HGVT

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PARAFFINALIA NEWSLETTER

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June 2021

The HGVT aims to provide a dynamic continuing education program in which all persons with an interest in Histology and Histotechnology are freely invited to participate.

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Committee Page

The members of the Histology Group of Victoria and Tasmania 2020 are:

Name	Institution
Kerrie Scott-Dowell	Dorevitch Pathology/Leica
Adrian Warmington	Dorevitch Pathology (Ballarat)
Mark Bromley	Sullivan Nicolaides Pathology
Elizabeth Baranyai	Cabrini Health
Alison Boyd	Northern Hospital
Kellie Vukovic	Melbourne Pathology
Yvette Beaber	Monash Health
Samantha Arandelovic	Mater Hospital Brisbane
Snejana Ursache	Alfred Hospital
Imogen Campbell	Alfred Hospital
Sukwinder Sohal(Romi)	University of Tasmania
Meghan Leo	Histolab
Bindi Bates	Peter Mac
Ola Aladassi	VCS
Yashi Xie	St. Vincent's Hospital
Cristina George	Holmesglen TAFE
Alex Johnston	Walter & Eliza Hall Institute

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President's Report – Behind the Bench

Welcome to another Paraffinalia lockdown edition,

Fortunately, I kept masks in every handbag and jacket pocket, so it was an easy transition back into restrictions. It was a little harder to remember how to NOT fog up my glasses during microtomy, but hopefully Victorians will not need to endure anywhere near the lockdown timeframe of 2020. It does of course mean that all meetings will remain in a virtual format for the foreseeable future and any group social events look unlikely.
Another casualty of COVID has been the National Histology Conference that was due to be held this year in NSW, and was postponed until next year, but has now be cancelled. It was no longer financially viable with the uncertainty around travel and restrictions. It certainly makes me appreciate my committee members, for their continuing support to deliver educational opportunities, as well as keeping us connected. Thank you team.
There are currently many employment opportunities within our industry, so don't be afraid of change, to grow, learn and make new connections. Today is all about taking on challenges, being flexible and appreciating the small things in life, while still dreaming of a holiday somewhere.

Take care out there,

Kerrie Scott
(Leica/ Dorevitch Pathology)
HGVT President

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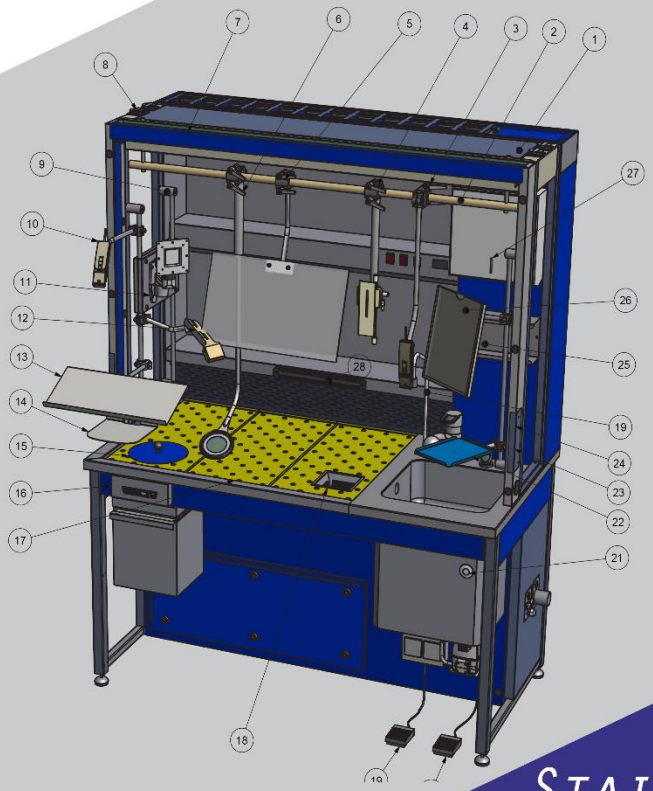

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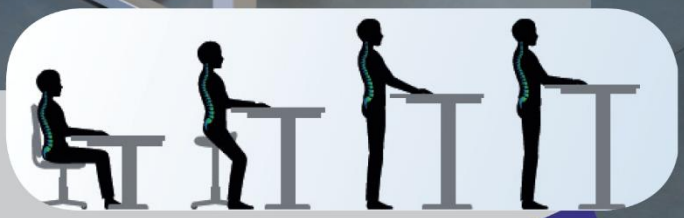

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“Virtual Meeting”
“Complex Cut Up”

Speakers: **Edward Kwan – “Kidney” (Monash Health)**

Andrea Whiteside – “ Colon” (Cabrini Health)

Date: **Thursday 24th June 2021**

Time: **18:45 – 19:00 – Joining the meeting**

19:00 – 20:30 - Presentation

Link: **Zoom Meeting**

Join Zoom Meeting
https://zoom.us/j/94725902485?pwd=TWdnbXFlRVNCb3hTNzNjaHVsbzVTdz09

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Attendance at this meeting contributes to APACE points

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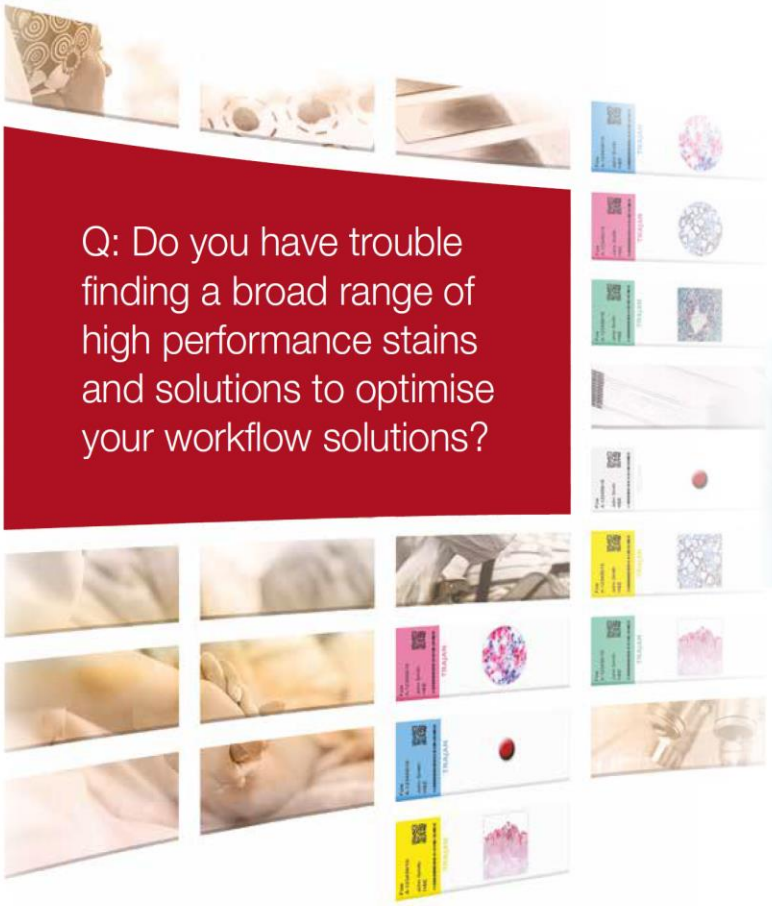

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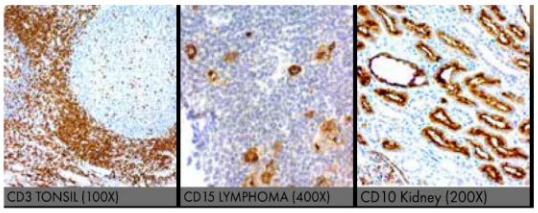

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AIMS is hosting it's National Scientific Meeting for 2021 with many accomplished speakers from all pathology backgrounds to be in attendance. Registration is still open for interested parties with the program available for viewing via this link https://aomevents.eventsair.com/aims-national-scientific-meeting-2021/

The event will not only to histology but other scientific disciplines so don't be shy and mention it to your friends in other areas of pathology!

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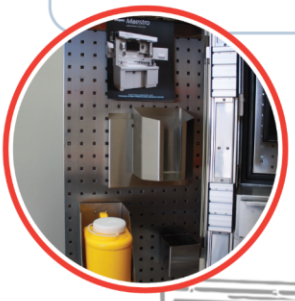

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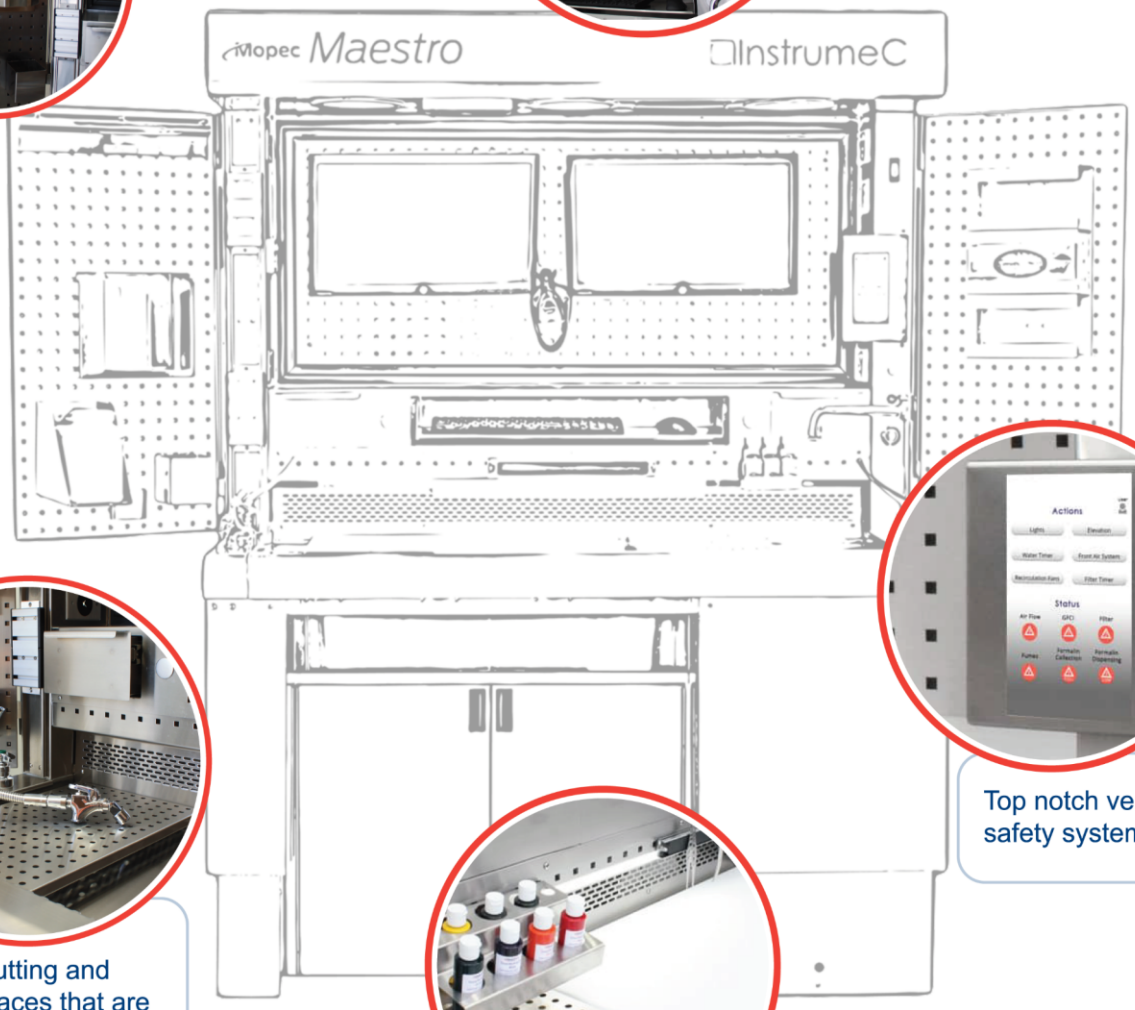

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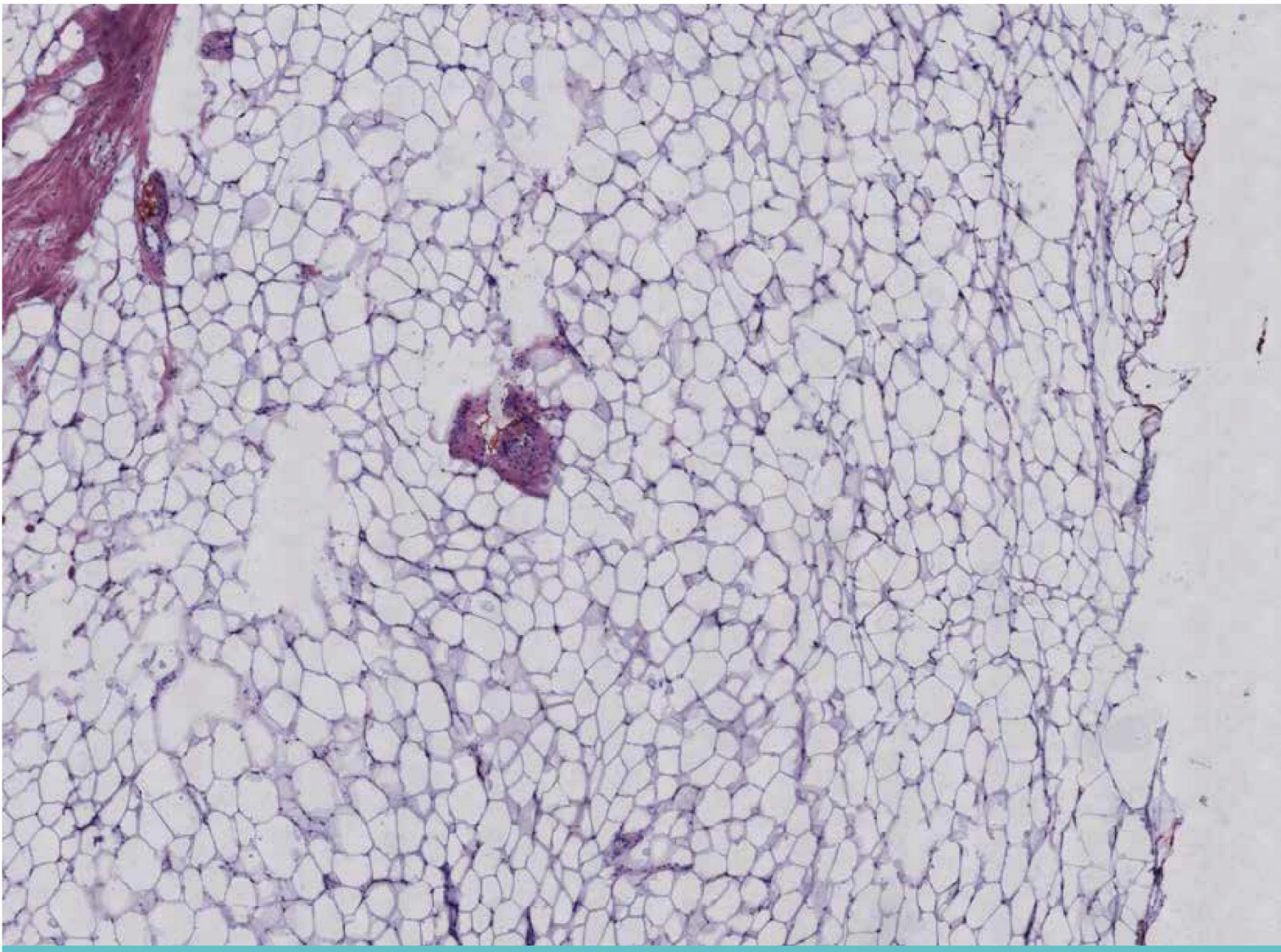

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UNDER THE MICROSCOPE WITH CRISTINA GEORGE

What was your first part-time job?
The education system in Romania does not encourage part time jobs during school, but I was making pocket money from doing things around the house, gardening, and other maintenance jobs at my mum's company during summer school holidays. Flirted during university years with being a cosmetics representative or insurance agent, but I was losing more money buying products than making. My first real job was after I finished my PhD, as an administrative assistant at a veterinary distribution company.

How long have you worked in histology?
I have been in histology since 2008 when I started my PhD, had no clue what's about or how my next four years were going to be like.
When I came to Australia, I continued applying the skills in histology and IHC, working at Monash University on animal (rats and sheep) preclinical trials for Bionic Vision, while teaching histology and laboratory techniques at Holmesglen TAFE, where I am today a full-time teacher and assessor for biological units.

When people ask, "So, what do you do?" How do you explain Histology?
I tell them that I cut very thin sections of organs, stain them and you can view their cellular structure under the microscope.

What is a skill you would like to learn and why?
I would really like to learn more about cut up and cytology. Due to the research background, I would also like to be as confident with a confocal or electron microscope as I am with a light optical microscope.
If I were to change career, I am inclined to go towards a craft, like silversmithing or jewelry making but for now I stick to my candle making hobby, that I discovered during the COVID year.

If money were no object, what would you do all day?
I would probably travel and pay for my family or friends to travel with me…and I would retire early.

What is an ideal weekend for you?
Spending time with my husband, going for a day trip somewhere and taking pictures…if we can take our cat with us, even better.

If you could take only THREE items with you to a deserted island, what would they be?
Fishing gear (I love it and I used to fish as a kid), a machete/axe and a pot.

What's on your bucket list this year?
Finishing the undergraduate degree that I am currently enrolled in.

Where do you most want to travel, but have never been?
Too many places… would love to see Japan in spring, Canada in summer…Indonesia, Tanzania, Antarctica, Norway … but also so many places that I would love to go back.

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Bednar Tumour: An Uncommon Entity

By Bindi Bates

Have you ever heard of a Bednar Tumour? If your answer is no then you're just like me! A Bednar tumour is a type of pigmented dermatofibrosarcoma protuberans (DSFP) that only occurs in 1-5% of all DFSPs. It is so rare that the majority of the Pathologists at PMCC have only seen them in literature or in their very early registra years in learning sets!

The specimen that was received at PMCC was a skin lesion of the natal cleft. The lesion was described with slight lobulation and swelling. Upon slicing the lesion was described as ill defined, haemorrhagic and lobulated invading into the subcutaneous tissue. DFSPs are known for occurring on the trunk, the upper and lower extremities of the body and the head and neck.

Microscopically, Bednar Tumour's are described as having pigment-laden melanocytic cells- and that's exactly what this lesion had (figure 1). The melanocytic cells are what distinguish it from being any other type of DFSP. In addition to the pigmented melanocytic cells, the Bednar Tumour is chararcterised by its spindled cells arranged in a tight storifirm pattern- it looks like the cells are doing a cartwheel (figure 2). These spindle cells possess slender nuclei with lightly vesicular nuclear chromatin and scant amounts of cytoplasm. The tumour is very infiltrative extending along the fibrous septa (figure 3) entraping adipocytes giving a honeycomb appearance (figure 4).

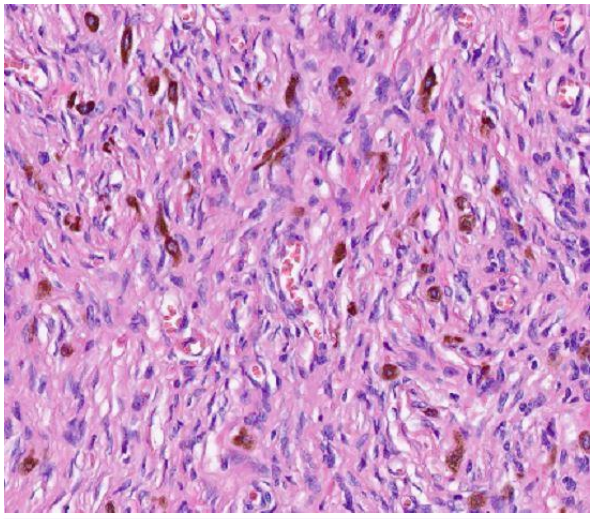

Figure 1: H&E. Focally pigmented melanocytic cells

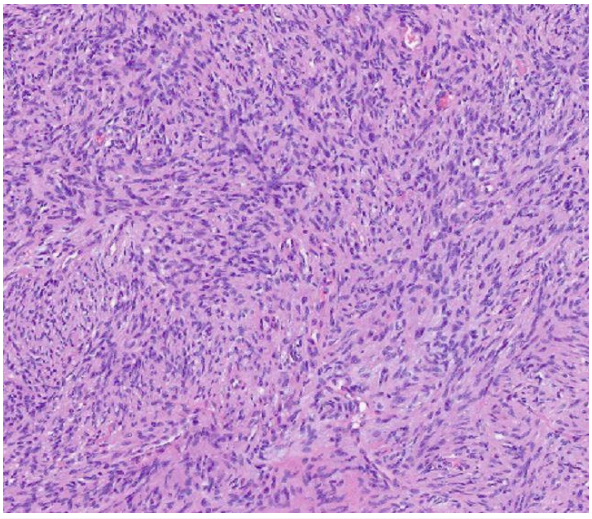

Figure 2: H&E. Storifom pattern

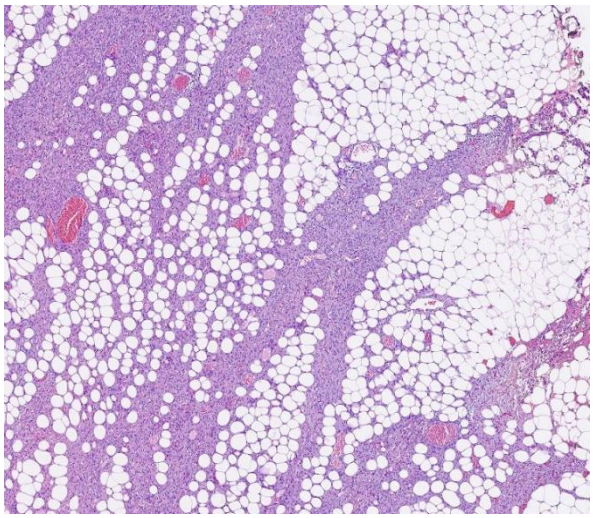

Figure 3: H&E of infiltrating tumour

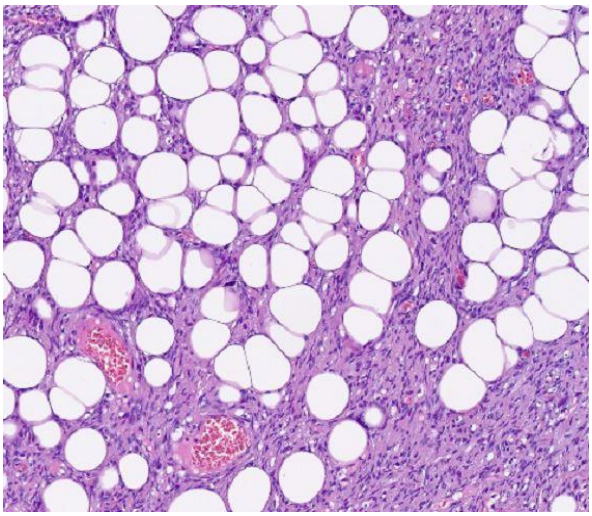

Figure 4: H&E. Honeycomb appearance of adipocytes.

On reporting, PMCC Pathologists ordered IHC using the Ventana OptiView DAB. Due to the pigmented melan ocytes, all IHC came back positive. Positive for CD34- a common marker used for all DSFPs (Figure 5), positive for Cytokertain marker AE1/3, melanoma marker SOX10 and S100, p40, desmin and SMA.
To aid the pathologists, the IHC markers (exc ept CD34) were performed again but using the UltraView Red Alkaline Phosphatase detetction kit. IHC returned as negative for p40, S100, SOX10, Desmin and AE1/3. Melanocytes were positive for melanoma markers S100 and SOX10 (Figure 6).

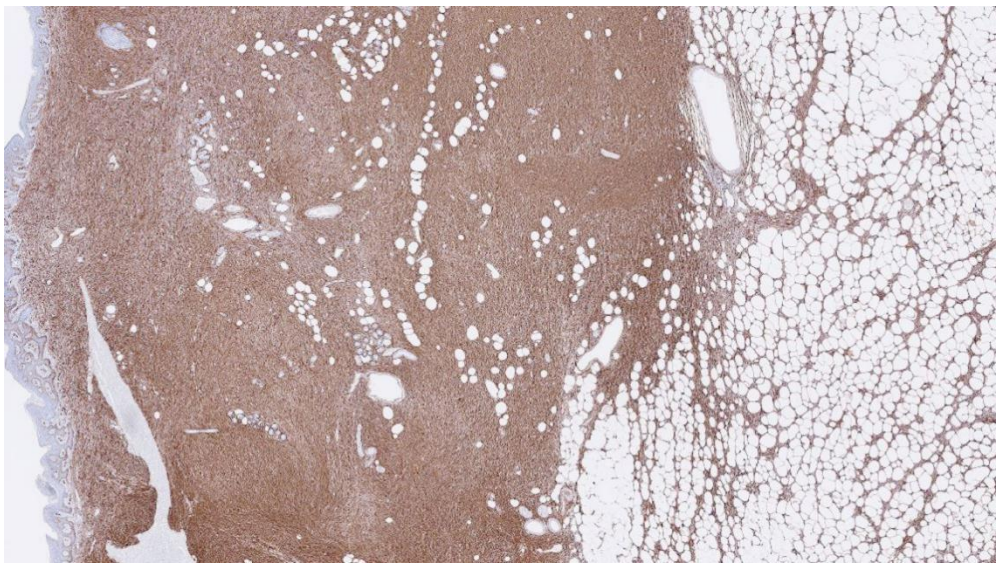

Figure 5: IHC CD34 positive for Bednar Tumour DSFP.

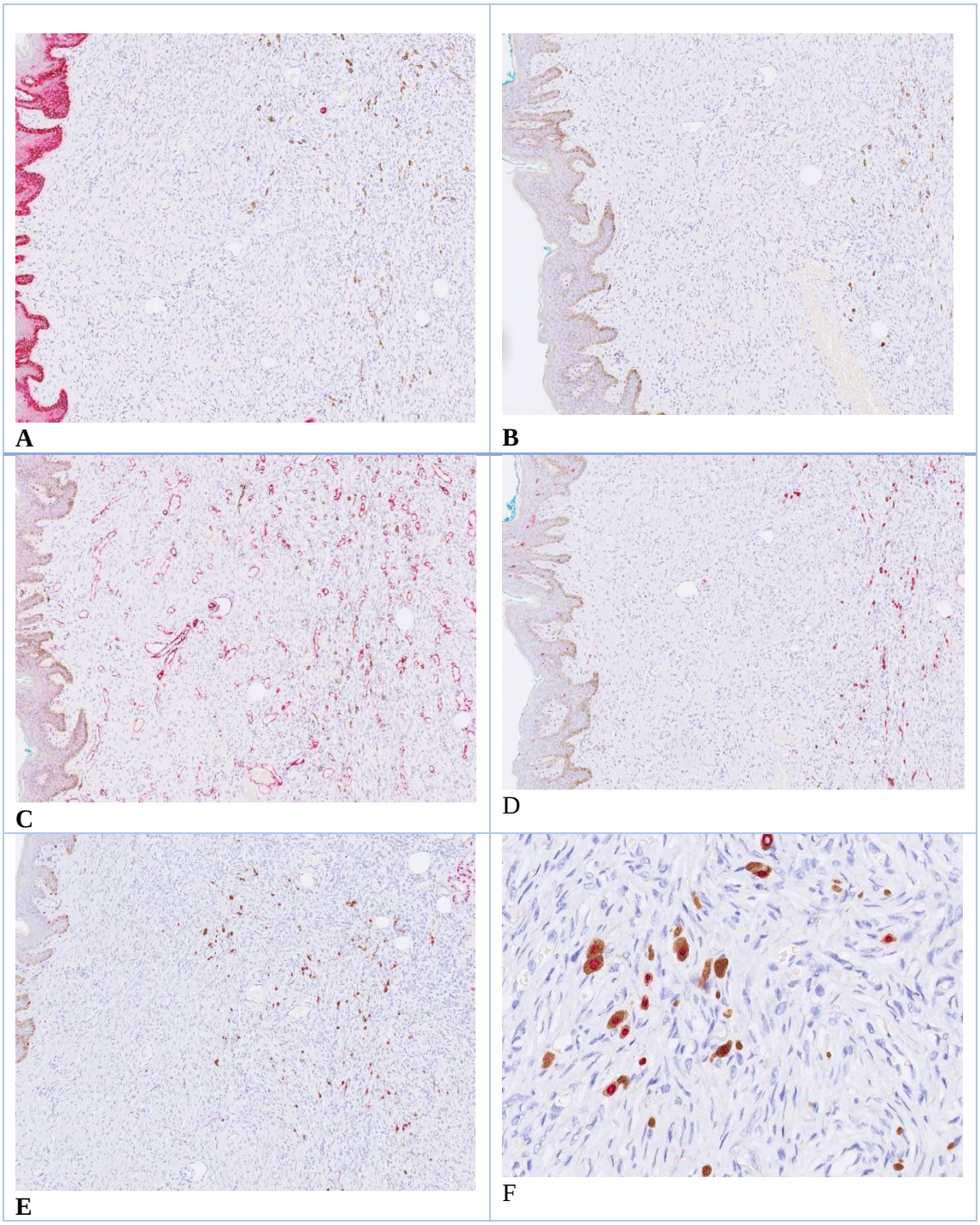

Figure 6: A AE1/3 negative. B Desmin negative. C SMA negative. D S100 negative. E SOX10 negative. F. SOX10 melanocytes positive but tumour negative.

The Bednar Tumour has a very low rate of metastases, but if not fully removed, due to its nature of being infiltrative, it can and will metastases. Unfortunately, the specimen that was received, the tumour went through a margin so the patient will need to get a deeper skin excision

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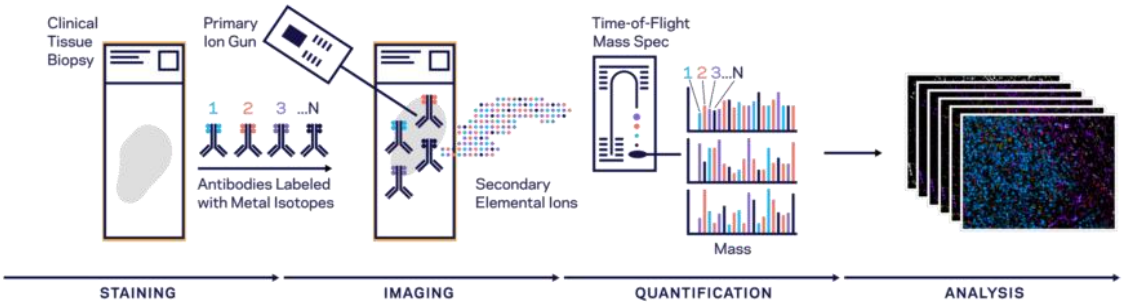
MIBI: A Metallic Multiplexing Marvel

By Alex Johnston

In any kind of science, it is the innovation and exploration of new horizons that sparks the intellectual curiosity in all of us.
For histology, many staples like microtomy, embedding and processing have been optimised to achieve both high efficiency and quality and as such, have remained relatively unchanged for some time. For downstream staining processes and testing however, the frontier of new and exciting applications to explore disease and pathology has continuously evolved.
One of these relatively new developments is multiplexed ion beam imaging (MIBI).

Conventional immunohistochemistry (IHC) utilises antibodies raised against specific antigen targets that are then tagged with enzymes or fluorescent probes to achieve a visualised coloured outcome. Currently this process can be performed for single antigen target or repeated several times on one tissue section with one of the main limitations being the number of available unique substates (for IHC) or fluorophores (for fluorescent staining). Sometimes targets may be weakly expressed which can make their initial detection tricky, particularly if also competing with an endogenous autofluorescence signal. Moreover, panel combinations developed during multiplexing can involve antigen targets that may co-localise within the same cell type which can potentially lead to issues disseminating true signal during analysis due to physical and (in the case off fluorescent staining) spectral overlapping.

MIBI attempts to address these limitations by employing the use of mass spectrometry of metallic elements (in a process called secondary mass ion spectrometry) as means a of demonstrating antigens in a sample. Instead of being conjugated to a substrate or fluorophore, antibodies are instead conjugated with an element from a group of metals known as lanthanides. [1]
Once prepared, all antibodies for a panel can applied to a sample simultaneously in a similar fashion to conventional direct IHC methods. The real difference comes in the imaging stage wherein an ion beam is aimed at the section, liberating the metallic ions from the antibodies bound to their antigen target which can then have their masses detected specifically by a mass spectrometer. As the ions pass through the detector, a corresponding image can be developed pixel by pixel according to the expelled ion profile for that specific microscopic point. [1, 2]



MIBI Workflow [2]

A considerable limitation with MIBI currently is the technique and preparation for a prospective panel can be an expensive exercise in regards to both time and money taken to achieve an outcome which serves as a limitation for potential wider adoption. It is possible that in the future with optimisation to reduce costs, techniques like this and more may be implemented systemically to have the same impact immunohistochemistry has had in our understanding and ability to overcome disease thus far. Irrespective of the outcome, MIBI is an example of both the beauty and innovation within science and serves to promote histology's advance further into technology aided analysis going forward.

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2. IonPath. Workflow of MIBI [Internet]. 2020 [cited 6 June 2021]. Available from: https://www.ionpath.com/mibi-technology/

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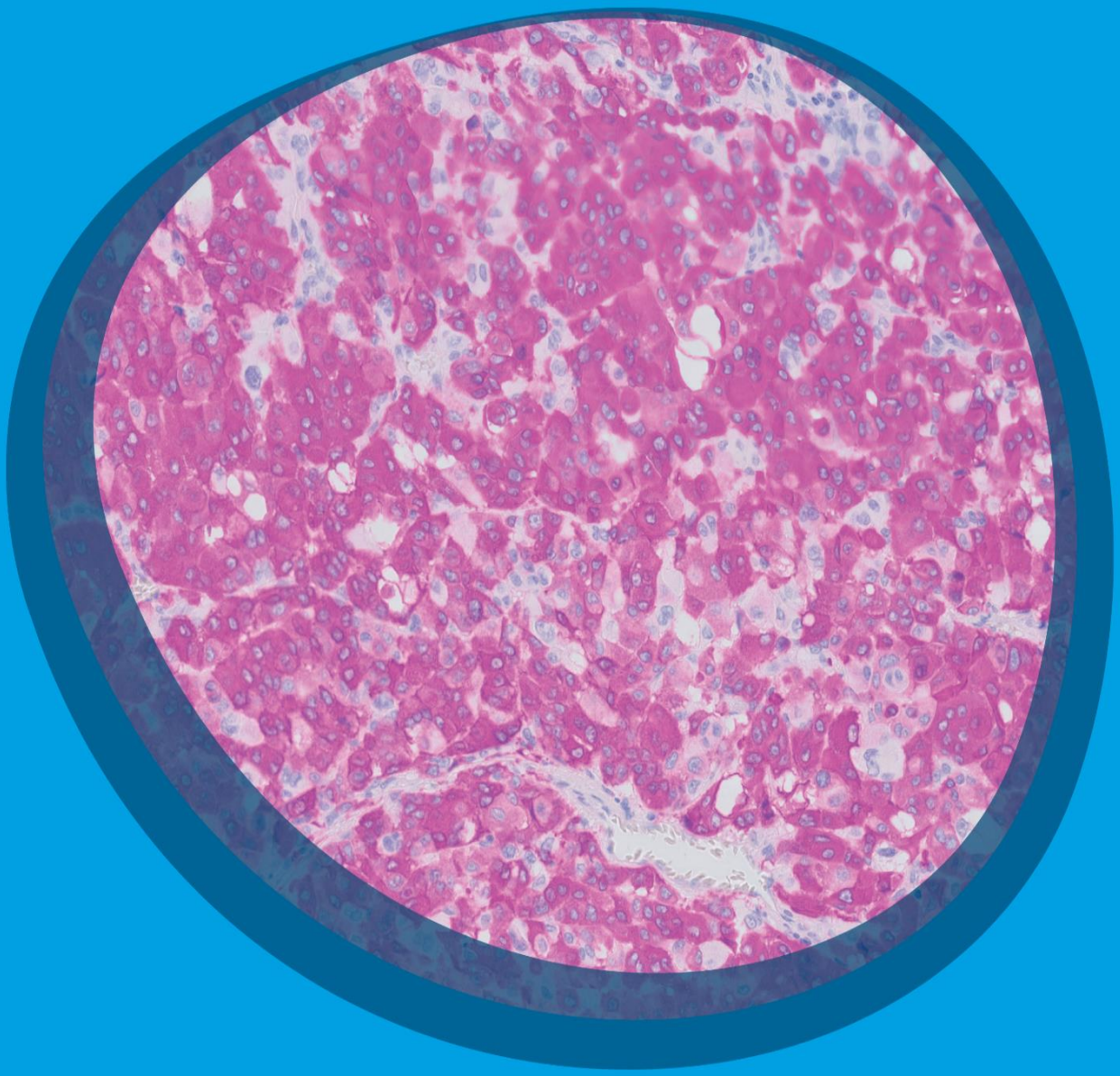

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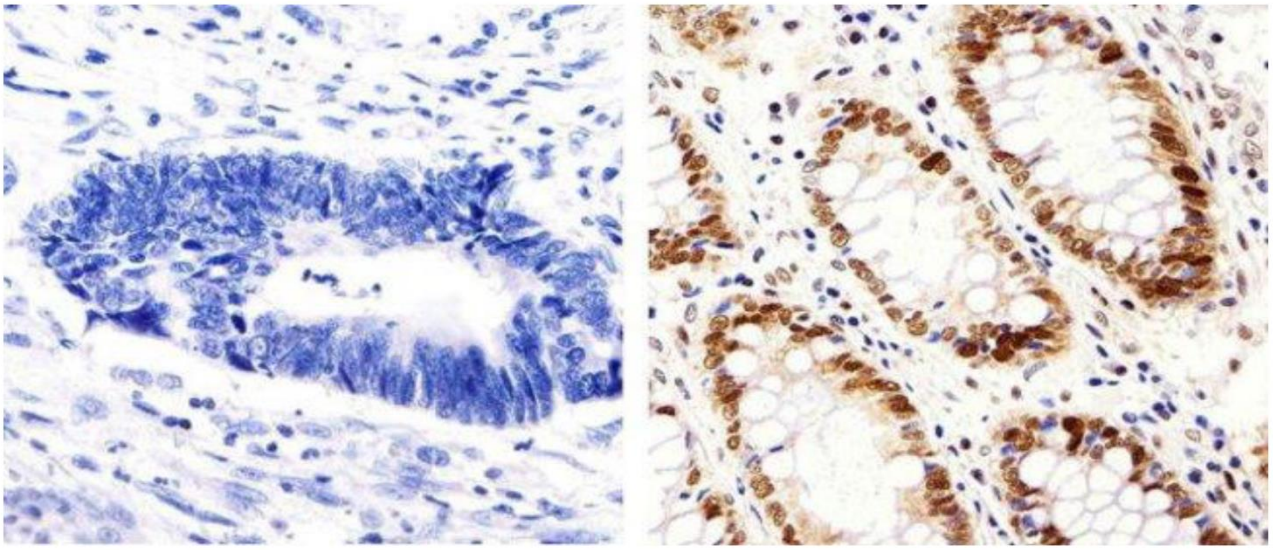
IHC ANTIBODY SPOTLIGHT: PCNA

PCNA (polymerase delta auxiliary protein) is found in the nucleus of the cell and is involved with several DNA processes associated with replication including 'cutting' and repair operations, helping to increase the processing of leading strand synthesis during DNA replication. It has potential in identifying the proliferation state of tumors being a marker for cells in early G1 phase and S phase of the cell cycle.

Being used as a tool for assessing proliferation status of tumours is useful as it can have implications for prognosis.

In response to DNA damage, PCNA is ubiquitinated and is involved in the RAD6-dependent DNA repair pathway. Two transcript variants encoding the same protein have been found for PCNA. Pseudogenes of PCNA have been described on chromosome 4 and on the X chromosome.[1]



Immunohistochemistry analysis of PCNA showing staining in the nucleus of paraffin-embedded human colon carcinoma (right) compared to a negative control without primary antibody (left).
Source:(https://www.thermofisher.com/order/genome-database/generatePdf?productName=PCNA&assayType=PRANT&productId=13-3900&detailed=true)

References

1. PCNA proliferating cell nuclear antigen - Gene - GTR - NCBI [Internet]. Ncbi.nlm.nih.gov. 2021 [cited 6 June 2021]. Available from: https://www.ncbi.nlm.nih.gov/gtr/genes/5111/

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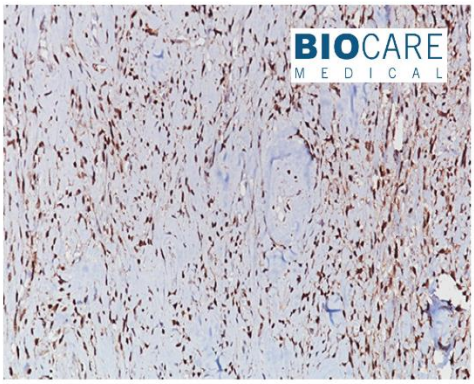
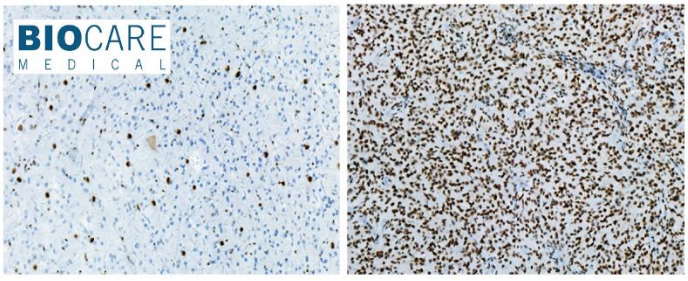
STAT6 [YE361]



STAT6 (signal transducer and activator of transcription 6) is a member of the STAT family of cytoplasmic transcription factors, which regulate gene expression by transmitting signals to the nucleus and binding to specific DNA promoter sequences. STAT6 is composed of a DNA-binding domain, a C-terminal transcriptional activation domain, and a SH2 domain. STAT signaling is critical for cellular processes such as embryonic development, immune tolerance and tumor surveillance, and regulation of cell differentiation, growth, and apoptosis.

For product information visit: https://biocare.net/product/stat6-ye361/



ATRX

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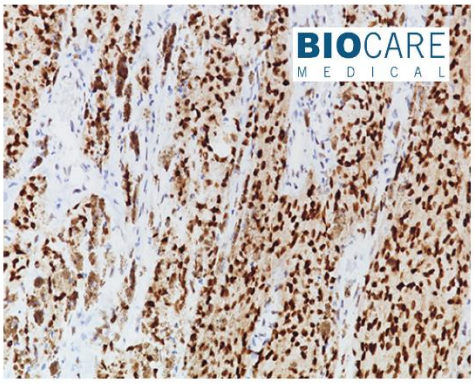
For product information visit: https://biocare.net/product/atrx/

PRAME [EPR20330]



PRAME (preferentially expressed antigen in melanoma) is located on chromosome 22q11.22 and encodes a 509 amino acid protein. PRAME is an autosomal cancer-testis antigen (CTA) gene. PRAME is expressed in melanoma, various nonmelanocytic malignant neoplasms, including nonsmall cell lung cancer, breast carcinoma, renal cell carcinoma, ovarian carcinoma, leukemia, synovial sarcoma, and myxoid liposarcoma. Normal healthy tissues are not known to express PRAME except for testis, ovary, placenta, adrenals, and endometrium.[1,2]

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ONLINE SCIENTIFIC MEETING REVIEW

22nd of April, 2021

By Kerrie-Scott Dowell

David Lu from Peter Mac spoke first on CDH1, a tumour suppressor gene. A mutation in the CDH1 gene gives increased risk of developing gastric cancer and other cancers associated with Hereditary Diffuse Gastric Ca. The genetic mutation is on the chromosome 16. CdH1 is a gene that codes for a protein Cadherin and is associated with 2 cancers HDGC and lobular ca of the breast and is an autosomal dominant mutation. It accounts for only 1% of all stomach cancers, however, it runs in families as half will inherent the mutation. It can also be a spontaneous mutation. Therefore, a lot of endoscopic biopsy monitoring is required if you have a family history, as well as lung, colorectal ca, prostate (for men) and breast (women) monitoring.
HDGC is a diffuse cancer, so not a solid tumour. It microscopically forms clusters of signet ring cells in gastric adenocarcinoma. It can be difficult to detect so a gastrectomy specimen a Peter mac will have 200-300 blocks, as it will be completely blocked out looking for the signet rings. Pas stains are useful as the mucin stains and Ck7 with a distinct staining pattern.

Kitty Feng from Monash Health discussed a case of an 11yo boy from Seaford who presented with a skin nodule for 8 weeks. The skin punch biopsy showed spongiosis in the epidermis and necrosis. Differential diagnosis was insect bite special stains included PAS, Wade Fite and ZN the latter 2 showing positive staining clusters indicating an atypical mycobacterial infection. PCR confirmed the presence of Mycobacterium Ulcerans bacterial DNA.
The epidemiology of this disease began in the 1940s in Bairnsdale and since then it has spread to the Mornington Peninsula. It is also called a Buruli Ulcer or Bairnsdale Ulcer. There are 2 stages to this infection - 1) pre-ulcerative stage, 2) the ulcerative stage. There is perivascular necrosis, and this is often not painful. The differential diagnosis at the second stage is SSC and as there are usually lower bacterial numbers than the first stage clinical features as well as histological features and PCR maybe required. Treatment is surgical and 3month antibiotics. Vaccinations are also in development and the transmission of this disease is still unknown whether it is animals, mozzies, or open wounds, but it is recognised that stagnant water plays a part.

Pranav Dorwal presented the 3rd talk about a new antibody INSM 1 (insulinoma associated protein 1).
INSM 1 is a transcription factor expressed in neuroendocrine tissue and has been identified in multiple tumours of NE and neuroepithelial origin. It has high sensitivity and specificity. NE tumours include tumours of the cervix, GIT, lung, head, and neck have shown to have INSM1 staining. It is a nuclear marker and considered to be better than chromogranin, synaptophysin, and CD56.
When looking for a target marker for small cell lung carcinomas and therapies, INSM 1 has better sensitivity for the high-grade tumours than traditional markers (CD 56 is less specific). In Small Cell Carcinomas of the head and neck, tumours of the GIT and pancreas as well as Merkel cell tumours, INSM1 is better than the other markers.

In myxoid, chondrosarcomas and CNS tumours, it can be a useful marker.
From all the reading, Pranav has done he thinks that it would be best practise to include INSM 1 in lung tumour and thoracic tumour panel. As this is a new marker, the test of time will show its true value as a good IHC marker.

Future Events: 2021

Org. No. A0035235F

Date: June 24th

Scientific Meeting

Topic: Complex Cut Up

Venue: Streamed live and recorded using Zoom

Trivia Night

On hold until further notice

Date: September 9th

Scientific Meeting

Topic: IHC Problems, New antibodies – panel discussion

Venue: Streamed live and recorded using Zoom

Date: October 14th

AGM/Scientific Meeting

Topic: TBA

Venue: Streamed live and recorded using Zoom

Date: November (date TBA)

Tasmanian Meeting

Topic: TBA

Venue: Streamed live and recorded using Zoom