Chapter 10

Summarizing Discussion and Future Perspectives
Summarizing discussion

Extensive progress has been made in the development of novel therapeutic human papilloma virus (HPV) vaccines to cure HPV-associated (pre)malignant lesions. In this thesis we focused on the preclinical and clinical development of alphavirus replicon vaccine candidates derived from Semliki Forest virus (SFV) targeting HPV type 16 E6 and E7. SFV replicon vaccines provide an intrinsic adjuvant property and the impressive preclinical results suggest these vaccines are rational approaches for clinical application. Yet, vaccine regimens may require further improvement to preemptively increase the chance of therapeutic efficacy in the clinic. Tolerance and immunosuppressive mechanisms counteract durable and long-lasting responses in cancer patients. We aimed to improve on the already-potent replicon vaccine through various design and delivery strategies for enhancing vaccine-induced immunity. These strategies aim to enhance anti-HPV immunity for more potent anti-tumor control. In addition, we present studies that have implications in predicting and monitoring the anti-tumor immune responses potentially elicited by immunotherapeutic vaccination.

In this chapter, the studies of this thesis will be discussed with subsequent important considerations to take into account as future perspective for the clinical application of HPV vaccines.

Development and clinical application of therapeutic HPV replicon vaccines

The discovery of high-risk HPV as an etiological factor for cervical lesions and cervical cancer provides justification for the myriad of immunotherapeutic HPV vaccines that have been evaluated in both preclinical and clinical studies. These vaccines range from protein, peptide, nucleic acid, viral- or bacterial-based vectors with most targeting HPV oncoproteins E6 and E7 for controlling HPV-associated disease.1,2,3 In this thesis, viral vector and DNA replicon vaccines based on SFV were studied. In particular, the viral vector strategy was previously extensively explored in preclinical studies. In these studies, a recombinant SFV replicon particle encoding a fusion protein of E6 and E7 of HPV 16 (rSFVeE6,7) demonstrated potent HPV-specific cellular responses that translated into effective eradication of established tumors.4,5,6 These studies point towards the potential of this strategy for successful treatment of HPV-associated disease in the clinic. To date, no therapeutic vaccines have been FDA-approved for HPV-induced (pre)-malignant lesions.

In Chapter 2 we present the clinical translation of rSFVeE6,7 replicon particles. The production method for clinical translation required the use of helper RNAs. The RNA encoding for the replicase and the fusion protein of E6 and E7 is co-transfected into Vero cells with helper RNAs for complementation in trans.7 The helpers RNAs separately encode the capsid protein and the envelope spike proteins.7 The first-generation helper was originally a single molecule encoding for both the capsid and envelope protein.7 Dividing the helper was imperative to decrease the chance of recombination for the generation
of wild-type replicating virus. This so-called split helper system was used for the clinical production rSFVeE6,7, termed Vvax001. Chapter 2 describes the scale-up production of Vvax001 in Vero cells, a suitable cell line for GMP production. Vvax001 was further tested for toxicity in mice and no adverse effects were observed. Furthermore, the vaccine demonstrated high anti-HPV cytotoxic lymphocyte activity concomitant with potent therapeutic efficacy in a TC-1 mouse model of cervical cancer. Vvax001 has recently been introduced in a phase I clinical trial. Despite the potential of this vaccine strategy, there are a few drawbacks associated with this replicon platform. These include the high production costs with the use of cell lines and instability in long-term storage with requirement of a cold chain reaction.

The drawbacks of viral replicon particles can be circumvented with DNA-based vaccination. DNA vaccines might be more favorable in the clinical setting as they can be manufactured to scale at low cost, possess a long shelf life and are more temperature-stable. However, first-generation DNA plasmids are associated with poor translation from animal models to humans with low levels of T cell and B cell memory observed in clinical studies. For this reason, in Chapter 3 we designed a next-generation DNA vaccine based on the SFV replicase. This DNA replicon vaccine (DREP) encodes for the same target antigen as for our SFV replicon viral particles (DREPeE6,7). We compared the ability of DREPeE6,7 with conventional DNA (pVAX) in eliciting E7-specific responses. Administration of the vaccines by intradermal injection followed by electroporation demonstrated that DREP was far superior to that of conventional DNA in both magnitude and functionality of E7-specific T cells. The induction of efficient cytotoxic lymphocyte (CTL) responses resulted in robust anti-tumor responses compared to either the conventional DNA or buffer control. The mice were immunized with various dosages of DREP to determine the dose-sparing effect on the anti-tumor response. Previous studies have demonstrated significant cellular responses by DREP vaccination with as low as 80 ng. DREPeE6,7 could be further reduced in dose to as low as 50 ng with approximately 70% of mice being tumor-free up to 15 weeks post tumor inoculation. Interestingly, immunization with a dose of 10 µg resulted in lower anti-tumor efficacy compared to 20 and 50 ng. Immunization with replicon vaccines results in an upregulation of type I interferon responses leading to an antiviral state inducing specific immune responses, thus hampering the response through various pathways that inhibit virus replication. This result is not unprecedented as a previous study demonstrates lower immunogenicity with DREP administered beyond a dose of 10 µg.

A concern in the clinic with regard to the safety of DNA vaccines is the potential of integration into cellular DNA. Although the risk of integration of current DNA vaccines tested is orders of magnitude lower the rate of spontaneous mutation, increasing the potency through design modification, adjuvantation and delivery could increase the risk. Integration of HPV DNA vaccines may lead to risk of cellular transformation by E6
and/or E7. To prevent this risk, point mutations may be introduced in E6 and E7 to disturb the binding of known cellular targets p53 and Rb, respectively. Alternatively, gene-shuffling can also prevent the binding to additional cellular targets without disturbing putative immunodominant epitopes.\textsuperscript{15,16} Therefore, in Chapter 3, a gene shuffled version of E7 (E7SH) was introduced in DREP.\textsuperscript{15,16} As our vaccine is an adjuvant in itself, we also determined whether immunogenicity of DREP could be further enhanced by increasing antigen stability and by adding CD4+ helper epitopes (PADRE, P30 and NEF).\textsuperscript{17} Although the frequency of E7-specific T cells was increased with these additional genes, the antitumor efficacy was not significantly improved at equal doses. These data indicate that the intrinsic potency of DREP is at a high enough threshold for therapeutic efficacy.

In summary the data presented in Chapter 2 and Chapter 3 indicate that our replicon vector systems based on SFV are potent for eliciting effective HPV-specific immune responses and strongly support the current and future development of these vectors in clinical trials.

Exploring design and delivery strategies to enhance anti-HPV immunity of SFV replicon vaccines

Immunotherapeutics regimens may need to be altered in their design to accommodate the translational barrier of poor clinical efficacy. To improve the outcome of these regimens, several strategies have been put in place. One such strategy is the inclusion of additional help in the form of immunomodulators as elements to further boost tumor-specific immunity. In Chapter 4 we provide a summary on the recent literature on various immunomodulators that further improve the immunogenicity of HPV-specific immunotherapeutic strategies. These include combination approaches with toll-like receptor adjuvants, cytokines and costimulatory molecules and molecules targeting immunosuppressive mechanisms. Regarding the last category, mechanisms that create a tolerized state in the tumor in the course of tumor progression include the presence of inhibitory molecules on both tumor and immune cells and the infiltration of immunosuppressive cells. Immunosuppressive cells include regulatory T cells (Treg) and myeloid-derived suppressor cells (MDSCs).\textsuperscript{16,19} Immunotherapeutic strategies have focused on blocking inhibitory molecules that negatively regulate T-cell function which include programmed cell death receptor 1 (PD-1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). Nivolumab and pembrolizumab are FDA-approved PD-1 inhibitors for the treatment of metastatic cancers and are currently under evaluation in clinical trials for HPV-associated malignancies.\textsuperscript{20,21} Yet, recent advances in cancer therapeutics have underlined the importance of combination approaches for an immunopotent tumor microenvironment.\textsuperscript{22} Current studies focus on the combination of checkpoint blockade with suppressor cell-depleting agents.\textsuperscript{23,24} Targeting these suppressive mechanisms in
combination with therapeutic vaccination may further increase the immunotherapeutic effect. Although rSFV has previously been shown to overcome immunological tolerance in an HPV-transgenic mouse model and Treg was not shown to be involved in T-cell tolerance, the functionality of antigen-specific T cells induced by immunization may be further enhanced in combination with other treatments. In Chapter 5 we report on a trimodal design approach consisting of depletion of MDSCs using a small molecular tyrosine kinase inhibitor (sunitinib), PD-1 blockade and SFVeE6,7 therapeutic immunization. We demonstrated that SFVeE6,7 as a standalone treatment enhances expression of PD-1 in T cells inducing T-cell tolerance. Combination of SFVeE6,7 with PD-1 blockade resulted in a reduction in tumor PD-L1 expression and infiltration of PD-1+CD8+ tumor-infiltrating lymphocytes (TIL). Strikingly, the combination of rSFVeE6,7 immunization with PD-1 blockade nor the trimodal treatment approach, improved the therapeutic efficacy of SFVeE6,7 in a mouse model for cervical cancer. In fact, additional treatment of PD-1 blockade with immunization seemed to have hastened tumor growth in 50% of mice. Our observations suggest a challenge in combining additional treatment with a potent immunization strategy. Yet, studies suggest that the timing of PD-1 blockade may drastically influence the treatment efficacy with one trial comparing the administration of Pembrolizumab during and after treatment with DNA vaccine against metastatic prostate cancer. It may be worthwhile to compare different treatment schedules as well as different dosages for the design of multimodal treatment regimens with current therapies that are becoming the standard-of-care.}

Expression of antigen in vivo directly correlates with the induction of vaccine-induced immunogenicity. Therefore improvement in delivery strategies may, in addition to immunomodulators, be a prerequisite for enhancing immunity to overcome the translational barrier of immunotherapeutic vaccination approaches. The site of vaccine delivery is a crucial factor for these approaches. In this thesis, in addition to the conventional route of administration being intramuscular, we explored the administration of SFV replicon vaccines via the intradermal route. The skin, being an immunocompetent organ, is populated with a myriad of antigen-presenting cells (APCs) such Langerhans cells and dermal dendritic cells that are efficient at cross-priming T cells. The commonly used method of intradermal injection utilizes a hypodermic needle. With this conventional method, administration of rSFV particles resulted in similar responses compared to intramuscular administration (unpublished observations). DREP was also delivered via the intradermal route. However, this was complimented with in vivo electroporation (EP) as prior studies with DREP show a dose-sparing effect of adding EP to intradermal delivery (Chapter 3). Other devices have been developed for efficient delivery. Examples include gene gun-assisted delivery of gold particles, microneedle injection and tattooing. All of these methods have been utilized for the delivery of DNA-based vaccines. Tattooing has shown to increase the magnitude of DNA vaccination by 10- and 100-fold in rhesus
macaques compared to animals vaccinated intramuscularly. Tattooing also induces danger signals with thousands of punctures resulting in local inflammation. For viral vector-based vaccines, the novel delivery methods have seldom been explored. In Chapter 6 we adopted tattooing as a delivery method for rSFV particles. Only one prior publication used this method for delivery of viral vectors. Tattoo injection resulted in an overall 10-fold lower antigen expression compared to intramuscular administration. Despite this difference, both delivery methods resulted in equal levels of anti-HPV immune responses. In addition, tattooing resulted in an approximately 20-fold increase in antigen expression in the draining lymph nodes compared to intramuscular injection. In the muscle rSFV readily infects myocytes. However, it still remains unknown which cell types are infected in the skin and whether the virus migrates to draining lymph node on its own or via cells. Due to the negligible rSFV infection of dendritic cells observed in vitro, it is conceivable that cells efficient at cross-presentation, including Langerhans cells and lymph node resident dendritic cells (CD8+ DCs), merely process and present rSFV-derived antigen from apoptotic bodies rather than being directly infected. This phenomenon has been suggested in previous studies. Tattooing is not a controlled delivery process, as most of the material is wasted due to application on the surface of the skin rather than injected with a syringe. However, the delivery method points towards a dose-sparing effect of injection at the skin site with 90% less rSFV required to elicit the same immunotherapeutic effect. This would have to be confirmed for other methods of intradermal administration.

Predicting and monitoring immune responses for the future of patient selection

Characterization of the tumor microenvironment in patients is key to understanding which tumor responses are protective and can aid selection for the most effective treatment approaches. Tumor heterogeneity with respect to genetic differences poses a major challenge for a one-size-fits-all approach. Detecting biomarkers with selected tools allows the characterization of the molecular signatures that are relevant for personalized therapy. Prognostic and predictive biomarkers provide information on the outcome of cancer treatment and on the chance of response to a certain treatment, respectively. Predictive biomarkers may also guide treatment decisions. It has been demonstrated that the presence of TIL is associated with prognosis in various cancers and predicts the outcome to conventional treatments which includes surgery, radiotherapy and/or chemoradiotherapy. For cervical cancer in particular, high infiltration of CD8+ TIL is associated with disease-specific and disease-free survival. The prognostic value of other TIL types, such as regulatory T cells is controversial with studies showing positive and negative associations with clinical outcome. Additionally, TIL in the epithelium is far superior to that in the stroma. In this thesis we demonstrate that TIL correlates with survival in cervical cancer patients (Chapter 7 & 8). Differential prognostic values are
observed when stratifying the patients for treatment type. More specifically, we assessed whether CD27+ and CD103+ TIL could be used as a biomarker in cervical cancer. The CD27+ TIL subset was associated with better prognosis benefit in early stage patients that were primarily treated with radiation as well as in patients treated with (chemo)radiation. CD27+ TIL is also predictive for response to (chemo)radiation. This particular TIL subtype demonstrated superiority above other TIL subsets (CD8+ or FoxP3+) as an independent prognostic factor for disease-specific survival. CD103+ TIL was also an independent prognostic marker for disease-specific survival. Furthermore, CD103+ TIL can be used as a marker to demarcate epithelial TIL form stromal TIL to predict the response to immunotherapy, as exemplified in a preclinical tumor model for cervical cancer. These results indicate that these tumor-reactive TIL subsets could be used for the stratification of patients according to particular treatment regimens, whether it be standard therapy, immunotherapy or possibly the implementation of both. It would also be of interest to evaluate the effect of treatment on TIL infiltration with before and after treatment.

Yet, determining the treatment-induced changes in tissue derived from patients is met with several limitations due to the invasiveness of the procedure, technical challenges and time constraints. New tools have developed to non-invasively determine changes in TIL infiltration and activation. In Chapter 9, T cell activation was monitored by the specific interaction with interleukin-2 (IL-2) receptors using a recently developed positron emission tomography (PET) tracer, N-(4-[18F]-fluorobenzoyl)interleukin-2 ([18F]-FB-IL2). Using [18F]-FB-IL2 PET, the noninvasive monitoring of tumor-infiltrating and systemic activated lymphocytes was achievable in tumor-bearing mice. The highest tracer uptake was measured in tumors, thymus, spleen and draining lymph nodes of mice treated with rSFV immunization and tumor irradiation compared to the non-irradiated or irradiated group alone. Furthermore, the infiltration of IL2+ TIL was dependent on CXCR4 as reduced tracer uptake was observed with CXCR4 antagonist AMD3100. This study provides proof that [18F]-FB-IL2 PET is a effective biomarker and may be used to non-invasively monitor the response to novel immunotherapeutics in the clinic.

Future perspectives

Replicon HPV vaccines of the future
May replicon-based vaccination be the cure for HPV-induced (pre)malignant lesions? The HPV replicon viral vector addressed in this thesis has been extensively studied preclinically in the past 15 years. As the vaccine was recently introduced in the clinic, whether the potent HPV-specific immune responses observed pre-clinically can be translated to clinical outcome has to be awaited. The results from the three clinical trials that have utilized the viral replicon system are encouraging.39-40 Yet, the viral vector replicon
platform, despite its potency and having been most extensively evaluated above other platforms, is also endowed with the disadvantage of a laborious production process. The GMP-production of viral vectors for large scale manufacturing in Vero cell lines is hindered by the relatively low titre yield in contrast to production in baby hamster kidney cell lines for preclinical use.8

Recombinant replicons can also be delivered as DNA. This platform would compensate for the hurdles associated with recombinant viral particles. In the 1990s, researchers observed antigen-specific immune responses towards DNA immunization.41-42 However, DNA vaccines have thus far only been FDA-approved for veterinary use.43 For HPV immunotherapy, most of the recent clinical studies in fact use DNA vaccination and they demonstrate impressive results with regard to induction of robust E6 and E7-specific immunity.44-45 One study demonstrated complete regression in seven out of nine patients.44 As we have shown, potent immunotherapeutic HPV-specific immunity was achievable in the pre-clinical setting with a dosage as low as 0.05 µg due to the intrinsic adjuvant properties of SFV. No previous study had achieved such HPV-specific immunity at such a low dosage before and the current HPV DNA vaccine trials fuel the optimism that our HPV replicon DNA vaccine could potentially succeed in the clinic.

The success of DNA vaccination in the clinic is in large part due to advances of delivery systems. In particular, in vivo electroporation has been used in the clinic to augment transfection efficiency and gene expression of DNA vaccines upon intramuscular injection. In this thesis, we delivered DREP intradermally coupled with in vivo electroporation as previous studies have demonstrated the enhanced potency of targeting the skin compared to the muscle.34,35 Intradermal injection would be more clinically tolerable given the non-invasive nature of the administration and causing minimal pain when coupled to electroporation than coupling with intramuscular injection. We propose that intradermal delivery should be evaluated in the clinic to replace intramuscular delivery. Therefore, it would be a great value to compare in a head-to-head study the administration the HPV DREP vaccine at different sites to determine the dose-sparing effect on therapeutic efficacy and rationalize the intradermal approach for clinical assessment.

Apart from DNA and recombinant virus particles, replicons can also be delivered as RNA. As replicon DNA vaccines, the development is still in its infancy. Yet the non-replicating RNA vaccination field has recently started to rapidly move forward. RNA vaccines possess the ideal trait of simplicity and cost-effective production to accommodate rapidly mutating infectious agents.46 They also provide a safety feature above DNA vectors such as eliminating the risk of integration into the host genome.47 RNA also merely requires access to the cytoplasm for translation of transgenes to occur. Not only would electroporation be ideal, but formulation in lipid nanoparticles has also shown to be an optimal delivery system for enhanced RNA stability of replicating and non-replicating RNA.48,49 Currently research is focusing more on a personalized therapy approach with neoantigen-guided
immunotherapy. RNA vaccines would be amenable to such therapy as a cost-effective strategy for treatment of individual patients. A large number of patient-specific antigens can be isolated from the tumor and amplified using polymerase chain reaction (PCR) for transcription to RNA.\textsuperscript{50,51} For instance, in one study, RNA encapsulated in lipoplexes induced robust immunity towards various tumor-associated antigens with DC-specific targeting.\textsuperscript{51} The robust CTL response stemmed from the waves of IFN-alpha by both DCs and macrophages generating long-lasting antitumor responses and overcome tumor-associated tolerance.\textsuperscript{51} Impressively, immune responses were observed with a smaller dose than that used in the mouse studies in three melanoma patients, although a larger cohort of patients is required to reach definitive conclusions.\textsuperscript{51,52} With the combination of cancer mutanome mapping based on next generation sequencing, the manufacturing of mRNA vaccines is underway in a process referred to as MERIT (mutanome engineered RNA immunotherapy).\textsuperscript{53} To keep up with these current trends in cancer treatment, it would be interesting to evaluate whether replicon RNA, with a suitable formulation, would be ideal for delivery of viral-and neoantigens.

**Considerations for combination treatment strategies targeting HPV-induced malignancies**

It is becoming increasingly more apparent that, as part of the design of vaccine regimens, combination therapy is a better treatment option compared to monotherapy for durable clinical responses. Even though replicon vectors may be potent as stand alone treatments, they could still be considered as part of a combination regimen. Combination regimens currently being explored include differing immunotherapeutic approaches or immunotherapy combined with conventional chemotherapy and radiation therapy. A more in-depth understanding is required when combining different therapies, such as dosing and scheduling, for rational therapeutic combinations.

The most successful immunotherapy in achieving durable responses is immune checkpoint inhibition with antagonists targeting CTLA-4 or the PD1-PDL1 pathway as a form of passive immunotherapy. Antibody targeting PD-1 has demonstrated durable responses in melanoma, renal cancer, lung cancer and colon cancer.\textsuperscript{54} Additionally, the monoclonal Ab displays low toxicity. The effectiveness of checkpoint blockade therapy still needs validation in HPV-associated malignancies in the clinic as stand-alone treatments. Few pre-clinical studies have demonstrated the synergism between HPV vaccines and checkpoint blockade targeting with variable results depending on the vaccine type.\textsuperscript{55} Clinical studies have shown that synergism is favored when checkpoint blockade is combined with potent vaccines such as DC-based vaccine or recombinant viral vectors.\textsuperscript{56,57} With the effectiveness of checkpoint blockade therapy correlating with neoantigen load, it is worthwhile to consider replicon nucleic acid vaccinaton encoding neoantigens, as mentioned previously, due to their rapid and cost-effective production as well as their
potency. Yet caution should be taken with regard to the timing of combination therapy, a concept that is seldom addressed in clinical trial design. For instance, the enhanced antitumor effect of a DNA vaccine was achieved when anti-CTLA-4 mAb was administered only with booster immunizations. Tumors may be administered with antibody blockade prior to vaccination, as this timing is adapted to the foreseeable future in the clinical setting where these antibodies are becoming the standard of care. Yet, we have demonstrated that timing of anti-PD1 mAb treatment may require adaptation with rSFV immunization due to the observable reduction in therapeutic efficacy. It may also be worthwhile to combine replicon vectors with CTLA-4 blockade. CTLA-4 blockade more effectively inhibits T cells with a higher avidity for antigen in contrast to PD-1 blockade and may be more favorable in a combination approach with rSFV.

Apart from passive immunotherapy, combinations with other vaccines as part of prime-boost regimens have demonstrated enhanced immunity for vaccination against HPV-induced malignancies. This is particularly the case for nucleic acid or peptide vaccination. Priming with DNA or RNA and boosting with a viral vector vaccine has demonstrated to result in enhanced HPV-specific immunity compared to a single modality treatment. In the case of replicon vectors, DREP having shown to be an optimal vaccine for priming T cell and antibody responses before a heterologous boost. Caution should be taken when selecting immunization protocols as the responses that are elicited may not always be relevant against the encoded antigen. For instance, homologous prime-boosting with SFV replicon particle results in higher central memory T cell frequencies compared to heterologous protocols. This may not be favorable for tumor vaccination as central memory T cells are required for long-term protection due to their recall proliferation capacity. Yet head-to-head studies between different prime-boost vaccine modalities targeting the same tumor antigen will have to be performed to extensively characterize the memory phenotype that contributes to immunotherapeutic efficacy.

Chemotherapy and radiotherapy have historically been acclaimed to solely damage DNA and block division of tumor cells. Nowadays, preclinical and clinical data suggest that conventional therapies are also immunostimulatory. For instance, radiotherapy can prime the immune response through release of tumor-associated antigens and damage-associated molecular patterns for effective DC activation. As these treatments are currently the used standard of care, it would be feasible to combine these approaches with immunotherapy. Enhanced HPV-specific immunity was demonstrated in numerous preclinical studies with combination approaches with conventional therapy. Yet, to include these agents as potential immunomodulators, requires important considerations such as potential side effects that are associated with systemic administration as well as the timing of the different treatments.
Patient selection for clinical development of HPV immunotherapy

Selection of an ideal patient population for HPV immunotherapy relies on several factors that include stage of disease, pretreatment with conventional therapy/surgery and biomarkers that predict response to therapy. Normally safety and efficacy are evaluated in patients with metastasis or those in late stage of disease. However, these patients are usually pretreated with conventional therapy. In our phase I clinical trial of Vvax001, the study population consists of patients with a history of having undergone primary surgery without addition adjuvant treatment. Interestingly, it has been observed in the majority of patients treated with surgery for HPV16-induced lesions that most responses that are HPV16-specific was not functional for production of IFN-γ or other proinflammatory cytokines. Interestingly, the absence of effective anti-HPV immunity leading to recurrence or persistence of disease is speculated to be associated with the surgical procedure itself. Generally, clinical vaccination trials include patients that have late-stage disease. As these patients are immunocompromised by previous treatment and/or by the disease itself, this results in limited vaccination efficacy. Moreover, stand-alone treatments for late-stage patients may not be as effective due to the accumulation of mutations over time not relevant for the antigen of interest. Early stage patients are most likely to respond to vaccination compared to late stage patients. This explains the success rate of a vaccine consisting of HPV16 E6 and E7-derived synthetic long peptides (SLP) with 47% of VIN 3 patients showing complete regression in contrast to the 3% of complete regression achieved in a study in end-stage cervical cancer patients. The clinical efficacy of Vvax001 in CIN 2 and CIN 3 patients are awaited upon initiation of a phase II clinical trial.

Due to inter-patient variation affecting the immunological response to a given vaccine, patient-specific therapy can be guided by evaluation of relevant prognostic and predictive biomarkers. Such biomarkers can help prioritize the most rational treatment approach to conventional therapy, immunotherapy or a combination of the two. Improvement in treatment response and survival towards immunotherapeutic approaches is reported to occur in a limited number of patients. Efforts continue to seek for better predictors of response prior to the start of therapy. This is usually accomplished by molecular and pathological techniques. Yet, markers, such as PD-1 and PD-L1, that were found to predict response towards antibody treatment turned out to not be robust as once thought due to limitations such as the lack of standardized methods. The assessment of predictive markers through monitoring a response after start of treatment is even more challenging. Systemically measuring the immune response is a rational approach for monitoring vaccine efficacy, yet it would be of value to monitor responses at the lesion site as well. For instance, after initiating anti-PDL1 mAb treatment in patients, an inflammatory response ensues, concomitant with imaging scans of increased tumor sizes as well as appearance of new lesions. Early immunotherapy trials would have removed such patients from the
study despite their tumors shrinking over time and with some patients even exhibiting complete tumor eradication. State-of-the-art imaging methods, such as with PET tracers, make it possible to clarify this “pseudoprogression” as an immune-related effect. Such techniques may also aid as predictive markers to determine TIL infiltration and function as a result of therapy as well as during disease progression. This would also determine effective timing of novel immunotherapeutic regimens. The application has been extensively explored preclinically yet clinical applicable is clearly needed as soon as possible to potentially prevent patients from continuously receiving treatments that show limited responses at an early time point.

**Conclusion**

In this thesis, we provide insight into the development of SFV replicon-based immunotherapeutic strategies for targeting HPV-induced (pre)malignant lesions. The preclinical testing of the past 15 years of rSFV replicon particles supports its recent clinical development. We also evaluated alternative design and delivery strategies of the currently optimized rSFV replicon vaccine. A DNA vaccine based on the SFV replicase was developed with potent HPV-specific therapeutic responses induced with dose-sparing effects. Requirement of low doses was also accomplished with intradermal methods of delivery. Combination treatments with current FDA-approved treatments did not enhance the immunogenicity of rSFV, although further optimization of dose and scheduling may be required. With the recent initiation of a phase I clinical trial testing the toxicity and immunogenicity of rSFVeE6,7, we will gain insight into whether this promising approach has translatable therapeutic efficacy in humans as well as provide clues to the potential of testing other SFV replicon platforms.
Summarizing discussion and future perspectives

References


Chapter 10


