

New opportunities to decrease the impact of head and neck cancer

Citation for published version (APA):

Demers, I. (2024). *New opportunities to decrease the impact of head and neck cancer: uncovering HPV awareness, viral integration, and treatment options in preclinical models*. [Doctoral Thesis, Maastricht University]. Maastricht University. <https://doi.org/10.26481/dis.20240327id>

Document status and date:

Published: 01/01/2024

DOI:

[10.26481/dis.20240327id](https://doi.org/10.26481/dis.20240327id)

Document Version:

Publisher's PDF, also known as Version of record

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

Take down policy

If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

Summary

Head and neck squamous cell carcinoma (HNSCC) is the most common type of head and neck cancer (95% of cases) and represents 5% of all cancer diagnoses worldwide with an overall mortality rate of approximately 50%. HNSCC covers a heterogeneous group of tumors, leading to several clinical challenges in its prevention and management, including increasing incidence of HPV-related tumors, high recurrence rates, substantial treatment-related side effects, and difficulties to predict treatment efficacy for individual patients. This thesis aimed to 1) assess and increase HPV awareness among the population and general practitioners (GPs) in The Netherlands with the goal to increase HPV vaccination coverage and stimulate early detection; 2) improve the knowledge on HPV genome integration and associated detection techniques in order to identify prognostic biomarkers; 3) investigate potential new targeted therapies to improve treatment options; and 4) explore the suitability and applications of tumor-derived culture models to guide personalized treatment for HNSCC patients (**Chapter 1**).

While the incidence of tobacco related HNSCC has declined in the past two decades, there is an increase in HPV-related oropharyngeal cancer (OPSCC) cases in Western countries. In contrast to patients with non-HPV-related OPSCC, patients with HPV-related OPSCC are generally younger, more often male, have a higher socioeconomic status, and are less likely to have a history of extensive tobacco and alcohol use. Knowledge about HPV and the association of HPV and OPSCC, both in the general population and among health care professionals, will promote HPV vaccination coverage, early detection of the disease, and ultimately patient outcome. In **Chapter 2**, the current knowledge on HPV and HPV-related OPSCC among the general population in The Netherlands was explored using a cross-sectional survey study. Our data revealed that 30.6% of the participants (N=1044) had heard of HPV. This awareness was significantly lower in males, participants older than 65 years of age, participants with low educational level and current smokers. Of the participants who had heard of HPV, 29.2% was aware of the causative link between HPV and OPSCC (11% of total population). In addition, almost 50% of participants knew about the existence of an HPV vaccine. Our findings indicate that targeted knowledge in the Dutch population is lacking and increasing the awareness of HPV is required to improve vaccination coverage and (primary) prevention. In this context, the results of this thesis have been used for the national 'Make Sense Campaign' by the Dutch Working Group on Head and Neck Tumors (NWHHT).

The HPV awareness among GPs in The Netherlands was assessed in **Chapter 3**. A total of 207 GPs throughout The Netherlands participated in our cross-sectional study, of which 72% recognized HPV as a risk factor for OPSCC and 76.3% was aware of the increasing incidence rates. Patient characteristics were less well recognized, with 35.7% of GPs knowing that patients with HPV-related OPSCC are more often male and just over half was aware of the younger age of patients. Our findings demonstrate that GPs are reasonably aware of HPV as a causative factor for OPSCC, however, a quarter of GPs is still unaware of this link. Specific knowledge on patient characteristics could be improved, to ensure early recognition of the disease in this relatively young patient group without classical risk factors. Further training in the form of regional and national meetings may contribute to better targeted knowledge and appropriate referral of patients to secondary care. To raise HPV awareness among GPs in The Netherlands, findings described in this thesis were summarized in an infographic and published in the Dutch Journal for GPs 'Huisarts en Wetenschap' (**Chapter 4**).

Despite the generally better prognosis of HPV-related OPSCC compared to their HPV-negative counterpart, a still unidentified subgroup of 10-20% of HPV-positive patients will develop recurrent disease after treatment. Integration of the HPV genome in the human genome has been suggested to have further reaching consequences for tumorigenesis of HPV-positive tumors, but studies on the relation between HPV integration and patient outcome have shown inconsistent results. In **Chapter 5**, we summarized the recent literature on HPV integration in OPSCC and observed that HPV integration prefers less protected and more accessible chromosomal regions, including highly transcribed (cancer) genes. In addition, several factors were described to promote viral integration, including reactive oxygen species, inflammation, and APOBEC expression. HPV integration could lead to constitutive expression of viral oncogenes and deregulation of cellular (cancer) genes, possibly conferring neoplastic pressure. Importantly, different techniques to detect HPV integration have been used in studies so far, which are often biased, insensitive and/or nonspecific. Together with the variety of described bioinformatic approaches for integration detection, this might explain the inconsistent data on integration percentage and clinical relevance. Furthermore, current detection techniques are generally unsuitable for formalin-fixed, paraffin-embedded (FFPE) tumor tissues. Therefore, in **Chapter 6**, we developed and validated a novel sequencing approach (cell-based TLA, FFPE-TLC), based on the ligation of DNA sequences in close proximity to each other. Seven HPV-positive cell lines and FFPE tissues of 27 HPV-positive OPSCC were used for HPV integration detection using this method. Our findings demonstrate that this approach enabled sequencing of hundreds of kb around the HPV integration site, detecting exact HPV integration loci, structural

variants, and chromosomal rearrangements in both HPV-positive cell lines and FFPE tissues of OPSCC. HPV integration sites were detected in 15/27 FFPE tumor tissues and confirmed by PCR analysis and Sanger sequencing in a subset of samples, showing simple and complex integration patterns, resulting in structural variations, which also may explain mechanisms underlying integration and clonal evolution. This FFPE-TLC method provides the opportunity for reliable and robust detection of HPV integration sites, specifically valuable for FFPE tissues, enabling further research on the clinical relevance of HPV integration in OPSCC, including clonality assessment of multiple tumors with HPV-involvement within a patient.

Over the last decades, survival rates for HNSCC patients have hardly increased, recurrence rates are still high, especially for HPV-negative HNSCC, and treatment-related side effects are substantial. Therefore, there is an urgent need for improved, more targeted treatment options. In **Chapter 7**, the antiproliferative effect of the antiviral agent cidofovir was investigated using HPV-positive and HPV-negative HNSCC cell lines. It was observed that cidofovir treatment resulted in inhibition of cell growth in all cell lines, and that DNA damage accumulated and activated the DNA damage pathway, leading to G2/M cell cycle arrest. Moreover, our findings suggest the occurrence of mitotic catastrophe, without the induction of apoptosis.

In addition to antiviral therapies, options for therapies targeting specific genetic alterations and/or deregulated cellular pathways have been a field of interest for the treatment of HNSCC patients. Cell cycle control genes, as well as the PI3K/Akt/mTOR pathway are commonly affected in HNSCC. In **Chapter 8**, the therapeutic efficacy of several CDK4/6 (palbociclib, ribociclib) and PI3K/Akt/mTOR pathway inhibitors (alpelisib, buparlisib, gedatolisib) was explored *in-vitro*. Both inhibitor types showed to inhibit their respective pathways and cell growth. The CDK4/6 inhibitors showed to be mainly effective in HPV-negative HNSCC cell lines, inducing cell cycle arrest in G1 phase without the induction of apoptosis. Treatment with PI3K/Akt/mTOR inhibitors resulted in inhibition of cell proliferation of both HPV-negative and HPV-positive HNSCC cell lines, the induction of apoptosis, and the attenuation of oxidative and glycolytic cellular metabolism. Furthermore, we observed that the combination of a CDK4/6 and a PI3K/Akt/mTOR inhibitor, i.e., ribociclib and alpelisib, synergistically decrease cell viability. Further research should elucidate whether (a combination of) these inhibitors are effective therapeutic agents for HNSCC patients.

Due to the heterogeneous nature of HNSCC, varying response rates to both standard-of-care and new treatments are observed between patients. Tumor-derived culture models

offer the chance to predict therapy response in a personalized setting. In **Chapter 9**, we summarized available culture models for HNSCC, and evaluated their application as a preclinical prediction model for therapy response. Results demonstrate that a wide range of primary culture models has been introduced for HNSCC, including monolayer cultures, spheroids, organoids, histocultures, xenografts, and microdevices. Technical aspects of these culture models were assessed, such as culture success percentage, culture duration and complexity, resemblance to the original tumor, and predictive value for patient therapy response. The histoculture model was most often used, and showed the best success rate, tumor resemblance, and prediction of patient response to chemotherapy with ~75% accuracy. Although radiotherapy, either or not in combination with chemotherapy, is an important treatment modality for HNSCC, evidence on the use of histocultures to predict radiosensitivity is limited. In **Chapter 10**, we aimed to characterize a short-term HNSCC histoculture model, derived from fresh tumor tissue, and evaluated its application to study repair capacity of irradiation-induced DNA damage as a measure for radiosensitivity. During short-term culture (48h), histocultures maintained tissue architecture, including different cell types, epithelial marker expression, and tumor cell proliferation. Cultures derived from HPV-positive tumors maintained their strong expression of the HPV surrogate marker p16. Ex-vivo irradiation of histocultures resulted in increased DNA double strand breaks, visualized by 53BP1 foci, and differences in repair capacity, assessed at 24 hours after irradiation, were observed between individual patients. Specifically, cultures derived from HPV-positive tumors showed significantly less efficient DNA repair. The combination of cisplatin and radiotherapy decreased DNA repair efficacy in 3 out of 4 tumors compared to radiotherapy alone. Sufficiently powered studies, with adequate comparison between ex-vivo response and patient response are required to elucidate whether histocultures can mature into useful clinical tools.

Finally, in **Chapter 11**, the results described in this thesis are discussed and reflected in the light of current knowledge in the field. The new insights presented in this thesis might provide new opportunities to decrease the impact of HNSCC and open doors for future research.