Summary

Human papillomavirus (HPV) is the most common sexually transmitted infection worldwide. Most HPV infections are benign and are cleared by the host within six to twelve months, although some infections can persist. A persistent infection with an oncogenic HPV type is required for the development of cervical cancer. In addition, oncogenic HPV type infections can lead to a number of other cancers in both females and males. The development from initial infection to cervical cancer can take decades and progresses through histopathologically well-defined pre-cancerous stages called cervical intraepithelial neoplasia 1–3 (CIN1–3).

Over 200 different HPV types have been described, of which only a limited number are capable of causing cervical cancer. These HPV types are considered oncogenic HPV types. Currently, three highly efficacious prophylactic vaccines are available to prevent HPV-related cervical disease. The bivalent vaccine protects against HPV16 and HPV18, potentially preventing up to 70% of all cervical cancer cases. The quadrivalent vaccine also protects against HPV16 and HPV18, but further includes protection against HPV6 and HPV11, thereby preventing most cases of genital warts. The more recently introduced nonavalent vaccine further extends protection of the quadrivalent vaccine by including HPV31, 33, 45, 52 and 58, which cause approximately 20% of all cervical cancer cases. All three vaccines convey strong protection against the targeted HPV types. Cross-protection against other, non-vaccine HPV types has been described for all vaccines, but has been found strongest for the bivalent vaccine.

In the Netherlands, prophylactic vaccination against cervical cancer was initiated in 2009 using the bivalent vaccine. Parallel to its introduction, a vaccine monitoring programme was initiated, to monitor vaccine effects. The earliest conventional marker to monitor vaccine effectiveness is the presence of persistent infections in vaccinated individuals. This thesis explores the value of HPV viral load measurements to assess (persistent) infections in both vaccinated and non-vaccinated individuals. In addition, long-term HPV vaccination could lead to selective pressure on the HPV population, potentially leading to changes in the prevalence of specific HPV genome variants. In this thesis, we attempt to gain insight into such events, by developing HPV whole genome sequencing assays and applying them to relevant cohort studies.
Part I: Viral load measurements in vaccinated and non-vaccinated settings

The value of viral load measurements is assessed in Part I of this thesis. In Chapter 2, we analyzed HPV16 and HPV18 viral load measurements obtained from a study of young, vaccine-eligible women (aged 16–29 years old). The viral load trends we identified from these young women were similar to trends described in previous studies including mature women. Baseline HPV16 and HPV18 viral load measurements were found to be significantly higher at the population level for infections that persist for at least one year compared to infections that clear within a year. However, these differences were not strong enough to generate discriminatory value between clearing and persistent infections at the individual level. Consequently, these viral load assays were used in order to analyze possible population-level effects of the bivalent HPV vaccine on viral load in Chapter 3. Here, viral load measurements were conducted for all oncogenic HPV types as well as HPV6 and HPV11. For the vaccine types HPV16 and HPV18, significantly lower viral loads were found in both breakthrough incident clearing and incident persistent infections in vaccinated individuals compared to infections in non-vaccinated individuals. This finding implies that breakthrough HPV16 and HPV18 infections in vaccinated individuals are potentially less likely to persist and cause cervical disease. Cross-protection was found against HPV31, 33, 35 and 45, but these effects could not be correlated with viral load measurements.

Part II: Development and application of (next-generation) sequencing assays in epidemiological and clinical contexts

In Part II of this thesis, we explore HPV16 and HPV18 sequence diversity in pre-vaccination and clinical studies. In Chapters 4 and 5, both persistent and clearing HPV16 and HPV18 infections were studied in a cohort of unvaccinated women. The resulting HPV16 and HPV18 populations were found to be remarkably diverse considering the conserved nature of the virus. Nearly all study participants were found to be infected with unique HPV16 or HPV18 variants, potentially opening research opportunities for transmission studies. In persistent infections, the HPV sequence was found to be strongly conserved over the three-year follow-up period of the study. In rare cases, the variant sequenced at baseline did not match with the variant sequenced at follow-up. This implies that over time, variant switching may occur. When persistent HPV type-specific infections are identified in vaccinated women, infection with a different variant of the same HPV type should be excluded. This finding may be relevant in vaccine monitoring studies.

The switching of variants over time raises the possibility that multiple HPV variants of the same type could be present at any one time. The presence of these so-called minority variants was explored in Chapter 6. A subset of the same study used in Chapters 4 and 5 was used to study HPV16 intra-host diversity. Using a novel, highly sensitive next-generation sequencing assay, a large number of intra-host minority variants were identified. The mutation patterns of these infections, identified in young women, appeared to be different when compared to mutation patterns of CIN2/3 infections. These differences potentially suggest that mutation processes
identified in HPV present in CIN2/3 lesions are not (yet) present in HPV infections not associated with CIN. These mutational signatures could be an indication of the biological processes driving HPV diversity generation.

In chapter 7, we assessed HPV16 variants from HPV 16 positive women who were treated for CIN2/3 and who were found to have HPV16 positive recurrent CIN (rCIN) during follow-up. Usually, the majority (90%-95%) of women who undergo ablative treatment (LEEP or LLETZ) after CIN2/3 detection are able to clear the infection. However, a minority of women (5%-10%) contract rCIN. Sequencing of both the baseline HPV16-associated CIN and rCIN samples one to two years later resulted in (nearly) identical sequences before and after treatment. The presence of the same HPV16 variant in both baseline and posttreatment rCIN suggests that the treatment procedure was inadequate or that these women are unable to clear these specific variants.

Vaccine cross-protection is currently being studied extensively, and although there is consensus that HPV vaccines can cause cross-protection against other non-vaccine HPV types, discrepancies between studies do exist. In chapter 8 of this thesis, we correlate HPV reference sequence data with HPV vaccine effectiveness data. Phylogenetic distance of a given HPV type to HPV16 and HPV18 vaccine protein sequence is shown to strongly correlate with vaccine effectiveness against that HPV type. Stronger cross-protection was inferred against HPV types phylogenetically closely related to vaccine HPV types. On the other hand, cross-protection against HPV types phylogenetically distant from the vaccine HPV types is unlikely. These findings might help to reconcile differences in findings between vaccine monitoring studies.

The studies described in this thesis have resulted in new methods to monitor the presence of persistent HPV infections in vaccinated women. Using viral load measurements, we were able to reinforce the high vaccine efficiency of bivalent HPV vaccination at the population level. Through various methods of sequencing, HPV was shown to be strongly conserved over time, yet highly diverse at the same time. Furthermore, HPV16/18 variant analysis may be useful to exclude repeated infections with different variants of the same HPV type in breakthrough infections in vaccinated women.