Variable Outcome of Immunotherapy in Advanced Multiple Cutaneous Squamous Cell Carcinomas in Two Patients with Recessive Dystrophic Epidermolysis Bullosa

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Cutaneous squamous cell carcinoma (cSCC) is a major complication of recessive dystrophic epidermolysis bullosa (RDEB) that has high morbidity and mortality rates and unmet therapeutic needs. The aim of this study was to evaluate the molecular pattern of cSCC and the clinical course of immunotherapy in 2 RDEB patients with multiple advanced cSCC. Clinical course and disease staging were evaluated retrospectively. The tumour tissues were subjected to immunohistochemical staining. DNA from the blood and cSCC samples was subjected to massive parallel sequencing, and somatic mutations were determined. Patient 1 survived for over 2 years as disease control was achieved with cemiplimab and intralesional interleukin-2. The target advanced cSCC demonstrated a high rate of somatic mutations and strong expression of the immune markers, indoleamine 2,3-dioxygenase, programmed cell death protein ligand 1, and lymphocyte-activation gene 3. The patient ultimately succumbed to complications of oesophageal carcinoma. Patient 2 had an undifferentiated cSCC on the foot, which displayed a low mutational burden and did not express immune markers. The tumour progressed quickly even with cemiplimab therapy. These 2 cases underscore the challenges of cSCC treatment for RDEB. Multiple tumours with different molecular and immune profiles occur concomitantly or sequentially, and surgical excision is not always possible because of the anatomical and tissue constraints imposed by the disease itself. In conclusion, programmed cell death protein 1 inhibitors are approved and effective in treating metastatic and locally advanced cSCC. Our experience and the literature suggest that cemiplimab is an option in patients with RDEB if surgery is not. Somatic mutations and the immune microenvironment should be characterized to predict therapeutic response, particularly in aggressive undifferentiated tumours.

Key words: collagen VII; epidermolysis bullosa; squamous cell carcinoma.

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Significance

Recessive dystrophic epidermolysis bullosa (RDEB) is a rare genetic disease with skin blistering, wounds with bacterial colonisation, and a high risk of cutaneous squamous cell carcinoma (cSCC), which metastasizes early and has a severe and often lethal course (1–7). cSCC develops at sites of persistent injury with chronic inflammation, and innate sensing of microbial products are important risk factors (8, 9). Endogenous mutation processes dominated by apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC), cause RDEB skin and tumours to rapidly acquire a high burden of mutations (10). Scarring, activation of fibroblasts, increased production of transforming growth factor beta-1, and other pathways involved in tissue repair contribute to the aggressive course of cSCC in RDEB (11). Inflammation is considered an important component in the pathogenesis of RDEB, but the immunological microenvironment of RDEB-associated cSCC has not yet been characterized.

Although cSCC represents the main cause of mortality in RDEB, little is known about adjuvant, neoadjuvant, or palliative therapies for RDEB-associated cSCC (5) and only single case reports are available (12–16). We report here the immunological microenvironment and somatic mutations of advanced cSCC in 2 patients with RDEB and the clinical course of treatment with cemiplimab, an inhibitor of programmed cell death protein 1 (PD-1).
**MATERIALS AND METHODS**

**Patients and samples**
cSCC samples obtained from 2 patients with severe RDEB were studied. The patients did not receive any therapy prior to tumour excision for therapeutic purposes. Histopathological characteristics were retrieved from pathology reports. After obtaining informed consent, genetic testing was performed by whole exome sequencing (Novogene, [https://en.novogene.com/](https://en.novogene.com/)) using the Agilent SureSelect All Exon V6 protocol for exome capture and Illumina HiSeq XTEN. This study was approved by the ethics committee of the University of Freiburg (EK-Freiburg/18 and 5/20) and was conducted according to the principles of the Declaration of Helsinki. The patients in this manuscript provided written informed consent for publication of their case details.

**Immunohistochemistry**

Immunohistochemistry (IHC) was performed on FFPE sections for indoleamine 2,3-dioxygenase (IDO), PD-1 ligand 1 (PD-L1), PD-1, lymphocyte-activation gene 3 (LAG-3), and T-cell immunoglobulin and mucin-domain containing-3 (TIM-3). AxioVision (Zeiss, Feldbach, Switzerland) was used for imaging. Three observers were blinded to the clinical parameters independently scored the IHC-stained sections. The histology score (H-Score) was calculated for IHC markers IDO, PD-1, TIM-3, and LAG-3 as a semi-quantitative approach and was measured using 3 different scores: combined positive score (CPS), tumour proportion score (TPS), and immune cell score (IC). Further details are provided in Appendix S1; Table S1.

**Whole exome sequencing and bioinformatic analysis**

Whole exome sequencing (WES) was performed by Novogene ([https://en.novogene.com/](https://en.novogene.com/)) using the Agilent SureSelect All Exon V6 protocol for exome capture and Illumina HiSeq XTEN. Bioinformatic analyses included quality control to filter out reads containing adapters or with low quality, alignment with the reference genome (Genome UCSC hg19), statistics of sequencing depth and coverage, single nucleotide polymorphism, insertion or deletion of bases (InDel) and copy number variation (CNV) calling, annotation, and statistics. The inclusion criteria for the variants were as follows: at least 8 reads per base and at least 2 reads supporting the variant; rare mutations (minor-allele frequency < 0.001, gnomAD); no “Black-listed” genes; and variant allele frequency > 10%.

This study only considered alterations that were recurrent in at least 2 samples, related to tumourigenesis or significantly mutated in RDEB-SCC, head and neck SCC, or UV-SCC, according to the literature.

To calculate recurrence percentages, we considered an alteration to be activating if it landed in a known oncogene and was either a known activating mutation based on a literature search or highly amplified. Similarly, alterations were considered to be inactivated if they were nonsense mutations or homozygous deletions. Missense or other nonsynonymous mutations in COSMIC ([https://cancer.sanger.ac.uk/cosmic](https://cancer.sanger.ac.uk/cosmic)) were considered mutations of unknown functional effects but potentially associated with cancer.

**RESULT**

**Clinical and molecular features**

Patient 1 had RDEB caused by the COL7A1 homozygous mutation c.425A>G. At the age of 36 years, he developed the first cSCC lesion on the right hand ([Fig. 1a, d]). Histopathology revealed a well-differentiated verrucous cSCC with a tumour thickness >12 mm. There were no loco regional or distant metastases, and the tumour was completely excised via micrographic surgery. The tumour recurred locally twice in the following 2 years ([Fig. 1a, d]) and could not be completely removed. The interdisciplinary tumour board recommended amputation, which was declined by the patient, who wanted to maintain his grasping capacity.

IHC staining of cSCC (second recurrence) showed strong IDO expression in stromal immune and tumour cells ([Fig. 2 and Appendix S1; Table SII]). Furthermore, intense staining for PD-1 and LAG-3 and moderate staining for TIM-3 were observed in the stromal cells. Tumour and immune cells in the stroma showed strong PD-L1-expression ([Fig. 2, Appendix S1; Table SII]). The cSCC contained 236 somatic mutations, resulting in a mutation rate of 10.04/Mb. The main driving variants were in the oncogenes HRAS, FGFR3, and NOTCH1 and the tumour suppressor genes CTCF, FAT1, CASP8, and KMT2B, and the dominant mutational signature was UV followed by APOBEC ([Table 1]).

Based on this molecular profile, the interdisciplinary tumour board recommended anti-PD1 therapy. Cemiplimab (350 mg every 3 weeks) was initiated and administered for 2 years. In addition, intraslesional interleukin-2 (IL-2) (6 MIU/every 3 weeks) was administered to a newly developed cSCC on the scalp ([Fig. 1b]). While receiving this regimen, the cSCC on the hand showed some reduction in hyperkeratosis and ulceration, while the scalp lesion regressed completely ([Fig. 1a, b]). Regular magnetic resonance imaging (MRI) scans were used to exclude metastases. The patient developed further cSCC on the foot, right shoulder, and arm but remained stable without evidence of disease progression ([Fig. 1c]). One year after cemiplimab initiation, oesophageal SCC with lymph node metastases was diagnosed ([17]). Collagen VII is expressed in mucous membranes making them prone to trauma and squamous cell carcinoma formation. This tumour responded well to radiotherapy ([17]), but the patient succumbed 1 year later at the age of 42 years due to complications including mucositis, scarring, and recurrence of the oesophageal tumour. At the last visit, the cSCC was stable and he had no metastasis.

Patient 2 had RDEB caused by the compound heterozygous COL7A1 variants c.425A>G and c.1837C>T, p.Arg613*. The clinical course in this patient was severe, with extensive chronic heavily bacterially colonized wounds and repeated episodes of fever, oesophageal stenosis, and cachexia (body mass index (BMI) 16.6 kg/m²). He developed his first cSCC on the right elbow at the age of 16 years, followed by several well-differentiated cSCC on his right forearm and a large ulcerative cSCC of the right foot ([Fig. 2a and b]). Ingual lymph node metastasis was
suspected on ultrasonography. At that time, the patient declined surgery.

IHC of cSCC from the right forearm showed strong IDO expression in stromal immune cells but remained negative in tumour cells (Fig. 3 and Appendix S1; Table S1). PD-1 and TIM-3 were strongly expressed in the stroma, whereas LAG-3 expression was low in the stromal cells. PD-L1 expression in tumour and stromal cells was moderate and graded lower than the cSCC of patient 1. The poorly differentiated cSCC of the right foot demonstrated a distinct pattern of immune markers: in stromal cells, IDO, PD-1, PD-L1, and TIM-3 expression was very low, IDO was not expressed by tumour cells, and LAG-3 expression was similar to that of the cSCC on the forearm (Fig. 3 and Appendix S1; Table SII). Importantly, the cSCC on the foot had a relatively low mutational rate of 3.41/Mbp, few TP53, FAT1, KMT2B, and NOTCH1 driving mutations, and a dominant APOBEC signature (Table I).

The multidisciplinary tumour board recommended treatment with cemiplimab (350 mg every 3 weeks). However, after administering cemiplimab for 3 months, the cSCC on the foot increased in size, while that on the forearm remained stable (Fig. 2a and b). The treatment was discontinued, and the patient agreed to undergo amputation of his right lower leg and resection of the...
right inguinal lymph nodes (Fig. 2c). Histopathology confirmed tumour infiltration of the inguinal lymph nodes; however, the resection was incomplete. Because of residual metastatic lymph nodes, cetuximab and celecoxib were initiated (12). Three months later, the postoperative wound in the right groin did not heal, and a large ulcerative cSCC developed and grew rapidly on the upper left leg. The therapy was stopped, and the patient died 2 months later from complications related to the non-healing wound.

**DISCUSSION**

The clinical course in patient 1 showed that PD-1 inhibition with cemiplimab may be a well-tolerated and effective therapy for disease control of cSCC in patients with RDEB. Intralesional IL-2 serves as an additional local treatment option for newly developed cSCC. The
Fig. 3. Immunohistochemical (IHC) staining of cutaneous squamous cell carcinomas (cSCC). Patient 1: IHC staining of the cSCC on the patient’s right hand showed a strong indoleamine 2,3-dioxygenase (IDO) expression in stromal immune cells and tumour cells. Intense staining for programmed cell death protein 1 (PD-1) and lymphocyte-activation gene 3 (LAG-3) and moderate staining for T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) was present in stromal cells. Tumour cells and immune cells in the stroma showed a strong PD-L1 expression. Patient 2: IHC staining of the tumour from the right forearm showed a strong IDO expression in stromal immune cells but in almost no tumour cells. Strong PD-1 and TIM-3 expression was present in the stroma. LAG-3 expression was low in the stromal cells. Programmed cell death protein ligand 1 (PD-L1) expression on tumour and stromal cells is moderate and lower than those in the cSCC of patient 1. Finally, the cSCC of the right foot showed much lower IDO, PD-1, PD-L1 and TIM-3 expression on stromal cells. IDO expressing tumour cells were not present. LAG-3 expression was similar to the cSCC of the right forearm. *Tumour islets of the cSCC. G1: well differentiated; G3: poorly differentiated. Scale bar=100 µm; Insert scale bar=25 µm.
high rate of somatic mutations and the immune microenvironment of cSCC with high expression of IDO and PD-L1 in tumour cells and LAG-3 expression on immune cells may predict a beneficial response to immunotherapy. PD-L1 and LAG-3 are markers for T cell exhaustion and are targetable via monoclonal antibodies, as is already being performed for malignant melanoma (18). IDO confers immunosuppressive functions in the tumour microenvironment (TME) and specific blockage via peptide vaccines is being tested in a clinical study on the oesophageal carcinoma in the current patient. Immune escape mechanisms under treatment with checkpoint inhibitors can cause secondary treatment failure/resistance, leading to the development of new cancer types (20). Therefore in the 2 patients with RDEB in this study it is not possible to differentiate between SCC development due to selection pressure/secondary resistance and spontaneous development due to the underlying disease, RDEB. There are few case reports on systemic immunotherapy with PD-1 inhibitors for cSCC in RDEB (Table II). Somatic mutations and the immune microenvironment should be characterized to predict therapeutic response and guide therapeutic decisions, particularly in aggressive undifferentiated tumours.

The major limitations of this study are the small number of patients and the evaluation of only the target lesions in patients with multiple tumours.

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REFERENCES