Materials and Methods

Study Population

From November 2011 to July 2017, all patients with confirmed diagnosis of lentigo maligna (LM) who had undergone therapy with Aldara® 5% cream (imiquimod; MEDA Pharma GmbH, Germany) and examination with reflectance confocal microscopy (RCM) were included in our study. LM was diagnosed according to the proven clinical and conventional histological criteria. Furthermore, RCM was performed in all patients. The following patient data were collected: age at the beginning of therapy, gender, Fitzpatrick skin phototype, clinical response during therapy and concomitant side effects, location and size of LM, recurrence of LM, time from the beginning of therapy to relapse, death, and other skin malignancies. Informed consent was given by all patients.

All procedures of this study were in accordance with the standards of the Ethics Committee of the Canton of Bern, Switzerland, on human experimentation (KEK No. 2016-00382) and with the Helsinki Declaration of 1975, as revised in 1983.

Procedures

In order to clinically map the potential LM lesions, pictures were taken of all subjects using the FotoFinder Imaging System under standardized conditions. After taking baseline clinical, dermatoscopy and RCM images, biopsy was performed on the lesions to confirm the diagnosis. Thus, every LM in our study was diagnosed both by clinical and histological examination.

When the diagnosis of LM was confirmed, therapy with imiquimod was started. Therapy consisted of one to two daily applications of imiquimod cream. Therapy was performed in all patients until weeping erosion appeared in the treated skin area. All patients underwent regular clinical follow-ups during and after imiquimod treatment.

At the first follow-up examination, the treated areas were inspected and the clinical and dermatoscopy images using FotoFinder Imaging System were taken. Then RCM imaging of the corresponding areas was carried out and stored in the system. At this time point, no histological examination by biopsy was performed.

Histology Evaluation

The biopsies of LM were fixed in formalin, embedded in paraffin and after preparation evaluated by at least two certified dermatopathologists analyzing both hematoxylin-eosin and immunohistochemically for melan A antigen-stained slides.

For cell counting, melan A immunohistochemical staining was chosen, where the number of melanocytes/each mm of epidermis instead of melanocytes/mm^2 were counted, because this approach is more reliable in elderly patients with sun-damaged skin due to the thin epidermis [15]. For each patient, 2 mm of epidermis were analyzed. Each count was performed 3 times and then the mean score was taken.

In vivo RCM Evaluation

All LM were examined and imaged with the Vivascope 1500 (Lucid Inc., Rochester, NY, USA). The most important features of LM are loss of the epidermic architecture (which is characterized through the honeycomb pattern), missing identifiability of the papillae (called nonedged papillae), nests of atypical melanocytes and the presence of nucleated cells in the epidermal layer [16]. All pictures (i.e., features) were analyzed according to the LM score of Guitera et al. [8] (Table 1). In this study, an LM score ≥ 2 was reported to be significant with a sensitivity of 85% and a specificity of 76% [8]. In the present study, according to LM score (Table 1), atypical cells were identified and depicted as numbers of atypical cells per mm^2. Figure 2 shows the different forms of atypical cells detected in this study. In each patient,

images of the RCM were analyzed before and after imiquimod treatment and repeated in case of LM recurrence.

RCM images of all patients enrolled in the study were preselected. Only images showing the epidermal area with the highest number of atypical cells were chosen. Every image was analyzed 3 times and the mean atypical cell count was taken. In each patient at least five RCM images from an area of $0.5 \times 0.5\ mm^2$ of epidermal skin were analyzed.