ISSN: 2754-4893

Journal of Dental Science Research Reviews & Reports

SCIENTIFIC Research and Community

Review Article

Open Access

Current Immunotherapy Treatments and Researches Regarding HPV-Positive HNSCC, a Revue of the Literature

Aurore Casse[1*], Julie Guillet[1,2], Céline Schwey[1], Romina Mastronicola[1,2], Sophie Cortese[1], Emilie Beulque[1], Bérengère Phulpin[1,2], Pauline Le Roux[1] and Gilles Dolivet[1,2]

[1]Institute of Cancerology of Lorraine ICL, 6 Avenue de Bourgogne, 54519 Vandoeuvre-lès-Nancy, France

[2]CRAN, CNRS, UMR 7039, University of Lorraine, 54519 Vandoeuvre-lès-Nancy, France

ABSTRACT

Human papillomavirus (HPV)-positive head and neck squamous cell carcinoma (HNSCC) represents a distinct and intriguing subset of HNSCCs. This comprehensive review article explores the genetic and clinical aspects of HPV-positive HNSCC, shedding light on its unique characteristics and clinical implications and its difference from HPV-negative HNSCC.

We delve into the etiological factors underlying HPV infection in HNSCC, emphasizing the role of high-risk HPV types, especially HPV-16. HPV-driven tumorigenesis through the E6 and E7 oncoproteins and their impact on key tumor suppressor genes, such as p53 and Rb, is discussed, highlighting the relatively lower mutation burden in these tumors compared to HPV-negative HNSCC.

This article also explores ongoing research efforts, genomic profiling, and emerging targeted therapies tailored specifically for HPV-positive HNSCC. A deeper understanding of the unique genetic landscape of these tumors offers promising prospects for personalized treatment strategies and improved outcomes.

This review provides a comprehensive overview of the genetic mutations, clinical features, and potential therapeutic avenues in the realm of HPV-positive HNSCC, emphasizing the need for continued research to advance our knowledge and treatment options in this distinctive subset of head and neck cancers.

***Corresponding author**

Aurore Casse, Institute of Cancerology of Lorraine ICL, 6 Avenue de Bourgogne, 54519 Vandoeuvre-lès-Nancy, France.

Received: October 25, 2023; **Accepted:** November 02, 2023; **Published:** November 09, 2023

Keywords: Human Papillomavirus, Carcinomas, Head and Neck

Introduction

For the last decade, a shift has been observed regarding the causes of head and neck squamous cell carcinomas (HNSCC). Oncogenic Human Papilloma Viruses (HPV) have been found to be a growing trigger in head and neck tumors. Nevertheless, its recurrency has allowed scientists to study its molecular aspects in order to find new ways of treatments for HPV-induced tumors. It includes comprehending the differences between HPV-related and non-related squamous cell carcinomas, as well as studying the different oncogenic pathways that lead to the creation of tumorous cells. Indeed, HPV-related and non-related HNSCC can be treated differently, as the two diseases appear to have more differences than it was originally thought. HPV-related HNSCC call for a less aggressive chemo- and radiotherapy and may be eligible to the use of specific or not specific immunotherapy.

This article will focus on describing Human Papilloma Viruses, its impact on the formation of a tumor, and the current ways of treatment that exist to this day as well as the curative molecules that are still understudy.

Quick Description of the Human Papilloma Virus

The Human Papillomavirus is a DNA virus that infects the mucous membranes and the skin. HPV is best known as a sexually transmitted virus. Indeed, most of the time it is transmitted by a sexual contact, making it the most common sexually transmitted infection [1]. Yet, other ways of transmission exist like deep open-mouthed kissing, self-inoculation or autoinoculation, vertical transmission from infected mother to her child, or horizontal transmission from breast to mouth [2] Many types of HPV exist, yet only high-risk HPV are linked to cervical cancer, or other types of cancers such as anal, vaginal, oropharyngeal cancers and so on. In fact, most of the time the virus is naturally eliminated by the body, making the infection temporary.

Many classifications exist regarding Human Papillomavirus. There are classifications according to its genome, its tropism, or its oncological risk. HPV are classified in two groups : the ones that have cutaneous tropism, and the ones that have mucous tropism. The last one is itself divided in two subgroups : HPV at low cancerous risk, and HPV at high cancerous risk [3]. Regarding HPV at high cancerous risk, the best known in that category would be HPV 16 and 18. Small lesions could be provoked by it and

grow for years before leading to cancer.

Statistically, 80% of the sexually active population is at risk of being contaminated with a Human Papillomavirus at least once in their life [4].

If this virus is mostly implicated in cervical cancer, the second most important cancer that is caused by it is oropharyngeal. In France, HPV, mostly the HPV 16, is the cause of 1/3 oropharyngeal cancer [5]. In 2015 in France, HPV was the cause of 4 to 34% of head and neck cancers, 91% of anus cancers, 23% of vulval and vaginal cancers, 27% of penial cancers and 100% of cervical cancers [5].

Epidermoid Carcinomas HPV-Inducted

The epidemiology of the head and neck squamous cell carcinoma (HNSCC) has changed over the last fifteen years. The increase of some kinds of HNSCC has been observed, like oropharyngeal carcinomas [6]. Moreover, even if head and neck cancers are often related to alcohol or tobacco, HPV-induced carcinomas usually occur in younger patients with less exposure to this kind of substances and overall, a better diet than HPV-positive patients. Clinically speaking, HPV-positive HNSCC tend to be less differentiated and are more likely to be related to a positive lymph node status [7].

A study conducted by Kunak S. Jain et al. exposed the fact that in the last decades, the etiologic causes of oropharyngeal HNSCC has shifted [8]. Indeed, the biggest cause of this type of cancer went from been primarily tobacco-associated, to primarily due to oncogenic human papillomavirus.

HPV-positive HNSCC in the oropharynx area are most of the time due to HPV-16. However, WHO classifies 14 mucosal HPV types as high carcinogenic risk (HPV-16, 18, 31, 33, 35, 39,

45, 51, 52, 56, 58, 59, 66, 68) [9]. This HPV situation often leads to an overexpression of p16, a decreased expression of the p53 and Rb genes, a decreased expression of EGFR and overall, a different expression pattern of the genes than patients with HNSCC that are HPV- negative [10]. All of those differences set the HPV-positive HNSCC apart. Moreover, patient with HPV-positive HNSCC usually have a better prognosis that could lead to a decrease of the treatment's intensity.

HPV-related HNSCC tend to be described as nonkeratinizing squamous cell carcinomas. Nowadays HPV-related HNSCC has even become itself a separate disease with its own prognosis and treatments [11].

It is also interesting to describe the virus-induced tumor regarding its oligoclonal aspect. The oligoclonal aspect of HPV-positive tumors in the head and neck refers to the presence of multiple distinct clones of cancer cells within the tumor mass. Oligoclonality means that the tumor is composed of a limited number of different cell populations, each with its own unique genetic and molecular characteristics.

Studies have demonstrated that HPV-positive head and neck tumors often display a more limited heterogeneity compared to HPV-negative tumors [7]. This means that the genetic and molecular profiles of the cancer cells within HPV-positive tumors are more similar to each other than in HPV-negative tumors, where there can be a greater diversity of cancer cell populations. HPV-positive tumor often has fewer genetic mutations in comparison to HPV- negative HNSCC. The E6 and E7 oncoproteins of HPV can disrupt key tumor suppressor genes, like p53 and Rb, which play a role in cell cycle regulation and DNA repair. As a result, mutations in these genes are less common in HPV-positive HNSCC, whereas HPV-negative HNSCC tend to have a higher frequency of genetic mutations in genes associated with cell cycle control, DNA repair, and tumor suppression. Common mutations may involve TP53 (p53 gene), NOTCH1, PIK3CA, and others.

Understanding the genetic and clinical differences between HPV-positive and HPV-negative HNSCC is important for tailoring treatment approaches. For HPV-positive HNSCC, targeted therapies and immunotherapies may be more effective, while conventional therapies such as surgery, radiation, and chemotherapy are often used for HPV-negative HNSCC. Additionally, ongoing research is continually uncovering more about the molecular characteristics of these cancers, which may lead to more personalized treatment options in the future.

The oligoclonal nature of HPV-positive head and neck tumors is thought to be related to the mechanism of tumorigenesis driven by HPV infection. The integration of the HPV genome into the host cell's DNA results in the expression of viral oncoproteins E6 and E7, which disrupt normal cell cycle regulation and promote cellular proliferation. These oncoproteins lead to the expansion of a limited number of genetically related cell clones, giving rise to the oligoclonal tumor structure.

Type of HNSCC	Oligoclonality
HPV-16-related	- Carcinogenesis due to viral genes E6 and E7 - Integration of hrHPV DNA into the host genome
	- Increased degradation of p53 and Rb - → cancerous cells - PIK3CA mutations (→improved outcomes) - Upregulation of the immune-checkpoint protein PD-L1 - On average, a few dozen to a couple of hundred mutations in their genomic DNA [12] - 2.8 mutations per megabase [13]
HPV-unrelated	- Often TP53 mutations - 9p21-22 deletion - On average, hundreds to thousands mutations in their genomic DNA - 4.83 mutations per megabase [13]

According to K. Kobayashi et al. when describing the pathological molecular mechanism in carcinogenesis, HPV-free patients will show p53 mutations, and deletion of 9p21-22 so the P16's function of tumor suppressing is stopped. This mechanism leads to an inhibition of the Rb phosphorylation by the formation of a complex between P16INK4a and CDK4/CDK6. As a result, the transcription factor E2F-related cell rotation is inhibited [11]. HPV-related HNSCC will have a more important effect on the E6 and E7 oncogenes, that will improve the degradation of p53 and RB tumor suppressor genes. P. Goone et al. state that the immortalization of oral keratinocytes and oral cells happen rapidly, even in vitro. They farther state that HPV-positive HNSCC showed mutations in the promoter/enhancer region that gave it growing activity, leading to a bigger expression of E6 and E7 [14].

Furthermore, the integration of the HPV DNA into genomic DNA is not a rare thing. Usually, it is followed by the deletion or disruption of HPV E1 or E2 open reading frame (ORF). Since E2 plays the role of a repressor of E6 and E7, the discontinuity of its activity leads to maintaining the immortalized phenotype [15]. However, the molecular pathway seems very heterogenous. Indeed, a study conducted by W. Koskinen et al. stated that out of 23 HPV16- positive samples, 48% showed integrated DNA, 35% showed episomal DNA and 17% showed mixed forms of DNA [16].

The oligoclonality of HPV-positive tumors has several implications such as in therapeutic targeting. The limited heterogeneity within HPV-positive tumors makes them potentially more amenable to targeted therapies that specifically exploit the molecular alterations caused by HPV infection. This could lead to more effective treatment strategies.

The prognostic significance also depends on the oligoclonality of the tumor. The presence of a dominant clone or a limited number of clones with more favorable genetic features could be associated with improved treatment responses and patient outcomes.

Oligoclonality could also lead to biomarkers development. The identification of specific genetic alterations or biomarkers associated with dominant clones in HPV-positive tumors could aid in the development of novel diagnostic and prognostic markers.

However, it's essential to remember that even though HPV-positive tumors have a more limited heterogeneity compared to HPV-negative tumors, they can still exhibit some degree of clonal diversity. Additionally, tumor heterogeneity, even within an oligoclonal tumor, can still influence treatment responses and disease progression.

Research on the genetic landscape and clonal architecture of HPV-positive head and neck tumors is an active area of investigation, and ongoing studies may provide further insights into the clinical implications and potential therapeutic opportunities related to their oligoclonal nature.

Moreover, HPV-induced carcinogenesis is linked to a lower number of DNA mutations as well as fewer chromosomal changes yet shows a higher percentage of epigenetic changes.

Indeed, HPV-positive HNSCC are associated with a significantly lower mutational rate next to HPV-negative HNSCC. All of that leads to the creation of oligoclonal tumors which are more chemo- and radiosensitive [6]. Regarding HPV-negative HNSCCs, which involve mutations of both oncogenes and tumor suppressor genes by substances like tobacco or alcohol, their mutation load is higher.

The number of mutations in HPV-positive head and neck squamous cell carcinoma (HNSCC) can vary widely from one tumor to another. However, in general, HPV-positive HNSCCs tend to have a lower mutation burden compared to HPV-negative HNSCCs [12].

On average, HPV-positive HNSCCs may have a few dozen to a couple of hundred mutations in their genomic DNA. This is relatively low compared to some other cancer types that can have thousands of mutations, such as melanoma or lung cancer, which are often associated with high levels of ultraviolet (UV) or tobacco-related DNA damage. The lower mutation burden in HPV-positive HNSCC is partially explained by the fact that the oncogenic HPV virus contributes to the development of these cancers by disrupting key regulatory pathways through its viral oncoproteins (E6 and E7) rather than causing extensive DNA damage. These oncoproteins interfere with the function of tumor suppressor genes like p53 and Rb, leading to cell cycle dysregulation and tumorigenesis [7].

It's important to note that the exact number of mutations can vary among individual cases of HPV-positive HNSCC, and ongoing research may provide more precise data on the mutation profiles of these tumors. Understanding the mutation landscape can be important for tailoring treatment approaches, especially in the context of emerging targeted therapies and immunotherapies.

In addition to that, it seems crucial to distinguish the difference between HPV-positive HNSCC and HPV-related HNSCC. Indeed, in HPV-related HNSCC it is the HPV-virus that initiates and sustains the entire carcinogenesis process. HPV-related tumors are defined by a low TMB, p16 overexpression, a wild-type p53 status, a wild-type status of the genes INK4, CCND1 and EGFR. The other category of HPV-positive tumors shows p53 mutations, INK4 mutations and CCND1 amplification as well yet they are associated with a poor prognosis like the HPV-negative tumors [6].

Weinberger et al. analyzed 80 oropharyngeal tumors regarding mutations of biomarkers like p16, p53 and pRB. This led them to classify the tumors in three groups. Class I was the HPV- negative ones (HPV16-negative and p16 nonexpressors). Class II was the HPV-positive ones (HPV16 positive/p16 nonexpressors). Class III was the HPV-related ones (HPV16 positive/p16 expressors) [17].

Epidemiology

As written earlier, a shift has been observed over the last decades regarding head and neck cancer around the world. Indeed, oropharyngeal cancer linked to HPV has been increasing visibly, especially in North America and northern Europe. This type of cancer is more seen in men than women, and also more seen in the younger men population in developed country; contrary to oral cavity and lung squamous cell carcinomas that appeared to decrease. On the other hand, oropharyngeal cancer, lung squamous cell carcinomas, and oral cavity cancers have been more and more common in women, been a direct consequence of the higher smoking rates in this population [18]. HPV16 is even more dominant in HPV-related HNSCC (from 85 to 95%) than in cervical carcinomas (from 50 to 60%) [14].

A study conducted by M. Blomberg et al. chose to classify head and neck tumor's location by their likability to be HPV-related. At the end of the study (which compared cases from 1978 to 2007), their conclusion was that incidence rates were increased for tumor in the oral cavity, tonsils, oropharynx and hypopharynx while lip cancer was decreasing. They also concluded that HPV-associated locations were globally growing in terms of cancers development whereas the locations that were unlikely HPV-related were diminishing in men or stagnating in women. It was also noted that the most present HPV-virus in head and neck cancers was HPV16 [19].

However, such observations have positive outcomes since HPV-positive cancers show improved outcomes according to A. K. Chaturvedi. Indeed, apparently this type of cancer is linked to a reduction of 28 to 80% of risks of death compared to HPV-negative cancer [20]. It is believed that it is also related to the younger age

and better lifestyle of HPV-positive patients.

Screening

Numerous techniques exist to detect viral DNA such as culture, the search for cellular modification, detection of viral proteins or nucleic acids (DNA, mRNA) in tissues, antibodies detection … However, papillomaviruses can unfortunately not be cultivated in vitro [21].

For years the main technique used to detect this type of viruses was the observation through a microscope. Scientists were looking for cellular modifications linked to the presence of HPV like the presence of koilocytosis, multinucleation, dyskeratosis and parakeratosis. Such method is quite safe regarding the cervical area yet not perfectly suited for the detection of HPV in the oral cavity. Indeed, there are elements that can complicate the identification of real koilocytosis like chronic mechanical irritations [21]. Moreover, the microscopic observation does not allow the medical team to determine which type of HPV is present [22].

About the detection of antibodies directed against viral antigens like anti-L1, anti-E6, anti-E7, it is not a reliable technique because of the inconsistent immune response [23]. Moreover, a positive result with this technique does not indicate anything about the location of the infection [24].

Molecular techniques based on the detection of viral protein or nucleic acids soon became reference methods to detect the presence of HPV in a lesion, allowing the medical team to distinguish HPV-induced tumors from HPV-free tumors [25]. Such analysis is performed on fresh tissue or tissue fixated with formol and paraffine after a biopsy, after the pathologist has realized the histopathological exam [26]. They can also be obtained after a smear [21].

Nowadays, PCR and in situ hybridization are used. The chosen technique is usually associated with the detection of the p16 protein through immunohistochemistry in order to increase the reliability of the results and so to improve the global medical care for the patient [11]. However, several detection kits are commercialized and there is a lack of standardization of protocols, making the development of comparative studies more complicated to realize [27].

Clinical Implications for HPV-Positive Head and Neck Squamous Carcinomas for Treatments

Regarding the increasing number of HPV-positive HNSCC in the population and the change of its prognosis, it is starting to be recognized as a different disease. The clinical aspect, biological aspect, and epidemiological aspect all seem different from other cancers. Even if HPV-positive HNSCC are often associated with locoregionally advanced tumors, the prognosis stays brighter than HPV-negative HNSCC. Indeed, they have an 80% or higher rate of three- year survival [22]. However, the HPV status was not incorporated yet in the staging classification of the cancer. The TNM classification had become unfaithful to the real prognosis of HPV-positive HNSCC cancers, studies like the one conducted by S. Hui Huang et al. suggesting that the American Joint Committee on Cancer/Union for International Cancer control TNM stage and prognostic groups for human papillomavirus-related oropharyngeal carcinomas should be reviewed and adapted [28]. Incorporating data like smoking history and HPV-status would allow a better understanding of the patient's prognosis. Since recently, the 8th Edition of TNM Classification for Head and Neck Cancer has been changed and updates in order to include data like HPV-linked cancer and soft tissue sarcoma of the head and neck [29].

As we described before, where HPV- HNSCC show mutations in P53 and in pRB, HPV+ HNSCC do not. These genes are intact, just inhibited by E6 and E7 from HPV. This observation means that the apoptosis could be restored if HPV E6 and E7 are removed from expression, making this tumor more likely to respond to chemotherapy [17].

Since HPV-positive tumor respond so well to chemo and radiotherapy, many studies are directed around the de-escalation of the treatments.

Ferris et al. conducted a study based on the use of neoadjuvant nivolumab for patients with resectable HPV-positive and HPV-negative squamous cell carcinomas of the head and neck in the CheckMate 358 trial [30]. It focused on patients with previously untreated resectable HNSCC who received, during the study, 240 mg of nivolumab on day 1 and 15 with the surgery planned on the 29th day. Results showed that even if they were more treatment- related adverse e□ects in patients with HPV-positive tumors (73.1% in the HPV-positive group against 53.8% in the HPV-free group), the radiographic response rate was of 12.0% in the HPV-positive group and 8.3% in the HPV-negative cohorts. The final conclusion of the study was that neoadjuvant nivolumab was generally safe and induces pathogenic regressions in HPV-positive (23.5% and HPV-negative (5.9%) tumors.

Woody et al. directed a study about the preservation of the regional control after dose de-escalating radiotherapy to involved lymph nodes in HPV-positive oropharyngeal cancer [31].

They retrospectively identified patients with HPV-positive oropharyngeal squamous cell carcinomas treated with definitive chemo- and radiotherapy (70-74.4 Gy) to the primary site and since a post-radiation neck dissection was planned, 54 Gy to the involved nodal areas.

The authors observed a five-years locoregional control, disease-free survival and overall survival of 96%, 81%, and 86% respectively. The conclusion was that regional lymph node control in HPV positive oropharyngeal cancer was not compromised by dose de-escalated radiotherapy to involved nodes in the setting of concurrent cisplatin-based chemotherapy. The goal of another study conducted by Palma et al. was to evaluate the toxic effects and survival in treatment de-escalation with radiotherapy compared with transoral surgery for HPV-associated oropharyngeal squamous cell carcinoma as primary approaches [32]. The study occurred on 61 patients that were randomized in 2 groups (30 in radiotherapy and 31 in transoral surgery) between 2018 and 2020. However, the results are not conclusive since the study's end is planned only in 2028 [33]. Like the studies described earlier, their results suffer of being only phase II or unfinished studies.

Current HPV-Inducted Epidermoid Carcinomas Treatments

Since HPV-positive patients are younger and usually have better chances of survival, long- term treatment outcomes have become an issue. In order to respond to this problem, ways of de-escalation of treatments are explored like lower the give dose or targeted therapeutics. Nowadays, patients are usually treated with single modality treatment like chemotherapy or radiation, or surgery alone. The choice of the treatment usually lies in the overall state of the patient and in the clinical outcomes. Regarding the surgery,

radiations could be added depending on the positivity of the margins, the positivity of the lymph nodes, or bone erosion [14].

A study from 2014 was able to prove that lower dose induction chemotherapy could have better results on HPV-positive patients with oropharyngeal squamous cell carcinoma. The study was designed with cetuximab-IMRT and various doses were given to patients. In the end the conclusion was that lower dose were able to produce high tumor control rates with minimal late toxicities, as well as 95% of 2-year survival rate [34]. Such study calls for a deeper knowledge of dose management.

With the absence of biomarker, it seems hard to define a precise treatment which progress can be followed and studied. That is why scientists chose to work on this topic, and it was discovered that tumor DNA could be detected and studied not only in the blood of patients with head and neck cancer, but also in their saliva regarding those with oral cavity cancer. In the study, it was observed that tumor DNA from the oral cavity appears most likely in the saliva, and this DNA was detected post surgically before clinical diagnosis of recurrence in three patients. This observation makes the detection of tumoral DNA in the saliva a promising biomarker for head and neck squamous cell carcinomas [35].

Immunotherapy

Immunotherapy is a type of cancer treatment that harnesses the body's own immune system to recognize, target, and destroy cancer cells. The immune system is a complex network of cells, tissues, and organs designed to protect the body from foreign invaders, including viruses, bacteria, and abnormal cells like cancer cells. However, cancer cells can sometimes evade the immune system's detection and continue to grow unchecked. Immunotherapy works by enhancing or restoring the immune system's ability to recognize and attack cancer cells. Immunotherapy in cancer find its use in the existence of "tumor-associated antigens" (TAAs), allowing an immune response to occur. In the clinical field, several immunotherapy strategies are available. HPV-driven HNSCC are often T-cells rich, showing the presence of HPV antigens which are very immunogenic.

PD-1/PD-L1 Based Therapies

One of the many possibilities of immunotherapy is to target immune checkpoint inhibitors. These drugs block certain proteins on the surface of immune cells or cancer cells, known as immune checkpoints, which regulate the immune response. By blocking these checkpoints, the immune system is unleashed, allowing it to mount a stronger and more e□ective attack against cancer cells. That is why the immunomodulation of the PD-1/PD-L1 immune checkpoint is a promising cure. A study conducted by T. Seiwert et al. tried treating HPV- positive HNSCC patients with pembrolizumab and analyzed their RNA expression profiling and survival data. Their conclusion was that pembrolizumab was a great therapy for patients with "inflamed phenotype" signature, meaning high level of tumor-infiltrating lymphocytes and expression of immune checkpoints [36]. To this day, 2 anti-PD-1 antibodies are approved for recurrent and metastatic HNSCC : nivolumab and pembrolizumab [37, 38]. While it seems like an interesting option for the patient, this treatment is useful only for less than 20% of the patients after the failure of classic chemotherapy [39].

Indoleamine 2,3-Dioxygenase Inhibitors

Other immunotherapy drugs have been studied such as indoleamine 2,3-dioxygenase (IDO1) inhibitors navoximod and epacadostat. IDO1 has an immunosuppressive role in tumor immune microenvironments. According to some studies, the upregulation of IDO1 inhibited the function of antitumor T-cells and a high expression of IDO1 was linked to a poor prognosis. That explains why IDO1 inhibitors are able to restore the T-cell function and by doing so, resulting in the tumor microenvironment immunogenicity [40]. According to the beginning of a phase I clinical trial, of the evaluable patients, 36% had stable disease with navoximod [41]. Another study forms the ECHO-202/ KEYNOTE-037 trail stated that epacadostat in combination with pembrolizumab was generally well tolerated and had encouraging antitumor activity in multiple advanced solid tumors [42].

TLR-Agonist

Molecules like motolimod have also been studied. Motolimob is a toll-like receptor 8 (TLR8) agonist that may stimulate innate and adaptative immunity. The Active8 study consisted of the administration of combination treatment with platinum (carboplatin or cisplatin), fluouracil, cetuximab, and either placebo or motolimob, each administrated intravenously every 3 weeks for a maximum of 6 chemotherapy cycles. Overall, the results of the study were that the addition of motolimob into the chemotherapy protocole was not useful for most of patients : it was well tolerated yet no improvement of the progression-free survival (PFS) or overall survival (OS) was noticed. However, regarding patients with HPV-positive tumors, significant benefit was observed. Indeed, the PFS was improved from 5.9 months for patients

who received a placebo to 7.8 months for patients who received motolimob. The OS was also respectively of 12.6 months and 15.2 months, highlighting the benefits of motolimob [43].

Adoptive T-cell Therapy

The transfusion of lymphocytes, also named as adoptive T-cell therapy, is another immunotherapy tested for cancer treatment [44]. Indeed, adoptive T cell therapy has the potential to enhance antitumor immunity, augment vaccine efficacy and limit graft-versus- host disease. T-cell therapy involve using a patient's own T-cells to treat cancer. These T-cells are collected from the patient's blood, modified or activated in the laboratory, and then infused back into the patient to enhance their ability to recognize and destroy cancer cells. However, adoptive T-cell therapy is still a relatively new and complex treatment approach.

Tumor-Infiltrating Lymphocytes

A related approach is tumor-infiltrating lymphocytes (TIL). Most recently, scientists have discovered a superior form of killer T-cell in patients who have successfully cleared end stage solid cancer. The researchers discovered that dominant, successful killer T-cells could recognize multiple different cancer-associated targets at the same time. This discovery occurred during their research regarding patients with late-stage solid cancer who were given TIL (tumor-infiltrating lymphocyte) therapy [45]. This study proves that the belief that individual killer T-cells only saw a single target on cancer cells is wrong.

CAR-T Cells

CAR-T cells is a denomination that stands for Chimeric Antigenic Receptor-T. It is classified as an immunotherapy that aims at curing cancer while using the patient's own immune system. CAR-T cells are modified T lymphocyte genetically modified in order to recognize before terminating cancerous cells. They are at the basis of a whole new approach of cancer treatment, which consist of collecting T-cells from the patient before genetically modifying them and reinjecting them to the patient. Since T-cells

are part of the white blood cells, a part of the immune system, in case of cancer occurring, their activation mechanism is altered by the tumor leaving cancerous cells free to proliferate [46].

Indeed, tumor cells usually provoke an immune response from the body. Yet, HPV-induced cancer can silence this response by creating an immunosuppressing microenvironment based on the interaction between the host and the tumor. Mechanisms to escape the immune system are developed, including the direct obstruction of T-cells through soluble or surface molecules leading to the enrolment of suppressive cell populations [47].

The goal in modifying T lymphocytes, is to allow them to recognize any tumorous cell thanks to a gene that is introduced into the core, giving them a chimeric antigenic receptor. The genetical modification also give the CAR-T cell the ability to activate itself with a function of costimulation. Once created, the CAR-T cells are duplicated and re-injected into the patient's body. Before this re-injection it seems important that the patient follows a three-days long chemotherapy so that his immune system weakens, allowing the CAR-T cells to have a better chance at replicating themselves through the immune system. It reduces the chances of a rejection occurring. Such therapy is especially used in case of blood cancers but numerous studies are undergoing for a variety of cancers since it is a promising technology [46].

In HPV-positive head and neck squamous cell carcinomas, it has been noticed that the number of Treg is higher than in HPV-negative HNSCC, as well as the number of Treg in tumor infiltrating lymphocytes is higher than in peripheral blood lymphocytes regarding HNSCC. Also, the number of Treg that express inhibitory molecules T-cell immunoglobulin mucin-3 (TIM-3), PD-1 and CTLA-4 are higher in tumor infiltrating lymphocytes compared to peripheral blood lymphocytes [48].

TCRs could be considered as a good way to target cancer cells, nevertheless many tumors have resistance mechanisms as described before, by using checkpoint blockade molecules weakening immune recognition and attack. Immunotherapy can reactivate T cells anyway to target tumor cells [49]. Few immunotherapies have yet been approved in order to treat HNC like immune checkpoint inhibitors [50].

Several studies have showed that HNSCC expresses specific molecules that could correspond to good candidate in targeted therapies like CAR-T cells. As an example, there are markers like CD276, MICA, MAGE-A4, EGFR, FAP, MICB, EPCAM, B4GALNT1, CD70 that could be potential targets [51]. According to the same study, when CAR-T cells were created to target CD70 in tumor cells, the ones that did not express this gene remained intact [51].

CAR-T cells that express HER2 receptors in order to direct the T-cells response towards the cancer cells is another strategy. HER2 is a tyrosine kinase contained in most epithelial cell layers and translates the cell di□erentiation. In a lot of cancers, HER2 is overexpressed leading to an over-replication of the cells, and because of that represents a promising target for CAR-T cells. A study conducted on HER2 CAR-T cells targeted therapy in HNSCC proved that the tumor decreased of 56% in size [52].
The molecule MUC1 is also a potential target since it is importantly expressed in cancer tissues compared to the healthy tissues around it. CAR-T cells that produce IL-22 are able to increase the expression of MUC1 in HNSCC and by doing so increase T cell function [53].

HPV Vaccination

Several vaccines against HPV exist to this day, like Gardasil 9®, which is a nonavalent vaccine, or Cervarix®, which is a bivalent vaccine.

The first one is composed of fragments of Human Papillomaviruses. It contains no alive germs. Studies have shown that antibodies were successfully produced up until five years after the vaccination, and precancerous lesions have been proven to be reduced concerning papillomaviruses type 6, 11, 16, 18, 31, 33, 45, 52 and 58 in vaccinated women [54]. Indeed, the vaccine is made out of virus-like particles from HPV 11 and 6, and of oncogenic strains from HPV16 and 18 [55]. The second one also contains no living germs and shows a production of antibodies up until five years after the vaccination. It prevents precancerous lesions from HPV type 16 and 18 in vaccinated women [56]. However, their e□ect on other types of Human Papillomaviruses has not been observed.

Yet, those vaccines are no treatment but a preventive way of limiting the spread of Human Papillomaviruses. Because of the foreign nature of the virus, vaccines are easier to create. However, at the end of 2021 in France only 45,8% of 15-year-old girls and only 6% of boys of the same age received at least a single dose of vaccine [57]. This numbers are one of the lowest in industrialized countries. Men having homosexual relationships are the most targeted by vaccination campaigns since they are the most at risk of being contaminated by an HPV, it being a sexually transmitted disease (by oral sexual activities in the case of head and neck cancer).

Since the vaccines campaigns were mostly focused on cervical, vaginal, anal and genital cancers, some studies have started regarding its efficiency in HPV-positive head and neck cancers. Results are suggesting that such therapeutics do have a prophylactic effect on this type of HPV-induced cancers, showing that Gardasil 4® or Cervarix create the production of IgG antibodies in the oral cavity and not only in the serum, even if their presence is lower there (2). Only a few studies were focused on studying their effect on oral infection. A recent study conducted from 2011 to 2014, related that the prevalence of the HPV was reduced of approximately 88,2% in vaccinated folks, without looking at age, sex or race [58]. Moreover, not enough studies were made in order to reflect the effects of the vaccines in the male population. The same study, happening from 2011 to 2014, observed that the prevalence of oral type HPV infections preventable by vaccine were reduced of 100% in men [58].

Recently, scientists have developed a mixed MS2-L2 based HPV candidate vaccine. Such technology is based on bacteriophage MS2 virus-like particles (VLPs). It was tested on mice, making them immune against six HPV types. It was reported that regarding the genital area, mice were protected against HPV53, 56, 11 and at the oral area they were protected against HPV16, 35, 39, 52, and 58, which confers them a shield against the HPV types that are associated to head and neck cancer at approximately 99% [59].

A study conducted on mice recently showed also that a new type of injection, of HPV16 E6/E7- expressing mEERL-95-cells, into the submental space could reduce the tumor growth. Scientists state that regarding HNSCC in mice, when NP-E7LP-vaccination was performed after mEERL95-cell injection; but before resection of primary tumors, no post-surgery relapse was encountered and all of the mice survived through the experiment that was 70 days-long [60]. It suggests that HPV-vaccine in case of HPV+ HNSCC could benefit the patient in reducing the risk of a relapse.

Conclusion

As HPV-related HNSCC are becoming more and more detected in the population, more adapted treatments must be found. The first step is to de-escalate common chemo- and radiotherapy protocols as these are tough treatment that come with lots of side effects. Such tumors seem like good responders to immunotherapy, depending on the oncogenic mutation that is involved. With the knowledge of which mutation is occurring, scientists indeed have the opportunity to adapt the patient's treatment, giving more positive outcomes.

Nevertheless, regarding the rise of the implication of HPV in HNSCC, immune-based treatments are still at their beginning. Targeted therapies like oncogenic pathways inhibitors are also very much under development like alpelisib, a PI3K inhibitor [22]. Future treatments for HNSCC may imply a combination of both traditional therapy (chemo- or radiotherapy) as well as immunotherapy.

References

1. High Authority of Health (2023) Questions and Answers on human papillomavirus (HPV) infection, cause of cervical cancer, and screening.: https://www.has-sante.fr/jcms/p_3146343/fr/questions-reponses-sur-l-infection-a-papillomavirus-humains-hpv-cause-de-cancer-du-col-de-l-uterus-et-le-depistage
2. Tumban E A (2019) Current Update on Human Papillomavirus-Associated Head and Neck Cancers. Viruses 11: 922.
3. Papillomavirus (HPV) infection (2023) cancer risks • Cancer Environment [Internet]. Environmental Cancer. https://www.cancer-environnement.fr/fiches/expositions-environnementelles/infection-a-papillomavirus-%20humaines-%20hpv/
4. Papillomavirus, what exactly is it? (2023) https://www.cancer.be/les-cancers/facteurs-de-risque/le-papillomavirus-quest-ce-%20exactement
5. Cancers caused by papillomaviruses | Papillomavirus.fr (2003) https://papillomavirus.fr/sinformer/cancers
6. Perri F, Longo F, Caponigro F, Sandomenico F, Guida A et al. (2020). Management of HPV-Related Squamous Cell Carcinoma of the Head and Neck: Pitfalls and Caveat. Cancers (Basel) 12: 975.
7. Qin T, Li S, Henry LE, Liu S, Sartor MA (2021) Molecular Tumor Subtypes of HPV- Positive Head and Neck Cancers: Biological Characteristics and Implications for Clinical Outcomes Cancers 13: 2721.
8. Jain KS, Sikora AG, Baxi SS, Morris LGT (2013). Synchronous cancers in patients with head and neck cancer: risks in the era of human papillomavirus-associated oropharyngeal cancer. Cancer 119: 1832-1837.
9. Lechner M, Liu J, Masterson L, Fenton TR. (2022) HPV-associated oropharyngeal cancer: epidemiology, molecular biology and clinical management. Nat Rev Clin Oncol. 19: 306-327.
10. Mendenhall WM, Logan HLC (2009) Human papillomavirus and head and neck cancer. Am J Clin Oncol 32: 535-539.
11. Kobayashi K, Hisamatsu K, Suzui N, Hara A, Tomita H, et al. (2018) A Review of HPV-Related Head and Neck Cancer. Journal of Clinical Medicine 7: 241.
12. Farah CS (2021) Molecular landscape of head and neck cancer and implications for therapy. Annals of Translational Medicine 9: 915-915.
13. Liu X, Gao X lei, Liang X hua, Tang Y ling (2016) The etiologic spectrum of head and neck squamous cell carcinoma in young patients. Oncotarget 7: 66226-66238.
14. Goon PK, Stanley MA, Ebmeyer J, Steinsträsser L, Upile T, et al. (2009) HPV & head and neck cancer: a descriptive update. Head & Neck Oncology 1: 36.
15. Zur Hausen H. (1999) Papillomaviruses in Human Cancers. Proceedings of the Association of American Physicians 111: 581-587.
16. Koskinen WJ, Chen RW, Leivo I, Mäkitie A, Bäck L, et al. (2003) Prevalence and physical status of human papillomavirus in squamous cell carcinomas of the head and neck. Int J Cancer 107: 401-406.
17. Weinberger PM, Yu Z, Haffty BG, Kowalski D, Harigopal M, et al. (2006). Molecular classification identifies a subset of human papillomavirus--associated oropharyngeal cancers with favorable prognosis. J Clin Oncol 24: 736-747.
18. Gillison ML, Chaturvedi AK, Anderson WF, Fakhry C. (2015) Epidemiology of Human Papillomavirus–Positive Head and Neck Squamous Cell Carcinoma. J Clin Oncol 33: 3235-3242.
19. Blomberg M, Nielsen A, Munk C, Kjaer SK (2011) Trends in head and neck cancer incidence in Denmark, 1978-2007: Focus on human papillomavirus associated sites. International Journal of Cancer 129: 733-741.
20. Chaturvedi AK (2012) Epidemiology and Clinical Aspects of HPV in Head and Neck Cancers. Head and Neck Pathol 6: 16-24.
21. Syrjänen S (2018) Oral manifestations of human papillomavirus infections. Eur J Oral Sci 126: 49-66.
22. Vokes EE, Agrawal N, Seiwert TY (2015) HPV-Associated Head and Neck Cancer. JNCI: Journal of the National Cancer Institute. 1 déc 107: djv344.
23. Eurofins Biomnis (2023) Genome detection of high-risk (HR) HPV, as part of primary screening for CCU. Eurofins Biomnis. https://www.eurofins-biomnis.com/services/referentiel-des-examens/page/HPV/#
24. Dulguerov P, Vourexakis Z (2011) Les cancers ORL HPV positifs. Rev Med Suisse 311: 1919-1922.
25. Augustin JG, Lepine C, Morini A, Brunet A, Veyer D, et al. (2020) HPV Detection in Head and Neck Squamous Cell Carcinomas: What Is the Issue? Front Oncol 10: 1751.
26. Salazar CR, Smith RV, Garg MK, Haigentz M, Schiff BA, et al. (2014) Human papillomavirus-associated head and neck squamous cell carcinoma survival: a comparison by tumor site and initial treatment. Head Neck Pathol 8: 77-87.
27. Badoual C, Péré H, Roussel H, Si Mohamed A, Tartour É (2013) Cancers of the upper aerodigestive tract associated with human papillomavirus. Med Sci (Paris) 29: 83-88.
28. Huang SH, Xu W, Waldron J, Siu L, Shen X, et al. (2015) Refining American Joint Committee on Cancer/Union for International Cancer Control TNM stage and prognostic groups for human papillomavirus-related oropharyngeal carcinomas. J Clin Oncol 33: 836-845.
29. Huang SH, O'Sullivan B (2017) Overview of the 8th Edition TNM Classification for Head and Neck Cancer. Curr Treat Options in Oncol 18: 40.
30. Ferris RL, Spanos WC, Leidner R, Gonçalves A, Martens UM, et al. (2021) Neoadjuvant nivolumab for patients with resectable HPV-positive and HPV-negative squamous cell carcinomas of the head and neck in the CheckMate 358 trial. J Immunother Cancer 9: e002568.
31. Woody NM, Koyfman SA, Xia P, Yu N, Shang Q, et al. (2016) Regional control is preserved after dose de-escalated radiotherapy to involved lymph nodes in HPV positive oropharyngeal cancer. Oral Oncol 53: 91-96.
32. Palma DA, Prisman E, Berthelet E, Tran E, Hamilton S, et al.

(2022) Assessment of Toxic Effects and Survival in Treatment Deescalation with Radiotherapy vs Transoral Surgery for HPV-Associated Oropharyngeal Squamous Cell Carcinoma: The ORATOR2 Phase 2 Randomized Clinical Trial. JAMA Oncol 8: 1-7.

33. Lawson Health Research Institute (2022) A Randomized Trial of Treatment De-Escalation for HPV-Associated Oropharyngeal Squamous Cell Carcinoma: Radiotherapy vs. Trans-Oral Surgery (ORATOR II). Clinicaltrials 111: 1324-1325.
34. Cmelak A, Li S, Marur S, Zhao W, Westra WH, et al. (2014) E1308: Reduced-dose IMRT in human papilloma virus (HPV)-associated resectable oropharyngeal squamous carcinomas (OPSCC) after clinical complete response (cCR) to induction chemotherapy (IC) JCO 35: 490-497.
35. Wang Y, Springer S, Mulvey CL, Silliman N, Schaefer J, et al. (2015) Detection of somatic mutations and HPV in the saliva and plasma of patients with head and neck squamous cell carcinomas. Science Translational Medicine 7: 293ra104-293ra104.
36. Seiwert TY, Burtness B, Weiss J, Eder JP, Yearley J, et al. (2015) Inflamed-phenotype gene expression signatures to predict benefit from the anti-PD-1 antibody pembrolizumab in PD-L1+ head and neck cancer patients. JCO 33: 6017-6017.
37. Research C for DE, Nivolumab for SCCHN (2023) FDA https://www.fda.gov/drugs/resources-information-approved-drugs/nivolumab-scchn.
38. Research C for DE, FDA (2019) approves pembrolizumab for first-line treatment of head and neck squamous cell carcinoma. FDA https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-pembrolizumab-first-line-treatment-head-and-neck-squamous-cell-carcinoma.
39. Von Witzleben A, Wang C, Laban S, Savelyeva N, Ottensmeier CH (2020) HNSCC:Tumour Antigens and Their Targeting by Immunotherapy. Cells 9: 2103.
40. Wang H, Zhao Q, Zhang Y, Zhang Q, Zheng Z, et al. (2021) Immunotherapy Advances in Locally Advanced and Recurrent/Metastatic Head and Neck Squamous Cell Carcinoma and Its Relationship with Human Papillomavirus. Frontiers in Immunology 12: 652054.
41. Nayak Kapoor A, Hao Z, Sadek R, Dobbins R, Marshall L, et al. (2018) Phase Ia study of the indoleamine 2,3-dioxygenase 1 (IDO1) inhibitor navoximod (GDC-0919) in patients with recurrent advanced solid tumors. J Immunother Cancer 6: 61.
42. Mitchell TC, Hamid O, Smith DC, Bauer TM, Wasser JS, et al. (2018) Epacadostat Plus Pembrolizumab in Patients With Advanced Solid Tumors: Phase I Results From a Multicenter, Open-Label Phase I/II Trial (ECHO-202/KEYNOTE-037). J Clin Oncol 36: 3223-3230.
43. Ferris RL, Saba NF, Gitlitz BJ, Haddad R, Sukari A, et al. (2018) Effect of Adding Motolimod to Standard Combination Chemotherapy and Cetuximab Treatment of Patients With Squamous Cell Carcinoma of the Head and Neck: The Active8 Randomized Clinical Trial. JAMA Oncol 4: 1583-1588.
44. June CH (2007) Adoptive T cell therapy for cancer in the clinic. J Clin Invest 117: 1466-1476.
45. Cardiff University (2023) Superior T-cell discovered in cancer survivors. Disponible sur: https://www.cardiff.ac.uk/news/view/2734440-superior-t-cell-discovered-in-cancer-survivors#:~:text=Published%20today%20in%20the%20journal,single%20target%20on%20cancer%20cells.
46. Gustave Roussy (2023) Les cellules CAR-T. Disponible sur: https://www.gustaveroussy.fr/fr/les-cellules-car-t.
47. Chakraborty P, Karmakar T, Arora N, Mukherjee G (2018) Immune and genomic signatures in oral (head and neck) cancer. Heliyon 4: e00880.
48. Damasio MPS, Nascimento CS, Andrade LM, de Oliveira VL, et al. (2022) The role of T-cells in head and neck squamous cell carcinoma: From immunity to immunotherapy. Frontiers in Oncology 12: 1021609. https://www.frontiersin.org/articles/10.3389/fonc.2022.1021609
49. Sarkizova S, Hacohen N (2017) How T cells spot tumour cells. Nature 551: 444-446.
50. Łuksza M, Riaz N, Makarov V, Balachandran VP, Hellmann MD, et al. (2017) A neoantigen fitness model predicts tumour response to checkpoint blockade immunotherapy. Nature 551: 517-520.
51. Park YP, Jin L, Bennett KB, Wang D, Fredenburg KM, et al. (2018) CD70 as a target for chimeric antigen receptor T cells in head and neck squamous cell carcinoma. Oral Oncology 78: 145-150.
52. Warren EA, Liu HC, Porter CE, Liao KS, Hegde M, et al. (2019) Abstract 574: Overexpression of HER2 in head and neck cancer represents a potential target for T cell immunotherapy. Cancer Research 79: 574.
53. Mei Z, Zhang K, Lam AKY, Huang J, Qiu F, et al. (2020) MUC1 as a target for CAR-T therapy in head and neck squamous cell carinoma. Cancer Medicine 9: 640-652.
54. VIDAL (2023) GARDASIL. Disponible sur: https://www.vidal.fr/medicaments/gammes/gardasil-30455.html.
55. Wang C, Dickie J, Sutavani RV, Pointer C, Thomas GJ, et al. (2018) Targeting Head and Neck Cancer by Vaccination. Frontiers in Immunology 9: https://www.frontiersin.org/articles/10.3389/fimmu.2018.00830.
56. VIDAL (2023) CERVARIX. Disponible sur: https://www.vidal.fr/medicaments/gammes/cervarix-33535.html.
57. Inserm (2023) Papillomavirus : faut-il généraliser la vaccination ? • Inserm, La science pour la santé. Disponible sur: https://www.inserm.fr/actualite/papillomavirus-faut-il-generaliser-la-vaccination/
58. Chaturvedi AK, Graubard BI, Broutian T, Pickard RKL, Tong ZY, et al. (2018) Effect of Prophylactic Human Papillomavirus (HPV) Vaccination on Oral HPV Infections Among Young Adults in the United States. J Clin Oncol 36: 262-267.
59. Zhai L, Yadav R, Kunda NK, Anderson D, Bruckner E, et al. (2019) Oral immunization with bacteriophage MS2-L2 VLPs protects against oral and genital infection with multiple HPV types associated with head & neck cancers and cervical cancer. Antiviral Res 166: 56-65.
60. Domingos-Pereira S, Roh V, Hiou-Feige A, Galliverti G, Simon C, Tolstonog GV, et al. (2021) Vaccination with a nanoparticle E7 vaccine can prevent tumor recurrence following surgery in a human papillomavirus head and neck cancer model. Oncoimmunology 10: 1912473.