DR CHERIE BLENKIRON (Orcid ID : 0000-0002-0217-3808)

Article type : Research Letter

Merkel Cell Polyomavirus is uncommon in New Zealand Merkel Cell Carcinomas

B. Woodhouse[1,2], T.J. Robb[1], J.I. Hearn[1], P.S. Houseman[1], G. Hayward[3], R. Miller[4], A.P. Restall[4], M. Findlay[2], B. Lawrence[2], C.G. Print[1], K. Parker[1,2], C. Blenkiron[1,5*]

1. Department of Molecular Medicine and Pathology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

2. Discipline of Oncology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

3. Waitemata District Health Board

4. Auckland District Health Board

5. Department of Obstetrics and Gynaecology, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand

***Corresponding author:** Cherie Blenkiron, The University of Auckland, Private Bag 92019, Auckland, 1142. Email: c.blenkiron@auckland.ac.nz

Merkel Cell Carcinoma (MCC) is a rare skin tumour with an incidence of 0.88 per 100,000 people in New Zealand (NZ), one of the highest in the world[1] and almost on par with close neighbours Australia[2]. Current local diagnostic guidelines do not require analysis of the oncogenic Merkel Cell Polyoma Virus (MCPyV), therefore it has never been questioned whether the NZ-MCC MCPyV rate is similar to North American and European studies (40-89%) or indeed lower, as reported in Australian cohorts (18-24%) in which a UV-mediated etiology is dominant[3-5]. To investigate the presence of the MCPyV in NZ-MCCs we established droplet digital PCR (ddPCR, Bio-Rad) assays to amplify viral gene *LTA*[6] and gene expression of *LTA*, in parallel with a viral protein immunohistochemistry (IHC) assay using the commercial CM2B4 antibody[4]. Methods for ddPCR and IHC are available from the authors on request. This study collated a cohort of patients diagnosed with MCC between 1998 and 2016 in the Auckland region. We collected 53 formalin fixed paraffin embedded (FFPE) tumours excised from 35 donors; representing 39 primary or locally recurrent skin

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/bjd.16903

lesions, 11 lymph node or parotid lesions, and 3 distant organ metastases. All patients were clinically well-defined.

Overall 8 of 35 individuals (22.9%) presented with MCPyV positive MCCs. All matched tumours from the same individual retained the viral status of the primary tumour, even when excised at a later date or from a different body location. Presence or absence of MCPyV DNA (all 35 cases tested), RNA (all cases) and protein (14 cases) was consistent across the majority of patients; however where very low *LTA* copy number was identified (<0.05 *LTA*/*TPO* gene ratio), *LTA* RNA expression was also negligible, supporting those MCCs as effectively being MCPyV negative. In three skin lesions with low *LTA* copy number, further IHC investigation showed LTA protein expression in the overlying skin and stroma rather than in the tumour itself which would have affected the ddPCR results. This supports the value of testing borderline low copy number ddPCR samples with IHC in a multimodal manner if tissue is available to combine method sensitivity with tumour expression specificity.

Based on prior accounts of differing gene expression signatures in MCPyV positive and negative tumours, we assessed the expression of *RB1* and *TP53* in a subset of 35 tumours, (one sample from each patient in the cohort). Unlike in prior studies[7,8] there was no linear correlation between expression of these genes and virus status (r^2=0.002) so expression of *RB1* and *TP53* do not appear to be of value as surrogate markers of MCPyV presence in our cohort.

Although a small cohort, in general positive primary tumours were located on the limbs whilst negative primary tumours were predominantly found on the head and neck (Fishers exact test p=0.102 Table 1). MCPyV positivity was significantly associated with older age (mean 83.3 years Vs 74.1 years, t-test p=0.040), with a trend toward association with female sex (Fishers exact test p=0.091). Interestingly, none of the MCPyV positive cases reported immunosuppression in clinical notes (renal transplant or lymphoma) and non-surgical treatment choices were equivalent between the groups. Due to the obvious challenges of statistical rigour using small cohorts we propose that an analysis combining cohorts from other countries is warranted, which would also provide the opportunity to include MCPyV negative cases that are limited in number in the Northern Hemisphere. We hypothesise that with increasing sun exposure of our aging populations, the incidence of MCPyV negative cases will further increase[2].

In summary, we have established assays in New Zealand for the detection of MCPyV in diagnostic FFPE tissues that combines the sensitivity of PCR with the visual cellular localisation of IHC. This study is the first to investigate MCPyV status in New Zealand and our findings align with Australian reports, suggesting that there may be an Australasia-wide propensity for the alternative ultraviolet (UV) light etiology[6]. Future genomic sequencing of a New Zealand MCC cohort will test this hypothesis through identification of somatic tumour suppressor mutations in genes such as *RB1* and *TP53,* and UV mutational signatures. Further investigation will determine whether this Australasian difference is due to higher UV exposure increasing the number of UV triggered MCCs, lower overall commensal MCPyV rates or even a different MCPyV clade. As current standards of care are based on

Northern Hemisphere studies and clinical trials, this difference warrants consideration for the provision of appropriate local diagnostic and treatment guidelines for New Zealand's predominantly MCPyV negative patients.

Cherie Blenkiron ORCID: 0000-0002-0217-3808

Kate Parker ORCID: 0000-0002-2574-0983

Tamsin Robb ORCID: 0000-0002-1326-0755

Acknowledgements: We would like to thank staff at the Auckland-region District Health Boards pathology laboratories for assistance with archived block access. A/Prof. Kathy Mountjoy and Lottery Health Research, NZ for access to a Bio-Rad droplet digital PCR system.

Conflicts of interest: None declared

References

1 Robertson JP, Liang ES, Martin RC. Epidemiology of Merkel cell carcinoma in New Zealand: a population-based study. *Br J Dermatol* 2015; 173: 835-7.

2 Stang A, Becker JC, Nghiem P *et al.* The association between geographic location and incidence of Merkel cell carcinoma in comparison to melanoma: An international assessment. *Eur J Cancer* 2018; 94: 47-60.

3 Santos-Juanes J, Fernandez-Vega I, Fuentes N *et al.* Merkel cell carcinoma and Merkel cell polyomavirus: a systematic review and meta-analysis. *Br J Dermatol* 2015; 173: 42-9.

4 Paik JY, Hall G, Clarkson A *et al.* Immunohistochemistry for Merkel cell polyomavirus is highly specific but not sensitive for the diagnosis of Merkel cell carcinoma in the Australian population. *Hum Pathol* 2011; 42: 1385-90.

5 Garneski KM, Warcola AH, Feng Q *et al.* Merkel cell polyomavirus is more frequently present in North American than Australian Merkel cell carcinoma tumors. *J Invest Dermatol* 2009; 129: 246-8.

6 Wong SQ, Waldeck K, Vergara IA *et al.* UV-Associated Mutations Underlie the Etiology of MCV-Negative Merkel Cell Carcinomas. *Cancer Res* 2015; 75: 5228-34.

7 Harms PW, Patel RM, Verhaegen ME *et al.* Distinct gene expression profiles of viral- and nonviral-associated merkel cell carcinoma revealed by transcriptome analysis. *J Invest Dermatol* 2013; 133: 936-45.

8 Sihto H, Kukko H, Koljonen V *et al.* Merkel cell polyomavirus infection, large T antigen, retinoblastoma protein and outcome in Merkel cell carcinoma. *Clin Cancer Res* 2011; 17: 4806-13.

Table 1. Association of clinical features with MCPyV status in a NZ-MCC cohort (n=35)

	Positive (n=8) n, (%)	Negative (n=27) n, (%)	Total (n=35) n, (%)
Age at diagnosis (years)			
mean	83.3	74.1	76.2
range	68-97	46-98	46-98
Sex			
Male	3/8 (37.5)	19/27 (70.3)	22/35 (62.9)
Female	5/8 (62.5)	8/27 (29.6)	13/35 (37.1)
Disseminated at Diagnosis			
Node	0/5* (0.0)	9/16 (56.2)	9/21 (42.8)
Distant metastasis	0/4 (0.0)	3/18 (16.7)	3/22 (13.6)
Primary location			
Head and Neck	3/8 (37.5)	17/27 (62.9)	20/35 (57.1)
Upper Limb	2/8 (25.0)	2/27 (7.4)	4/35 (11.4)
Trunk/Torso	0/8 (0.0)	2/27 (7.4)	2/35 (5.7)
Lower Limb	3/8 (37.5)	2/27 (7.4)	5/35 (14.3)
Unknown	0/8 (0.0)	4/27 (7.4)	4/35 (11.4)
Normalised gene expression (mean, range)†			
MCPyV *LTA*	4.20 (0.06-14.80)	0.00	0.96 (0.00-14.80)
TP53	0.018 (0.006-0.024)	0.016 (0.001-0.041)	0.016 (0.001-0.041)
RB1	0.058 (0.000-0.121)	0.039 (0.005-0.093)	0.043 (0.000-0.121)
Normalised *LTA* gene copies (mean, range)•			
MCPyV *LTA*	5.596 (0.70-39.4)	0.00 (0.00-0.014)	1.513 (0.00-39.4)

* Information not stated in patient notes so n is lower

† Gene expression normalised to sum of *ATOH1* and *ACTB*

• Gene expression normalised to *TPO*