Treatment choice for locally advanced head and neck cancers on the basis of risk factors: biological risk factors

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Patients with locally advanced head and neck squamous cell carcinoma often experience relapse, the cause of poor survival statistics. Relapse occurs following the three main types of treatment, surgery with or without post-operative (chemo)radiotherapy, or chemoradiation (containing cisplatin). Cancer relapse can result from (i) outgrowth of residual tumour cells, sometimes with a number too small to be detected by routine histopathology or (ii) development of another carcinoma in a field of pre-neoplastic cells that has remained after treatment of the primary carcinoma. At this moment, clinical staging is not enough to identify patients who will develop relapse and who need tailored treatment. This review describes the latest knowledge of mechanisms of cancer relapse, addresses the biomarkers of potential interest detectable in the tissue of the tumour or its surgical margins and discusses three biomarkers, human papillomavirus, TP53 and epidermal growth receptor in more detail. Once a marker panel has been established, treatment should be focussed on the patients at risk of relapse by improved tailoring of existing treatment modalities. Also, the implementation of more targeting therapies based on the characteristics of the discovered markers should lead to better survival rates.

Key words: biomarker, head and neck cancer, oral cancer, prognosis, recurrence, relapse

Head and neck squamous cell carcinoma (HNSCC) arises in the oral cavity, oropharynx, larynx and hypopharynx, and is the sixth leading cancer by incidence worldwide [1]. The overall 5-year survival for the advanced stages is ∼50% and disappointingly, this has not markedly improved in the last decades, because of the fact that patients frequently develop relapse at the primary site, distant metastases and second primary tumours (SPT) [2].

About one-third of patients present with early-stage disease, and these patients are treated with surgery or radiotherapy and have a favourable prognosis. Patients with locally advanced tumours of the oral cavity are mainly treated by surgery combined with post-operative (chemo)radiotherapy. In the last decade, organ-preservation protocols using combined chemoradiation (combined systemic treatment with cisplatin and loco-regional radiation) has become the standard of care for locally advanced oropharyngeal, hypopharyngeal and laryngeal carcinomas. The very advanced hypopharyngeal and laryngeal carcinomas are treated by surgery followed by post-operative (chemo)radiation.

At this moment, clinical staging and pre-treatment analysis of the biopsy do not allow identification of the patients who will experience relapse. There is the hope that further unravelling of the molecular carcinogenesis of HNSCC will lead to the application of biomarkers, aiming to improve tailoring of existing treatment modalities for the individual patient and to develop novel therapies. This review describes details on HNSCC relapse and the current biomarkers with a potential role in the prediction of relapse.

mechanisms underlying cancer relapse

Relapse is generally designated to be local, if it develops within 3 years and within 2 cm in relation to the primary carcinoma and SPT, if these criteria are not met [3]. The clinical decision-making on the type of relapse is imperfect as it is often not capable of discriminating an SPT from a distant metastasis in the lung, and it has been discovered that the molecular origin of a local relapse as well as an SPT in the head and neck region is twofold. Comparison of genetic markers involving the primary lesion enables discrimination of an SPT from a distant metastasis. To understand the two ways by which a local relapse and an SPT can develop and the implications this has, some more explanation is provided.

The first and most obvious explanation for the development of a local relapse is that cancer cells grow out again despite therapy, be it surgery, (chemo)radiotherapy or combinations. Residual malignant cells are then the origin of such a local relapse. When after surgery the margins have been proved to be free of cancer by histopathology, there is still the possibility that a very small number is the cause of local...
relapse; this phenomenon is known as minimal residual cancer (MRC).

There is a second explanation for local relapse, being in line with the so-called 'field cancarization' process. Using genomic and protein markers, it was shown that a significant proportion of oral and oropharyngeal carcinomas are surrounded by mucosal epithelium that can be considered pre-neoplastic [4–7]. Specific information of genetic markers, e.g. allelic imbalance at certain locations and type of TP53 mutation, has revealed that there often is a clonal relationship between the primary tumour and the tumour-adjacent pre-neoplastic epithelium [4, 7]. This knowledge together with the results of molecular risk assessment studies of leukoplakia [8, 9] have led to the proposition of the field-carcinogenesis model [10]. The basic principle of this model is that during carcinogenesis, a field of pre-neoplastic epithelium precedes the development of cancer. This field of cells with genetic alterations develops and expands by lateral displacement in a process of 'Darwinian' clonal selection at the expense of uninvolved non-neoplastic tissue [11]. The majority of these fields are not visible and do not give any symptoms despite their sometimes large dimensions. In the course of time, a cell within the field may develop into a cancer cell as a result of a series of crucial genetic hits, and this cell may evolve into an invasive carcinoma. Importantly, when after diagnosis and excision of a primary HNSCC, the precursor field remains, there is the continuous, and in theory life-long, threat for relapse at or near the site where the primary tumour was located. Such another tumour in a field that also caused the index tumour has been labelled second field tumour (SFT) to emphasize its unique origin [10]. It has to be realized that it may take some time before an SFT develops. In that case, some physicians are inclined to designate it as what is clinically known as an SPT (definitions have already been given). The term ‘SPT’ has been proposed for tumours that arise in a manner that is completely independent from the way the primary tumour has developed [3]. Figure 1 shows the various types of cancer relapse in HNSCC.

Two studies assessed the origin of local relapses, clinically defined as earlier, in patients who underwent histologically radical surgery and found that approximately half of the cases were actually from a field that remained behind, and the other half was the result of the outgrowth of MRC [12]. This study concerned treatment of the primary HNSCC with surgery alone or surgery with post-operative radiotherapy. Also, after radiotherapy of laryngeal cancer as a single treatment modality, local relapses appeared to have developed via these two same mechanisms [13]. Some, on the basis of a 100% genetic similarity, could be considered local relapses from residual cancer cells and others that share a part of the genetic markers with the index tumour could be considered SFT from the precursor field that was apparently not eradicated by irradiation.

Figure 1. Field cancarization and relapse. The relationship between field cancarization and types of relapse is shown. On the basis of recent molecular findings, field cancarization is defined as the presence of one or more mucosal areas consisting of epithelial cells that have cancer-associated genetic or epigenetic alterations. A precursor field (or field; shown in light blue) is monoclonal in origin and does not show invasive growth or metastatic behaviour, which are the hallmarks of an invasive carcinoma. A field is pre-neoplastic by definition; it may have histological aberrations characteristic of dysplasia, but not necessarily. An important clinical implication of a field is that it may be the source of local relapses and second primary tumours after surgical resection of the initial carcinoma. These two possibilities can be distinguished clinically on the basis of their distance from the index tumour or the time interval after which they develop (whereby a local relapse is <2 cm away from and occurs within 3 years of the primary tumour; a second primary tumour is >2 cm from or occurs >3 years after the primary tumour). Additional genetic changes are needed to transform a field into a new carcinoma. The field and primary tumour share genetic alterations should be considered as having a common clonal origin. Tumours that do arise in a non-resected field have been described as ‘second field tumours’ as opposed to true local relapses (which develop from residual tumour cells) or true second primary tumours (which have an origin that is independent of that of the first tumour). This process has been summarized in an animation that can be found in the VU Medical Center website http://www.vumc.nl/afdelingen/kno/1463998/1839021/4871476/4871481/. Reprinted and adapted with permission from the publisher [2].
margin analysis with surgery as primary treatment

To identify patients who will experience local relapse, biomarker research can be done on the carcinoma itself (also after radiotherapy or chemoradiation, see below), but it is most attractive to perform this type of research on the resection margins, which are the location closest to the site at risk. According to standard histopathology protocols in most institutions, the mucosal margins are investigated for the presence of carcinoma and dysplasia. Similarly, the deep margin is examined in one or more central sections of the tumour. The margins are labelled ‘clean’ or ‘clear’ when there is a distance of >5 mm between carcinoma and the margin. These are classified as ‘involved’ when there is cancer in or within 1 mm of the margin. There is also a gray area that is known as ‘close’, which refers to a distance of 1–5 mm between the tumour and the margin. Most publications demonstrate a relation between involved or close margins and the development of local relapse [14].

There are two situations where the standard histopathology has its limitations and the introduction of biomarkers is urgently needed. First, this is the case when routine histopathology does not detect cancer in the margin, but MRC may be present. To detect MRC-sensitive molecular methods, for instance based on sensitive detection of a specific TP53 mutation, have been proven to be of value [14]. With this technique, it is possible to reach 100% sensitivity in identifying the HNSCC that had a local relapse in two independent studies [15, 16]. There is, however, also a downside with this assay, namely the considerable workload and the relatively low specificity of 40%.

There is a second situation where the standard pathology shows its shortcomings, in case a pre-neoplastic field has remained behind and dysplasia in the mucosal margins may have been diagnosed. The diagnosis itself has problems, regarding objectivity and reproducibility, and there is no consensus on whether treatment should be adopted. Here, there is an important potential role for biomarkers for better risk assessment. Various biological markers have been investigated mostly in mucosal margin samples and a considerably large panel with a potential application for local relapse risk assessment has been reported in the literature. These are protein markers, detected by immunohistochemistry, with p53, cyclinD1 and p16 as the most prominent examples. DNA-based techniques measure DNA-copy number changes (such as interphase FISH, CGH and DNA-ploidy), promoter-methylation (e.g. p16), allelic imbalance (e.g. at 3p, 9p and 17p) and mutation analysis (e.g. TP53). For a more detailed information, one is referred to a recently published review that describes the performance of these markers and recommendations for future studies [14]. In addition to that review, a 4-gene profile was recently reported that was highly predictive for local relapse of oral cancer [17].

Not only from the conceptual, but also from the therapeutic point of view, discrimination between a field and MRC as a risk of the development of cancer relapse is important. When after primary surgery, MRC is detected, immediate post-operative radiotherapy or a second resection is warranted. In case of a field-at-risk, the situation is less clear. Surgery of the field is possible but in general not easy, because of possibly large dimensions of the field and the fact that the majority is not visible to the naked eye. The decision to give post-operative radiotherapy is the topic of discussion. There are some arguments for a contra-indication of radiotherapy. A field concerns pre-neoplastic tissue and radiotherapy might in theory accelerate the carcinogenic process. In support of this, it has been reported that post-operative radiotherapy of a primary HNSCC increases the risk of a SPT [18, 19]. Nevertheless, there are also reports that present arguments favouring radiotherapy as treatment of a field, showing a similar or a less frequency of SPT after radiotherapy [20, 21]. Unfortunately, it is hard to estimate the value of the evidence from these four publications as these are clinical observational studies without information on the genetic make-up of the field at risk and the genetic relation between the primary and the secondary carcinoma.

Therapy for fields is a challenge and represents a new area of translational and clinical research. Biomarker validation studies are indispensable to identify patients most at risk and monitor the disease process and the treatment outcome. Biomarker information is also crucial when setting up new treatment studies, as they provide leads for targeting drugs. Unfortunately, no biomarker has arrived at the stage of clinical application at the moment.

tumour analysis for relapse prediction

Locally advanced HNSCC is sometimes treated with chemoradiation, ideally a combination of systemic cisplatin and loco-regional radiotherapy. In recent years, therapy targeted at the epidermal growth receptor (EGFR) has been introduced. EGFR-targeted therapy with the antibody erbitux in combination with radiotherapy was shown to be more active than radiotherapy alone [22]. Patients treated with chemoradiation can relapse from a field or from MRC in a similar way to patients who are primarily treated with surgery. Unfortunately, it is not possible to study margins in this first group; one normally has to rely on a tumour biopsy to study biomarkers of risk. Though possible in theory, it will be difficult from the ethical point of view to gather a sufficient number of biopsies from the macroscopically normal tissue before treatment is started. When studying this type of relapse in a primary chemoradiation setting, one always has to keep in mind that the therapy may have exercised a selective pressure on the cancer cell phenotype, making it possible to identify markers in the pre-treatment biopsy that are associated with treatment resistance, known as ‘predictive’ markers. In that respect, additional information can be derived from marker analysis of the relapse, for instance to assess whether treatment has selected cells with a specific geno- or phenotype.

Many studies have been performed on establishing the prognostic value of biomarkers in pre-treatment HNSCC tissue (as reviewed in [23] and [24]). An important variable in this type of studies is related to the patient: the choice of the end point (overall, disease-specific, and relapse-free survival), the tumour site and therapy (surgery, surgery with radiotherapy, radiotherapy or various types of chemoradiation). But also, at
the biomarker detection level, there is variation regarding the method and interpretation of the data. Markers can be investigated for expression (at the mRNA- or the protein level, the latter often with immunohistochemistry, methylation, DNA copy number alterations or specific DNA mutations). A recurring theme in this type of investigations is the pathways that contain the markers of interest: DNA-repair (e.g. XRCC-genes), cell motility and invasiveness (e.g. MMP-genes and keratins), metabolism, cell cycle (e.g. CDKs and cyclins) and signal transduction (growth factor receptors and downstream kinases). In addition to markers related to proteins encoded by genes, initial data suggest that expression of miRNAs is of potential interest for prognosis of HNSCC, as reviewed by Wua et al. [25].

It is not the scope of this review to discuss in detail all potential markers. Most markers can be considered promising and their real value must be clarified after confirmation in additional studies, taking into account the variation regarding patient characteristics and detection methods. Three biomarkers and one environmental factor will be described in more detail; they have been studied extensively, and rather convincing evidence for their prognostic and predictive value considering relapse has been established for two of them: human papillomavirus (HPV) and smoking tobacco; for two of them, TP53 and EGFR, there is still controversy regarding their role.

**risk factors for HNSCC relapse**

**tobacco consumption**

Cigarette smoking is a well-known risk factor for HNSCC, and there is a direct relationship between the total amount of tobacco consumed and the cancer risk [26]. In addition, the cumulative amount of tobacco consumed is directly related to relapse-free survival, i.e. the higher the exposure the shorter the survival [27]. This effect is particularly observed when the patients are treated with chemoradiation containing cisplatin [28-30]. The biological basis for this effect has not been discovered yet. Tobacco contains a plethora of toxic compounds, and it is conceivable that long-term exposure to these compounds has made cancer cells resistant to the DNA damage imposed by cytotoxic drugs such as cisplatin.

**HPV**

In a subgroup of HNSCC patients, HPV plays a causative role [2]. The virus is particularly common in cancer of the oropharynx, where the percentage of HPV-positive tumours varies between 20% and 80%, depending on the geographic location and the time period of analysis. It is well established that patients with a HPV-positive carcinoma have a better prognosis, in particular, after radiotherapy and chemoradiation [31, 32]. The improved prognosis may to a large extent be attributed to a better loco-regional control [28]. The biological explanation for the better prognosis for patients with a HPV-positive HNSCC remains to be established. Increased sensitivity to radiotherapy or anticancer drugs, or immune-related factors may play a role.

**EGFR**

Despite the fact that sufficient evidence is available that EGFR can be considered an established cancer gene in HNSCC [2], controversy over the relation between EGFR and relapse-free survival exists. In general, 60% of the studies show an association between over-expression and outcome, whereas 40% do not [2]. This may be related to the difference in protocols of the immunostaining procedures, as exemplified by the large variation in the reported prevalence rates of over-expression HNSCC [2]. An alternative and potentially a more objective method for measuring EGFR alteration is to assess gene amplification, for instance with FISH, and this may be a better prognostic than protein expression [33]. The association between EGFR gene amplification and response to chemotherapy of cisplatin/5-fluorouracil (5-FU) with erbitux, an antibody directed to the receptor, has been studied only in a single study [34]. Unexpectedly, no association of EGFR copy number with overall survival, progression-free survival or best overall response was found for patients treated with cetuximab and platinum/5-FU. It has to be added that these were patients with recurrent or metastatic disease, and it cannot be ruled out that previous treatment played a role. More studies with the measurement of EGFR gene amplification are needed to establish the definitive importance of this biomarker.

**TP53**

Because of its important role in carcinogenesis, a mutation in TP53 in tumour tissue has extensively been investigated as a marker of prognosis in HNSCC. Despite a large number of studies, no conclusive evidence has been obtained [35]. This could be related to patient- (low numbers and variation in tumour sites and stage) and technique-related factors (subjective immunohistochemical scoring). Recent studies provide evidence that the type of mutation is important for prognosis prediction. Disruptive mutations [36] and, in particular, the mutations that result in a truncated protein are most discriminative [37]. More studies with type of mutation classification are needed to establish the value of TP53 as a biomarker.

**future outlook**

Currently, the value of the use of biomarkers for relapse prediction is insufficient for implementation in a clinical setting. More attention should be focussed on the robustness and accuracy of the assays. The ultimate validation should be done in multiple independent patient groups, with strict assay protocols and fixed cut-off levels. An integral part of such research should be the detection of whether the anticipated marker panel differs by the type of endpoint, whether a relapse is from residual cancer or from a remaining field. Once a marker panel has been established, treatment focus should be laid on the patients at risk.

Improved tailoring of existing treatment modalities and the implementation of target therapies on the basis of the characteristics of the discovered markers should lead to better survival rates.
disclosure

The authors have not declared any conflicts of interest.

references