homogeneous and nucleolar. Clinical details in this group also included SLE and joint pain.

This investigation demonstrates that IFA testing alone for PCNA may miss clinically relevant cases due to a strong dominant ANA pattern. Clinical characteristics and correlation with IFA is required to validate the immunoblot PCNA result.

EVALUATION OF A VIRTUAL ANA EXTERNAL QUALITY ASSURANCE CHALLENGE

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Background: Provision of an antinuclear antibody (ANA) EQA program which challenges laboratories with a range of potential autoantibody patterns is often limited by access to sufficient donor serum. RCPAQAP introduced a 'virtual sample' in its 2019 ANA program with the aim of widening the scope of potential patterns on offer.

Methods: A commercial control (Euroimmun IIFT Control for antibodies against PCNA Cyclin 2 IgG) was sent to a reference laboratory to prepare slides (using an Immuno Concepts Hep 2000® kit). The slides were returned to RCPAQAP and scanned in-house using a Zeiss Axio Imager Z2 microscope with Meta-Systems scanning and imaging platform. An image was then loaded to the myQAP portal for participating laboratories to access and submit their comments.

Results: 69/77 (90%) of participants correctly identified the expected pattern as cell-cycle related proliferating cell nuclear antigen (PCNA-like) antibodies.

Discussion: The addition of a virtual sample with related images to the RCPAQAP ANA surveys has been well received by participating laboratories. These are also seen as a valuable educational resource to ensure the competency of staff performing ANA testing across the range of ANA patterns.

UNDERSTANDING THE TUMOUR IMMUNE MICROENVIRONMENT (TIME) AT DIFFERENT SITES OF MELANOMA METASTASES (METS)

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Background/Aim: The site of melanoma metastases influences response to immunotherapy (IT), and also has prognostic impact; liver particularly is a uniquely IT-resistant site.^{1,2} We aimed to characterise and compare the TIME at different sites of metastases.

Methods: Multiplex immunofluorescence was performed on FFPE samples from 139 untreated metastatic melanoma pts, from 5 anatomical sites [liver $n=20$; lung $n=23$; brain $n=40$;

subcutaneous $n=21$; lymph node (LN) $n=35$]. T-cell (CD3, FoxP3, Tim-3, CD103, PD1) and myeloid-derived cell (CD14, PDL1, CD68, CD56, CD16) markers were stained and analysed.

Results: CD3+ T-cell densities were significantly lower in liver (median=154.3 cells/mm²) vs lung (median=434.7 cells/mm²) and LN mets (median=504.8 cells/mm²) ($p<0.01$). The proportion of PD1+ T cells was lower in liver (0.7%) vs subcutaneous (7.5%), LN (7.4%) and lung (3.0%) mets ($p<0.01$). Tim-3+ T-cells were higher in liver (10.7%) compared to LN (1.2%) and subcutaneous (1.1%) mets ($p<0.001$). Liver mets presented higher CD68+ macrophage densities compared to LN mets (median=290.6 vs 99.75 cells/mm²; $p<0.05$).

Conclusion: Melanoma liver mets have less immune T cell infiltration compared to other sites, and might hold unique mechanisms of immune tolerance. These data provide insights into the biology of melanoma mets at different sites and response to therapies.

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BIOMARKERS FOR PREDICTING CLINICAL RELAPSE IN REFRACTORY PEMPHIGUS PATIENTS TREATED WITH RITUXIMAB

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Background: Rituximab is an effective treatment for inducing remission in pemphigus vulgaris (PV) refractory to conventional immunosuppression. Re-treatment is required on relapse, but there are currently no reliable biomarkers in predicting clinical relapse.

Method: We prospectively followed 18 PV patients previously treated with rituximab at Westmead Hospital over a 2-year period. Disease activity was assessed by pemphigus disease activity index (PDAI) along with desmoglein (dsg), intercellular cement substance (ICSA) antibody titres, total B-cell and memory B-cell (CD19+CD27+) counts.

Results: 9/18 patients relapsed requiring further rituximab administration. Of these, relapse was preceded by dsg elevation in 5/9 and B-cell return in 2/9 patients. Chronic elevation in dsg antibodies and B-cells were seen in the remaining 2/9 patients with no relationship to PDAI. Of the 9 patients in clinical remission, 5 had normal dsg antibody levels. Review of their clinical data prior to the study suggests correlation of dsg titre elevation with disease relapse. The remaining 4 patients had chronic elevation of dsg antibodies and B-cells. There was no additional information gained from measuring