

HPV8-associated actinic keratosis identifies immunosuppressed patients at high risk of developing squamous cell carcinoma

William Tuckwell,¹ Huw J Morgan,¹ Boris Y Shorning,¹ Su-Yi Liew,² Marcelle McDonald-Leslie,² Nitya Malladi,² W John Watkins,³ Majid Rashid⁴ and Girish K. Patel¹

¹ European Cancer Stem Cell Research Institute, School of Biosciences, Cardiff University, Cardiff, UK

²Welsh Institute of Dermatology, Glamorgan House, University Hospital of Wales, Cardiff, UK

³ Department of Infection and Immunity, School of Medicine, College of Biomedical & Life Sciences, Cardiff University, Cardiff, UK

⁴Department of Pathology, Aneurin Bevan University Health Board, Newport, UK

Corresponding author: Dr William Tuckwell

Email: will.tuckwell30@gmail.com

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Dear Editor, cancer prevention strategies, such as cervical screening, rely on the detection and treatment of intraepithelial neoplasia. In the skin, actinic keratoses (AKs) are readily recognisable. In a high-risk group of veterans, over a 4-year period, keratinocyte carcinoma (KC) frequently arose at the site of a previously photographed AK¹. Furthermore, treatment of skin fields containing multiple AKs with 5-fluorouracil twice daily for a month reduced the rate of invasive SCC by 75% in the following 12 months². Recently, we determined HPV8 reactivation as a cause for AKs in 70% of individuals, among whom there was an increased risk for subsequent invasive SCC (OR 32.0, $p < 0.001$) and BCC (OR 4.5, $p < 0.01$)³⁻⁵. Since immunosuppressed patients, such as solid organ transplant recipients, have an increased risk for AKs and SCC, we sought to determine their frequency of HPV8-associated AK and relative risk for SCC.

Following ethical (19/NS/0012) and NHS R&D approvals, 105 patients with histologically confirmed AK and no antecedent history of KC were recruited. Among these, 25 immunosuppressed patients were identified based on ongoing haematological disorders ($n=9$), immunosuppressive treatments for inflammatory disease ($n=9$), and immunosuppression in the context of solid organ transplantation ($n=7$). Tissue sections from a pathologist-defined AK were analysed for HPV8 by reverse *in situ* hybridisation and the presence of koilocytes, as previously described^{4,5}.

In the 10 years following the pathologist's diagnosis of AK, 36% ($n=9$) of the immunosuppressed patients developed an invasive SCC, compared to 12.5% ($n=10$) in the immunocompetent group (OR 3.9, 95% CI 1.4-11.3, $p < 0.05$). There was no difference in age (68 ± 9 years versus 70 ± 10 years) or AK body site, although the immunosuppressed group had more males (68% versus 40%, $p < 0.02$). All the SCCs that developed in immunosuppressed patients, but not in the immunocompetent group, met at least one Bingham and Women's Hospital high-risk criterion. Thus, as previously reported,

1 immunosuppressed patients had a greater risk of developing SCC associated with a poor
2 outcome.

3
4 The immunosuppressed group had HPV8-associated AK more frequently (84% versus 60%,
5 $p < 0.05$). Immunosuppressed patients with HPV8-associated AK were more likely to
6 develop invasive SCC (38%) than immunocompetent patients with HPV8-associated AK
7 (17%, $p = 0.0029$, Figure 1), even though there was no difference in age, gender or body site
8 of the initial AK. In contrast, there was no difference in BCC frequency between
9 immunosuppressed and immunocompetent patients with HPV8-associated AKs. Hence,
10 the higher rates of HPV8-associated AK in immunosuppressed patients may specifically
11 contribute to their elevated risk of KC, and immunosuppression may further augment SCC
12 risk through tumour immune evasion.

13
14 To determine the role of HPV8 in promoting KC, we were unable to undertake a direct
15 comparison with immunosuppressed patients without HPV8-associated AKs due to the
16 small cohort size ($n=4$). Instead, we compared immunosuppressed patients with HPV8-
17 associated AKs and immunocompetent patients without HPV8-associated AKs. The groups
18 were similar in age (68 ± 9 versus 69 ± 10 , N.S.) and AK locations, but the
19 immunosuppressed patients with AKs had a higher proportion of males (71% versus 25%,
20 $p < 0.005$). In the ensuing 10 years, immunosuppressed patients with HPV8-associated AKs
21 were more likely to develop invasive SCC (OR 9.2, 95% CI 1.7-49.6; $p < 0.01$). Following
22 adjustment for age and sex using multivariable logistic regression, this remained an
23 independent risk factor for SCC development (OR 6.9, 95% CI 1.0-46.3; $p < 0.05$). Patients
24 who developed multiple KC in this period ($n=4$) were exclusively immunosuppressed with
25 HPV8-associated AKs ($p < 0.05$). Hence, HPV8-associated AK represents an important risk
26 factor for SCC development in immunosuppressed patients.

1 Virus reactivation-associated malignancies, such as Hodgkin's lymphoma and Kaposi's
2 sarcoma, represent a basis for the increased cancer risk among immunosuppressed
3 patients⁶. Here, we show that immunosuppressed patients are more likely to develop
4 HPV8-associated AKs. As previously described, HPV8 reactivation within the hair follicle
5 leads to proliferation of keratinocyte stem cells lacking melanosome protection that
6 occupy the overlying epidermis, recreating the AK pathology and rendering them
7 susceptible to UV-induced transformation⁴. Hence, the findings suggest a viral basis for KC
8 in the context of immunosuppression.

9
10 This study once more demonstrates the association between HPV8-associated AKs and a
11 greater risk for subsequent KC. Among immunosuppressed patients, SCC subsequently
12 developed in 38% (n=8) of those with HPV8-associated AK and in one of four (25%) patients
13 without HPV8-associated AK. Compared with immunocompetent patients with HPV8-
14 associated AK, immunosuppressed patients had a 3.1-fold greater risk of developing SCC;
15 this may be due in part to the higher number of immunosuppressed males in this cohort.
16 We similarly observed an increase in the frequency of SCC in HPV8 transgenic mice when
17 crossed with the immunosuppressed Rag2 knockout transgenic mouse, which
18 accentuated the mutagenic effect of UV light⁷. Intriguingly, immunosuppression did not
19 augment the HPV8-associated risk for BCC development. Once SCC develop, immune
20 evasion also facilitates tumour growth, accounting for the poor outcomes observed in
21 immunosuppressed patients. In summary, virus reactivation is more common in
22 immunosuppressed individuals, suggesting that immunosuppression both increases the
23 likelihood of HPV8 reactivation and promotes tumour formation.

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1 **Figure legend**

2
3 **Figure 1.** Kaplan-Meier Analysis of subsequent squamous cell carcinoma in
4 immunosuppressed and immunocompetent patients presenting with HPV8 associated AK.
5 Log-Rank $p=0.0029$.
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