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LETTER TO THE EDITOR

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Distinguishing recurrence and new primary tumor as well as the origin of neck metastases in head and neck cancer clinical trials by targeted DNA sequencing

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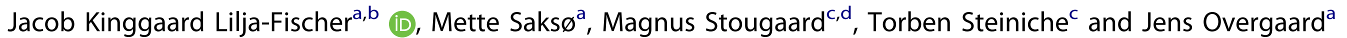

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Introduction

Patients with head and neck squamous cell carcinoma have a high incidence of recurrences and new primary tumors (NPT) [1,2].

Distinguishing between recurrent disease and NPT at the primary tumor site is often impossible using clinical examination or imaging [3]. In patients with synchronous or metachronous head and neck squamous cell carcinoma, the origin of a neck lymph node metastasis is also not possible to establish with certainty. In some cases, histologic differentiation and immunohistochemistry may be of some value, but most often squamous cell carcinomas are moderately differentiated, which may change during tumor progression, and most cases of recurrence or NPT are p16-negative. This distinction is mostly of less clinical importance, since treatment decisions are mostly determined by feasibility of surgical resection or re-irradiation.

However, in the setting of a clinical trial, this distinction affects assessment of treatment efficacy. In randomized clinical trials, risk factors for the development of NPT should be balanced between treatment arms and are thus less likely to directly affect efficacy estimates. However, it could lead to reduced statistical power and imprecise absolute estimates of recurrence rates. Smaller clinical trials are less likely to be balanced and obviously have less statistical power to establish treatment effect, and are consequently more damaged by the confounding effect of NPT.

In this paper, we describe how to utilize the increasing availability of DNA sequencing to distinguish between local recurrence and NPT, as well as to determine the origin of a neck metastasis. This technique could be especially useful in a clinical trial setting.

Methods

In the DAHANCA 24 clinical trial, 40 patients were treated with curatively intended radiotherapy. The trial aimed to establish the prognostic effect of hypoxia imaging using a pretherapeutic FAZA PET/CT, which was demonstrated with a median follow-up of 19 months [4]. Recently, the cohort was updated with long-term follow-up [5].

One patient was initially treated in the DAHANCA-24 trial with primary radiotherapy for a squamous cell carcinoma of the right supraglottic larynx, staged T4a N1 M0 (UICC 7th edition). One and a half years after treatment, the patient developed a squamous cell carcinoma of the left side of the oral cavity, which was considered a new primary tumor and was treated with local surgical resection. After a further six months of follow-up the patient developed a squamous cell carcinoma lymph node metastasis to the left side of the neck, which was treated with an ipsilateral neck dissection.

The oral cavity tumor was most likely an NPT, and the lymph node metastasis most likely originated from this, but the precise distinctions had no clinical implications. However, in the context of the DAHANCA 24 clinical trial, we wished to increase the strength of this interpretation, to determine whether the patient had a treatment failure of radiotherapy. To aid in this distinction, we performed cancer-gene targeted sequencing of the 22 genes included in the Ion AmpliSeq Colon and Lung Cancer Research Panel v2 using the Ion Torrent S5 platform (Thermo Fisher Scientific). Briefly, DNA was isolated from formalin-fixed, paraffin embedded tumor samples. Library preparation, amplification, and sequencing were performed according to the manufacturer's instructions. The Ion Reporter software version 5.6 (Thermo Fisher Scientific) was used for variant calling.

We found that the first laryngeal primary tumor, treated with radiotherapy in the DAHANCA 24 trial, harbored a *TP53* mutation, c.833C > A translating in to p.P278H. The second tumor of the oral cavity and the subsequent neck lymph node metastasis were both found to have in common another *TP53* mutation, c.538A > T (p.I195F), see Figure 1. Both mutations are outside hotspot areas, but are considered pathogenic. Eight common polymorphisms were identified in all three samples, confirming that they were from the same patient. We thus determined that the oral cavity tumor was an NPT and not a recurrence, and that the neck recurrence originated from this NPT.

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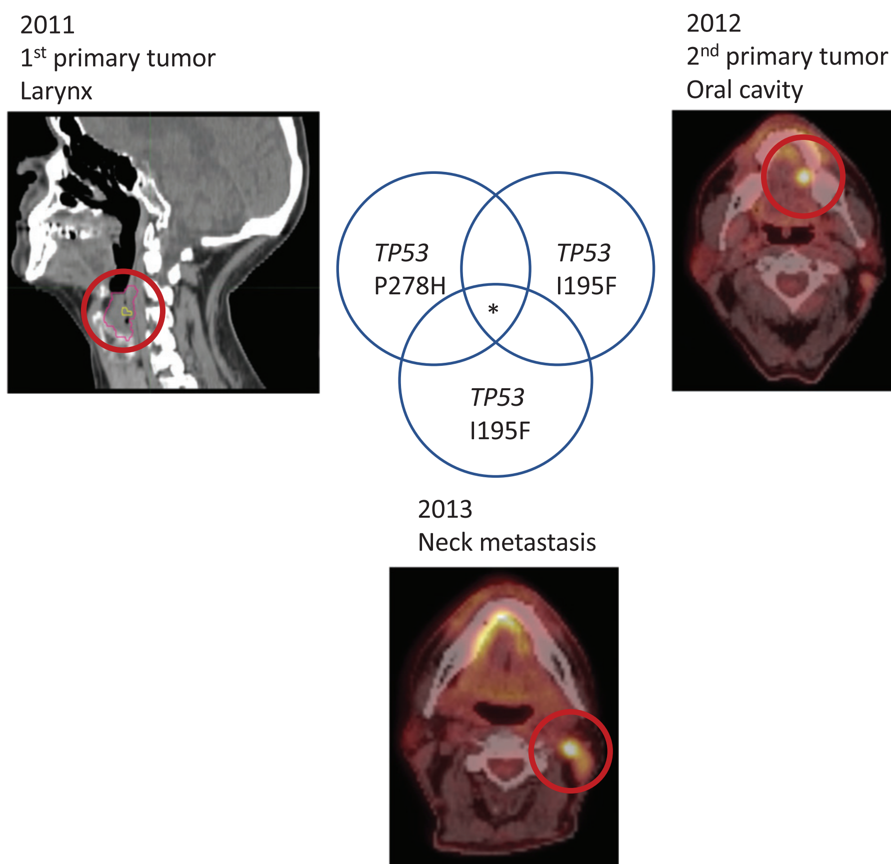

Figure 1. Relation between two primary head and neck tumors and one nodal metastasis. Gross tumor volume and FAZA-hypoxic volume are depicted in the CT-scan of the 1st primary tumor. The two other scans are FDG-PET/CT. (*) Eight single-nucleotide polymorphisms were present in all three samples.

Based on these results, we concluded that the patient had not had a recurrence from his original laryngeal cancer treated with radiotherapy in the DAHANCA 24 trial.

Discussion

TP53 mutations are very frequent in non-HPV-related head and neck squamous cell carcinoma, affecting 86% in the TCGA study and are as such an attractive target for mutation analysis [6]. As a driver mutation, *TP53* mutations are likely to be retained in all tumor cells regardless of subsequent tumor heterogeneity. Although there are hotspot regions, the most frequent *TP53* mutation in the COSMIC database (c.524G > A, p.R175H) only comprises less than 5% of mutations. (COSMIC 20/12-18: 87 of 3016 samples with *TP53* mutations). This translates in to a risk of less than 0.2% that two unrelated tumors would contain the same *TP53* mutation by chance only. Although previous studies have found perfect concordance between *TP53* mutations in primary tumors and corresponding lymph node metastases, we plan to confirm our findings in a larger study to further inform the precise interpretation of sequencing results in individual patients [7]. Inclusion of other defining mutations in the gene panel may help further discrimination.

In conclusion, tumor mutation analysis is an attractive technique to distinguish between recurrent or new primary head and neck cancer, as well as to determine the origin of neck metastases. This may have particular interest in the context of clinical trials.

Disclosure statement

The authors report no conflicts of interest.

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