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Fluorescence molecular imaging using cetuximab-800CW in cutaneous squamous cell carcinoma surgery: a proof-of-concept study

Dear editor, cutaneous squamous cell carcinoma (cSCC) has a cure rate of 95%. However, due to its high incidence, it is associated with more disability-adjusted life-years than melanoma.[1] The primary treatment of cSCC is surgical excision, with the most common approach being standard excision, where the tumor is removed with a predetermined margin of healthy tissue. Alternative approaches, such as Mohs micrographic surgery (MMS), have been proposed to improve surgical and cosmetic outcomes. In MMS, the complete resection margin is assessed intraoperatively through frozen sampling. Up to 100% margin control and lower recurrence rates have been reported with MMS, but it is resource-intensive and time-consuming.[2] The need for real-time information has led to increasing interest in optical imaging techniques that enable intraoperative tumor visualization, possibly supporting surgical decision-making. For example, fluorescence molecular imaging (FMI), a novel imaging method that uses tumor-specific tracers to highlight tumor tissue, has shown potential for intraoperative margin assessment.[3,4] A common target for FMI is epidermal growth factor receptor (EGFR), a transmembrane receptor overexpressed in up to 90% of cSSCs.[5]

In this proof-of-concept study, we explored the potential of FMI using cetuximab-800CW for discrimination between cSCC and adjacent tissue. Ten patients with histology-confirmed cSCC, scheduled for conventional excision or MMS were included. Two to three days before surgery, patients were intravenously administered 75 mg cetuximab, followed by 15 mg of cetuximab-800CW one hour later.[3] FMI was performed intraoperatively for *in vivo* tumor visualisation and *ex vivo* margin assessment of the surgical specimen.[6] Surgical specimens were processed according to standard of care; additional EGFR immunohistochemistry was performed on all tissue slices. FMI was performed on tissue slices to cross-correlate fluorescence signal with final histopathology. Mean fluorescence intensities of the tumor and the background were calculated to determine a tumor-to-background ratio (TBR).[3]

A total of twelve lesions were identified, of which six were treated with MMS and six with conventional excision. One lesion was unexpectedly diagnosed as basal cell carcinoma, and for three other lesions the diagnosis of keratoacanthoma was suggested on final histology. The two conventional excisions containing cSCC showed TBRs of 2.86 and 2.35. The MMS specimens showed a mean TBR of 2.24 (1.82-2.62). The TBR of the tumor versus adjacent tissue in the deep margin (i.e. mostly fat) was 3.07 (1.85-4.39). Excised cSCC specimens were

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analysed on the back-table, including two conventional excisions and six MMS cases. In one conventional excision, a patient with a high-risk cSCC that was previously irradically excised, we observed a fluorescent lesion at the deep resection margin, corresponding to a tumor-positive margin on final histopathology (Fig. 1A). The other conventional excision did not show fluorescent signal, and the minimal deep margin was 4.8 mm. In 5/6 first stage MMS specimens, we observed a fluorescent lesion at the deep resection margin (Fig. 1B). In these five cases, three tumor-positive margins were identified. In the other two cases, the margin was not tumor-positive, but the fluorescent signal co-localised with tumor tissue on additionally obtained tissue sections located closer to the skin. As such, the false positive signal resulted from limited depth information of the fluorescence signal, leading to detection of tumors localized under the surface. No tumor was present on histopathological examination in the MMS specimen that did not show a fluorescent lesion. The three MMS patients with a tumor-positive margin required an additional excision (i.e. after stage 1). None of these showed fluorescence signal, and all were tumor-negative on final histopathology. We determined the overall performance of FMI for margin assessment using all conventional excisions and all MMS excisions. We obtained 100% sensitivity, 63% specificity, 100% negative predictive value and 57% positive predictive value. The overall accuracy was 75%.

In the basal cell carcinoma, we found a TBR of 2.22. The three keratoacanthomas showed TBRs of 0.77, 1.66 and 1.69. One patient with two keratoacanthomas had a long history of immunosuppression use and showed substantial actinic damage. This resulted in high fluorescence in the background, and the tumors did not show increased fluorescence signal compared to this background signal during *in vivo* imaging. EGFR immunohistochemistry showed weak to strong expression in all cSCCs, co-localising with fluorescence signal. Low EGFR expression was found in the basal cell carcinoma. Keratoacanthomas showed no EGFR-expression as reported earlier.[7]

This proof-of-concept study demonstrates that FMI using the fluorescent tracer cetuximab-800CW can differentiate between tumor and adjacent tissue with high contrast. FMI detected all tumor-positive margins intraoperatively within seconds. FMI could be valuable for patients with a large or complex cSCC, where obtaining 100% intraoperative margin control is anticipated to be critical but seems less useful for keratoacanthoma-like cSCC or in patients with excessive actinic damage. Future studies should determine the clinical value of FMI in surgery of high-risk cSCCs, ideally with new imaging methodologies that deliver improved depth information.[8]

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Conflicts of interest: GMvD is a member of the scientific board of SurgVision BV, founder, shareholder, and CEO of TRACER Europe BV (Groningen, the Netherlands).

Data availability: All data are available upon reasonable request

Ethics statement: The study protocol was approved by the institutional review board (METc 2019/183) and the Dutch competent authority.

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Figure 1: Fluorescence imaging during standard excision and Mohs micrographic surgery. A) Fluorescence imaging before standard excision of a temporal, subdermal tumor that was earlier irradically removed. *Ex vivo* imaging of the specimen showed a fluorescent lesion at the deep resection margin, correlating with a tumor-positive margin on histopathology (red arrows). B) Fluorescence imaging during a MMS procedure. *In vivo* imaging shows a sharply demarcated fluorescent lesion. *Ex vivo* imaging shows a fluorescent lesion at the deep resection margin, which co-localises with tumor on H&E histopathology. The second MMS round did not show any remaining fluorescence signal, and no tumor was found on histopathology. Abbreviations: H&E; Haematoxylin and Eosin, MMS; Mohs Micrographic Surgery.

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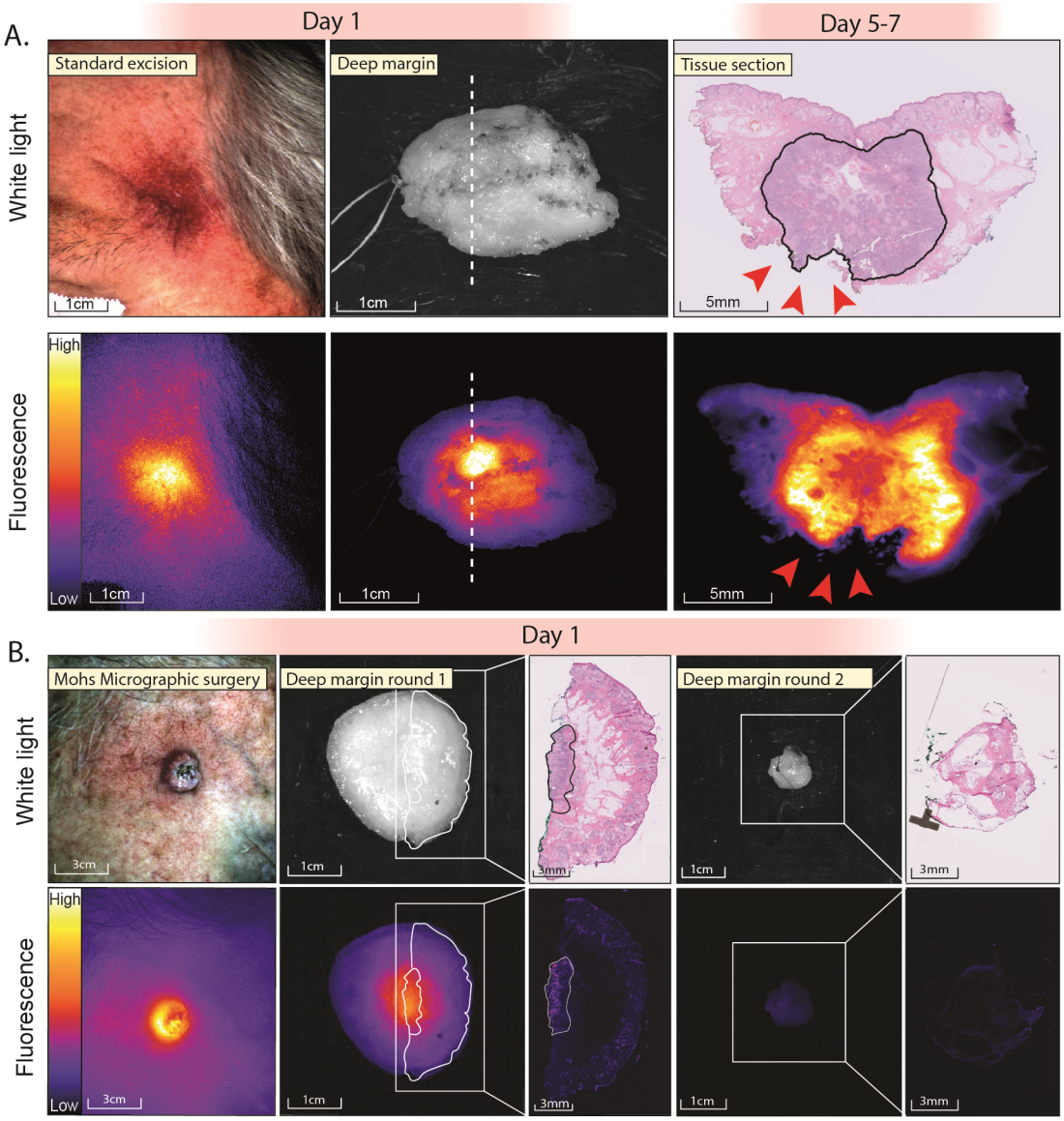

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