SUPPLEMENTARY MATERIAL AND METHODS

This retrospective study (EK1689/2015) was approved by the ethics committee of the Medical University of Vienna, Austria, and conducted in accordance with the Declaration of Helsinki.

Formalin-fixed, paraffin-embedded tissues from 18 patients with squamous cell carcinomas (SCCs) \((n = 9)\) and/or Bowen’s diseases (BDs) \((n = 28)\) located on the skin of the hands and wrists were included. The patients’ and tumour characteristics are shown in Table S1. Ten patients (55.6%) presented with a single, 8 (44.4%) patients with multiple tumours over time. If tumours reappeared at the same localization after complete surgical removal, either due to de novo development or local recurrence, only tumours that arose at intervals longer than a year were included.

DNA was extracted from the tissues using the EZ1 DNA Tissue Kit (Qiagen, Hilden, Germany) and analysed for the presence and respective papillomaviral genotype by real-time PCR (HPV Genotypes 14 Real-TM Quant kit, Sacace Biotechnologies, Como, Italy), which detects the high risk-human papillomavirus (HR-HPV) types 16/18/31/33/35/39/45/51/52/56/58/59/66/68. RNA in situ hybridization was performed using RNAScope® for HPV16 (Advanced Cell Diagnostics, Newark, CA, USA) according to the manufacturer’s instructions. Hybrid capture-2 tests (Digene Inc., Gaithersburg, MD, USA) of skin swabs taken from the tumour surfaces detect the presence of one or more HPV types belonging to the group of mucosal HR-HPVs (represented by HPV16/18/31/33/35/39/45/51/52/56/58/59/66/68) and/or to the group of mucosal low-risk HPVs (represented by HPV6/11/42/43/44), but not of cutaneous HPVs. Results, given in relative light units (RLUs) with 1 RLU being equivalent to 100,000 HPV copies/ml, allow a semi-quantitative estimate of viral loads. Immunohistochemical (IHC) analyses were performed with primary antibodies to p53 (rabbit polyclonal; dilution 1:200; Abcam, Cambridge, UK), p16 (CINtec® p16 Histology Kit; clone E6H4; Ventana, Roche, Basel, Switzerland), p21 (rabbit monoclonal; dilution 1:100; Cell Signaling Technology, Frankfurt/Main, Germany), and epidermal growth factor receptor (EGFR) (clone E30; dilution 1:50; Dako, Santa Clara, CA, USA). Immunoreactivities in tumours were semi-quantitatively scored: the p53 staining pattern were classified “positive”, when distinct nuclear staining was found in ≥10% of tumour cells, or “negative”, when <10% of tumour cells stained for p53. P16 expression was “positive” when strong nuclear and diffuse cytoplasmic staining was observed in the cells of the basal and parabasal cell layers, with or without involvement of the intermediate and superficial layers, as typically seen in HR-HPV-induced cervical neoplasia. The intensities of immunopositivity were graded: weak staining and positivity in <10% of tumour cells (grade 1), distinct positive staining in 10–49% (grade 2) and ≥50% of tumour cells (grade 3). Tumours classified as “negative” showed focal, non-continuous or lack of p16 staining. P21 expression was considered “positive” in the case of distinct nuclear staining or “negative” otherwise. Distinct membranous staining of EGFR in ≥10% of tumorous areas was considered “positive”, in <10% “negative”. Numbers and percentages of immunopositive cells per mm² in the tumours were quantified using the HistoQuest software (TissueGnostics GmbH, Vienna, Austria).

Statistical analyses were performed using GraphPad Prism, version 6.0, and \(p\)-values <0.05 considered statistically significant.