Increasing awareness and therapeutical options to improve prognosis of HPV positive and HPV negative head and neck cancer

Summary

While the incidence of tobacco related cancer has declined in the past two decades, because of a reduction in the prevalence of smoking in most high-income countries, there is an increase in HPV associated oropharyngeal squamous cell carcinoma (OPSCC). More specifically, the prevalence of HPV in OPSCC increased from 21.4% in 2004 to 50% in 2011 in Maastricht University Medical Center. An HPV vaccine has been available for some time now, which may reduce not only the incidence of uterine cervical cancer but also HPV associated head and neck squamous cell carcinoma (HNSCC). In the Netherlands, the HPV vaccine is since 2010 available for girls and since 2022 for boys from the age of ten. In order to maximize the potential benefits of HPV vaccination, it is necessary to get the vaccination coverage as high as possible. Therefore, it is important that patients and health care professionals are aware of the human papillomavirus, the association of the virus with cancer and the availability of an HPV vaccination. Until now it was unclear what the knowledge is about the role of HPV in OPSCC under the general population and general practitioners in the Netherlands.

Chapter 2 shows the results of a study examining the awareness of HPV associated OPSSC among a representative sample of the Dutch population. 30.6% of the participants had heard of HPV and only 29.9% of these participants knew about the association between HPV and OPSCC. 49.7% of the participants knew that there is an HPV vaccine available. The results of this survey indicate that the public awareness of HPV and the association with OPSCC is lacking. The awareness about HPV, the HPV vaccine and the link of HPV with OPSCC was greater among women and suggest that this knowledge is primarily due to awareness of the role of HPV in the development of uterine cervical cancer. Greater awareness of the role of HPV infection in OPSCC is necessary to improve vaccine uptake, in women but especially also in men.

The awareness of the association between HPV and OPSCC is much higher under the general practitioners in the Netherlands (72%), but more than a quarter of them is unaware of HPV as a causative factor for OPSCC. The results in **chapter 3** show that there was limited awareness among the general practitioners regarding gender, age and prognosis of patients with HPV associated OPSCC. Only 35.5% of the participating general practitioners was aware that HPV associated OPSCC patients are more often male, and just over half of the participants knew that these patients are generally younger of age. Interventions to increase awareness of HPV and its association with non-uterine cervical cancer should be considered, which might help to increase the HPV vaccination uptake and earlier diagnosis of this disease leading to improved survival.

HPV vaccination and increased awareness of HPV related OPSCC are possibilities to improve the prognosis of these cancers. Most HPV positive tumours have a more favourable prognosis in comparison with HPV negative tumours, however a subgroup of HPV positive tumours shows less favourable prognosis with a greater risk of recurrence or developing a second primary tumor. In order to improve the prognosis of HNSCC patients, there are new therapeutical strategies necessary. There are different pathways whose deregulation play a role in the development of HPV positive and -negative HNSCC and thus may be potential targets in the treatment of HNSCC. Therefore, we studied whether or not medication targeting some of the most important pathways are able to improve treatment.

Because HNSCC is associated with activation of the PI3K/Akt/mTOR pathway and also with deregulation of the core cell-cycle regulatory machinery, we investigated the in vitro antiproliferative effects of several PI3K/Akt/mTOR pathway inhibitors (alpelisib, buparlisib and gedatolisib) and CDK4/6 inhibitors (palbociclib and ribociclib) in HPV positive and -negative HNSCC cell lines (**chapter 4)**. In addition, we compared the inhibitors with the growth inhibitory effect of cisplatin, which is the current, most widely used chemotherapeutical treatment option in HNSCC. PI3K inhibitors and CDK4/6 inhibitors proved to efficiently inhibit their respective pathways and HNSCC cell growth in vitro, the latter only in HPV negative cell lines. Whereas PI3K inhibition especially induces apoptosis and attenuates cellular metabolism, CDK4/6 inhibition particularly leads to cell cycle arrest. Further research should elucidate whether (a combination of) these inhibitors may be effective therapeutic agents for HNSCC patients.

Another possible therapeutic option for HNSCC is the antiviral agent Cidofovir (CDV), which is an acyclic nucleoside phosphonate targeting DNA viruses that encode for their own DNA polymerase. Besides direct antiviral effects in DNA viruses CDV has also demonstrated antiproliferative properties against HPV positive and HPV negative malignancies in vitro and in vivo. The molecular mechanism underlying the efficacy of CDV is not completely understood, as HPV uses the host DNA polymerase for replication. Therefore, in **chapter 5,** the antiproliferative effects of CDV were investigated in HPV positive and HPV negative HNSCC cell lines and the normal oral keratinocyte cell (NOK) cell line. We investigated whether the antiproliferative effect was caused by a difference in response to DNA damage. CDV inhibited the cell growth of all the HPV positive and -negative HNSCC cell lines. Treatment with CDV caused DNA damage by means of DNA double strand breaks (DSBs) and as a result the DNA damage response pathway became activated. There was more DNA damage visible in the HPV positive cell lines showing the strongest inhibition as compared to the HPV negative cell lines showing significantly less

inhibition by CDV. CDV treatment resulted in G2/M phase arrest, but apoptosis did not appear to occur. Rather our data indicate the occurrence of mitotic catastrophe.

Following an HPV infection, the virus can remain in its episomal form, or the HPV genome become eventually integrated into the host cell genome. So far, there is little evidence that viral integration may have impact on prognosis and it is unclear if there is a biological consequence of viral integration. Therefore in **chapter 6**, by comparing HPV16 positive OPSCC harboring episomal or integrated virus using mRNA microarray expression profiling, we identified a unique signature of differentially expressed human mRNAs in relation to viral physical state. The tumors with viral integration showed deregulated expression of genes involved in metabolic pathways, frequently including upregulated Aldo-keto-reductase 1C1 and/or 1C3 (AKR1C1 and AKR1C3) expression. Survival analysis of 141 additionally immunostained OPSCC (HPV positive as well as HPV negative) showed unfavorable survival rates for tumors with upregulation of AKR1C1 or AKR1C3.

If these results could be confirmed in larger studies, than AKR1C1 and AKR1C3 may be considered to be included in prediction models in OPSCC, independent of HPV status. Low risk groups (for example HPV positive OPSCC tumor without AKR1C upregulation) could then potentially profit from de-intensification of treatment protocols, whereas intermediate and high risk groups could be selected for other therapeutic options, such as inhibitors of the PI3K and NRF2 pathways including AKR1C.