

# TISSUEPAPER



## PRESIDENT'S REPORT - MARK BROMLEY

Welcome to our winter 2023 edition of TissuePaper.

I see myself write the word "winter" but let's face it, winter really isn't winter at all up in Queensland! We're the envy of every other state with our blue sunny skies.

Unfortunately, a last minute change of seating policy at our usual scientific meeting venue left us homeless for our last meeting, and despite a frantic scramble for an alternative venue, we were forced to abandon it. We will continue looking for alternatives, but for now the Pineapple Hotel in Kangaroo Point has thrown us a lifeline for the joint AIMS/HGQ meeting on the 24th of August. The topic is Forensics, so put it in your diaries!

Another date for the diary is the upcoming trivia night on Friday 28th of July, again at the Pineapple Hotel. Only a few weeks to go, so get your table booked and your brains in high gear for what I'm sure will be an evening of fun and frolics.

And then, as if trivia isn't enough to stoke the flames of inter-lab rivalry, we have our inaugural axe throwing competition on Saturday September 2<sup>nd</sup> in Newstead. This will be a LOT of fun!

Please also join me in congratulating Kailan Bilney, who won the Histotechnology Group of Queensland QUT Award for the highest academic results in the third year Bachelor of Medical Laboratory Science Histology unit.

So until the next edition, enjoy the fabulous winter sun, unless you're one of our interstate readers, in which case stay warm!



### In This Issue:

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Scientist focus

Save the date



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# Out Damned Spot

Article from the Registrars presentations given at the Mater Hospital Anatomical Pathology Dept.

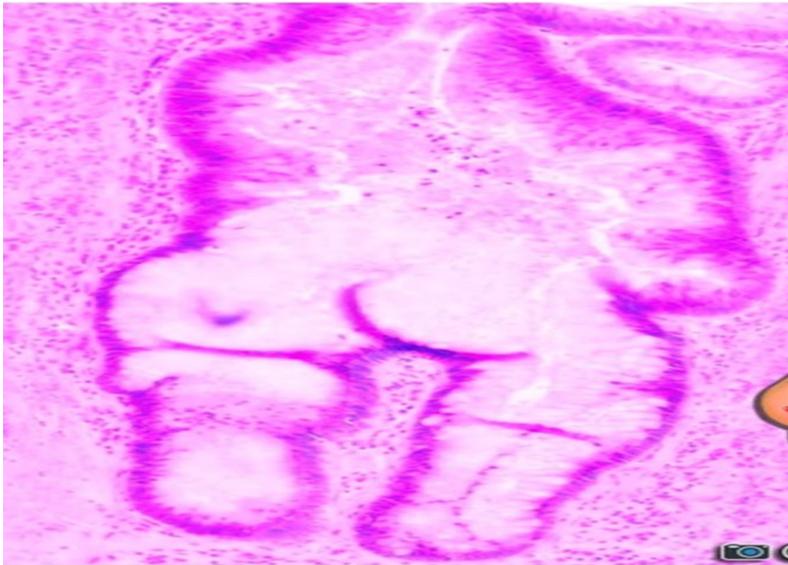
Dr. Shelley Verma, 04/04/2023

Article summary taken from the presentation given by Dr Verma.

Firstly, Shelley took time in outlining the talk and presented the following points.

## Outline

- Meet the Melanocytic lesions
  - Clinical Concern
  - Histology
  - Immunohistochemistry
  - Molecular techniques
- A word on sentinel lymph nodes



Firstly, Shelly dived into the brief history of Melanoma, giving everyone a lesson in how long we humans have been aware of Melanoma and how our understanding has grown.

## A brief history of melanoma

- First described by Hippocrates in the 5<sup>th</sup> century BCE
- Widely metastatic disease found in Peruvian mummies from 2400 years ago
- Given its name in 1838 (previously “fungous tumour”) by Carswell
- The first melanoma to be surgically removed, in 1787, is preserved in a London Museum
- Also in the early 1800’s:
- Mostly seen in pale skinned people
- Can be hereditary
- Only way to survive is to resect the disease early
- And so, it has remained, until 2011 when Immunotherapy became viable



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### Other Dates

- **Anaesthesia** was invented in 1846
- **Antisepsis** first used in 1865
- **Hereditability** - Mendel published in 1865
- **DNA** discovered by Miescher in 1860

Back to present Day!

- Melanoma is the 10<sup>th</sup> most common cause of cancer death
- #19 in top 20 causes of death in Australians between 25-44 (young people!)
- 1400-ish people die of melanoma of the skin in Australia every year
- Or 6.4 people per 100 000



What Shelley described as Melanocytic Minions (lots of sub-groups of Melanocytic lesions) are laid out in the following tables.

As you can see there are heaps of groupings subsets.

### 3. Melanocytic neoplasms

---

Introduction

Genomic landscape of melanoma

#### Melanocytic neoplasms in intermittently sun-exposed skin

Naevi

Junctional, compound, and dermal naevi

Simple lentigo and lentiginous melanocytic naevus

Dysplastic naevus

Naevus spilus

Special-site naevi (of the breast, axilla, scalp, and ear)

Halo naevus

Meyerson naevus

Recurrent naevus

Combined naevus

Melanocytomas

WNT-activated deep penetrating/plexiform melanocytoma (naevus)

Pigmented epithelioid melanocytoma

BAP1-inactivated melanocytoma

MITF pathway-activated melanocytic tumours

Melanoma intermittently sun-exposed skin

Low-CSD melanoma (including superficial spreading melanoma)

#### Melanocytic neoplasms in chronically sun-exposed skin

Melanoma in chronically sun-exposed skin

Lentigo maligna melanoma

Desmoplastic melanoma

#### Spitz tumours

Spitz naevi

Pigmented spindle cell naevus (Reed naevus)

Spitz naevus

Spitz melanocytoma

Spitz melanocytoma (Atypical Spitz tumour)

Spitz melanoma

Spitz melanoma

#### Melanocytic tumours in acral skin

Acral naevi

Acral naevus

Acral melanomas

Acral melanoma

#### Genital and mucosal melanocytic tumours

Mucosal and genital naevi

Melanosis

Genital naevus

Mucosal melanomas

Mucosal melanomas

#### Blue naevus and related tumours

Blue naevi and melanocytoses

Naevus of Ito and naevus of Ota

Congenital dermal melanocytosis

Blue naevus

Melanomas arising from blue naevi

Melanoma arising in blue naevus

#### Congenital melanocytic tumours

Congenital naevi

Congenital melanocytic naevus

Proliferative nodules in congenital melanocytic naevus

Melanomas arising in congenital naevi

Melanoma arising in giant congenital naevus

### Clinical Assessment

This portion of Shelley's talk related to the presentations of Melanoma and "pre-Melanoma" – Melanocytic lesions. See the below images of some textbook presentations of Nevi.

These images demonstrate just how varied they present on our skin. And also, just how important it is to have an expert check your skin for these types of lesions.



Dr Verma stressed the importance of having checks, and having your skin checked regularly.

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## DERMOSCOPY

The local doctor / GP skin clinic and specialised dermatologist will enlist these devices to visualise, measure and record their patients' lesions. All are very important tools that everyone's GP or health provider can utilise to help detect melanocytic lesions early.

- Dermoscopy uses a hand-held magnifier with a light source, ideally polarised, to allow the practitioner to see into the skin
- Fancy ones have cameras and mapping software
- Basic ones come as an iPhone attachment



### What you typically expect to see from a Melanocytic Lesion?

- A – Asymmetry of shape and colour
- B – Border: irregular, ill-defined, smudgy
- C – Colour variation or change
- D – Different – the ugly duckling sign
- E – Evolving or Elevated
- F – Firm to touch



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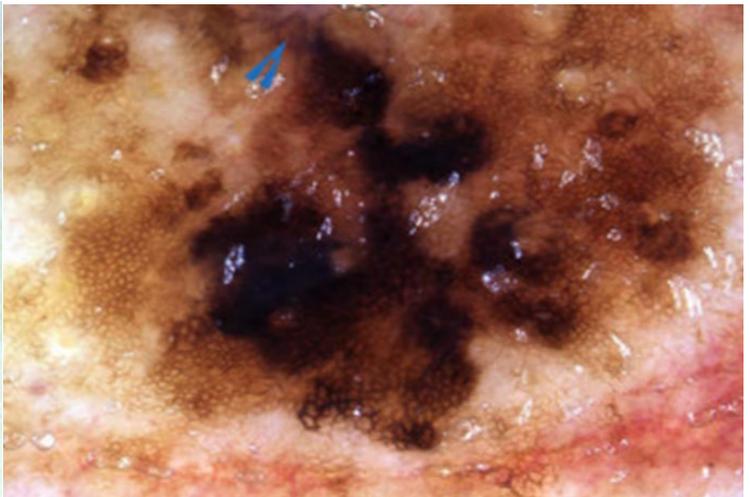
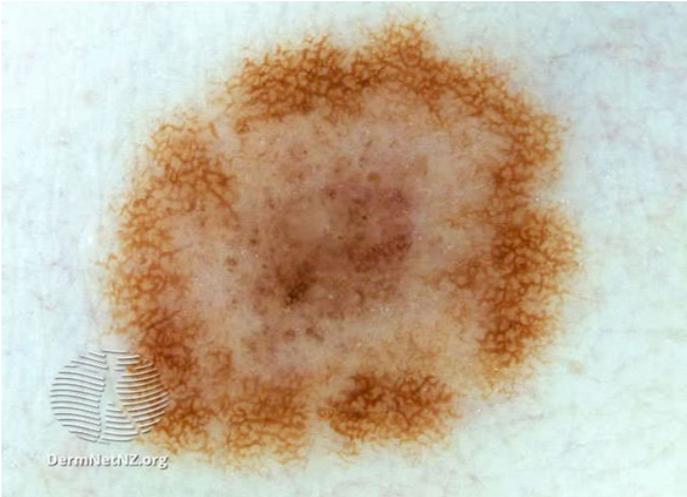
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See below examples





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Here we have some more detail on what our physician/s look for with assessing the lesions they are presented with.

### The Nature of Pigmented Lesions

- Dysplastic naevus
  - Usually has a junctional and a dermal component
  - Should have architectural and cytological atypia
  - But not as much as melanoma

### Architectural features

- Nests part way up rete ridges
- Bridging of adjacent rete
- Upwards scatter of melanocytes
- Increased density of non-nested melanocytes

### Cytological features

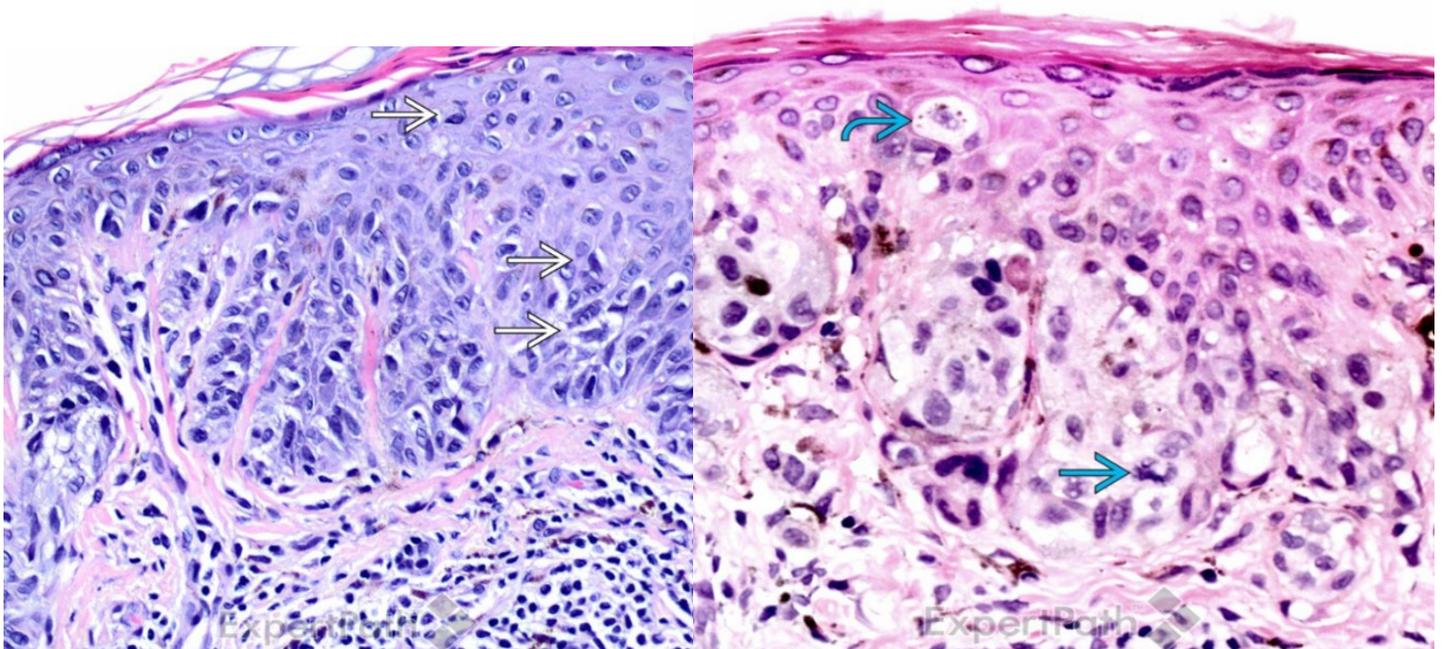
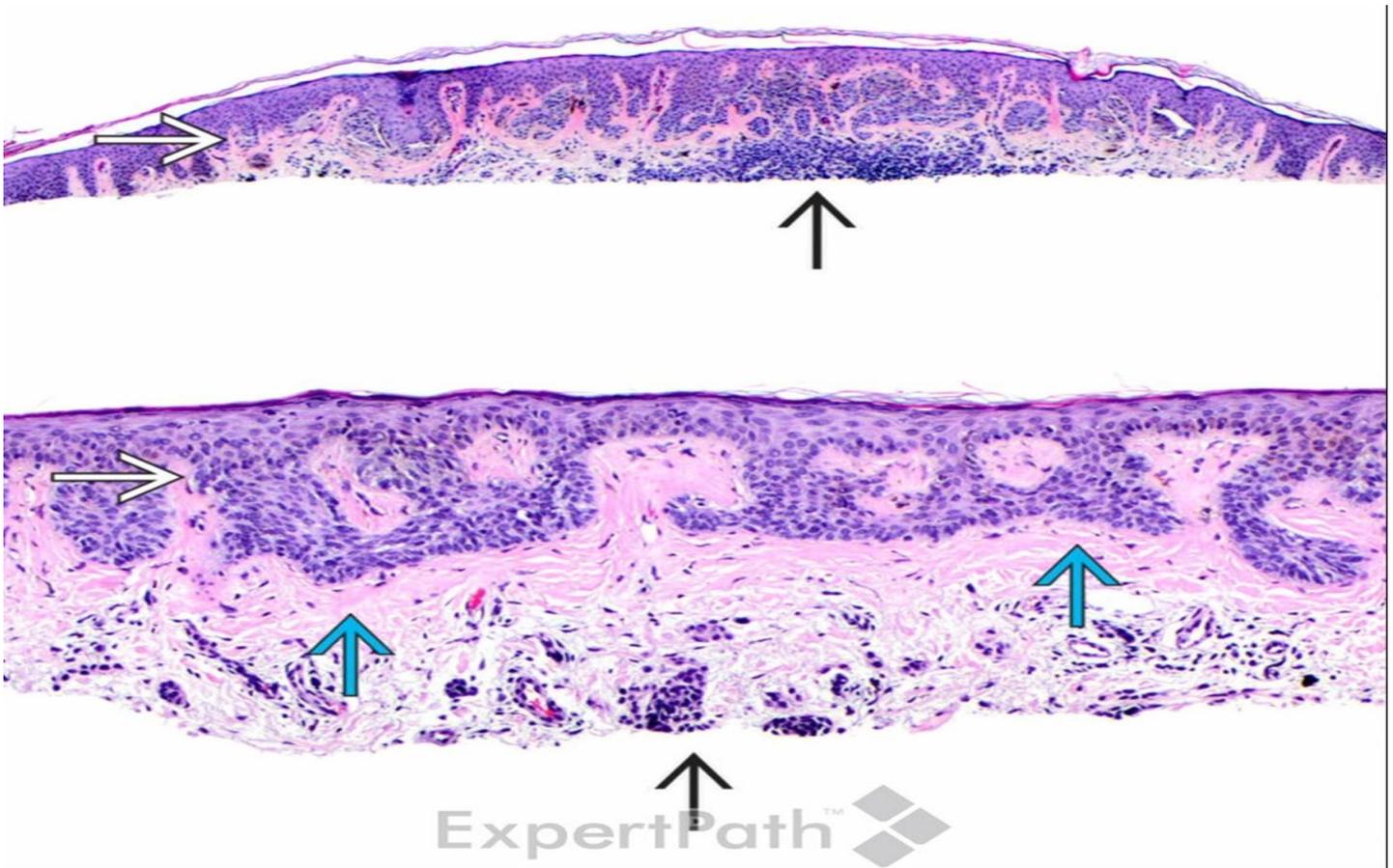
- Nuclear size larger than keratinocyte
- Hyperchromatism, coarse chromatin
- Nuclear pleomorphism
- Nucleoli prominent/lavender



### Other Clues for Melanoma

- Lack of maturation
- A cytologically distinct population within a naevus
- Intra-epidermal or intradermal mitotic figures
- Especially at the leading/deep edge
- Ulceration
- Destruction of adnexal structures
- Large size
- Histologically asymmetrical
- Infiltrating lymphocytes

Below images of architectural and cytological features



Then Dr Verma discussed the use of the important and highly useful tool “Immunohistochemistry” and how we utilise panels of Antibodies to indicate positive and neg staining and what these can indicate.

## Immunohistochemistry

SOX10 – to highlight Pagetoid spread

HMB45 – tends to be lost with maturation (so will fade with depth in benign naevi)

The malignant cocktail

- P16 (looking for loss)
- Ki67 (expect <5% within dermal nests)
- PRAME

### The New Kid on the Block – PRAME

PRAME was initially identified as a target antigen on tumor specific lymphocytes in a patient with metastatic melanoma

IHC was initially developed to select potential cases for immunotherapy, NOT as a marker of malignancy

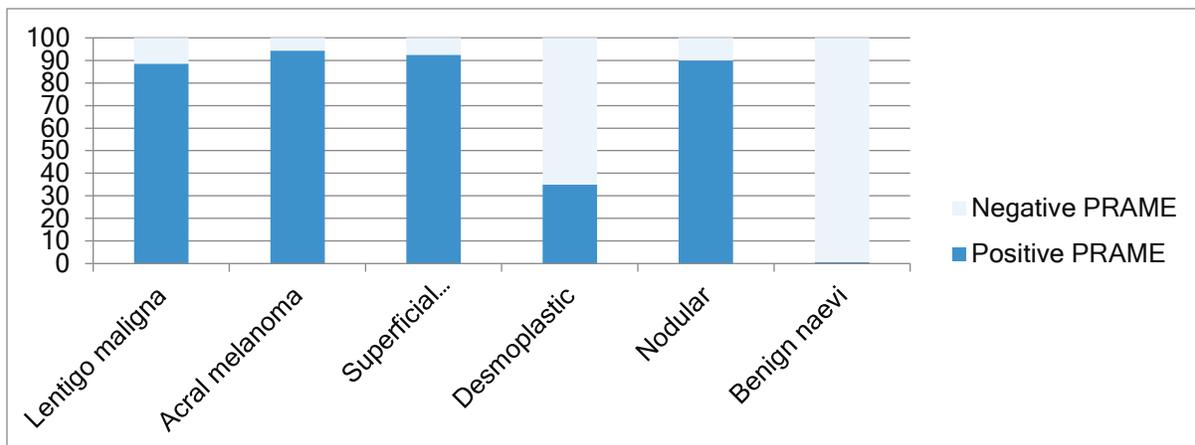
The first PRAME paper aimed to validate the IHC, use in determining malignant potential was a happy coincidence

ORIGINAL ARTICLE

#### PRAME Expression in Melanocytic Tumors

*Cecilia Lezcano, MD,\* Achim A. Jungbluth, MD,\* Kishwer S. Nehal, MD,†  
Travis J. Hollmann, MD, PhD,\* and Klaus J. Busam, MD\**

Graph of PRAME specificity for the different tumours



BUT!! (why is there always a but)

Not as good in histologically ambiguous lesions

Only weak to moderate agreement with well-validated molecular tests in this setting

#### PRAME

Interpretation: any nuclear positivity in >75% of lesional cells (original described cut off point)

Usage:

In small melanomas arising within naevi (to show a distinct population)

In lesions too superficial for molecular analysis

In distinguishing nodal mets from nodal naevi (near 100% sensitive and specific in this circumstance)

Margin assessment in known PRAME positive melanomas

CAUTION – isolated PRAME positive cells are often seen in severe sun damaged skin

Then Dr Verma moved into the Molecular Genetics techniques we can use to help identify the precursor subtypes, see below for some tables and descriptions of molecular techniques.

#### MOLECULAR TECHNIQUES

For determining melanocytic lesion subtype

For assessing malignant potential

CGH

FISH

Next Dr Verma described the differences of her Melanocytic minions with molecular alterations.

#### Molecular hallmark

Lesion type	Molecular hallmark	Technique
Spitz naevus	ALK fusion ROS fusion NTRK fusion	IHC and FISH
Deep penetrating naevus	CTNNB1 mutation (B catenin)	IHC
BAP1 inactivated melanocytic tumours	BAP1 mutation	IHC
Blue naevus spectrum	GNAQ, GNA11 activating mutations	NGS IHC (NEGATIVE for BRAFv600e)

# A

## ssessing malignant potential: A Caveat

Histology remains the gold standard for differentiating benign from malignant  
 Most studies are done in definite benign v malignant tumours  
 We need these tests for lesions with borderline histology  
 None of the tests have been around long enough for long term follow up of borderline lesions.

## CGH

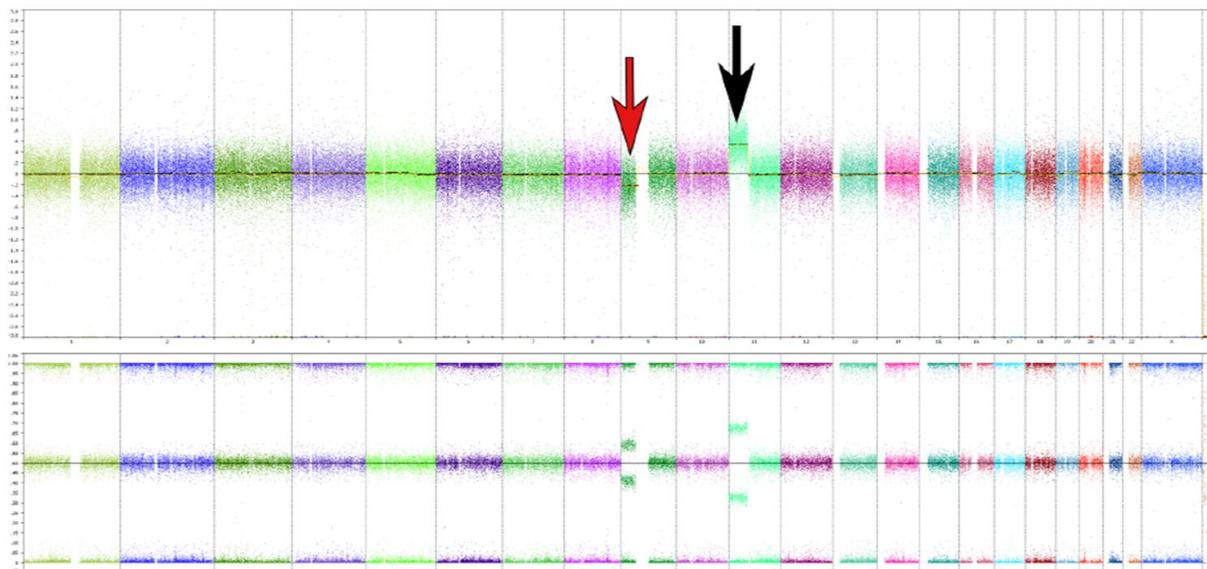
### Comparative Genomic Hybridisation

#### The principle

New kits analyse thousands of loci across the genome, output is mapped by chromosome  
 Deflection away from the baseline indicates loss or gain of allele  
 Gives both an indication of chromosomal instability but can also provide:  
 Information about specific cytogenetic abnormalities (eg: 11p gain in desmoplastic spitz naevus, ALK or ROS translocations in other spitz naevi)  
 Presence of specific mutations (homozygous deletion of CDKN2A is high risk for malignant behaviour even in the absence of other mutations)

#### Question for us;

How many copy number changes make malignancy? Sensitivity and specificity changes depending on cut offs



**Figure 1.** Typical SNP array output. The upper panel shows copy number status (log ratio on the vertical and chromosome locus on the horizontal). Gains and losses are reflected by deflections of the average yellow line above or below 0, respectively. Red arrow indicates a one copy number loss of chromosome 9p and black arrow a gain of chromosome 11p. The lower panel shows the B-allele frequency for each SNP on the array (B-allele frequency on the vertical and chromosome locus on the horizontal). In the normal state this track consists of three lines, a middle heterozygous line and two outer lines which are homozygous for the A and B alleles. Red and black arrows indicate LOH events on chromosomes 9p and 11p.

## CGH V. MULTIPLEX FISH

Multiplex FISH targets a much more limited number of loci (most studies use 4, but up to 6). See number of signals per nucleus and ratio of signals.

Benefits of FISH	Downsides of FISH
Requires fewer cells	Less sensitive and specific
Does not have a minimum tumour purity	Lower genomic coverage
Cheaper/more readily available	Cannot give specific mutations
Faster	Requires expertise to interpret

*Molecular testing for melanocytic tumors* 161

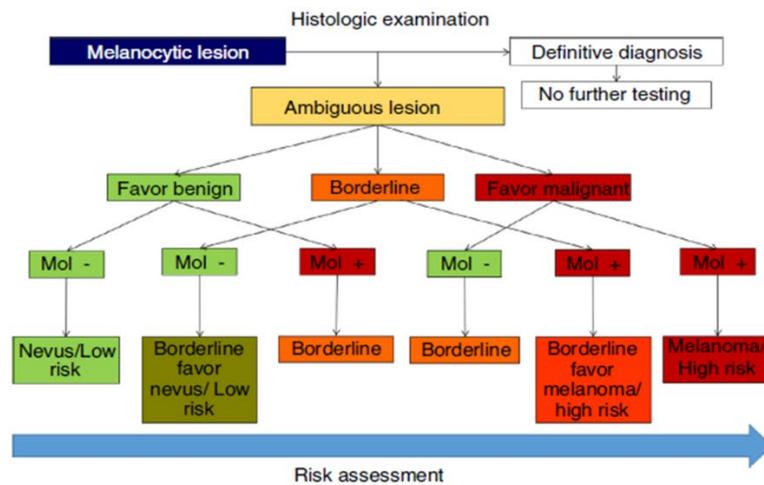


Figure 6. Algorithm for integrating histology with molecular data in the diagnosis of melanocytic tumors.

Then to wrap up Dr Verma's talk she led us to further discussions on Sentinel Lymph nodes.

And explained the importance of the sourcing and investigations of sentinel nodes in the patients journey from cancer sufferer to survivor.

## Sentinel Lymph Nodes



### Purpose of the Sentinel Node Biopsy

Mostly prognostication

A large randomised controlled trial showed no survival benefit or distant disease recurrence rate between groups with a positive sentinel node followed by regional nodal dissection and those who did not have a sentinel node biopsy performed

Strongest predictor of melanoma specific survival

Indicated if:

Primary melanoma >1mm thickness

Clinically node negative

### What guidance do we have?

There is no consensus on how to cut sentinel nodes, how many H&E sections to make, at what interval or what IHC should be used

Australian guidelines suggest:

Bivalve along longitudinal axis or breadloaf

“Pathologists should examine multiple haematoxylin-eosin and immunohistochemically-stained sections from each SLN. Sections from each slice of all SLNs should be stained with both H&E and immunohistochemistry for melanoma associated antigens. HMB-45, S100, SOX10, Melan A and tyrosinase have all been utilised as immunohistochemical stains.”

Here's some guidance from the European Organisation for Research and Treatment of Cancer (EORTC) Comparison Trail

In the trial:

Bivalve the node longitudinally

10 sections of each half

S100, melanA, HMB45

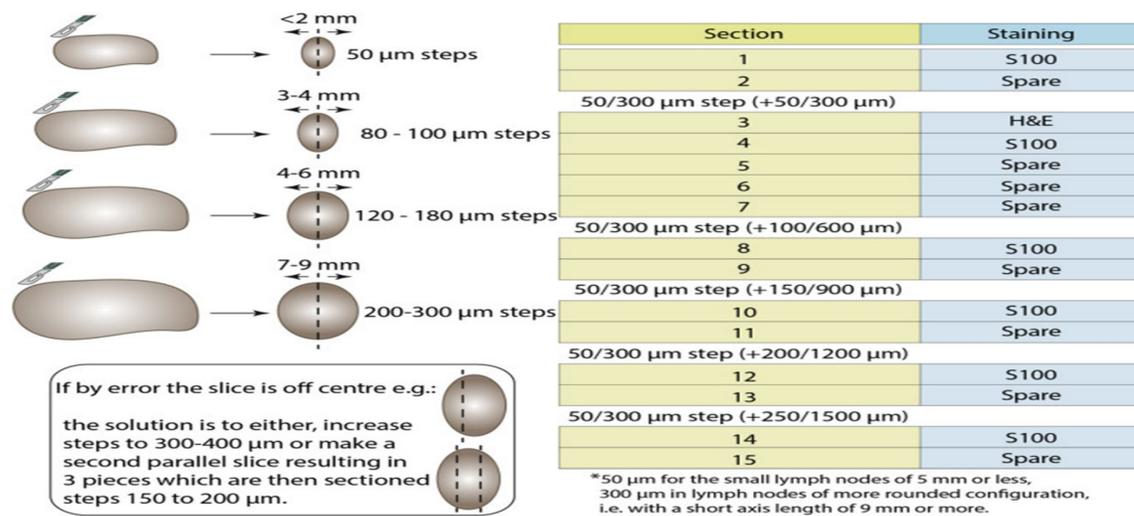
In 2008:

Bivalve longitudinally

4 sections

Stain 1 and 4 with H&E

Stain 2 and 3 with S100 and HMB-45



## Take Home Message

Melanocytic lesions are tricky for everyone  
Evidence is evolving!

**If in doubt, cut it out!**

Thanks to Dr Shelley Verma for supplying her presentation for the QLD Histotechnology Group to make into this article. Thanks to DermNet, ExpertPath and ihearthisto for the images used in this talk.

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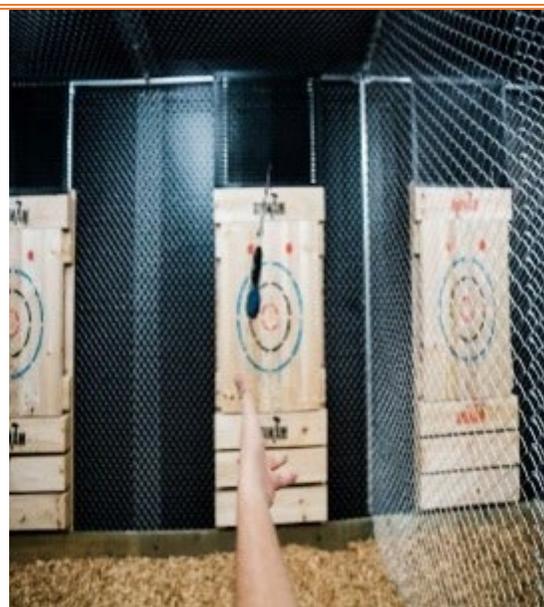
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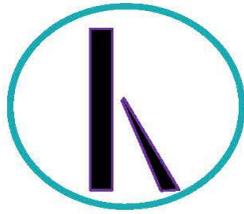
<https://www.maniax.com.au/>



Please transfer payment to: [Histotechnology Group of Queensland Inc](#), BSB 084 009 A/c 198048439

Email: [secretary@hgq.org.au](mailto:secretary@hgq.org.au) with your team name, members and payment details.

**Saturday 2<sup>nd</sup> September**



# HISTOTECHNOLOGY SOCIETY OF NSW



We are getting together in-person again! Join us for

## Histo at the Beach

**Saturday 29<sup>th</sup> July 2023**

**First presenter announced: 'Forensics on the foreshore'**  
Dr Allan Cala, Forensic Pathologist, NSW Health Pathology

**Get out and about! Face to Face 10am till 3.30pm**

**Don't forget the food! 2 course lunch & drinks**



**Register now!**

<https://histonsw.org.au/event/histo-at-the-beach/>

Click [here](#), use the QR code or visit our website to register!\*

Registrations close 17<sup>th</sup> of July 2023

# Forensic Pathology

## Save the Date

A COMBINED SCIENTIFIC MEETING BROUGHT TO YOU BY THE  
HISTOTECHNOLOGY GROUP of QUEENSLAND  
&  
AIMS QUEENSLAND STATE BRANCH

**Thursday 24th August 2023**

The Plantation Room @  
The Pineapple Hotel  
706 Main St, Kangaroo Point QLD 4169

**6.30pm – 8.30pm**

**RSVP by 22/8/2023 @ 3pm AEST**

**FREE** for AIMS and HGQ members/student members



# Contributions welcomed!

Journal, scientific article and antibody reviews all accepted!

Know someone who should be featured?

Something exciting happening in your lab?

Want to do a birthday shout out?

Have a photo you want to share?

Let us know!

**We are always looking for contributions of scientific articles and news, or if you have improvements and techniques that make a difference in your lab!**

**Submissions can be sent to [HGQ Tissue Paper](#) in digital format**

