) by using two proteomics strategies: antibody-based proteomics and liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based proteomics. They found that insulin-like growth factor-binding protein (IGFBP)2 and IGFBP3 have the ability to discriminate IDACP patients at an early stage from healthy controls, and IGFBP2 appeared to be increased in conditions such as intraductal papillary mucinous neoplasms. Further, diagnosis of IDACP using the combination of carbohydrate antigen 19-9 (CA19-9), IGFBP2 and IGFBP3 was significantly more effective than CA19-9 alone. This suggests that IGFBP2 and IGFBP3 may serve as compensatory biomarkers for CA19-9.

MUC-1

Muc-1 is a transmembrane mucin glycoprotein and is another biomarker that is associated with the most invasive forms of PC.¹²⁹ Muc-1 levels are elevated in the majority of patients with PC, and Muc-1 plays a key role that affects oncogenesis and the motility, metastasis, metabolism, and growth of cancer cells.¹³⁰ Gold et. al.,¹³¹ showed that Muc-1 is overexpressed in PC both in the cytoplasm and in the cell membrane, compared with most chronic pancreatitis tissues and normal pancreatic tissues in which Muc-1 is only expressed in the cell membrane with no cytoplasm expression. Thus, there is a direct relationship between high invasiveness and poor PC prognosis.¹³² The PAM4 antibody against Muc-1 is more

specific for pancreatic cancer than antibodies to the other Muc-1 antigens that are observed in other tumours. In a recent study, the authors found that the PAM4-reactive Muc-1 epitope was not detected in the normal pancreas but was expressed in 87% of invasive pancreatic adenocarcinomas. Additionally, Muc-1 acts as a master regulator of the metabolic program that can also help tumour cells survive and proliferate in hypoxic environments.¹³³ Many studies have demonstrated that Muc-1 can be used as an ideal target in the diagnosis and treatment of pancreatic cancer.^{134,135}

CIRCULATING MICRORNA PROFILING STUDIES

Several studies of differentially expressed miRNAs as candidate biomarkers of PC have been conducted, however, these have been mainly performed in single laboratory settings. A meta-analysis of circulating mRNA profiling studies in PC was undertaken.¹³⁶ The analysis demonstrated that multiple miRNA profiles were more accurate for diagnosing PC than a single miRNA, and future studies are still needed to confirm the diagnostic value of these pooled miRNAs.

Currently, more than 20 miRNAs have been proven to be associated with PC.¹³⁷ miRNA-21 has been considered to be the miRNA most closely related to cell proliferation, metastatic ability, and poor overall survival.¹³⁸

Moreover, miRNA-21 has been demonstrated to be significantly overexpressed in both PC cell lines and tissues relative to normal pancreatic tissue.¹³⁹ Additionally, some other miRNAs (13Ob¹⁴⁰, 196a¹⁴¹, 92a¹⁴², 198¹⁴³, 221¹⁴⁴, 23b¹⁴⁵, and 29a¹⁴⁶) have also been shown to have important roles in PC. In a recent study, Nagano et al.¹⁴⁷ established seven miRNA-based biomarker models (miR-20a, miR-21, miR-24, miR-25, miR-99a, miR-185, and miR-191) for PDAC diagnosis and found that these biomarkers exhibited high sensitivity and specificity in the discrimination of PC and chronic pancreatitis patients. Therefore, the identification of the miRNAs suggests that they can also be used as potential tools for the screening of early-stage PC.

DIAGNOSTIC ACCURACY OF MRI, PET SCAN

A Cochrane review¹⁴⁸ was undertaken to determine the diagnostic accuracy of MRI, PET scan, and EUS performed as an add-on test or PET-CT as a replacement test to CT scanning in detecting curative resectability in pancreatic and periampullary cancer. The review found no evidence to suggest that it should be performed routinely in people with PC or periampullary cancer found to have resectable disease on CT scan.

MOLECULAR IMAGING TECHNOLOGY

Molecular imaging is a medical imaging technique that combines molecular biology, chemistry, material science, radiation medicine, and computer science.^{149,150} In contrast

with traditional imaging techniques that are primarily based on gross anatomy structures, molecular imaging can: identify pathological changes at the molecular and cellular level, determine the qualitative properties of the diseases, enable objective monitoring of the efficacy of treatment, and predict disease development. Molecular imaging research primarily includes two aspects, the first of which is the choice of imaging equipment. Molecular MR imaging has become a novel technique for assessing specific cellular or subcellular events, and is becoming one of the core integrative technologies in biomedicine because many of the parameters that are used to produce contrast, such as the spin-lattice relaxation (T 1) and spin-spin relaxation (T 2) times, are dependent on the local chemical structure of the molecules being imaged¹⁵¹.

Additionally, some protein markers have newly been discovered. CEACAM-1¹⁵², CEACAM-6¹⁵³, CD133¹⁵⁴, S100A4¹⁵⁵, and midkine¹⁵⁶ have been shown to be biomarkers that are also expressed in PC and are significantly associated with invasion and metastasis in PC and PC prognosis. Therefore, these markers also have the potential to become the imaging and therapy targets for PC.

A recent study¹⁵⁷, found that using the MRI technique, these investigators demonstrated that ScFvEGFR-IO specifically bound to and was internalized by EGFR-expressing cancer cells. Additionally, the use of ScFvEGFR-IO as a molecular imaging agent was demonstrated with MRI in an orthotopic human PC mouse xenografted model.

UPAR is a biomarker of PC that is highly expressed in tumour and stroma cells, and the active retention of these nanoparticles is increased in many target cells in tumour masses. In 2013, Lee et. al.,¹⁵⁸ engineered urokinase plasminogen activator receptor- (UPAR-) targeted magnetic iron oxide nanoparticles (IONPs). The results revealed that UPAR can act not only as the imaging probe but also as the therapy carrier for PC.

MR molecular imaging appears to be a promising imaging modality for the early detection of PC. This imaging modality also facilitates the study of the pathological changes associated with PC at the molecular and cellular levels.¹⁵⁹ At present, many studies¹⁶⁰ have conducted in vivo experiments and provided evidence of the feasibility of these targeted contrast agents. However, there are still some studies that have not conducted in vivo experiments. Therefore, this issue is worthy of extensive research because these issues have great significance for targeted molecular imaging and therapy of PC.

PREDICTIVE BIOMARKERS

CURRENT AND EMERGING THERAPIES IN METASTATIC PC

Targeted therapies and immunotherapy have changed the face of multiple solid malignancies, including metastatic melanoma and lung cancer, but no such therapies exist for pancreatic ductal adenocarcinoma (PDAC) despite the knowledge of key mutations and an increasing understanding of the tumour microenvironment. Until now, most clinical studies have not been biomarker driven in this highly immunosuppressive and heterogeneous cancer. Ongoing basic and translational studies are better classifying the disease in the hope of identifying critical pathways that distinguish the unique PDAC subtypes, which will lead to personalized therapies.

Manji et al's review¹⁶¹ discussed the current treatment options for metastatic PC and highlighted current ongoing clinical trials, which aim to target the stroma and the immune microenvironment either alone or in combination with standard chemotherapy. Identifying biomarkers and key resistance pathways and targeting these pathways in a personalized manner in combination with chemotherapy are likely to yield a more immediate and durable clinical benefit.

VANDETANIB WITH OR WITHOUT GEMCITABINE

An RCT¹⁶² demonstrated improved overall survival in patients receiving erlotinib in addition to gemcitabine for locally advanced or metastatic PC. Patients with elevated levels of receptor tyrosine-protein kinase erbB-2 (HER2) expression had improved overall survival when treated with erlotinib compared to placebo.

Vandetanib plus gemcitabine versus placebo plus gemcitabine in locally advanced or metastatic pancreatic carcinoma (ViP) was examined in a randomised, double-blind, multicentre Phase 2 trial.¹⁶³ Vandetanib is a novel tyrosine kinase inhibitor of VEGFR2, RET, and EGFR, all of which are involved in the pathogenesis of PC. Middleton et al¹⁶⁴ investigated the clinical efficacy of vandetanib when used in combination with gemcitabine in patients with advanced PC. The addition of vandetanib did not improve overall survival.

PEPTIDE COCKTAIL THERAPY

A Phase 2 clinical trial of peptide cocktail therapy for patients with advanced PC was undertaken.¹⁶⁵ The study demonstrated that this therapeutic peptide cocktail might be effective in patients who demonstrate peptide-specific immune reactions although predictive biomarkers are needed for patient selection in its further clinical application.

PROSTATE CANCER

BIOMARKERS FOR PROSTATE CANCERS

DIAGNOSTIC BIOMARKERS

- Active surveillance
- Evidence for the use of biomarkers in the early detection
- Clinicopathologic variables and biomarkers for risk stratification
- MicroRNAs
- Validation of Diagnostic Tumor Biomarkers

PROGNOSTIC BIOMARKERS

- Potential prognostic biomarkers
- Prognostic immunotherapies
- PTEN genomic deletion
- Analysis of PHI, 4Kscore, MIPS, GPS, Prolaris, Decipher
- AE37 peptide vaccination
- Retinoic Acid Pathway in Early Prostate Cancer
- Serum biomarkers of bone metabolism

PREDICTIVE BIOMARKERS

- Effective predictors of tumor recurrence
- Urinary EN-2
- Hormone Therapy with Salvage Radiation Therapy
- Effect of Early Switch From Docetaxel to Cabazitaxel or Vice Versa
- Biomarkers for castration resistant cancer

Prostate cancer (PCa) is a leading cause of death in men. After radical prostatectomy (RP), nearly 30% of men develop clinical recurrence with high serum PSA levels. Active surveillance (AS) is a conservative management approach, conducted for those patients with localised disease, which avoids long-term adverse effects on the patient's quality of life. AS is broadly appropriate for men with a Gleason score of 6 or less and a PSA level of less than 10 ng/mL. An essential element of the AS approach is early recognition of higher-risk disease, which is diagnosed by systematic biopsy in 30% of patients who initiate AS with low-risk disease. In addition, a small group of patients have molecular alterations that can cause progression to more aggressive disease; these men can be switched to immediate treatment if such progression is detected.

DIAGNOSTIC BIOMARKERS

When PCa is suspected, tissue biopsy remains the standard for diagnosis. However, the identification and characterization of the disease have become increasingly precise through improved risk stratification and advances in MRI and functional imaging, as well as from the emergence of biomarkers.

Multiple management options now exist for men diagnosed with prostate cancer.

EVIDENCE FOR THE USE OF BIOMARKERS IN EARLY DETECTION

The use of PSA is controversial; cohort studies have found that screening only marginally influences mortality from prostate cancer. This was confirmed by the Lamy et al¹⁶⁶ systematic review of clinical trials and studies assessing PSA and other biomarkers in the early detection of PCa. PSA can be used for early PCa detection, however, mass screening is not recommended. Studies on other biomarkers suggest that they could be used, individually or in combination, to improve the selection of patients with elevated PSA levels for biopsy, but RCTs assessing their impact on prostate cancer management and mortality are needed.

Many groups have identified alternative biomarkers for PCa screening, and to distinguish potentially lethal from indolent tumours, and to guide treatment decision. Lamy et al¹⁷³ have analysed these indicators for their diagnostic and prognostic potential. They identified 380 markers from the literature. The most interesting ones appeared to be claudin 3 (CLDN3) and alpha-methylsacyl-CoA racemase highly expressed in prostate cancer and filamin C (FLNC) and keratin 5 with highest expression in normal prostate tissue. However, to date, none of the markers are more specific than PSA.

CLINICOPATHOLOGIC VARIABLES AND BIOMARKERS FOR RISK STRATIFICATION

A systematic review was undertaken by Loeb et al.¹⁶⁷ Many studies found that a lower percentage of free PSA, a higher Prostate Health Index (PHI), a higher PSA density (PSAD), and greater biopsy core involvement at baseline predict a greater risk of progression. Limitations of these studies include varied definitions of progression and limited follow-up. There is increasing literature on patient characteristics, biopsy features, and biomarkers with potential utility in active surveillance. Several PSA-based tests (free PSA, PHI, PSAD) and the extent of cancer on biopsy can help to stratify the risk of progression during active surveillance.

MICRORNAS

Short non-coding RNAs known as microRNAs (miRNAs) influence a wide range of biologic processes and are often deregulated in cancer. They constitute potentially valuable markers for the diagnosis, prognosis, and therapeutic choices in PCa, as well as potential drugs (miRNA mimics) or drug targets (anti-miRNAs). A systematic review by Fabris et al¹⁶⁸ was undertaken to assess currently available data on miRNAs as biomarkers in PCa. They found that a common expression profile characterizing each tumour subtype and stage has still not been identified for PCa, probably due to molecular heterogeneity as well as differences in study design and

patient selection. They concluded that further studies are necessary to validate the translational potential of miRNAs in PCa management.

Yin et al's¹⁶⁹ systematic review and meta-analysis evaluated the use of circulating microRNAs as novel biomarkers in the diagnosis of PCa. The results confirmed the potential use of circulating miRNAs in early diagnosis, especially the combination of multiple circulating miRNAs. However, they concluded that large-scale prospective studies are still needed to further validate their findings.

VALIDATION OF DIAGNOSTIC TUMOUR BIOMARKERS

In prior retrospective studies, Pollack et al¹⁷⁰ had assessed a number of prostate tumour tissue biomarkers that were associated independently with the clinical outcome of men treated with radiotherapy (RT) ± androgen deprivation therapy (ADT). In this report, the associations of selected biomarkers with biochemical or clinical disease failure (BCDF) were prospectively evaluated in men with T1-T3 prostate cancer on a randomized hypofractionation trial. In this prospective study, multiple biomarker analysis in men with prostate cancer treated with RT±ADT, found both Ki-67 and bcl2&bax were independently related to early BCDF. However, Ki-67 alone is indicated to be the most clinically meaningful by C-index analysis and is universally available.

PROGNOSTIC BIOMARKERS

PCa stratification is based on tumour size, pre-treatment PSA level, and Gleason score, but it remains imperfect. Current research focuses on the discovery and validation of novel prognostic biomarkers to improve the identification of patients at risk of aggressive cancer or of tumour relapse.

POTENTIAL PROGNOSTIC BIOMARKERS

Lamy et al¹⁷¹ conducted a study of proposed prognostic markers. They used data sets sampling 152 prostate tissues, data sets with 281 prostate cancers analysed by microarray analysis and a study of integrated genomics on 218 cases to develop a multigene score. However, they found that the score did little to add to the Gleason score to aid in prognosis.

PROGNOSTIC IMMUNOTHERAPIES

Several types of immunotherapy have had encouraging results. There is a need to identify immune biomarkers to select patients who will benefit from such therapies. These predictive biomarkers could also be used as surrogates for overall survival. A pilot study in PCa patients treated with the AE37 li-key-HER-2/neu polypeptide vaccine suggested that HLA-A*24 and HLA-DRB1*11 alleles may be prognostic and predictive biomarkers for clinical benefit. Anastasopoulou et al¹⁷² found an association between immunologic parameters

and clinical outcome in prostate cancer patients who had been vaccinated with a HER-2/neu hybrid polypeptide vaccine (AE37) and received one booster 6 months post-primary vaccinations. Findings from their study suggest that HLA-DRB1*11 and HLA-A*24 are likely to be predictive factors for immunological and clinical responses to vaccination with AE37.

PTEN GENOMIC DELETION

PTEN (10q23.3) is a negative regulator of the phosphatidylinositol 3-kinase (PIK3)/Akt survival pathway and a tumour suppressor frequently deleted in PCa. PTEN genomic deletion is among the most common aberrations seen in PCa. The prognostic value of PTEN genomic deletion is unclear. Wang et al¹⁷³ performed a systematic review and meta-analysis to clarify the association between PTEN genomic deletion and a higher Gleason score or a higher possibility of capsular penetration. They found that PTEN genomic deletion in operable localised prostate cancer is associated with a higher Gleason score and a higher probability of capsular penetration, indicating a worse prognosis.

ANALYSIS OF PHI, 4KSCORE, MIPS, GPS, PROLARIS, DECIPHER

This systematic review by the Intergroupe Coopérateur Francophone de Recherche en Onco-urologie¹⁷⁴ analysed new evidence on the analytical validity and clinical validity and utility of six prognostic biomarkers (PHI, 4Kscore, MiPS, GPS, Prolaris, Decipher). On the basis of the available evidence, some biomarkers could help in discriminating between aggressive and non-aggressive tumours with an additional value compared to the prognostic parameters currently used by clinicians. Blood biomarkers (PHI and 4Kscore) have the highest ability to predict more aggressive PCa and could help clinicians to manage patients with localised PCa. The other biomarkers show a potential prognostic value, however, they should be evaluated in additional studies to confirm their clinical validity.

AE37 PEPTIDE VACCINATION

One challenge in immunotherapy for prostate cancer is the establishment of biomarkers that can predict patients' responsiveness to treatment. One Phase 1 study examined the immunologic and clinical responses of vaccination therapy with an li-key-modified HER-2/neu peptide (li-key/HER-2(776-790) or AE37). They found that biomarkers at the time-points measured offer promise for evaluating immunologic and clinical responses to AE37-based vaccinations.¹⁷⁵

RETINOIC ACID PATHWAY IN EARLY PCA

The International Cancer Genome Consortium have presented a computational strategy to systematically rank and investigate a large number of clinically testable gene sets,¹⁷⁶ using combinatorial gene subset generation and disease-free

survival. Two genes, OGS (CYP26A1 and RDH10) were found to be strongly associated with ALDH1A2 in the retinoic acid (RA) pathways, suggesting a major role of RA signalling in early PCa progression.

SERUM BIOMARKERS OF BONE METABOLISM

Elevated markers of bone turnover appear to be prognostic for poor survival in castration-resistant PCa. The predictive role of these biomarkers relative to bone-targeted therapy is unknown. One study¹⁷⁷ prospectively evaluated the prognostic and predictive value of bone biomarkers in sera from patients treated on a placebo-controlled Phase 3 trial of docetaxel with or without the bone targeted endothelin-A receptor antagonist atrasentan (SWOG S0421). They found that the serum bone metabolism markers had independent prognostic value. Importantly, they found that a small group of patients with highly elevated markers of bone turnover appear to preferentially benefit from atrasentan therapy.

PREDICTIVE BIOMARKERS

EFFECTIVE PREDICTORS OF TUMOUR RECURRENCE

An important challenge in prostate cancer research is to identify effective predictors of tumour recurrence. A meta-analysis¹⁷⁸ of six available miRNA expression datasets was undertaken to identify a panel of co-deregulated miRNA genes and overlapping biological processes. The study found that some of these miRNAs have an established prognostic significance in other cancers and can be actively involved in tumour growth. It was shown that the combination of DE miRNAs can assist in the more specific detection of the cancer and prediction of biochemical recurrence. They found that the identified miRNAs are candidate predictive markers for recurrent PCa after radical prostatectomy.

URINARY EN-2

A systematic review and meta-analysis on urinary EN-2 to predict PCa was undertaken by Rosa et al¹⁷⁹ to evaluate the accuracy of engrailed-2 protein (EN2) in urine as a prostate cancer biomarker. The EN2 test showed high specificity (89%) and low sensitivity (66%).

HORMONE THERAPY WITH SALVAGE RADIATION THERAPY

Salvage radiotherapy (SRT) is a standard of care for men who recur post-prostatectomy, and recent RCTs have assessed the benefit and toxicity of adding hormone therapy (HT) to SRT with differing results. Spratt et al¹⁸⁰ performed a systematic review of randomized Phase 3 trials of the use of SRT ± HT and generated a framework for the use of HT with SRT.

EFFECT OF EARLY SWITCH FROM DOCETAXEL TO CABAZITAXEL OR VICE VERSA

The TAXYENERGY¹⁸¹ trial evaluated the clinical benefit of early taxane switch and circulating tumour cell biomarkers to interrogate mechanisms of sensitivity or resistance to taxanes in chemotherapy-naïve, metastatic, castration-resistant PCa. The early taxane switch strategy was associated with improved PSA response rates versus TAX327. Taxane-induced shifts may serve as an early biomarker of clinical benefit in patients treated with taxanes.

BIOMARKERS FOR CASTRATION RESISTANT CANCER

Biomarkers of therapeutic response and prognosis are needed to assist in the sequencing of treatments for metastatic castration-resistant PCa. In a Phase 1 discovery study, Lin et al¹⁸² identified 14 circulating miRNAs that were associated with response to docetaxel therapy or overall survival. They performed a Phase 2 validation study to verify these findings. The association of circulating microRNAs with overall survival suggests their involvement in disease progression.

Cabozantinib is an orally available inhibitor of tyrosine kinases including VEGFR2 and c-MET. Leibowitz-Amit et al's¹⁸³ study aimed at finding associations between select plasma biomarkers and treatment response in patients with metastatic castration resistant PCa who received cabozantinib as part of a Phase 2 non-randomized expansion cohort. They did not find plasma biomarkers to be associated with response to cabozantinib.

LUNG CANCER

PREDICTIVE BIOMARKERS FOR LUNG CANCER

- Potential predictive biomarkers
- EGFR
- ALK
- ALK diagnosis
- ALK inhibitors
- Efficacy of alectinib
- Crizotinib
- Ceritinib
- Interstitial lung disease risk with ALK inhibitors
- Tumour biomarkers and clinical response with erlotinib
- PD-1

Lung cancer is the leading cause of cancer-related mortality. Over 80% of all cases are non-small cell lung cancer (NSCLC) and about 5% of NSCLC patients are positive for ALK gene rearrangement or fusion with echinoderm microtubule-associated protein-like 4 (EML4). NSCLC patients with positive ALK-EML4 gene fusion are highly sensitive to ALK-inhibitors. While the efficacy of the ALK-inhibitors in the treatment of NSCLC has been established, a limited number of randomized, large-scale clinical trials have been undertaken.

PREDICTIVE BIOMARKERS

POTENTIAL PREDICTIVE BIOMARKERS

A clinical study on differential gene expression profiles in lung adenocarcinoma subtypes was undertaken in 2017.¹⁸⁴ The researchers analysed the differences in the genetic expression of adenocarcinoma subtypes according to a new classification (Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification). Microarray gene expression analysis was performed. Lepidic-predominant adenocarcinoma was the only pattern that exhibited a marked gene expression difference compared with other predominant histologic patterns, revealing genes with significant expression. In addition, the researchers identified 13 genes with specific differential expression in the lepidic-predominant adenocarcinoma that could be used as a gene signature. Additionally, they identified a gene expression signature of 13 genes that have a unique behavior in the lepidic histologic pattern.

EGFR

Abernethy et al¹⁸⁵ conducted a systematic review and meta-analysis to assess EGFR gene copy number as a potential biomarker of survival for patients with advanced NSCLC receiving single-agent treatment with EGFR tyrosine kinase inhibitors (TKIs). Among TKI-treated patients, increased EGFR gene copy number appears to be associated with improved survival outcome. The effect appears to be limited to patients of non-Asian descent.

These findings are supported by another systematic review and meta-analysis.¹⁸⁶ Subgroup analysis found that in a population of patients who were primarily Caucasian, a higher EGFR gene copy number was also associated with increased survival. The results were similar in a population of Asian patients, except that a higher EGFR gene copy number was not associated with improved overall survival. It is likely that EGFR gene copy number is a biomarker for response to EGFR-TKI therapy in patients with advanced NSCLC.

ALK

A meta-analysis of lung cancer mutation profile of EGFR, ALK, and KRAS, undertook a comparison of never and ever smokers.¹⁸⁷ It found that there were significantly increased odds of presenting the EGFR and ALK-EML4 mutations in adenocarcinomas compared to NSCLC, and never smokers compared to ever smokers. The prevalence of EGFR mutations was higher in Asian women compared to other women. As the smoking history increased, there was a decreased odds for exhibiting the EGFR mutation, particularly for cases >30 pack-years.

ALK DIAGNOSIS

A systematic review and meta-analysis¹⁸⁸ on ALK immunohistochemistry for ALK gene rearrangement screening in NSCLC was undertaken. The aim was to investigate the diagnostic accuracy of ALK immunohistochemistry (IHC) for ALK gene rearrangement in NSCLC. The results suggested that ALK IHC equivocal cases should not be considered as IHC-negative in screening for ALK gene rearrangement. Additional detailed criteria for ALK IHC equivocal cases are necessary to determine how to best apply this approach in daily practice.

A systematic review and meta-analysis was undertaken on the efficacy of D5F3 IHC for detecting ALK gene rearrangement in NSCLC patients.¹⁸⁹ The researchers compared the efficacy of an immunohistochemistry (IHC) assay using the D5F3 antibody with that of fluorescence in situ hybridization (FISH) for detecting ALK gene rearrangement in NSCLC patients. The analysis showed that specimen type was a source of heterogeneity for specificity, and specimen type and FISH signal distance were sources of heterogeneity in the joint model. They concluded that the D5F3 IHC assay was nearly as effective as FISH for detection of ALK gene rearrangement in NSCLC patients.

ALK INHIBITORS

A systematic review and meta-analysis of the effect of ALK-inhibitors in the treatment of NSCLC has been recently published.¹⁹⁰ It was designed to review all phases of clinical trials in which ALK-inhibitors were used to treat NSCLC. It found that ALK inhibitors significantly improved the survival in NSCLC patients, especially of ALK or ROS1 gene fusion-positive cases. ALK inhibitors appear to be safe and effective in the treatment of NSCLC patients, especially those with positive ALK-EML4 gene fusion or rearrangement.

ALK gene rearrangements are oncogenic drivers of NSCLC. Brigatinib (AP26113) is an investigational ALK inhibitor with potent preclinical activity against ALK mutants resistant to crizotinib and other ALK inhibitors. In 2016 researchers conducted an activity and safety of brigatinib in ALK-rearranged non-small-cell lung cancer and other malignancies in a single-arm, open-label, Phase 1/2 trial.¹⁹¹ The aim was to assess brigatinib in patients with advanced malignancies, particularly ALK-rearranged NSCLC. Brigatinib showed promising clinical activity and has an acceptable safety profile in patients with crizotinib-treated and crizotinib-naïve ALK-rearranged NSCLC. These results support its further development as a potential new treatment option for patients with advanced ALK-rearranged NSCLC.

EFFICACY OF ALECTINIB

Purpose Alectinib is an ALK-specific kinase inhibitor that seems to be effective against NSCLC with a variety of ALK mutations. Central nervous system (CNS) progression is

common in patients with anaplastic lymphoma kinase-positive (ALK+) NSCLC receiving crizotinib. Therefore, a clinical trial of the efficacy of alectinib in CNS metastases in crizotinib-resistant ALK-positive non-small-cell lung cancer was undertaken.¹⁹² Findings suggest that Alectinib demonstrated promising efficacy in the CNS for ALK+ NSCLC patients pre-treated with crizotinib.

Further evidence supports alectinib from a three-year follow-up of an alectinib Phase 1/2I study in ALK-Positive Non-Small-Cell Lung Cancer.¹⁹³ Most cancer symptoms were relieved early, and medication for symptoms was decreased during alectinib therapy. The study concluded that alectinib was effective with a favorable safety profile over a long administration period in ALK-positive NSCLC without previous ALK inhibitor treatment.

A recent RCT compared alectinib versus crizotinib in untreated ALK-Positive NSCLC. Peters et al¹⁹⁴ investigated alectinib as compared with crizotinib in patients with previously untreated, advanced ALK-positive NSCLC, including those with asymptomatic CNS disease. It found that compared with crizotinib, alectinib showed superior efficacy and lower toxicity.

CRIZOTINIB

Crizotinib was approved to treat ALK-positive NSCLC by the FDA in 2011. A systematic review of clinical trials and retrospective studies to compare the efficacy and safety of crizotinib with chemotherapy was undertaken.¹⁹⁵ This systematic review revealed improved objective response rate and increased disease control rate in the crizotinib group compared to the chemotherapy group. Crizotinib treatment would be a favorable treatment option for patients with ALK-positive NSCLC. ALK inhibitors and may have future potential applications in other cancers driven by ALK or c-MET gene mutations.

CERITINIB

Ceritinib is a next-generation (ALK) inhibitor, which has shown robust anti-tumour efficacy, along with intracranial activity, in patients with ALK-rearranged NSCLC. Ceritinib versus chemotherapy in patients with ALK-rearranged NSCLC previously given chemotherapy and crizotinib (ASCEND-5): a randomised, controlled, open-label, Phase 3 trial¹⁹⁶ was undertaken. In Phase 1 and 2 studies, ceritinib has been shown to be highly active in both ALK inhibitor-naïve and ALK inhibitor-pretreated patients who had progressed after chemotherapy (mostly multiple lines). In this study, Shaw et al¹⁹⁷ compared the efficacy and safety of ceritinib versus single-agent chemotherapy in patients with advanced ALK-rearranged NSCLC who had previously progressed following crizotinib and platinum-based doublet chemotherapy. Their findings show that patients derive significant clinical benefit from a more potent ALK inhibitor after failure of crizotinib,

and establish ceritinib as a more efficacious treatment option compared with chemotherapy in this patient population.

A 2017 clinical study was undertaken to assess the potential for drug-drug interactions between ceritinib and proton pump inhibitors in healthy subjects and in patients with ALK-positive NSCLC.¹⁹⁸ The impact of proton pump inhibitors on the pharmacokinetics and efficacy of ceritinib was evaluated. The researchers concluded that long-term administration of ceritinib with protein pump inhibitors does not adversely affect the pharmacokinetics and efficacy of ceritinib in ALK-positive cancer patients.

INTERSTITIAL LUNG DISEASE RISK WITH ALK INHIBITORS

A recent systematic review and meta-analysis¹⁹⁹ was undertaken to assess the overall incidence and risk of interstitial lung disease (ILD) and QTc prolongation associated with ALK-tyrosine kinase inhibitors (-TKIs) in NSCLC patients. It found that the use of ALK-TKIs significantly increases the risk of developing high-grade ILD and QTc prolongation in lung cancer patients.

TUMOUR BIOMARKERS AND CLINICAL RESPONSE WITH ERLOTINIB

A multicentre clinical Phase 2 gene expression profiling study was undertaken of the putative relationship between tumour biomarkers and clinical response with erlotinib in NSCLC.²⁰⁰ Identification of appropriate markers for predicting clinical benefit with erlotinib in NSCLC may be able to guide patient selection for treatment. The study supports the use of erlotinib as an alternative to chemotherapy for patients with relapsed advanced NSCLC.

PD-1

Nivolumab and pembrolizumab are IgG4 monoclonal antibodies targeting the immune checkpoint inhibitor programmed death-1 (PD-1) expressed by T cells, whilst atezolizumab is a monoclonal antibody targeting the ligand PD-L1 which is expressed by T cells and tissues such as tumours and metastases displaying inflammation. They have demonstrated improved outcomes and quality of life in NSCLC, being granted FDA approval in 2014.²⁰¹⁻⁴

OESOPHAGEAL CANCER

BIOMARKERS FOR OESOPHAGEAL CANCERS

- Exploratory biomarker study of dacomitinib

EXPLORATORY BIOMARKER STUDY OF DACOMITINIB

A Phase 2 clinical and exploratory biomarker study of dacomitinib in recurrent and/or metastatic oesophageal squamous cell carcinoma²⁰⁵ was undertaken. It aimed

to investigate the clinical activity, safety and predictive biomarkers of dacomitinib, an irreversible pan-HER inhibitor, in patients with recurrent or metastatic oesophageal squamous cell carcinoma (R/M-ESCC). Dacomitinib showed clinical efficacy with manageable toxicity in platinum-failed R/M-ESCC. The authors concluded that screening of ERBB pathway-related gene expression profiles may help identify patients who are most likely benefit from dacomitinib.

OVARIAN CANCER

BIOMARKERS FOR OVARIAN CANCERS

DIAGNOSTIC BIOMARKERS

- Transcriptome data for ovarian cancer
- Serum markers in epithelial ovarian cancer
- HE4 for ovarian cancer recurrence and in its early detection
- Diagnostic values of osteopontin
- Platelet-to-lymphocyte and Neutrophil-to-Lymphocyte
- Tumor associated antigens
- Combined biomarker panels
- Frozen section diagnostic accuracy
- Open laparoscopy
- Vitamin D receptor gene polymorphisms
- Gene expression profiles

PROGNOSTIC BIOMARKERS

- Single gene prognostic biomarkers
- Matrix metalloproteinase 2 (MMP-2)
- Elevated plasma fibrinogen levels
- Therapeutic potential of PD-1
- Inflammatory markers

PREDICTIVE BIOMARKERS

- Bevacizumab with carboplatin and weekly paclitaxel as first-line adjuvant therapy

There is an urgent need for biomarkers for the early detection of ovarian cancer (OC). In spite of various treatment options currently available, ovarian cancer (OC) still remains a major cause of death in women world-wide. Since OC is symptomless in the early stages, most women present too late for effective curative treatment. Thus, diagnosis at an early stage is one of the most important factors that determines survival. Unfortunately, current diagnostic tools have limited efficacy. Several new molecular OC biomarkers have recently been identified and are subject to validation.

DIAGNOSTIC BIOMARKERS

TRANSCRIPTOME DATA FOR OVARIAN CANCER

A recent meta-analysis²⁰⁶ of transcriptome data for OC was undertaken. Comparative and integrative analyses yielded reporter biomolecules (genes, proteins, metabolites, transcription factors, and micro-RNAs), and unique or common signatures at protein, metabolism, and transcription regulation levels, which might be beneficial to uncovering the underlying biological mechanisms behind the disease. These signatures were mostly associated with formation or initiation of cancer development, and pointed out the potential tendency of polycystic ovary syndrome (PCOS) and endometriosis to tumourigenesis. Molecules and pathways related to MAPK signalling, cell cycle, and apoptosis were the mutual determinants in the pathogenesis of these diseases.

SERUM MARKERS IN EPITHELIAL OVARIAN CANCER

The European Group on Tumour Markers guidelines are based on a systematic review for serum markers in epithelial ovarian cancer (OC)²⁰⁷ and were last updated in 2017. The guidelines state that because of its low sensitivity and limited specificity, cancer antigen 125 (CA125) is not recommended as a screening test in asymptomatic women. The Risk of Malignancy Index, which includes CA125, transvaginal ultrasound, and menopausal status, is recommended for the differential diagnosis of a pelvic mass. Because human epididymis protein 4 has been reported to have superior specificity to CA125, especially in premenopausal women, it may be considered either alone or as part of the risk of ovarian malignancy algorithm, in the differential diagnosis of pelvic masses, especially in such women. The guidelines recommend that CA125 should be used to monitor response to first-line chemotherapy using the previously published criteria of the Gynecological Cancer Intergroup, that is, at least a 50% reduction of a pre-treatment sample of 70 kU/L or greater.

The value of CA125 in post-therapy surveillance appears to be less clear. Although an RCT concluded that early administration of chemotherapy based on increasing CA125 levels had no effect on survival, the guidelines suggest that monitoring with CA125 in this situation should occur, especially if the patient is a candidate for secondary cytoreductive surgery. At present, CA125 remains the most important biomarker for epithelial OC, excluding tumours of mucinous origin.

HE4 FOR OC RECURRENCE AND IN ITS EARLY DETECTION

A recent systematic review examined OC recurrence and early detection in terms of how HE4 plays a key role.²⁰⁸ New biomarkers studied included human epididymis 4 (HE4), primarily expressed in the reproductive and respiratory tract. The review found that HE4 had good sensitivity and specificity in detecting OC.

This evidence is supported by a recent meta-analysis.²⁰⁹ The aim of this study was to determine whether the Risk of Ovarian Malignancy Algorithm (ROMA) is more accurate than HE4 or CA125 biomarkers with respect to the differential diagnosis of women with a pelvic mass. ROMA showed good sensitivity and specificity and further, the ROMA log DOR results were better than HE4 and CA125 log DOR results especially for the early-stage patient group. The results support the use of ROMA to improve clinical decision making in patients with early OC.

DIAGNOSTIC VALUES OF OSTEOPONTIN

Osteopontin (OPN) is currently one of the most studied serum biomarkers of OC. A recent meta-analysis of the diagnostic values of osteopontin combined with CA125 for ovarian cancer was undertaken.²¹⁰ It found that OPN is a useful tumour biomarker in future screening tests of OC and can be a promising adjunct to CA125.

PLATELET-TO-LYMPHOCYTE AND NEUTROPHIL-TO-LYMPHOCYTE

Platelet-to-lymphocyte (PLR) and Neutrophil-to-Lymphocyte (NLR) ratios have been extensively investigated in cancer. However, to date, actual guidelines concerning OC are missing. Thus a systematic review was undertaken to summarize the available evidence for the diagnostic efficacy of PLR and NLR in OC.²¹¹ According to the findings of this study, both PLR and NLR seem to be promising screening and prognostic factors of epithelial ovarian cancer. The actual diagnostic cut-off values remain, however, undefined until now. Despite their limited sensitivity and specificity, they might be useful in the future as adjunct biomarkers for the detection and surveillance of the disease.

TUMOUR ASSOCIATED ANTIGENS

A systematic review aimed to summarize known tumour-associated antigens (TAAs) or anti-TAA autoantibodies and their diagnostic values in ovarian cancer.²¹² It found that serum TAAs or anti-TAA autoantibodies are promising diagnostic biomarkers in the detection of OC. A customized mini-array of multiple TAAs may enhance the detection of anti-TAA autoantibodies in OC.

COMBINED BIOMARKER PANELS

A recent case control study found that an RA combined biomarker panel showed improved sensitivity for the early detection of OC allowing the identification of the most aggressive type II tumours.²¹³ The purpose of this study was to assess whether changes in serum levels of lecithin-cholesterol acyltransferase (LCAT), sex hormone-binding globulin (SHBG), glucose-regulated protein, 78kDa (GRP78), calprotectin and insulin-like growth factor-binding protein 2 (IGFBP2) are observed before clinical presentation, and to assess the performance of these

biomarkers alone and in combination with CA125 for early detection. Combined biomarker panels outperformed CA125 up to 3 years pre-diagnosis, identifying cancers missed by CA125, providing increased diagnostic lead times for Type I and Type II OC. The model identified more aggressive Type II cancers, with women crossing the threshold dying earlier, indicating that these markers can improve on the sensitivity of CA125 alone for the early detection of OC.

VITAMIN D RECEPTOR GENE POLYMORPHISMS

Vitamin D receptor (VDR) gene polymorphisms and the risks for various breast and ovarian cancers have been reported in many epidemiological studies, however, the association between these polymorphisms and the risk for each type of cancer is unclear. A meta-analysis²¹⁴ was undertaken to evaluate these associations in female reproductive cancers. The results indicate that the FokI polymorphism was related to increased risks for breast and ovarian cancers, whereas the BsmI polymorphism was associated with a decreased risk for developing these cancers. The authors suggest that the FokI and BsmI VDR gene polymorphisms may be significantly associated with gynecological cancers.

GENE EXPRESSION PROFILES

It was hoped that the introduction of microarray techniques in cancer research would enable the detection of biomarkers that would improve patients' treatment; however, the results of such studies have been found to be poorly reproducible and critical analyses of these methods are rare. A recent clinical trial has explored sequential gene changes in epithelial OC (EOC) induced by carboplatin via microarray analysis.²¹⁵ The study found that c-Jun and CCNB1 may be the prognostic biomarkers of EOC treated with carboplatin, and certain pathways (such as p53 signalling pathway, cell cycle and mitogen-activation protein kinase signalling pathway) may be involved in carboplatin-resistant EOC.

A review of gene expression analysis in OC has been published.²¹⁶ The main goal of this study was to delineate the molecular background of OC chemo resistance and find biomarkers suitable for prediction of patient prognosis. The researchers found that histological tumour type was the major source of variability in gene expression. Analysis of clinical endpoints found results that were not confirmed by validation either on the same group or on the independent group of patients. CLASP1 was the only gene that was found to be important for disease free survival in the independent group, whereas in the preceding experiments it showed associations with other clinical endpoints and with BRCA1 gene mutation; thus, it may be worthy of further testing.

A meta-analysis of gene expression profiles associated with histological classification and survival in OC samples²¹⁷ found that transcriptomic analysis of global gene expression in ovarian

carcinoma can identify dysregulated genes capable to serve as molecular markers for histology subtypes and survival. The aim of this study was to validate previous candidate signatures in an independent setting and to identify single genes capable to serve as biomarkers for OC progression. The study found that over 90% of subtype-associated genes were confirmed. Overall survival was effectively predicted by hormone receptors (PGR and ESR2) and by TSPAN8. Relapse-free survival was predicted by MAPT and SNCG.

PROGNOSTIC BIOMARKERS

SINGLE GENE PROGNOSTIC BIOMARKERS

To discover novel prognostic biomarkers in ovarian serous carcinomas a systematic review and meta-analysis were undertaken.²¹⁸ Twelve genes with high mRNA expression were prognostic of poor outcome with an FDR <.05 (AXL, APC, RAB11FIP5, C19orf2, CYBRD1, PINK1, LRRN3, AQP1, DES, XRCC4, BCHE, and ASAP3). Twenty genes with low mRNA expression were prognostic of poor outcome with an FDR <.05 (LRIG1, SLC33A1, NUCB2, POLD3, ESR2, GOLPH3, XBP1, PAXIP1, CYB561, POLA2, CDH1, GMNN, SLC37A4, FAM174B, AGR2, SDR39U1, MAGT1, GJB1, SDF2L1, and C9orf82).

MATRIX METALLOPROTEINASE 2 (MMP-2)

A meta-analysis was undertaken to evaluate the association of tumour-derived matrix metalloproteinase 2 (MMP-2) and stromal-derived MMP-2 expression with the prognosis of patients with ovarian cancer.²¹⁹ The results suggested that positive tumour-derived MMP-2 expression could predict a lower overall survival rate and could be an independent dangerous prognostic factor in patients with ovarian cancer.

ELEVATED PLASMA FIBRINOGEN LEVELS

To evaluate the effect of elevated plasma fibrinogen levels on the prognosis of EOC a cohort study and meta-analysis was undertaken.²²⁰ Crude and subgroup meta-analyses demonstrated that elevated plasma fibrinogen levels were associated with impaired survival in patients with all stage EOC. Elevated plasma fibrinogen levels appear to be more important for predicting survival than serum CA-125 levels, NLR and PLR in patients with EOC, in advanced-stage disease.

THERAPEUTIC POTENTIAL OF PD-1

A recent systematic review examined the significance and therapeutic potential of PD-1 and its ligands in OC.²²¹ The review confirmed that blocking PD-1 and its ligands in OC is feasible and valid both in animal models and patients. It suggested that immunotherapy might play a significant role in the future clinical management and improve the prognosis of OC.

INFLAMMATORY MARKERS

A 2016 systematic review and meta-analysis of inflammatory markers of CRP, IL6, TNF α , and soluble TNFR2 and the risk

of OC was undertaken.²²² There has been growing evidence showing that inflammatory markers play an important role in the development of OC. The researchers examined the associations between circulating levels of C-reactive protein (CRP), interleukin 6 (IL6), tumour necrosis factor α (TNF α), and soluble TNF α receptor 2 (TNFR2), and the risk of OC. This meta-analysis provides evidence that elevated levels of CRP, but not circulating IL6, TNF α , or soluble TNFR2, are significantly associated with an increased risk of ovarian cancer. These results suggest that circulating CRP may play a role in the aetiology of ovarian cancer.

PREDICTIVE BIOMARKERS

BEVACIZUMAB WITH CARBOPLATIN AND WEEKLY PACLITAXEL AS FIRST-LINE ADJUVANT THERAPY

A recent clinical trial was undertaken to assess the tolerability and efficacy of bevacizumab with carboplatin and weekly paclitaxel as first-line adjuvant therapy for advanced stage ovarian cancer.²²³ This Phase 2 trial enrolled patients with stage III or IV EOC after primary cytoreductive surgery to treatment with carboplatin (AUC 5), weekly paclitaxel (80 mg/m²), and bevacizumab (15 mg/kg) every three weeks for at least six cycles. It found that adjuvant bevacizumab with dose-dense chemotherapy is associated with acceptable toxicity and a high likelihood of completing four cycles of therapy. Dynamic changes in Flt-3L may represent a predictive marker to treatment response.

BREAST CANCER

BIOMARKERS FOR BREAST CANCER

DIAGNOSTIC BIOMARKERS

- Acolbifene in Premenopausal Women at High Risk for Breast Cancer
- Seriological Diagnostic Biomarkers

PROGNOSTIC BIOMARKERS

- SPAG5

PREDICTIVE BIOMARKERS

- Endocrine Therapy for Hormone Receptor-Positive Metastatic Breast Cancer
- Latum/paclitaxel-based treatment
- Biomarkers for adjuvant trastuzumab
- Guiding the use of adjuvant systemic therapy

Breast cancer is a leading cause of morbidity and mortality worldwide. Although mammography screening is available, there is an ongoing interest in improved early detection and prognosis.

DIAGNOSTIC BIOMARKERS

ACOLBIFENE IN PREMENOPAUSAL WOMEN AT HIGH RISK FOR BREAST CANCER

A 2015 clinical trial was undertaken of acolbifene in premenopausal women at high risk for breast cancer.²²⁴ Its purpose was to assess the feasibility of using the selective oestrogen receptor modulator (SERM) acolbifene as a breast cancer prevention agent in premenopausal women. It found that acolbifene was associated with favourable changes in benign breast epithelial cell proliferation and oestrogen-inducible gene expression but minimal side effects.

SERIOLOGICAL DIAGNOSTIC BIOMARKERS

A recent (2017) study of breast cancer serum biomarkers for early detection and prognosis in pre-diagnosis samples was undertaken.²²⁵ Significant associations with clinico-pathological features related to prognosis were found for several candidates (CA15-3, HSP90A and PAI-1). However, there were no consistent differences between cases and controls for any candidate in the lead up to diagnosis. This study using unique pre-diagnosis samples showed that CA15-3, HSP90A and PAI-1 have potential as early prognostic markers and warrant further investigation. However, none of the candidates or combinations

PROGNOSTIC BIOMARKERS

SPAG5

A study of SPAG5 as a prognostic biomarker and chemotherapy sensitivity predictor in breast cancer was undertaken.²²⁶ SPAG5 is a novel amplified gene on Ch17q11.2 in breast cancer. The study found that transcript and protein products of SPAG5 are independent prognostic and predictive biomarkers that might have clinical utility as biomarkers for combination cytotoxic chemotherapy sensitivity, especially in oestrogen receptor-negative breast cancer.

PREDICTIVE BIOMARKERS

ENDOCRINE THERAPY FOR HORMONE RECEPTOR-POSITIVE METASTATIC BREAST CANCER

The American Society of Clinical Oncology has published systematic review-based guideline for endocrine therapy for hormone receptor-positive metastatic breast cancer (MBC).²²⁷ The review recommends that sequential hormone therapy is the preferential treatment for most women with HR-positive MBC. Except in cases of immediately life-threatening disease, hormone therapy, alone or in combination, should be used as initial treatment. It suggests that patients whose tumours express any level of hormone receptors should be offered hormone therapy.

LATINUM/PACLITAXEL-BASED TREATMENT

A study has been published on integrated analysis of gene expression profiles associated with response of latinum/paclitaxel-based treatment in epithelial ovarian cancer.²²⁸ A meta-analysis was used to screen overlapping differentially expressed miRNAs (DEmiRNAs) in three studies. The miRanda was used to identify target genes related to overlapping DEmiRNAs. The Gene Ontology (GO) and Encyclopaedia of Genes and Genomes (KEGG) database was applied to further predict the function of these target genes. The researchers obtained seven overlapping miRNAs and six significantly over-represented GO terms closely related to breast cancer. Their findings suggest that the altered levels of miRNAs might have great potential to serve as novel, non-invasive biomarkers for early detection of breast cancer.

BIOMARKERS FOR ADJUVANT TRASTUZUMAB

Integrative proteomic and gene expression analysis can identify potential biomarkers for adjuvant trastuzumab resistance, according to analysis from a Fin-her phase III randomised trial.²²⁹ Trastuzumab is an effective therapy for patients with HER2-positive breast cancer. However, not all women with high levels of HER2 benefit from trastuzumab. By integrating mRNA and protein expression data from Reverse-Phase Protein Array Analysis (RPPA) in HER2-positive BC, the researchers developed gene expression metagenes that reflect pathway activation levels. The researchers concluded that in HER2-positive BC, some proteins are associated with distinct gene expression profiles.

GUIDING THE USE OF ADJUVANT SYSTEMIC THERAPY

A study addressed the use of MammaPrint²³⁰ to guide decisions on the use of adjuvant systemic therapy. The publication of the Phase III randomised MINDACT (Microarray in Node-Negative and 1 to 3 Positive Lymph Node Disease May Avoid Chemotherapy) study to evaluate the MammaPrint assay in women with early-stage breast cancer provided a signal. The recommendations include:

- Recommendation 1.1: If a patient has ER/PgR–positive, HER2-negative, node-negative, breast cancer, the MammaPrint assay may be used in those with high clinical risk per MINDACT categorization to inform decisions on withholding adjuvant systemic chemotherapy due to its ability to identify a good prognosis population with potentially limited chemotherapy benefit.
- Recommendation 1.1.2: If a patient has ER/PgR–positive, HER2-negative, node-negative, breast cancer, the MammaPrint assay should not be used in those with low clinical risk per MINDACT categorization to inform decisions on withholding adjuvant systemic chemotherapy, because women in the low clinical risk

category had excellent outcomes and did not appear to benefit from chemotherapy even with a genomic high-risk cancer.

- Recommendation 1.2.1: If a patient has ER/PgR–positive, HER2-negative, node-positive, breast cancer, the MammaPrint assay may be used in patients with one to three positive nodes and at high clinical risk per MINDACT categorization to inform decisions on withholding adjuvant systemic chemotherapy due to its ability to identify a good prognosis population with potentially limited chemotherapy benefit. However, such patients should be informed that a benefit of chemotherapy cannot be excluded, particularly in patients with greater than one involved lymph node.
- Recommendation 1.2.2: If a patient has ER/PgR–positive, HER2-negative, node-positive, breast cancer, the MammaPrint assay should not be used in patients with one to three positive nodes and at low clinical risk per MINDACT categorization to inform decisions on withholding adjuvant systemic chemotherapy. There are insufficient data on the clinical utility of MammaPrint in this specific patient population.
- Recommendation 1.3: If a patient has HER2-positive breast cancer, the clinician should not use the MammaPrint assay to guide decisions on adjuvant systemic therapy. Additional studies are required to address the role of MammaPrint in patients with this tumour subtype who are also receiving HER2-targeted therapy.
- Recommendation 1.4: If a patient has ER/PgR negative and HER2-negative (triple negative) breast cancer, the clinician should not use the MammaPrint assay to guide decisions on adjuvant systemic chemotherapy.

SURVEY OF EXPERTS

A survey of Australian oncologists and researchers in the biomarker field was undertaken in late 2017. Contact details of the target population were provided through: societies such as the Medical Oncology Association of Australia; cancer research networks; list supplied by the sponsors; and the authors of this report. Potential respondents were emailed with an information sheet explaining about the study, and a link to the SurveyMonkey website for accessing the survey. The data were then converted to SPSS 24 for analysis. The initial question on the survey asked whether the respondent gave formal consent for the data provided to be used for research purposes. All but three people agreed, and the records of those not consenting were deleted. This left 116 remaining questionnaires for analysis. Although the exact size of the target population is unknown, we believe that approximately 400 people were contacted. The response rate is therefore approximately 29%. This is sufficient to provide at least ± 10 accuracy for any questionnaire item.

CANCER SITES

Is the cancer research you do site-specific?

	Frequency	Percent
Yes	37	33.9
No	72	66.1
Total	109	100.0

Those that said Yes, covered a very broad range of cancer sites, with 48 respondents answering No, stating that they worked with multiple cancer sites.

SPECIALITY OF RESPONDENTS

What is your main area of research?

	Frequency	Percent
Cancer epidemiology	8	7.3
Clinical oncology	40	36.7
Cell biology	27	24.8
Omics	8	7.3
Other	16	14.7
Not a researcher	10	9.2
Total	109	100.0

Those that said Other, covered a wide range of fields including behavioural research, translational research, health economics, health technology assessment, pathology and statistics.

USE OF BIOMARKERS

Do you currently use biomarkers or biomarker tests?

	Frequency	Percent
Yes	73	81.1
No	17	18.9
Total	90	100.0

The majority of respondents said Yes. When asked why they said No, several said that they were developing biomarkers at present. Some of the other comments are listed below:

“no good ones exist”

“scepticism as to the utility of biomarkers to predict outcome”

“everyone uses biomarkers of some form”

“there aren’t any used for these cancers”

EASE OF ACCESS TO BIOMARKERS

Do you have easy access to biomarkers that can guide treatment in your area?

	Frequency	Percent
Yes	53	58.9
No	20	22.2
Unsure	17	18.9
Total	90	100.0

At least half of respondents had easy access to biomarkers.

EFFECTIVENESS OF BIOMARKERS

Do you think that there are any effective biomarkers currently in use?

	Frequency	Percent
Yes	66	73.3
No	9	10.0
Unsure	15	16.7
Total	90	100.0

The majority of respondents said Yes. For those that said Yes, a very wide number and type were mentioned. They included: ALK and EGFR for lung cancer, KRAS/NRAS for bowel cancer, PSA for prostate cancer, HER 2 for lung and gastric cancer, MGMT in GBM, ALK/ROS/NTRK fusions, BRCA 1 and 2 for breast cancer, BRAF for melanoma, DRG mutations in ovarian cancer, PD-1 and PDD-L1 in lung cancer, blood cholesterol levels, blood glucose levels, and many others. A full list of the suggested biomarkers is provided in the Appendix.

PATIENT ISSUES

Respondents were then asked about patient issues. The most common comments were:

- Patient discomfort
- Difficulty of obtaining an adequate sample
- Patient concerns about research
- Lack of genetic counselling
- Cost to patient
- Risk of complications
- Need for repeat biopsies
- The need for multiple biomarkers from limited samples

RELIABILITY OF BIOMARKER TESTS

In general, how reliable do you think biomarker tests are?

	Frequency	Percent
Reliable	43	47.8
Unreliable	20	22.2
Unsure	27	30.0
Total	90	100.0

Just under half the respondents thought that biomarker tests are reliable. When asked why they were not reliable, many respondents pointed out that some were very reliable and others not. Other comments included:

Many studies are under-powered

- Lack of standardisation between pathology labs
- Different tissues may produce dissimilar results, and may also differ from the initial trials
- Poor diagnostic accuracy, including sensitivity, specificity, NPV and PPV
- Lack of a biological basis for associations found
- Some do not fully predict response to treatment
- More work required on how to normalise miRNA to remove collection, storage, processing and amplification variability
- Lack of a definable target

- Use of results beyond the capability of the test
- Issues of reproducibility with respect to a single sample versus multiple samples, or sampling at different time points

A complete list of responses is provided in the Appendix.

SENSITIVITY OF BIOMARKER TESTS

Do you see any problems with the sensitivity of biomarker tests?

	Frequency	Percent
Yes	34	37.8
No	23	25.6
Unsure	33	36.7
Total	90	100.0

Many respondents were unsure about this topic. When asked to explain their answer, a wide range of comments were received. Many respondents said that it depends on the test and testing conditions. Other comments were:

- For early diagnosis, there are sub-optimal amounts of biomarker available
- Technology such as ctDNA is not sensitive enough
- Heterogeneity for PD-L1 levels, False positives for plasma BRAF and T790M etc
- HPV positive but P16 negative
- Mutations develop, isoforms exist
- Not able to detect early disease stage resulting in false negatives
- Some assays under-report V600K and BRAF mutations
- Some biomarkers like NGS and ddPCR are too sensitive
- Tumour heterogeneity, so biopsy samples may miss specific cancer regions

SPECIFICITY OF BIOMARKER TESTS

Do you see any problems with the specificity of biomarker tests?

	Frequency	Percent
Yes	46	51.1
No	19	21.1
Unsure	25	27.8
Total	90	100.0

Clearly, respondents were more concerned about test specificity than sensitivity. A very large number of comments were received for this item, and these are detailed in the Appendix.

BIOMARKER PRIORITIES

In your opinion, which 5 cancers do you think should be prioritized for biomarker development and what would be the role of the biomarker? (eg. prostate cancer, screening)

The table below shows a breakdown of suggested priority cancer sites and uses of the biomarker.

Cancer site	General	Screening	Diagnosis	Prognosis	Treatment choice	Monitoring Tx response	Immuno-therapy
Any cancer site		1			1		6
AML	1						
Bladder		4				1	1
Bone		1					
Brain		4	1		1	2	
Breast	10	6		3	9	3	
Cervical		3				1	
Colorectal	7	6		2	8	6	
Gastric	4					1	
GBM	1	1			2	2	
Glioma					2		
Head and neck	1	1			1	1	1
Liver	1	2			2		
Lung	11	8	2	2	5	4	6
Lymphoma							1
Melanoma	1	2			4	4	3
Mesothelioma		1				2	
Neuroblastoma				1			
Neuroendocrine				1			
Oesoph./Barrett's		2					
Osteosarcoma						1	
Ovarian	4	14			3	3	
Pancreatic	11	11	1	2	6	4	
Prostate	8	14	4	5	6	3	
Rare cancers	6	1			1		
Renal		2					
Sarcoma					1		
Stomach	1		1	1			
Testicular	1						
Thyroid			1				
Unknown primary		1				1	
Uterus	1						

The six most mentioned cancer sites with the number of mentions were prostate (40), lung (38), pancreas (35), breast (31), colorectal (29), ovarian (24). Suggestions for the use of biomarker tests were dominated by screening (85), and followed by treatment selection (52) and treatment monitoring (39). Notably, the cancer sites that were suggested to have greatest priority were those that are either relatively common with good survival (prostate, breast, colorectal), or those that were rarer but had comparatively poor survival (lung, ovarian, pancreatic).

In terms of site and use, the top priorities were: Screening for ovarian, prostate, pancreatic, and lung cancer, and aiding treatment choice for breast and colorectal cancer.

TECHNOLOGIES

In your opinion, what technologies are required to develop biomarkers? [Multiple response item]

	Frequency	Percent
Proteomics	52	22.5
Cell biology	47	20.3
DNA profiling	47	20.3
DNA testing	46	19.9
Lipidomics	19	8.2
Other	20	8.7
Total	231	100.0

Proteomics was the technology most frequently suggested as being required.

Other suggestions included:

- Circulating tumour cells, exosomes, microRNA
- Circulating tumour DNA
- Computer science, novel ideas
- Experimental work to functionally validate potential biomarkers
- Functional genomics
- Genomics and other profiling linked to potential drug actions
- Imaging
- Immune response
- Immunohistochemistry, gene expression profiling
- Ingenuity
- Possibly other omics: metabolomics, gut microbiome
- Metabolomics, pathology, immunohistochemistry
- Multidisciplinary - also need trials
- Possibly other omics: metabolomics, gut microbiome

- Radiotracer developments for theranostic approaches
- RNA profiling
- RNA tests in some settings, methylation/epigenomics
- Transcriptome profiling
- Transcriptional profiling, nanostring

IMPORTANCE OF BIOLOGICAL PLAUSIBILITY

How important is it to link a biomarker to a biological process?

	Frequency	Percent
Very important	35	41.7
Important	34	40.5
Neutral	9	10.7
Not important	5	6.0
Not at all important	1	1.2
Total	84	100.0

Notably, over 80% of respondents felt that it was important or very important for biomarkers to be linked to the biological process in the development of the cancer.

INFORMATION ON BIOMARKERS

Where do you find your information on biomarkers? [Multiple response item]

	Frequency	Percent
Conferences	61	25.6
Online research papers	57	23.9
Pubmed	60	25.2
Professional development events	25	10.5
Companies	12	5.0
Email	11	4.6
Pamphlets	2	0.8
Other	10	4.2
Total	238	100.0

Most respondents found their information on biomarkers from conferences, followed by published papers. Other responses were: Colleagues, My cancer genome – online site, Personal communication, Professional liaison with basic scientists, and Seminars at work.

RESEARCH FUNDING

Who should be the primary funder of biomarker research?

	Frequency	Percent
Government	43	51.2
Industry	11	13.1
Private	5	6.0
Public	4	4.8
Other	21	25.0
Total	84	100.0

Clearly, most respondents thought that the government should primarily be responsible for biomarker research. Many respondents who selected Other, said that all of these categories be responsible. Other comments were:

- All of the above - everybody should be involved. Government should not be alone being the primary funder.
- Collaborations and consortia between government and industry related to drug budgets.
- It depends on the phase of research. Industry are likely to profit from biomarker research, and should therefore fund it where this is the case. For rarer diseases or cancers, joint funding with the government may be required. In some cases, joint funding (govt / industry) may be reasonable to negotiate to help bring therapies to market, as long as there are returns for government investment.
- Not sure there should be a primary funder. Probably depends on the biomarker context e.g screening vs guiding use of a specific drug.

INTERVIEWS WITH KEY STAKEHOLDERS

Several stakeholders who are experts in the biomarker and oncology fields were asked to participate in a short interview. In the interview, participants were asked to give their opinion of cancer biomarker related topics such as leaders in the field, policy and regulations, barriers and implications of developing and implementing biomarkers.

FUNDING AND SHARING OF RISKS

When asked how should biomarker development and implementation be funded and the risks be shared, it was remarked that the current state of funding is chaotic. This is because the standard pathway for developing a biomarker precedes the drug with both commercial and semi-commercial tests employed (for example with Herceptin, Glivec, PARP inhibitors and BRCA, MSI and pembrolizumab).

Stakeholders agree that biomarker trials should be encouraged. Stakeholders are aware that the ideal scenario would be a patented “companion” biomarker for a new targeted agent. One major benefit could be population screening; which could be possible if multiple cancers could be detected with the same biomarkers and the benefit of funding a test would be to ensure access to the drugs for the targets found. Equally a provider of lab equipment could be a major contributor/benefiter of the pairing of a new drug and biomarker test; although in the field of genetics, this have proven more challenging, as shown by the Myriad/BRCA issues. Further, it was noted, if an academic or clinical group pioneers a novel technology, then they should benefit.

The major barrier with this process is the price point. If the tests were affordable for individuals (e.g. \$200 per test) then many individuals would fund their own tests. Another barrier has been the path to standardisation; although stakeholders believe there may be some merit in standardisation, there has been not much evidence of this working well to date.

POLICY

Stakeholders believe that policies should be in place to facilitate biomarker development from regulatory agencies, such as the Therapeutic Goods agency (TGA). The TGA encourages exploration of biomarkers or mandated for many novel therapeutics. However, a more flexible approach such as that used for the alternative medicines register would be ideal. An example of a suggested difference would be not requiring full evaluation of efficacy and toxicity. It was also suggested, there should be a policy for rare cancers - registering drugs on the genetic targets rather than each individual histology.

In the stakeholders' opinions, a drug should be able to register based on biomarker results with a few restrictions. Where a relevant biomarker improves patient selection, it should be mandatory for registration. Exploration of biomarkers in Phase IV could be a condition of registration or PBS listing. There also needs to be evidence in the form of meta-analyses – one stakeholder said a minimum of five. Stakeholders agree that meta-analyses currently provide the best evidence of biomarker efficacy (biomarker guided vs non-biomarker guided treatment) which is evidence for the benefit of precision medicine.

The Medical Services Advisory Committee (MSAC) process mechanism was considered the best process for approving biomarkers. Stakeholders noted that it provides the validation, rigorous evaluation, quality control, reproducibility, and the sustainability of the test in terms of long-term availability. The only suggestions were that the committee does not have to be the Australian regulator; also, some tests are validated by professional bodies (pathologists usually, sometimes biochemists). It was also identified by one stakeholder that having the assessment by MSAC, which does not include a link to the intervention that will improve health outcomes, is a barrier with the current process.

The stakeholders perceived several other barriers to effective biomarker development and implementation. Evidence from well-controlled studies with clearly defined outcomes that clearly indicates the benefit of implementing biomarkers are what are necessary as mentioned above. However, the design and interpretation of these studies is very challenging. This challenge is increased by the process where most approvals to date are only of individual markers rather than multi-testing genomic panels. One stakeholder also stated that it can be difficult that platforms have different thresholds for activity in different situations.

IMPACT ON PATIENT OUTCOMES

Stakeholders are very positive regarding the positive impact access to effective biomarkers would have on patient outcomes. Benefits mentioned by stakeholders resulting from biomarkers-based better patient selection include:

- reduced cost,
- reduced toxicity
- less wasted opportunity in the treatment of cancer.

Further, another stakeholder mentioned that in paediatric patients, there have been anecdotes of dramatically good outcomes when biomarkers have been used to guide treatments in desperate situations where conventional therapy does not exist or has failed to control the tumour.

LEADERS

To gain insight into key influencers, stakeholders were asked to identify key leaders/experts in cancer biomarker fields in Australia and the equivalent overseas. Within Australia, it was first stated that naturally the leader that comes to mind depends on the type of cancer. The stand out leaders mentioned as examples, included:

- Richard Scolyer, Melanoma pathology. Professor Richard Scolyer is currently Co-Medical Director and Consultant Pathologist at Melanoma Institute Australia; Senior Staff Specialist, Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital, Sydney; and Clinical Professor, The University of Sydney.
- David Bowtell Ovarian cancer. Professor Bowtell is based in Victoria at the Peter Cancer Centre. He is the Head of the Cancer Genomics and Genetics Program at the Peter MacCallum Cancer Centre and holds a Joint appointment as a Group Leader at the Garvan Institute of Medical Research, Sydney. He is a Visiting Professor at Dana Farber Cancer Institute, Boston.
- Anna Defazio, Ovarian cancer. Professor at the University of Sydney, Sydney West Chair in Translational Cancer Research, Obstetrics, Gynaecology and Neonatology, Westmead Clinical School, The Westmead Institute for Medical Research.
- Stephen Fox, Tumour Angiogenesis & Microenvironment Group Leader, Peter MacCallum Cancer Centre.
- Ben Solomon, Lung, Head & Neck Cancer. Professor at the Victorian Peter MacCallum Cancer Centre. Professor Ben Solomon is a medical oncologist in the Lung Service and the Head & Neck service. He is the Group Leader of the Molecular Therapeutics and Biomarkers Laboratory in the Research Division.
- David Ziegler, Paediatric Oncology. Associate Professor David Ziegler is a Group Leader at the Children's Cancer Institute where his preclinical research focuses on novel therapies for childhood brain tumours.
- Anthony Joshua, Prostate and Melanoma Cancers. Dr Joshua is Conjoint Associate Professor, Director of Oncology at St Vincent's Clinical School, Faculty of Medicine, UNSW Sydney.

Overseas, it was thought largely too difficult to narrow down to overall leaders. However, a couple of names were mentioned including:

- Paul Boutros, based in University of Toronto, Canada as an Associate Professor, Dr Boutros is considered a global leader in prostate cancer.
- Boris Bastian, Professor at the UCSF School of Medicine in the United States, is considered a global leader in melanoma biomarkers.

DISCUSSION AND RECOMMENDATIONS

In the era of personalised medicine and genomics and the shift from non-specific cytotoxic drugs to small targeted molecules, monoclonal antibodies and immunotherapies, biomarkers increase the efficiency of targeted drug treatments by indicating the presence of treatment targets. This allows selection of the optimal treatment and avoidance of therapies that are unlikely to be effective. However, to take advantage of this enrichment of the patient population, the regulatory and reimbursement process must align approval of the drug with approval of the measurement of the biomarker target. The patient outcome could then be considered in the approval process of the biomarker test. The ideal would be to have both approved by the same agency.

Moreover, cancers with similar genomic abnormalities and therefore the same targets for treatment should be able to have the appropriate drug approved to treat each of them and not have to have separate approvals for each cancer based on histopathology, which is the current practice. This would incidentally benefit rare cancers that are often not subject to clinical drug trials and where numbers do not make large trials feasible. However, if the rare cancer had the same genomic abnormalities of a more common cancer where a trial of a targeted therapy had shown efficacy, there would be a good rationale for that therapy to be used in the rare cancer.

A further efficiency to be gained from biomarkers is if the biomarker can be shown to be a surrogate endpoint for survival in a randomised clinical trial. The surrogate endpoint would be reached much earlier than a survival endpoint, allowing an earlier provisional approval of a drug. The continued approval would depend on eventually demonstrating the survival advantage. A standard trials methodology for evaluating the predictive ability of a biomarker, often a randomised clinical trial, should be defined and formal evaluation of biomarkers be undertaken. Strong bioinformatics capabilities will allow linkage and analysis of large genomic datasets.

The pharmacogenomic characteristics of a drug may guide drug dosing and avoid drug interactions. Biomarkers can also be used to follow the progress of anticancer therapy and may subsequently indicate relapse.

There is a definite need for biomarkers that have sufficient specificity and sensitivity to allow the early diagnosis of a cancer, or even screening for asymptomatic disease. Biomarkers, or more likely a panel of biomarkers, may be utilised for this purpose. Even more useful are biomarkers that offer prognostic information about the future course of a disease. The efficacy of these markers can be ascertained from well-conducted trials and retrospective data. The biomarkers

must be able to be freely developed and patent law should not be allowed to be a barrier.

Many biomarkers have been identified for various disease types. They must be carefully evaluated. PSA testing in prostate cancer is a good example of where use of biomarkers should be evidence-based. Screening studies show only a small survival benefit to early detection, but many more patients are over-diagnosed and over-treated, since PSA cannot definitively answer the question of whether prostate cancer can be placed under surveillance or needs immediate treatment.

The survey of experts showed a high use of biomarkers but only just under half of the respondents thought they were reliable. The experts were particular concerned about the specificity of biomarker testing. This simply underscores the need for better trials to demonstrate the efficacy of biomarkers. The experts agreed that biomarker development and validation would be more successful if the biomarker can be shown to be part of the underlying pathological process of tumour development.

More detailed interviews with stakeholders focussed on the funding issues of pairing a drug to its biomarker. They commented on the potential for risk sharing amongst those who would benefit from this pairing, such as those who developed the testing technology. They certainly were interested in regulatory models that would mandate pairing a targeted drug with its biomarker as a condition of funding the drug. The extent of patent protection of biomarker technology could influence their development and availability. They also recognised the difficulty of designing and interpreting biomarker trials and this is certainly an area requiring direction and further funding.

The patient outcome of reduced cost, toxicity and less wasted treatment when biomarkers are used is the most important outcome to be weighed when evaluating a biomarker.

Australia has several leading experts in the biomarker field and has taken steps to align the MSAC approval of biomarkers with drug TGA registration and PBS funding. It has also planned for provisional licensing prior to the conclusion of full Phase 3 trials. However, there is still a long way to go to approve drugs on their genomic rather than their histopathological characteristics, and to address the issue of funding the co-dependent drug and its biomarker.

RECOMMENDATIONS

1. Allow approvals and reimbursement of targeted drugs to be based on the genomic similarities of cancers expressing the target, rather than approving drugs only on histopathology.
2. Align the approval and funding of a targeted drug with that of its co-dependent biomarker preferably by the same agency where end-user benefit can be a part of decision-making.
3. Allow provisional drug approval based on surrogate biomarker endpoints.
4. Develop standards for evaluation of biomarkers as predictive tools.
5. Develop bioinformatics capabilities to analyse large genomic datasets.
6. Develop electronic health records and laboratory systems to allow for capturing and linking biomarker tests and data.
7. Develop guidelines for the use of biomarkers.
8. Ensure that patent law does not restrict biomarker development.

APPENDIX – OPEN-ENDED RESPONSES FROM SURVEY

EFFECTIVENESS OF BIOMARKERS

Biomarkers that are effective
BRCA and specific mutations in P52, PTEN etc.
BRAF
PSA is widely used in the clinic for prostate cancer screening and treatment management. While not perfect, it is very useful.
ER PR HER2 Kras EGFR
p16
Blood cholesterol levels for heart disease. Blood glucose levels for diabetes.
Those with good predictive/prognostic ability.
Molecular detection of leukemia-specific gene rearrangements / fusions; PSA in some cases
In gliomas: helpful in prognosis; less important for treatment selection; but some exceptions to this. Important in other selected settings (eg RAS mutations in colon; EGFR mutations in lung; etc)
Estrogen Receptor, HER2
BRAF V600E mutation in cutaneous melanoma & some KIT mutations in acral & mucosal melanoma help stratify patients for treatment with specific inhibitors
BRAF mutations for targeted therapy
EGFR mutations BRAF mutations c-kit Estrogen receptor
KRAS for EGFR targeted agents in CRC, EGFR/ALK/ROS mutations in lung cancer, ER/PR/Her2 breast cancer
Best example would be tumour markers in testicular cancer, and monitoring post CRC surgery - but there are many. I am not sure of your definition of what a biomarker is, so I am presuming tumour markers are. You could also site, ALK and EGFR for lung cancer, KRAS bowel cancer, HER 2 lung and gastric cancer etc etc, MGMT in GBM
HER2 for breast cancer EGFR and ALK for lung cancer RAS for colorectal BRAF for melanoma HRD for ovarian cancer
T790M LDH Alb
PSA, CEA, CA 15.3, CA 19.1, CA 124, BHCG, AFP, LDH, bcr abl, FLT3, NPM 1, CEPBA
Braf
Very few are truly effective eg HCG/AFP. The use of PSA, CA125 can guide treatment. Others are more indicative of activity but do not think are accurate enough for treatment decisions. The biological ones such as HER2, RAS, BRAF, EGFR etc are all useful predictive tests but not as monitoring
ER, HER2
EGFR mutations in lung cancer KRAS mutations in colon cancer Braf mutations in melanoma t790M mutations in lung cancer
BRAF, Rad, MSI, Her2, Braca1 and 2, PD1
BRAF for melanoma, KRAS/NRAS for CRC, EGFR/ALK/ROS1 for lung adenoca, DRG mutations prostate ca, DRG mutations ovarian ca, BRCA1/2 breast ca, her2 gastric ca.
HER-2, RAS, EGFR mutations, ALK mutations, BRAF mutation, MMR deficiency
BRAF, EGFR, ALK
EGFR, K-RAS, ALK, HER-2, B-RAF
PSA (for certain indications (eg treatment responses), but definitely not in all cases (eg prognosis))
Her 2, MSI, MMR, EGFR, RAS, BRAF

ki67 in combination with histopathological features HER2 and ER/PR
MMR, KRAS/NRAS, HER2
Hormone receptor expression, DNA mismatch repair enzyme expression, EGFR/KRAS/BRAF/ckIT (etc) mutations, chromosomal gain/loss (e.g. uveal melanomas), methylation
Breast cancer - Her2, ER, PR Gastric cancer - Her2 Lung cancer - PD1 (somewhat effective) Colorectal cancer - KRAS, BRAF Melanoma - BRAF
Ras testing COLORECTAL CANCER, EGFR/alk in NSCLC, HER-2ISH, BRAF
RAS mutation, EGFR mutation, ALK translocation, mismatch repair deficiency
PD1, PDL1, Her2, ER, PR
BRAF, ER, PR HER2
Many! EGFR for 1st line NSCLC ALK for 1st and later line NSCLC HER2 for breast cancer PD-L1 (either tumour or combination tumour + immune cell) for NSCLC (for both PD-1 and PD-L1 inhibitors) - and likely in other indications BRAF in melanoma. Issue is, what do you mean by "effective". Do some biomarkers predict treatment outcomes? Yes. Are they necessary for making treatment decisions? Sometimes. Are they necessary for making cost-effective decisions for reimbursement? Sometimes. There are many ways to use a biomarker, so effective is perhaps too imprecise.
ER, HER2, EGFR, ALK, RAS, BRAF
RAS, EGFR, ALK, BRAF, ER, PR, Her2
BRCA1/2 as a guide to PARPi susceptibility; but not sufficiently sensitive or specific
EGFR status in non-squamous NSCLC, RAS status in colon cancer, BRAF status in melanoma, ALK and ROS1 status in non-squamous NSCLC, PD-L1 status in NSCLC, ER/PR and HER2 in breast cancer. All these are effective predictive biomarkers. Some also provide some prognostic information.
KRAS in CRC. EGFRm in NSCLC BRCA in breast and ovary HER2 in breast and stomach HRDness in prostate (research) PD-L1/PD-1 expression in lung BRAF in melanoma
too many to list!
PSA (for advanced disease)
This question could take pages to answer, and its not clear what "effective" means? Targetable? Stratifying patients? But biomarkers in some form or another have always been integral to cancer treatment. Eg in NMYC in neuroblastoma; BRAF mutations; SHH mutations in medulloblastoma; H3.3 mutations for stratification; BCR-ABL, and MLL rearrangements in leukaemia, etc.
Cdx2
There is strong evidence that endosome biology is altered in prostate cancer and that proteins expressed by endosomes that impact endosome biology have altered expression in prostate cancer. These proteins have a great deal of potential as biomarkers. However, these data are pre-clinical.
Biomarkers that clearly identify a biological rationale for a particular therapy, e.g.: ER/PR/HER2 IHC EGFR mutations ALK/ROS/NTRK fusions
CA19-9 EGFR mutant ALK KRAS
PSMA PET for prostate cancer
BRCA2
ER HER2 KRas mutation status Mutant EGFR ALK translocations Bcr-Abl
Her2neu, egfr, alk, RAS, ER, BRAF etc
Insulin, BRAC1, EGFR
RAS mutation for anti-EGFR mabs in mCRC, EGFR mutations for EGFR inhibitors in NSCLC, HER2 expression for anti-HER2 therapy in mBC
CXCL9 EOMES GZMA GZMB CD8A IFNG PRF
EGFR, ALK (not using PD-L1 routinely yet but could be useful) ER, PR, HER2

ER/PR, Her 2, KIT, ALK, EGFR, ROS mutations- PD-L1 limited, BAP1 diagnostic, some prognostic

ER, PR, HER2, BRAF, EGFR, BRCA1, BRCA2 amongst others

faecal test, plus new DNA biomarker

Some are, depends what they are being used for. Least reliable in the area of screening but more are useful in monitoring or deciding on treatment. would not rely only on a biomarker. most will complement other clinical information

RAS, RAF, MMR

Genetic variants for targeted therapy in cancer

PATIENT ISSUES

What patient issues do you think there might be when obtaining samples?

Concern over tests and their ability to predict disease outcomes
Not relevant because result is essential to decide on PBS funded treatment
Samples should be easy to collect e.g. by patients themselves of a procedure which is as non-invasive as possible eg blood test.
Discomfort risk of collection
Recruitment
Patient discomfort if samples hard to collect such as tissue biopsies.
Patient discomfort / need for anaesthesia for collection of bone marrow aspirate for leukemia
Usually straightforward to collect; costs / availability of some tests sometimes an issue
Patient consent, collection, patient discomfort.
Surgery or needle biopsy is required to take tissue samples for molecular testing. This can cause, pain, discomfort & stress
Difficulty of sample collection
Sample sometimes does not have enough tissue left for biomarkers, particularly with small lung biopsies- lung
Difficulty if this requires re-biopsy - pt discomfort/risk of significant complications/risk of unsuitable sample
These are minor
Risks of biopsy - patient discomfort practical difficulty of acquiring existing tissue (requests to other laboratories, age of tissue makes them unsuitable)
Some markers e.g. Flts require bone marrow aspirate
Time. Possible pain. Possible complications
Blood markers are easy and can be repeated but lack sensitivity in general. Biopsy drive markers to follow molecular changes is another issue as biopsy can be dangerous so risk benefit issues
Repeat specimen/testing after neoadjuvant therapy - repeat testing after progression of metastatic disease. Difficulty arranging biopsy.
Complications of biopsy - pain concern about use of tissue concern about research
If repeat bx needed some pain
Pain and procedural complications. Difficulty obtaining tissue if metastasis sites are difficult to access or bone only disease (eg in prostate ca)
Repeat biopsies, waiting for results to guide treatment
Utility of the test - whether it's sufficient to affect treatment decision; likelihood of yielding results vs risk of test eg invasive biopsies
Patient discomfort, biopsy can be difficult
Discomfort from biopsy
Sampling of tumour material for advanced disease is challenging to justify
Sometimes hard to re-biopsy, patients very receptive if may change management
Liver biopsy is rare and becoming less common due to risks and discomfort. Also, biopsy samples vary in their readouts.
Adequate tissue, rebiopsies.
Difficulty collecting samples and timeliness of collection.
Patient discomfort only sometimes, fear if something negative is found
Patient discomfort, hard to collect sample, risks of pneumothorax or bleed
Risk of biopsy related complications of cite biopsy required
Patient discomfort and procedural risk.

Fresh sample, size of tissue
Hard to collect sample, much goes to pathology
Multiple biomarkers being taken from limited tumour samples (egfr, alk, pd-l1 etc from nsclc). The need for re-biopsy (safety) due to lack of tissue, or because the biomarker tends to change with lines of therapy, such that a biomarker test at baseline might not reflect the biomarker following progression (particularly when there is heterogeneity in tissue / metastases).
Pathologists required to do increasingly more tests on increasingly smaller samples > either need bigger samples, or panel-based testing
Adequate pathological specimen
Feasibility of biopsy if tissue not accessible lack of funding for biopsy unless clinical benefit has been proven time taken to obtain tissue at diagnosis of progressive disease ctc/ctdna analysis in blood is not yet widely available - once it is, this will improve accessibility. But needs labs capable of rapid turn around
Tissue biopsies (eg core biopsies) better than cytology samples, this could potentially have slightly higher risk to patients of biopsy associated side effects.
All of the above
If needing to repeat biopsy there are issues
Tissue biopsies can be uncomfortable, and can result in sampling error, even if the tissue is accessible.
Collection requires endoscopy, mild discomfort.
Collection and testing
Consent and ethical barriers.
Patient discomfort (biopsies)
Sample collection
Collection - if this is tumour tissue
Wishing not to consent - ill health
Difficulty and cost to access pet scanner
Inadequate genetic counselling to support the decision and the implication if results are positive inadequate information about the implications for insurance etc
Depends on cancer type and site. If (multiple) tumour biopsies are required then repeat procedures, discomfort etc
Should be no significant problems
Minimal
Sample collection should be non or minimally invasive. Fecal assays have low acceptance
Inconvenience, resources, interpretation
There may be discomfort associated, for some cancers a biopsy may be particularly difficult to obtain e.g. Lung
Patient discomfort repeated biopsy
Lung - nightmare to get a decent sample.
We test material that would be taken for diagnosis. Liquid biopsy may be way to go for some. I feel there are some underutilised samples- i.e. Pleural fluid, which is readily and relatively painlessly available and gives information about the tumour environment as well
Discomfort, cost.
Hard to obtain
Ease of collection, patient discomfort
Yes discomfort, frequency of tests, whether the use of biomarkers actually provides any advantage in terms of improved outcomes or just adds worry.
Cost, time.
Patients are generally happy to be involved. Biopsies and other invasive procedures can be uncomfortable and inconvenient for patients

RELIABILITY OF BIOMARKERS

Why biomarkers tests are unreliable

Specificity and sensitivity as is the case for PSA specificity. Many others not properly validated or used in inappropriate way e.g. PSA for screening

This depends on what the biomarker is. Some are reliable, others are not.

Variability in test quality sensitivity and specificity predictive vs prognostic impact

Poor specificity. But it can be useful for identifying a potential problem for further investigation and validation with other tests.

Usually reliable in determining level of biomarker; uncertainty in some settings as to its clinical relevance

Question is too broad: depends on biomarker

Issues with: heterogeneity of the tumour sampled, poor clinical correlation in some trials, poor access to tests

Some are more reliable than others

Mentioned above - may be predictive for some but not useful over time

Do not fully predict response to treatment, even with well validated ones such as ER. Need better markers to decide to treat, or not to treat, and which drugs.

Big variation in biomarkers - some excellent whereas others very vague with low positive and negative predictive value.

ki67 have issues with variability between pathologist ER and PR are now reliable issues with liquid biopsy EGFR mutation testing for T790 - need to understand quality assurance issues with test and whether need to do solid biopsy

No single marker is useful in all patients. No agreement on a combination or a move towards using DNA in blood.

In my area there are none because of the uncertainty about causes

Many biomarker tests are very reliable, some are unreliable due to operator dependency for results interpretation (e.g. methylation), use of results that are beyond the capability of the test (e.g. NGS), heterogeneity of tumour

Varies, but issues with reproducibility on 1 sample VS multiple samples at 1 point in time VS sampling at different time periods. Quite reliable for BRAF-Her2-ER-PR but not for PD1

Depends on the marker and the methods for testing it.

Assays used across path labs are not necessarily standardised - when more sensitive assays are used, greater numbers of patients will be detected (ie T790M mutations) - and this may not reflect the population in the key trials of the targeted agents when a different / less sensitive assay was used - therefore the clinical benefit will be uncertain. Different tissues (tumour sampling vs blood sampling) will not result in similar populations and may be different than the original trials of the targeted therapy. Immunohistochemistry requires subjective assessment. Biomarkers defined by a threshold require subjective assessment (ie >50% PD-L1).

Some proposed biomarkers (ie immunohisto-chemistry for PD-L1) is very iffy (heterogeneous/subjective): concern over denying patients treatment on the basis of suboptimal biomarkers

a few biomarkers have improved indications for therapy eg BRCA1/2, ALK fusions, EGFR mutations but we need more biomarkers that are relevant for the many patients without the ones listed above. Many biomarker studies are under-powered and unreliable

Lots of issues, but some include: lack of definable target, variation in methods / instrumentation, lack of standardised procedures, lack of interest in biomarker research and funding (major issue).

Biomarkers linked to "outcome" that are derived from screens or data mining are often not reproducible, have no biological basis, and do not translate into the clinic.

I work on circulating microRNAs. In this area, the key issue is how to normalise miRNA quantification to remove collection/storage/processing/amplification variations.

This answer can't be answered in general terms. Some tests are extremely reliable, others e.g. PSA for prostate cancer are difficult to interpret

Many biomarker tests are unreliable due to poor sensitivity/selectivity, insufficient validation,

Need further evaluation, but the term unreliable is too pejorative. Prefer exploratory.

Biopsy quality Percentage tumour content. Full tumour representation Sample amount

Mind you with HER2, I have concerns about borderline ones and the QAP with it.

It depends on the biomarker- e.g. PDL1 tumour heterogeneity, change in biomarker status over time, query different at different sites. Some issues relating to type of assessment- e.g. immunohistochemical reading- variable assessment methods/cutoffs e.g. for PD-L1, but even some lack of good kappa values for ER/PR; techniques used, type and amount of material- city versus histology- ? liquid biopsy

Not accurate enough

SPECIFICITY OF BIOMARKER TESTS

Problems with the specificity of biomarker tests

False positives in patients with no disease and false negatives in patients who have the disease. This is an issue with PSA testing

Again, this depends on the test

Quality varies between labs

Not specific for the disease resulting in false positives.

Not 100%

The specificity of any biomarker needs to be validated - this has been done for most current markers for acute lymphoblastic leukemia. CA125 and PSA work well for some tumours/ patients. But newer biomarkers often need more work

The quality of tests being used and whether standardized. Quality of techniques used. Site-site variations due to procedural differences,

A positive test in the case of some somatic mutations does not necessarily indicate that a patient will respond to a particular inhibitor since their tumour may have intrinsic resistance to that drug

Might depend on the quality of the sample

Question is too broad: depends on biomarker

Different test platforms, different reagents - and also few relate to just one disease entity.

Some biomarkers are very specific and these tend to be the most useful eg ras mutation in colorectal cancer essentially excludes benefit from some drugs. A lot of the immunotherapy biomarkers are far too non-specific.

No test is 100%

Many biomarkers are not cancer specific e.g. CA 125 may be falsely elevated in CCF, ascites etc.

Most are not specific so indirectly useful

Some patients who are positive will not respond to biomarker directed therapy. The tests are not specific enough for certain therapies, ie choice of chemotherapy.

PD1 does not discriminate in some tumours

Rare mutations with uncertain responsiveness to targeted therapies

Potential to miss patients who need treatment

Lack of discrimination

Variability between patients and tumours.

Too many results in NGS panels leading to abuse of PubMed and Google, and attribution of prognostic or predictive potential of mutation in a particular cancer type when it has only been evaluated for a different unrelated cancer type. It drives me nuts.

PD1 highly variable in predictive value across tumours

Although a patient's cancer may have a biomarker positively associated with treatment response, they often will still not respond to treatment ie., biomarkers are not 100% specific to clinical response or lack of response.

Does the Her2 marker works in cancer other than breast, gastric and bowel? PDL1 might be negative, but immunotherapy works.

These are very general questions. Any issue with the specificity of the test, that may result in patients who do not have the biomarker being treated with a targeted therapy MAY result in harm for no benefit, or at least forgone benefit if standard care is effective. However, where a targeted therapy is better than standard care irrespective of the biomarker, and the testing of the biomarker is only to enable the provision of treatment to those in whom it is most cost effective, a false positive is not likely to be problematic (except financially).

None is a perfect predictor of response.

Similar to above.

A tumour may have a specific biomarker, but other causes of drug resistance may be present, particularly if the analysis is in the relapsed (or multiply relapsed) setting as is often the case

False negatives can be a problem

I think there is a sliding scale / balance problem - the better we want a biomarker to be, the less generalisable it will become

If biomarkers are proteins then the ELISAs or antibodies used to detect such proteins must have high specificity. A great deal of validation must occur to ensure high specificity.

In the field of miRNAs, the key issue is that a lot of miRNAs may be common for several cancer types.

The specificity of biomarkers that are in development can be unknown

None perfect

Many antibodies used for ELISAs are not properly validated and lack specificity. Additionally, due to the heterogeneity of many diseases, it is now realised that panels of biomarkers may be required

Both false positives and negatives- depending on techniques, validation and training of reader.

There are few biomarkers that give definitive answers to clinical questions. Results usually need to be interpreted in clinical context.

Unable to separate indolent from aggressive tumours

The need for validation

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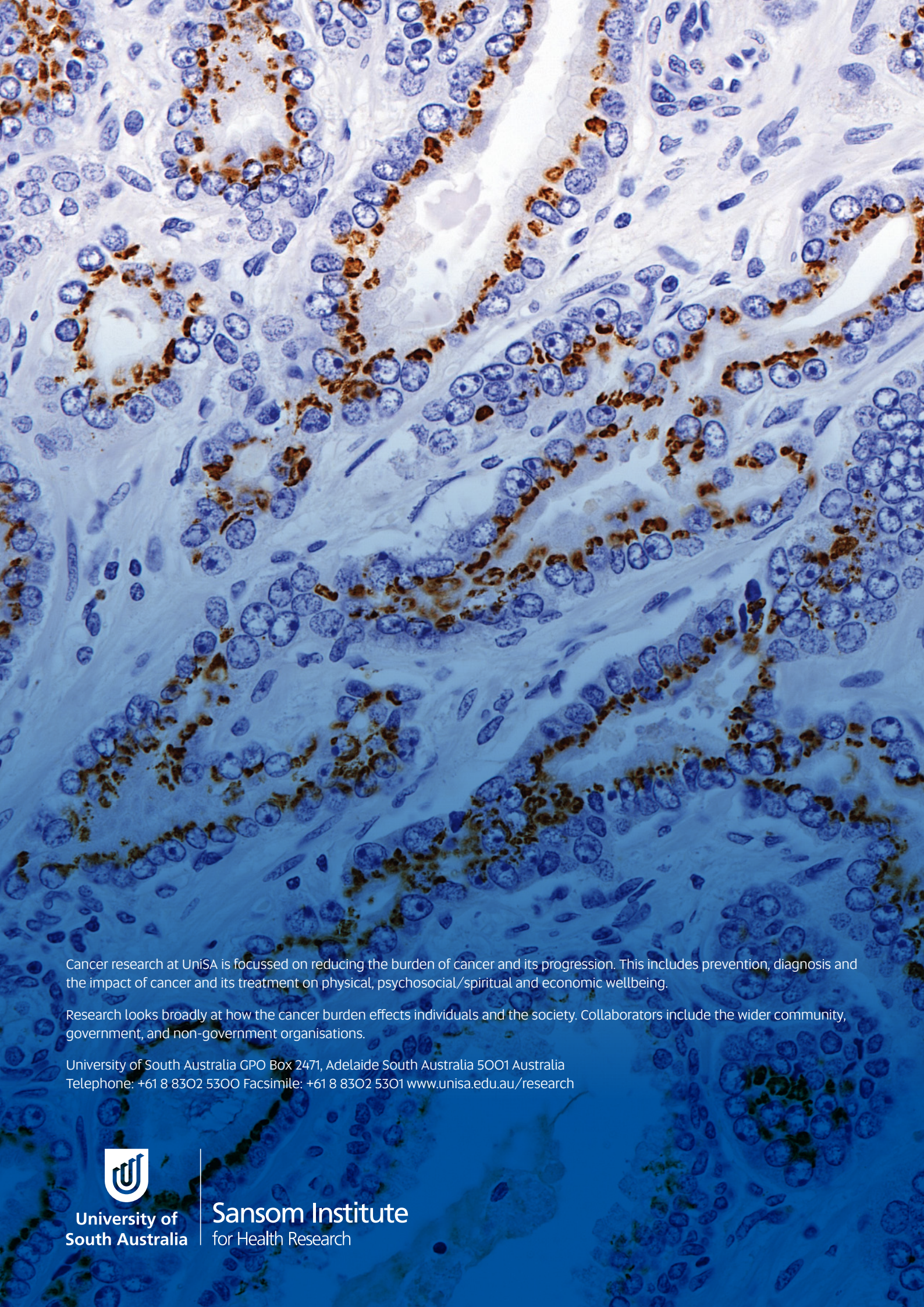
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Cancer research at UniSA is focussed on reducing the burden of cancer and its progression. This includes prevention, diagnosis and the impact of cancer and its treatment on physical, psychosocial/spiritual and economic wellbeing.

Research looks broadly at how the cancer burden effects individuals and the society. Collaborators include the wider community, government, and non-government organisations.

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