

Durable Response of a Recurrent, Irresectable, Locally Advanced, Microsatellite Instable Cutaneous Squamous Cell Carcinoma of the Head and Neck Treated with Cetuximab

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ABSTRACT

Cutaneous Squamous Cell Carcinoma (cSCC) of the head and neck is etiologically associated with age, skin tone, UV light exposure, and also with medical conditions that cause immunosuppression. In most cases, patients with a primary cSCC of the head and neck can be cured by surgical excision and/or radiation therapy. However, in recurrent or advanced tumor stages curation with Radiotherapy (RT) or a surgical approach might be impossible. For these clinical settings, systemic treatment options are available. Palliative platin-based chemotherapy may be harmful to the elderly and/or immune-compromised cSCC patients with comorbidities. For this subgroup, biologicals that target for example the immune-suppressed microenvironment (e.g. the immune checkpoint inhibitors cemiplimab and pembrolizumab) or dysregulated tumorigenic pathways (e.g. the chimeric Immunoglobulin (Ig) G1 monoclonal antibody cetuximab that binds the extracellular domain of EGFR has shown to increase curation rates in unresectable, recurrent and/or metastasized cSCC (R/M cSCC) and is moving into the standard of care. Despite the fact that cetuximab has shown durable clinical responses in cSCC patients, the lack of biomarkers that predict cetuximab response and the unknown mechanisms of resistance are unmet medical needs. In this case report, we show that strong EGFR expression without an underlying EGFR gene mutation or amplification may be a sign of a favorable response to cetuximab treatment in cSCC. The presence of Microsatellite Instability (MSI) might have contributed to the favorable response, but may also be implicated in the final treatment resistance. Because both MSI and high PD-L1 expression were detected in this cSCC case, combined or sequential treatment of cetuximab with immune checkpoint inhibitors should be considered in cSCC management.

Keywords: Cutaneous squamous cell carcinoma; Epidermal growth factor receptor; Microsatellite instability; Cetuximab; Programmed death-ligand 1

Abbreviations: cCR: Clinical Complete Response; cSCC: Cutaneous Squamous Cell Carcinoma; CT: Computed Tomography; DNA: Deoxyribonucleic Acid; EGFR: Epidermal Growth Factor Receptor; FFPE: Formalin-Fixed, Paraffin-Embedded; ICI: Immune Checkpoint Inhibitor; IgG1: Immunoglobulin G1; MMR: Mismatch Repair; Mlh: MutL Homolog; Msh: MutS Homolog; MSI: Microsatellite Instability; NGS: Next Generation Sequencing; PCR: Polymerase Chain Reaction; PD-L1: Programmed Death Ligand-1; R/M: Recurrent/Metastasized; RT: Radiotherapy; UV: Ultraviolet; VAF: Variant Allele Frequency

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INTRODUCTION

Cutaneous Squamous Cell Carcinoma (cSCC), most frequently occurring in the head and neck region, is the second most common skin cancer, which is increasing in incidence worldwide over the last decades due to an aging population, UV exposure, and increasing number of organ transplant recipients [1]. In unresectable R/M cSCC only a limited number of systemic treatments have been shown to increase the overall survival in this prognostically unfavorable patient subset. Conventional, mainly platin-based, chemotherapy schedules have shown only moderate tumor responses in R/M cSCC [2]. Targeted agents with curative potential are limited in R/M cSCC. Only Immune Checkpoint Inhibitors (ICI), creating an immune permissive microenvironment and cetuximab (chimeric IgG1 monoclonal antibody), inhibiting EGFR signaling, have been shown to induce and sustain Clinical Complete Responses (cCR) in a subset of R/M cSCC [3-5]. Although phase III randomised clinical trial results are still lacking, ICIs (e.g. cemiplimab and pembrolizumab) may in the future be considered as first-line treatment of R/M cSCC based upon promising phase II trial results [3,5]. However biomarkers are urgently needed to not only select 'ICI responders' but also define R/M cSCC patient subsets that will benefit from sequential treatment with cetuximab before initiating or after finalizing treatment with ICI. Since resistance mechanisms are still unclear, it is currently unknown if resistance to cetuximab or ICI can be prevented by concurrent treatment with both compounds. Here, we present an elderly patient, diagnosed with a recurrent irresectable cSCC successfully treated with cetuximab. Molecular analysis of the cSCC at the time of the first recurrence and at the time of progression during cetuximab treatment allowed us to hypothesize about potential biomarkers of response to cetuximab, mechanisms of resistance, and treatment options for cetuximab resistant cSCC.

CASE REPORT

A 75-year old Caucasian male was diagnosed with a cSCC (cT1N0M0) in the left pre-auricular region in May 2011. After an irradical primary excision of the tumor, margins were reported to

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be negative after re-excision. In December 2011 however, a crust developed in the scar, of which biopsies showed cSCC recurrence. In February 2012 a second re-excision with total parotidectomy was performed showing a cSCC with tumor-free margins ≤ 1 mm, peri- and intraneural invasion of the facial nerve, and a lymph node metastasis in level II (rpT3N1M0). Resection was followed by postoperative local and regional RT (51.2 Gy and 66 Gy respectively). Clinical evaluation four months after finalizing RT showed cCR. In November 2012 biopsy of a painful pre-auricular cutaneous lesion showed a poorly differentiated cSCC, indicating a new local recurrence (Figures 1A and 1B); without evidence of hematogenous or lymphogenous spread. Neither resection nor re-irradiation of the cSCC recurrence was feasible and therefore palliative chemotherapy was suggested. However, due to cardiac and renal comorbidity, a platin-containing chemotherapy schedule was contraindicated. Cetuximab treatment was started in February 2013 and dosed weekly (first dose 400 mg/m², followed by weekly dose of 250 mg/m²) [1]. One month after initiation, the CT-scan showed Stable Disease (SD). At the end of 2013, almost a year after start of the therapy, the pre-auricular tumour showed complete clinical clearance, and cetuximab was continued (Figure 1C), until progression in December 2014, when a pre-auricular tumour reoccured during cetuximab treatment (Figure 1D). Cetuximab was discontinued in March 2015.

MATERIALS AND METHODS

We analyzed three different cSCC tissue samples during the period from February 2012 until February 2015. The first sample consists of tissue from the first cSCC recurrence pre-auricular left, obtained by surgical resection before the start of radiation. The second sample was derived through biopsy from the recurrence in November 2012, before the start of the cetuximab. The last sample was taken in early 2015 and contains tissue of the progression during cetuximab treatment. Immunohistochemistry on 3 um thick Formalin-Fixed, Paraffin-Embedded (FFPE) tissue sections for EGFR (a mixture of clone EGFR.384 (Novocastra) and E30 (Dako)), Mismatch Repair (MMR) proteins (clones Msh2, Msh6, Mlh1, Pms2 (Dako)), and PD-L1 (clone 22C3 (Dako)) expression was performed on Dako



Figure 1: CT-scans from commencement of Cetuximab to discontinuation. A; B) January 2013, baseline CT-scan before the start of Cetuximab, showing large ulcerating defect parotid region and bone destruction of the mandibula. C) End of 2013 with a complete clinical clearance under Cetuximab, showing a decrease in ulceration in the parotid loge. D) December 2014, progression of the tumor during Cetuximab with ulceration and destruction of the surrounding soft tissue and bone.

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Link48 autostainers according to the manufacturer's protocol. For molecular analyses, tumor and normal DNA was isolated by microdissection of 3um sections from routine formalin-fixed and paraffin-embedded tissues. Indicated by a pathologist, tumor DNA was derived from about 30% neoplastic and 70% normal cells. MSI testing was performed by PCR amplification of the monomorphic microsatellite markers BAT25, BAT26, CAT25, NR21, NR22, NR24. Fluorescence *In Situ* Hybridization (FISH) using the Vysis EGFR LSI CEP7 dual-color probe mixture (Abbott Molecular) on 3 um thick FFPE slides was performed according to the manufacturer's instructions. Finally, next-generation sequencing with custom-made amplySeq gene panels including the EGFR and MMR genes (information available upon request) were run on an Ion Torrent NGS instrument (Ion GeneStudio S5 Prime System) according to the manufacturer's instructions (Life Technologies).

RESULT

The pre-auricular recurrent cSCC (2012) showed strong membranous EGFR immunostaining at the start of cetuximab treatment, which was preserved at progression under treatment (2015) (Figures 2). No EGFR gene mutation or amplification was found both by NGS and FISH (not shown). The same MSI pattern was present in the primary tumor (not in the normal tissue), as well as in its recurrences, as demonstrated by PCR (not shown), in combination with loss of Msh2 and Msh-6 expression (Figure 2 and Figure 3). Two pathogenic somatic mutations in the MSH2 gene, i.e. MSH2 exon 6: c.1013G>A;p.G338E, Variant Allele Frequency (VAF) 17% and MSH2 exon 13: c.2038C>T; p.R680X, VAF 23% could be identified in the recurrent cSCC (2012). Interestingly, the recurrent cSCC showed only focal weak PD-L1 expression in 5% of tumor cells before the administration of Cetuximab (2012) (Figure 2), while the Cetuximab-resistant recurrence more than two years later showed strong membranous PD-L1 expression in 100% of tumor cells (2015) (Figure 2).

DISCUSSION

In this case report we describe the molecular analyses of a recurrent cSCC of the pre-auricular skin of an elderly patient, who was successfully treated with an EGFR inhibitor, cetuximab (chimeric IgG1 monoclonal antibody). After initial cCR, the cSCC locally recurred after 2 years of cetuximab treatment. This

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long and durable tumour response is quite exceptional. In a phase II trial the efficacy of cetuximab was assessed in 36 patients with unresectable cSCC [4]. In this prospective study, disease control rate was reported as 67% and the median duration of partial or complete response was 6.8 months. In only two of those complete responders, the cCR could be retained up to 2 years; comparable to the current case report. Biomarkers to select cetuximab responders are currently lacking in cSCC. Our data indicate that high EGFR expression without an underlying gene alteration might be a sign for a favourable response to cetuximab treatment (Figure 2 and Figure 3).

Only limited data of MSI high cSCC of the head and neck are available and it is suggested that this molecular entity is correlated with a favourable prognosis [6]. Our analysis of the primary cSCC of the reported patient showed loss of Msh2 and Msh6 protein expression. Also, MSI was found which is associated with an increased rate of mutations due to defects in the MMR system [7]. Analysis of the local recurrence showed preservation of loss of MSH2 and MSH6 expression (Figure 3). By NGS two MSH2 mutations were found in the tumor DNA indicative of bi-allelic inactivation of the MSH2 gene in the tumor cells. It should be emphasized that these MSH2 mutations were somatic and this patient was neither known with Lynch syndrome nor Muir-Torre syndrome. One could hypothesize that the MSI in the tumor tissue induced an immunogenic microenvironment in which cetuximab induced antibody-dependent, cell-mediated cytotoxicity is facilitated and the development of cytotoxic lymphocytes was stimulated. Resistance mechanisms of tumor cells to cetuximab treatment are currently not known in EGFR Wild Type (wt) overexpressing cSCC. In those tumor cells with MSI, treated with cetuximab, T-cell exhaustion, and thus an immune-suppressive tumor microenvironment could be induced by upregulating PDL-1 expression on tumor cells [8]. In this patient we show a high level (TPS>50%) of PD-L1 expression by the cSCC cells, already at primary diagnosis; increasing in intensity at the time of progression (Figure 3). Our observations warrant further investigation into MSI-deficient DNA MMR and PD-L1 expression in EGFR wt overexpressing cSCC. Both MSI and high PD-L1 expression are tumor agnostic predictive markers for ICI treatment response. For irresectable R/M cSCC, ICIs (e.g.



Figure 2: Recurrent cSCC before administration of anti-EGFR therapy (November 2012). Top row (from left to right): Representative HE (100x); Strong membranous EGFR expression in tumor cells (magnification 400x); Partial and weak PD-L1 expression in 5% of tumor cells (400x); Bottom row: Loss of MSH2 and MSH6 expression in tumor cells (with positive internal lymphocytes control), whereas PMS2 shows retained nuclear expression (100x). Nuclear counterstaining was performed with hematoxylin.



Figure 3: Recurrent cSCC during anti-EGFR therapy (2015); Top row (from left to right): representative HE (100x) of moderately strong uniform membranous EGFR expression in tumor cells (400x); strong and uniform membranous PD-L1 expression in 100% of tumor cells (400x). Bottom row (100x): Loss of MSH2 and MSH6 expression in tumor cells (with positive internal lymphocytes control); PMS2 shows retained nuclear expression.

cemiplimab, pembrolizumab), have shown promising results in phase II clinical trials [3,5]. Whether cetuximab will increase ICI response, when administered in a combined treatment schedule in cSCC, by increasing tumor antigens and upregulating PD-L1 expression, remains to be investigated.

AUTHOR CONTRIBUTIONS

Dr (s) Ann Hoeben and EJM Speel, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Ann Hoeben, EJM Speel, Acquisition, analysis, and interpretation of data: Ann Hoeben, Postma AA, van den Hout MFCM, Dinjens WNM, EJM Speel, Drafting of the manuscript: Ann Hoeben, Hartgerink DEJ, EJM Speel, Critical revision of the manuscript for important intellectual content: All authors. Obtain funding: Ann Hoeben.

CONFLICT OF INTEREST

None.

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