Material and Methods

This retrospective study included 98 patients from the Skin Cancer Centre Hannover with 102 metastatic and non-metastatic cSCC that were chosen according to the following criteria:

- cSCC patients (metastatic, non-metastatic) with archived, formalin-fixed, paraffin-embedded cSCC samples with significant tumour amount, previously confirmed by haematoxylin-eosin staining
- According to patients' files and pathological reports well-documented clinicopathological parameters (age, sex, date of cSCC diagnosis/metastasis, localisation, tumour thickness, horizontal diameter and histological grading)
- Risk factors (organ transplantation)
- Four patients developed multiple primary tumours; in this case, samples of 2 primary tumours were immunohistochemically analysed; these 4 patients did not suffer from a recurrence/metastasis or were immunosuppressed

The approval to conduct this study was obtained from the ethics committee of the Hannover Medical School (vote No. 2071-2013).

Archived formalin-fixed paraffin-embedded samples from metastatic and non-metastatic cSCC (22 and 76, respectively) and corresponding skin and lymph node metastases (14 and 4, respectively) underwent immunohistochemical staining. This was performed on 7- to 10-µm-thick microtome-cut tissue sections. Polymer-based immunohistochemistry was used (DakoEnVision+ Systeme-HRP, Dako North America Inc., Carpinteria, CA, USA) involving primary monoclonal antibodies for E-cadherin (anti-human, clone NCH-38, Dako) and podoplanin (anti-human, clone D2-40, Dako). Isotype controls were included in every staining procedure showing appropriate results. The sections were semi-quantitatively judged and evaluated with respect to an evaluation system that was applied to all samples (suppl. Table 3, suppl. Fig. 1 and 2). The evaluation was conducted blindly by a dermatologist, a dermatopathologist and a medical student from the Hannover Medical School, respectively, and by a dermatologist from the University Medical Centre Essen.

The analyses were performed with SPSS 22.0 (SPSS Inc., Chicago), using the χ^2 test for correlations between categorical variables. Univariate analysis of relapse-free survival and multivariate analysis were conducted with Kaplan-Meier estimate and Cox regression. Relapse-free survival was defined as time between first diagnosis of cSCC and diagnosis of skin and/or

lymph node metastases. Staining results of primary cSCC and corresponding skin and lymph node metastases were compared using the paired Student *t* test with GraphPad Prism 5.02. We did not correct for multiple testing. *p* values <0.05 were considered statistically significant.

cSCC of Organ Transplant-Recipients

In our retrospective analysis of immunohistochemical expression of E-cadherin and podoplanin in cSCC, we included a total number of 23 patients who were organ transplant recipients (OTR), engrafted between 1979 and 2009, and under immunosuppressive therapy. They were compared to 75 non-OTR regarding clinicopathological parameters and immunohistochemical expression of E-cadherin and podoplanin. OTR were followed up for a mean of 23.5 months (median: 12.1 months). The mean age at diagnosis was 66 years in OTR and 80 years in non-OTR ($p < 0.001$). OTR showed divergent, not necessarily sun-exposed primary tumour locations like the trunk ($p < 0.001$). Specimens of OTR presented a lower risk profile with few metastatic cSCC (8.7%) and low invasion depth (OTR <5 mm: 78.3%, $p = 0.037$) which can be illustrated by the means of invasion depth of 3.9 mm in OTR and 5.9 mm in non-OTR ($p = 0.017$). The immunohistochemical expression of E-cadherin and podoplanin displayed no significant differences in cSCC between OTR and non-OTR. Both OTR and non-OTR developed predominantly G1 and G2 tumours. Metastasis occurred in 8.7% of OTR, affecting only the skin. We hypothesised that this might be due to early diagnosis and thus low invasion depth in the context of intense follow-up care after transplantation as the patient collective originates from a university hospital. However, the metastatic risk of OTR is estimated to be between 7 and 16% and distinctly higher than in immunocompetent patients (0.5–5%). Prior studies revealed cSCC of OTR to be more likely to metastasise and display an aggressive invasional behaviour that we could not confirm in our analysis. The divergent results might be due to selection bias of patients who benefitted from intense follow-up care and the overall small number of OTR (data not shown).