Danielle T. Greenblatt,[1] Lynne Hubbard,[1] Christopher Bloor,[1] David Card,[2] John A. McGrath[1] and Jemima E. Mellerio[1]

[1]St John's Institute of Dermatology, Guy's and St Thomas' NHS Foundation Trust, London, UK; and [2]Nutristasis Unit, Viapath, Guy's and St Thomas' NHS Foundation Trust, London, UK
Email: danielle.greenblatt@gstt.nhs.uk

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

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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 tumour 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 tumour visualization, possibly supporting surgical decision making. For example, fluorescence molecular imaging (FMI), a novel imaging method that uses tumour-specific tracers to highlight tumour 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 were included with histology-confirmed cSCC, scheduled for conventional excision or MMS. Two to three days before surgery, patients were intravenously administered cetuximab 75 mg, followed by 15 mg of cetuximab-800CW one hour later.[3] FMI was performed intraoperatively for *in vivo* tumour visualization and *ex vivo* specimen-driven margin assessment.[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 the fluorescence signal with the final histopathology. Mean fluorescence intensities of the tumour and the background were calculated to determine a tumour-to-background ratio (TBR).[3]

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

In total, 12 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 (range 1·82–2·62). The mean TBR of the tumour vs. adjacent tissue in the deep margin (mostly fat) was 3·07 (range 1·85–4·39). Excised cSCC specimens were analysed on the back table, including two conventional excisions and six cases of MMS. In one conventional excision, in 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 tumour-positive margin on final histopathology (Figure 1a). The other conventional excision did not show a fluorescent signal, and the minimal deep margin was 4·8 mm.

In five of six first-stage MMS specimens, we observed a fluorescent lesion at the deep resection margin (Figure 1b). In these five cases, three tumour-positive margins were identified. In the other two cases, the margin was not tumour-positive, but the fluorescent signal colocalized with tumour 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 tumours localized under the surface. No tumour was present on histopathological examination in the MMS specimen that did not show a fluorescent lesion. The three patients with MMS with a tumour-positive margin required an

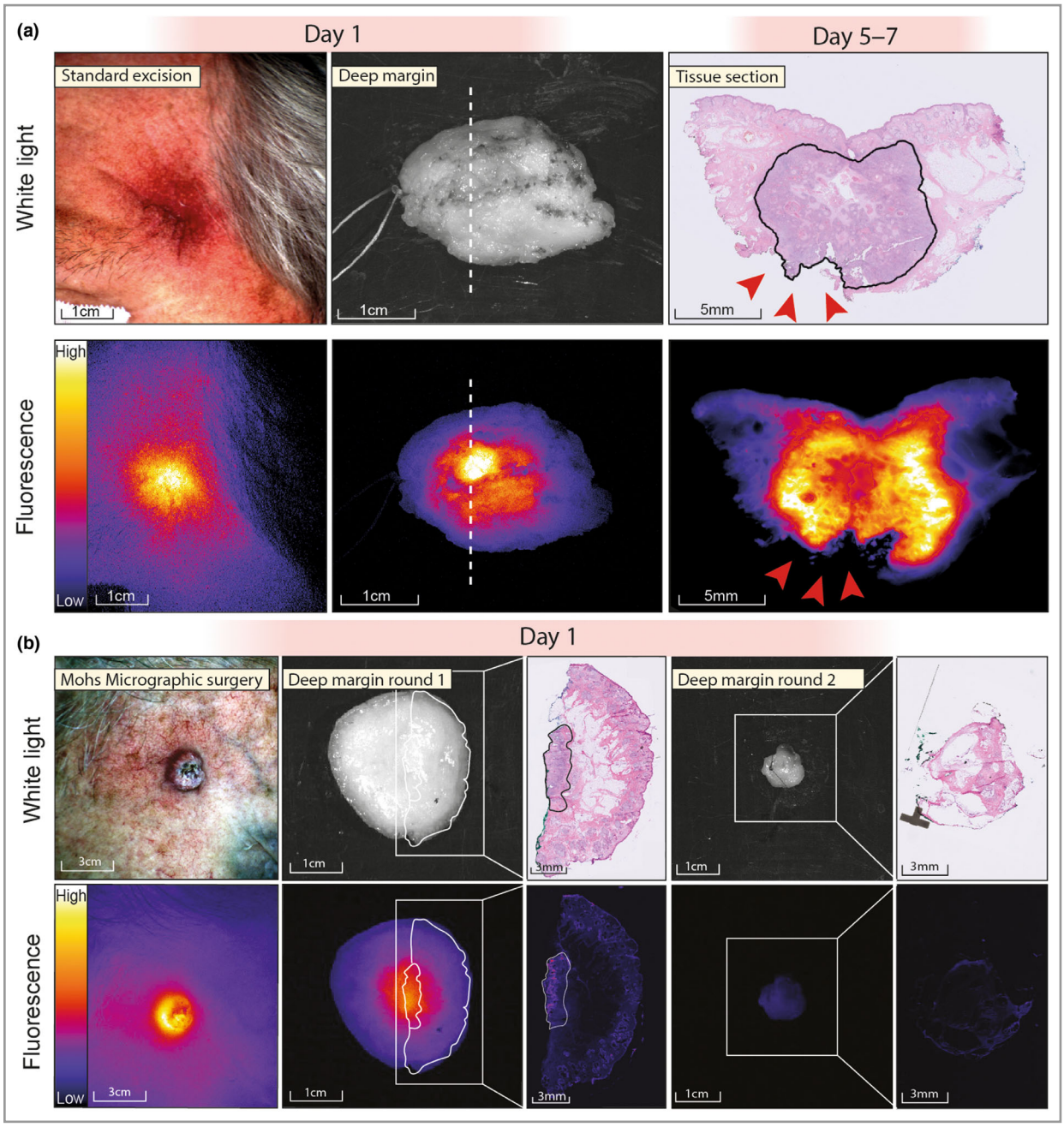

Figure 1 Fluorescence molecular imaging during standard excision and Mohs micrographic surgery (MMS). (a) Fluorescence molecular imaging before standard excision of a temporal, subdermal tumour that was earlier irradically removed. *Ex vivo* imaging of the specimen showed a fluorescent lesion at the deep resection margin, correlating with a tumour-positive margin on histopathology (red arrowheads). (b) Fluorescence molecular imaging during an MMS procedure. *In vivo* imaging shows a sharply demarcated fluorescent lesion. *Ex vivo* imaging shows a fluorescent lesion at the deep resection margin, which colocalizes with tumour on haematoxylin and eosin histopathology. The second MMS round did not show any remaining fluorescence signal, and no tumour was found on histopathology.

additional excision (i.e. after stage 1). None of these showed a fluorescence signal, and all were tumour-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 tumours did not show increased fluorescence signal compared with this background signal during *in vivo* imaging.

EGFR immunohistochemistry showed weak to strong expression in all cSCCs, colocalizing with the fluorescence signal. Low EGFR expression was found in the basal cell carcinoma. The keratoacanthomas showed no EGFR expression, as reported previously.[7]

This proof-of-concept study demonstrates that FMI using the fluorescent tracer cetuximab-800CW can differentiate between tumour and adjacent tissue with high contrast. FMI detected all tumour-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. However, it 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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Jasper Vonk,[1] Jaron G. de Wit,[1] Floris J. Voskuil,[1,2] Marjolein Koldijk,[3] Emőke Rácz,[3] Wouter T.R. Hooghiemstra,[4] Jan J. Doff,[2] Gilles F.H. Diercks,[2] Gooitzen M. van Dam,[5,6] Max J.H. Witjes[1] and Sebastiaan A.H.J. de Visscher[1]

[1]*Department of Oral & Maxillofacial Surgery;* [2]*Department of Pathology & Medical Biology;* [3]*Department of Dermatology;* [4]*Department of Clinical Pharmacy and Pharmacology;* [5]*Department of Nuclear Medicine and Molecular Imaging, University of Groningen, University Medical Centre Groningen, Groningen, the Netherlands; and* [6]*AxelaRx/TRACER BV, Groningen, the Netherlands*

Correspondence: Sebastiaan A.H.J. de Visscher.

Email: s.a.h.j.de.visscher@umcg.nl

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Data availability: All data are available upon reasonable request.

J.V. and J.G.dW. contributed equally to this work and share first authorship.

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Bread loaf sections provide useful information on more than 0·5% of surgical margins

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Dear Editor, Some authors assert that 'bread loaf' analysis of specimens with step sections assesses <0·5% of the surgical margin and that conventional histological resection margin control is an illusion.[1,2] The high cure rates of >90% reported for simple excision of small, low-risk basal cell carcinomas led us to consider the mathematics further.[3] Useful information about margins is inferred 'by proxy' when clear sections are obtained. When the percentage of margins evaluated by proxy is considered, findings more closely align with the excellent results that follow excisional surgery.

Keratinocyte carcinomas (KCs) do not cleave arbitrarily leaving skip areas. Rather, they exhibit cellular adhesion and grow as aggregated cell clusters. Energetic considerations influence three-dimensional growth patterns.[4] KCs typical originate in the epidermis or appendages and grow according to rules of fluid dynamics. If we perform a thought experiment in which a KC is entirely nested in a cube removed by an elliptical excision, we can envision a situation where information can be inferred regarding >40% of the margin (Figure 1). The face of the cube that corresponds to the surface of the skin is not a margin where recurrence can develop, so there are only five clinically relevant faces to consider. As bread loafing proceeds from the centre of the cube outwards to the two lateral faces perpendicular to the long axis, the first tumour-free sections 'north and south' along the long axis predict that any further distal slices will very likely be clear by proxy. Therefore, two of the five relevant faces of the cube (40% of the margin) are assessed even before considering the two lateral faces and the deep face (Figure 1). To check these