CHAPTER 9

Summary

SUMMARY

This thesis focuses on the role of the immune system in cervical cancer. Despite availability and improvements in screening and vaccination, cervical cancer continues to affect more than half a million women worldwide, and is still the fourth most common cause of cancer-related deaths in women. Early-stage cervical cancer is typically amenable to cure, unfortunately, advanced, recurrent, or persistent disease is often incurable. The immune system plays an important role in the development, maintenance and expansion of cervical cancer. Carcinogenesis often leads to an immunosuppressive environment that promotes tumour growth and protects the tumour from immune attack. When tumour cells overcome the immune control, they can proliferate, infiltrate and finally kill the host. Many different mechanisms of immunosuppression can impair the host defence against tumours such as secretion of immunosuppressive cytokines, proliferation and accumulation of immunosuppressive cells and alterations in antigen-presenting cell subsets. Although these abnormal immune responses accompany many cancers, they may also provide an opportunity for novel treatment strategies such as immunotherapy. In **chapter 1** we give a general introduction on cervical cancer, HPV and immunology, and immunotherapy, and we describe the aim and outline of this thesis. Through the studies in this thesis, we gained more knowledge on the local and systemic immune responses in patients with cervical cancer.

In **chapter 2** we studied the microenvironment of tumour-draining lymph nodes of cervical cancer patients, by flow cytometry-based phenotyping and enumeration of immune-cell subsets in tumour-negative (LN-) and tumour-positive (LN+) lymph nodes. We found important differences in the immune cell subsets between LN+ and LN- cervical cancer patients. In LN+ cervical cancer patients there was a profound immune suppressive microenvironment. This immunosuppressive environment is most likely able to negate a successful antitumour response and thereby enable metastatic spread. The presence of these suppressive factors and regulatory immune subsets can impede therapeutic vaccination efficacy.

In **chapter 3 and 4,** we studied different immune escape mechanisms in cervical adenocarcinoma (AC), adenosquamous (ASCC) and squamous cell carcinoma (SCC), to further unravel the biological and immunological behaviour of the different histological subtypes. Down-regulation of major histocompatibility complex class I chain-related

molecule A (MICA) and up-regulation of human leukocyte antigen G (HLA-G), are known immune escape mechanisms for different epithelial tumours. In chapter 3 we measured, by enzyme-linked immunosorbent assay (ELISA), soluble MICA and HLA-G in pre-treatment sera of cervical cancer patients. Cervical AC patients with high soluble MICA levels showed to have an increased disease-free (DFS) and disease-specific survival (DSS). There was no association found between survival and SCC, emphasizing the differences between the histological subtypes in cervical cancer.
Furthermore, HLA class II molecules, including HLA-DRA, have been described in a variety of tumours. For cervical cancer, the role of HLA class II expression and its clinical relevance is still unknown. Therefore, we studied in chapter 4 the pattern of HLA-DRA expression in cervical cancer patients, using immunohistochemistry. We found that up-regulation of HLA-DRA is associated with a decreased recurrence rate and an increased DSS in cervical AC patients. No association between survival and HLA-DRA up-regulation was found in SCC. In conclusion, in both chapter 3 and 4, we show that AC and SCC of the cervix are indeed immunologically different. These findings are potentially of great importance with the rise of cancer immunotherapy: should we treat AC and SCC differently?

Chapter 5 presents the study protocol of the European Union (EU) FP7-funded collaborative BIO-RAIDs study, which is the first prospective molecular profiling clinical study in cervical cancer, recruiting patients in 6 EU countries. From patients with previously non-treated cervical cancer stages IB2-IV tumour biopsies and blood samples are collected at defined time points. Patients receive standard primary treatment according to their stage of disease, and country of residence. The aim of the study is to define a set of stratification criteria based on molecular profiling. The implication of this project for the clinical practice is to stratify cancer patients, based on specific tumour deregulations, for the best treatment option, which is more specific and less toxic. Thereby, improving their prognosis and quality of life.

In **chapter 6** we describe the challenges that impeded the effective implantation of the BIO-RAIDs study, as described in chapter 5. In this era of precision medicine, biobanking studies are increasing. The BIO-RAIDs study is, as stated above, the first prospective molecular profiling study in cervical cancer across the EU. We identified multiple hurdles that lead to delays in clinical trial initiation. There was a lack of uniform international legal and ethical standards across the EU countries. Furthermore, complexities in clinical and molecular data management, and difficulties in determining the right technical platforms

and data analysis techniques, lead to great delays. We feel, that there is a need for standardisation in terms of regulatory rules and practises across the EU. Moreover, international working groups who recommend regulatory bodies, governmental funding agencies, and academic institutions, could be of great interest to achieve a proficient biobanking programme throughout EU countries.

We studied a therapeutic HPV16 E7 DNA vaccine (TTFC-E7SH) with a novel administration strategy, in which DNA is delivered via a tattoo, in patients with HPV-positive vulvar intraepithelial neoplasia (VIN). VIN is a precursor lesion for vulvar cancer. This study was initially designed to study HVP DNA vaccination in cervical cancer patients, but unfortunately the ethics committee did not agree to that, because they found patients with cervical cancer too vulnerable for the testing of a new treatment. Therefore, we decided to study the HPV DNA vaccination in an HPV-related precursor lesion. In **chapter 7** the results of the trial are described. The trial was designed to test the safety, immunogenicity and clinical response of TTFC-E7SH in VIN patients. Two dose levels were tested. DNA tattoo vaccination showed to be safe, as only grade I-II adverse events were observed upon vaccination. Unfortunately, only a limited vaccine-induced immune response and no clinical response was observed.

Finally, in the general discussion in **chapter 8,** the findings presented in this thesis are discussed and we focus on future prospects of (immunotherapy for) cervical cancer. In brief: cervical cancer remains a significant worldwide health problem. To illuminate effective new therapies for cervical cancer, one of the most important things is gaining a better knowledge of the microenvironment of the primary tumour and metastatic LNs. Furthermore, a better insight on molecular level will give us the opportunity to give tailored treatment, also called precision medicine. However, we have to realise that before these targeted therapies will be available for the population, a long road is ahead.