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ADELAIDE PATHOLOGY

Doctors' Newsletter

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It is my great pleasure to welcome you to our 2018 Doctors' Newsletter.

This being the first issue since my appointment as CEO, I have taken the liberty to introduce myself and to share an important value for which I stand.

From the start of my career at Clinpath Laboratories almost 17 years ago, through my time at Adelaide Pathology Partners (APP) and now having returned following the merger of the two laboratories, one constant has been patient-focused care.

The shared values of both laboratories resonated strongly with my colleagues and I, and guided us in our decision, to merge with Clinpath. Clinpath, a foundation laboratory of Sonic Healthcare, is a medical practice founded on the principle of Medical Leadership. It is Medical Leadership that provides Clinpath with the critical insight and appreciation of clinical practice and medical management, which collaboratively ensures the best possible patient outcomes. Just like you, the patient is central to all that we do and it is this value which I strongly believe is our unique point of difference. Medical Leadership is providing expert advice.

I hope that you enjoy reading our Doctors' Newsletter and the contributions from our clinical team are of value to you and your patients.

Please don't hesitate to contact me with any questions or feedback in relation to our services.

Dr Fergus Whitehead
BMBS, FRCPA, FIAC, Dip Cytopath RCPA
CEO – Clinpath Laboratories

Cover: Red blood cells

Rusty genes

Please pause for a moment (by all means, sip your coffee) while I take you back to your moment of conception. The fertilised egg that was you contained two copies of the entire genetic code. You will be familiar with the iconic helical DNA that represents the encoded genetic information. What is less widely appreciated is the length of this DNA. At conception, there were two linear metres of DNA in the fertilised cell. That is, you are as tall as the length of DNA in a single human cell. This simple observation leads to consequences that are both staggering and relevant in healthcare.

Professor Graeme Suthers
BSc (Med), MBBS, PhD, FRACP, FRCPA, GAICD
Director of Genetics, Sonic Healthcare (Australia)

DNA in a single cell must be copied before the cell divides. This occurs in a tiny space and is repeated billions of times, so it is not surprising that errors occur. The error rate is staggeringly low (10^{-9} per nucleotide) but, given the length of your DNA, this amounts to six new DNA sequence errors generated at every cell division.

At an early stage in development, a new mutation may compromise an entire lineage of cells. By the end of the first week of development, approximately 60% of embryos will have major chromosome abnormalities. These abnormalities occurred either in the formation of the sperm or egg, or, more commonly, reflect new mutations which occurred after conception. The great majority of these chromosomally abnormal embryos are so functionally compromised that they are lost by spontaneous miscarriage, often before a woman is aware she is pregnant. The new mutations, whether they be abnormalities in chromosome number or mutations in individual genes, contribute to the burden of congenital malformations identified at birth or within the first few years of postnatal life.

An adult human consists of approximately 100 trillion cells containing, in total, 200 trillion metres of DNA i.e. you contain approximately 10 light-days of DNA. It is important to note this incredible length of DNA is not a homogeneous strand, like a piece of string, but is a highly ordered sequence of nucleotides. It is impossible to create a highly structured strand this long without creating errors. Furthermore, it is impossible to sustain the integrity of the genetic code over this extraordinary distance. Even if further cell divisions did not occur, the sheer length of our entire genetic code dictates that the sequence must become corrupt over time.

The Second Law of Thermodynamics describes a fundamental property of energy and matter: energy must be expended to maintain order. The amount of energy required to maintain the precise order of nucleotides along a length of 10 light-days is mind-boggling. The net effect is that the integrity of your genetic code has been corroding since the moment of your conception. Your genetic code may have been compromised by genetic errors already present in the egg or sperm from which you developed; this is the basis of heritable predisposition to disease. But even if you had perfect genes at conception, fundamental physical forces dictate that your genetic code becomes 'rusty' throughout your lifetime.

Two consequences arise from the development of genetic rust. First, the accumulation of genetic errors (particularly in mitochondrial DNA) eventually compromises cell function. If sufficient cells within an organ have compromised function, we recognise this clinically as age-related organ failure. Second, genetic errors in a single cell can disrupt the control of cell division and result in that cell growing out of control i.e. cancer. From this genetic perspective, issues of infertility, miscarriage, malformations, cancer, organ failure, and ultimately mortality, can all be attributed to the single underlying mechanism of rusting genes.

The significance of these genetic processes, both heritable and acquired, is reshaping our understanding of the biological basis of disease and the investigations that can inform our clinical decisions. Throw in a good dash of technological advances, and we have a revolution in pathology that is providing us with unprecedented information. Genetic testing is changing the face of modern pathology.

How's the coffee? Gone cold?

Targeted cancer therapy determined by PD-L1 immunohistochemistry

Dr Craig James
MBBS, FRACP, FRCPA
Histopathologist

Immune checkpoint pathways activate regulatory signals for the physiologic T cell immune response. Accordingly, inhibitors targeting PD-1 and its ligands have demonstrated clinical benefit in several cancers. Clinpath Laboratories now offer PD-L1 immunohistochemistry to aid in selecting eligible patients with non-small cell lung carcinoma for anti PD-L1 targeted therapy.

The Programmed Death 1 (PD-1) receptor is a transmembrane protein expressed on T cells, that along with its ligands Programmed Death Ligand 1 (PD-L1) and Programmed Death Ligand 2 (PD-L2), play an important part in helping the immune system differentiate between normal and abnormal cells. The binding of PD-1 to PD-L1, which is found on many normal cells such as dendritic cells and macrophages, down-regulates the immune response by inhibiting T cell proliferation, cytokine production and cytolytic activity[1,2]. PD-L1 expression, which can be detected using immunohistochemistry, has been observed in many types of cancers and most widely studied in non-small cell lung cancer (NSCLC), melanoma and urothelial carcinoma[3]. PD-L1 expression by cancer cells, as shown in Figure 1, can inhibit the T cell's ability to kill the cancer cells and protects the cancer from immune elimination[1].

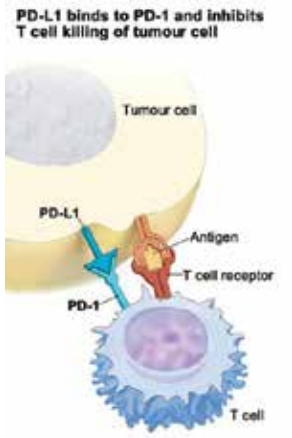


Figure 1. Tumour cell PD-L1 expression and interaction with the T cell[4].

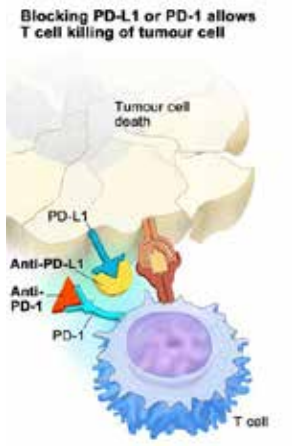


Figure 2. The blocking of PD-L1 and PD-1 ligands with immunotherapy drugs[4].

Anti PD-1 and PD-L1 immunotherapy drugs have been developed to block the binding of PD-1 to PD-L1, thereby allowing the bound T cell to kill cancer cells (Figure 2)[4]. KEYTRUDA® (pembrolizumab) and OPDIVO® (nivolumab) are two such drugs that have been approved in Australia for patients with metastatic NSCLC with positive PD-L1 expression. Both these drugs have shown improved 12-month overall survival for patients with metastatic NSCLC when compared to patients treated with standard treatments (Figures 3-4)[6,7,8].

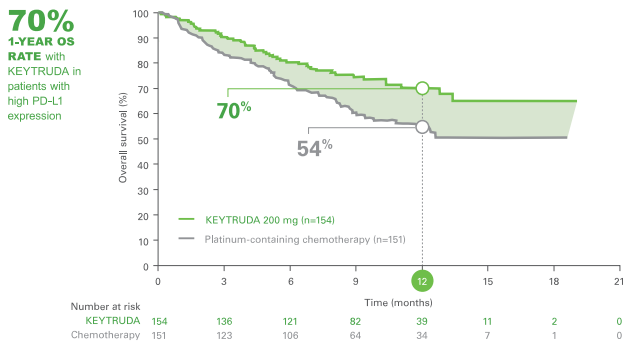


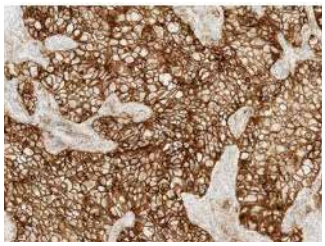
Figure 3. Kaplan-Meier estimates of overall survival (OS) of first-line single agent KEYTRUDA vs platinum-containing chemotherapy in metastatic NSCLC patients with high PD-L1 expression [tumour proportion score (TPS) ≥ 50%)][6].

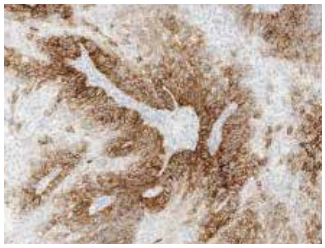
Figure 4. Kaplan-Meier estimates of overall survival (OS) of second-line single agent KEYTRUDA vs docetaxel in metastatic NSCLC patients with PD-L1 expression [tumour proportion score (TPS)] of ≥ 1%[7].

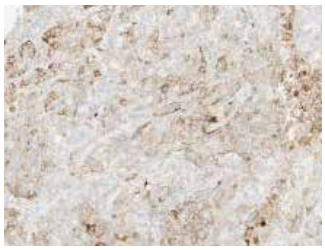
PD-L1 immunohistochemistry is conducted at Clinpath Laboratories on formalin-fixed paraffin-embedded samples using the VENTANA PD-L1 (SP263) Assay (on board the automated VENTANA BenchMark Ultra staining platform). For accurate reporting, the sample must contain at least 100 viable tumour cells. Patient samples are evaluated for the percentage of tumour cell positivity.

Therapy	PD-L1 expression – Therapeutic line
KEYTRUDA®	≥50% TC – First line
	≥1% TC – Second line
OPDIVO®	≥1%, ≥5% and ≥10% TC – Second line

Table 1. PD-L1 expression cut off point (tumour proportion score) for therapeutic treatment with KEYTRUDA and OPDIVO[5].







Figures 5–7. Examples of various membrane and cytoplasmic staining of NSCLC tumour cells and immune cells stained with the VENTANA PD-L1 (SP263) Assay[1].

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Requesting PD-L1 immunohistochemistry testing

Sample requirements

Tissue samples sent in 10% neutral buffered formalin.

Medicare rebate

Item number 72846.

How to request PD-L1 testing

PD-L1 testing may be requested at the time the patient sample is sent to Clinpath Laboratories. Additionally, requests for PD-L1 testing can be faxed to Histopathology Administration on (08) 8363 1648.

This exciting new test offers real hope to improve survival times in patients with a variety of aggressive malignant tumours.

For more information please contact:

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Deanna Wallis-Hill (Medical Scientist/Immunohistochemistry Team Leader) is acknowledged for her significant contributions in developing this article.

Diagnostic utility of flow cytometry in clinical practice

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Director of Haematology and Transfusion Medicine Services

Flow cytometry is a technology that is used to analyse the physical and chemical characteristics of particles/cells in a fluid as it passes through at least one laser. Cell components are fluorescently labelled with antibodies and then excited by the laser to emit light at varying wavelengths. The fluorescence emitted is measured and interpreted based on light scattering properties and an expression of cluster differentiation antigens (CD).

Flow cytometry has numerous applications in medicine and has become an important component in the diagnosis and monitoring of patients with a diverse array of diseases.

These include:

- Quantification of CD4 cell count or T cell subsets to monitor antiretroviral drug therapy in HIV
- Management of multiple sclerosis with newer immunotherapeutic agents
- Chronic infection, immunodeficiency
- Clearly differentiate persistent lymphocytosis into reactive or neoplastic process
- Diagnosis of lymphomas like CLL, Mantle cell lymphoma, follicular lymphomas, hairy cell leukaemias, lymphoplasmacytic lymphomas
- Myelomas and other paraproteinaemias
- Acute myeloid leukaemias and acute lymphoblastic leukaemia
- Myelodysplastic syndrome

We are the only private laboratory in South Australia to offer flow cytometry testing.

- Our turnaround time is 24-48 hours.
- We are the only laboratory where haematologists report flow cytometry in conjunction with clinical presentation and clinical history and offer advice on further management.

At Clinpath, we offer comprehensive lymphoma diagnosis as both histopathology and haematology/ flow cytometry testing are performed at our main laboratory at Kent Town.

Case study

A 60-year-old female patient with oral lesions, cervical lymphadenopathy and fatigability was referred by an ENT surgeon to a haematologist, with results as indicated below.

Parameter	Results	Reference range*
Haemoglobin	108	119–160g/L
WBC	18.0	4.0–11.0 x 10^9/L
Neutrophils	1.3	1.7–7.5 x 10^9/L
Lymphocytes	16.7	1.0–4.0 x 10^9/L
Platelets	80	150–450 x 10^9/L

***Ref:** Clinpath Laboratories reference ranges, correct at time of printing.

Investigations

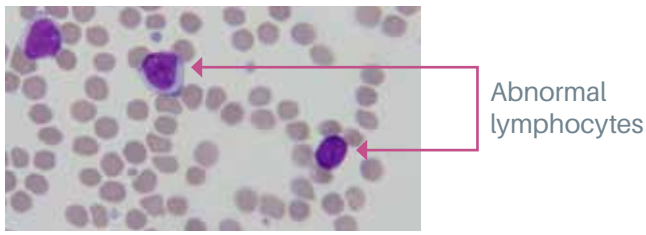


Figure 1. Peripheral blood film shows abnormal lymphocytes, as indicated.

The peripheral blood film showed mature lymphocytosis with clumped chromatin pattern, suggestive of lymphoproliferative disorder. In view of marked lymphocytosis and associated cytopenias, a subsequent bone marrow biopsy was performed.

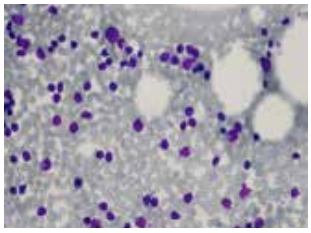


Figure 2. May-Grünwald Giemsa (MGG) staining of bone marrow aspirate showing marked lymphocytosis.

Figure 3. Haematoxylin and eosin (H&E) stained bone marrow trephine showing multifocal lymphoid aggregates.

Figure 5. H&E sections of lymph node.

Figure 6. CD20 immunohistochemistry.

Bone marrow

Review of the bone marrow, in conjunction with the flow cytometry report, was consistent with moderate involvement by chronic lymphocytic leukaemia (Figures 2-4).

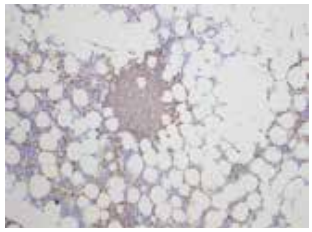


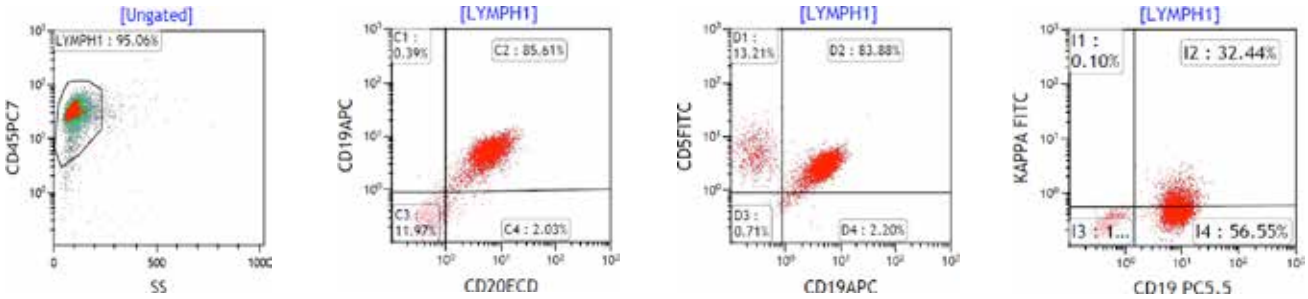
Figure 4. CD20 immunohistochemistry highlights abnormal B cells.

Lymph node

Lymph node biopsy was also performed and sent for histopathological analysis and flow cytometry. Histopathology on the lymph node biopsy showed monotonous population of small B cells, consistent with chronic lymphocytic leukaemia, with no evidence of large cell transformation (Figures 5-6).

Results

Flow cytometry on lymph node



The flow cytometry report shows presence of monoclonal B cells which are CD5, CD23 pos and identifies kappa light chain restriction. Features are consistent with chronic lymphocytic leukaemia.

Sample requirements

- Peripheral blood – EDTA(preferable) and/or Lithium heparin
- Bone marrow aspirate – Lithium heparin
- Bone marrow trephine (if intended for flow cytometry) - fresh in a urine pot without any formalin
- Tissues – fresh/in Hank's medium/saline. REMEMBER IF YOU NEED FLOW TO BE PERFORMED DO NOT SEND THE SAMPLE IN FORMALIN
- All FNA's – Hank's medium
- CSF – Fresh in sterile container

Highlights

- Within 48 hours, this patient had a diagnosis paving the way for the haematologists to plan on further management.
- We can perform flow cytometry on all types of samples ranging from blood, bone marrow, lymph nodes, tissues, body fluids (inclusive of CSF), bronchoalveolar fluids, FNA, breast implants etc.
- We are always available 24 hours a day and 7 days a week for consultation.

For all enquiries with regard to flow cytometry, please contact Dr Lakshmi Nath, Director of Haematology and Transfusion Medicine Services on (08) 8366 2057.

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Our Doctors' Newsletters contain articles written by our pathologists which focus on current issues and recent developments in pathology. Suggestions from you, which we invite wholeheartedly, are the best guarantee that our Doctors' Newsletter becomes a resource of maximum possible interest, information and relevance. If you have any topics you would like to suggest please feel free to contact Simone Hogarth (Business Development Manager) at busdev@clinpath.com.au.

Please note, this Newsletter can also be viewed on our website.

We look forward to hearing about your topics of interest and encourage your participation.

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