Formulation of peptide-based cancer vaccines

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CHAPTER 1

Introduction

VACCINES

The development and large-scale administration of vaccines has had a large influence on modern day society. The introduction of vaccination programs has practically eliminated a large number of devastating infectious diseases, such as polio, diphtheria and measles (1, 2). The current SARS-Coronavirus type 2 outbreak clearly showcased the scenario when no vaccine is available: more than 4,5 million deaths (as of September 2021), a number which would have been much higher if the world had not been locked down (3). Vaccines are designed to induce an immune response that is able to recognize, clear and remember pathogens, such as bacteria and viruses. In the last centuries prophylactic vaccines have been developed against infectious diseases and more recently vaccines have been used to induce tumor specific immunity. In order to induce a protective immune response in combination with immunological memory, the vaccine needs to instruct and activate the adaptive immune system.

Edward Jenner, an English physician (1749-1823), is seen as 'the godfather of vaccination' by successfully inducing immunity against the small-pox virus in an 8-year old boy. Edward hypothesized that exposure to the related, but milder, cowpox virus induced immunity for the small-pox virus, since farmers, working with cattle, were often not affected during smallpox outbreaks. The hypothesis was tested by scratching pus from a cowpox pustule into the skin of the 8-year old boy, when exposed to the smallpox virus several weeks later he did not get infected. This immunization procedure by Jenner was eventually named vaccination after the Latin name for cow: *vacca* (1, 4, 5). During the following century Louis Pasteur (1822 – 1895) made use of weakened or dead pathogens (disease causing bacteria or viruses) to induce immunity against anthrax and rabies (6). Building on this knowledge vaccines against a wide range of infectious diseases (e.g., diphtheria, typhoid, tetanus and influenza) were developed based on attenuated bacteria, viruses or whole pathogens during the following century (1, 7). Despite of the success of these attenuated pathogen-based vaccines, such vaccines were able to induce illness in immunocompromised individuals or even revert back to their pathogenic form.

Scientific and technical advancements in multiple fields (genetics, immunology and biotechnology) have allowed precise antigen identification of pathogens and enabled development of more safe and well defined types of vaccines, such as subunit-, whole inactivated-, and split-vaccines. In these vaccines only the inactivated pathogen, the antigen or part of the antigen is included and therefore there is no more infection risk of the vaccine (1, 7). Whole inactivated- and split-vaccines contain the whole or a part of the inactivated pathogen while the structural elements important for immune recognition are maintained (1). The advantage of these vaccines is their good immunogenicity profile, since multiple elements of the pathogen are part of the vaccine. One of the major disadvantages of such vaccines is that they are hard to fully define, from a chemical and pharmaceutical point of view. Modern vaccines circumvent this problem by only

including the antigen of interest instead of the pathogen (e.g., the spike protein in COVID-19 vaccines), which allows production of a well-defined vaccine. However, additional immune stimulating molecules are required to sufficiently activate the immune system upon vaccine administration (1).

MODERN VACCINE COMPOSITION

An effective vaccine is composed of multiple elements: antigen, adjuvant, delivery vehicle and formulation excipients. All ingredients together form the final vaccine formulation in its primary container (e.g., vial, syringe) that can be administered. In modern vaccines the antigen can be just a small part of the pathogen that is recognized by the immune system. The antigen can be incorporated as a synthetic replication or as a small string of genetic code (mRNA/DNA), the latter allowing production of the antigen after vaccination (1). In order to efficiently activate the immune system modern vaccines are adjuvanted by incorporation of a delivery system (to ensure the vaccine is engulfed by specific immune cells), immune modulators (molecules that trigger a specific kind of immune response) or both. Additionally, the delivery vehicle can protect the antigen from degradation and inactivation. Formulation excipients ensure that the vaccine stays intact upon storage, transport and administration.

VACCINE INDUCED IMMUNITY

The immune system is a sophisticated army of different cell types that protect the body from outside, e.g., bacteria, fungi, and viruses, and inside treats, such as cancer and intracellular pathogens (8, 9). Two types of immune responses can be distinguished: a fast but nonspecific “innate” response and a more slow but highly specific “adaptive” response. Both arms of the immune system work closely together and are required to clear and prevent infection or disease (1, 9). Within the adaptive immune system three cell types play a major role in vaccine induced immunity: 1. Dendritic cells 2. B-cells and 3. T-cells. The majority of vaccines are prophylactic agents that aim to prevent infection and do so by inducing antibody producing B-cells. The induced antibodies are specific for the pathogens’ antigens and bind the pathogen when detected in the body. The antibody binding results in neutralization of the pathogen and subsequent clearance by innate immune cells and hereby prevent infection. In order to establish an optimal B-cell response both dendritic cells and T-cells are required.

Upon administration the vaccine is internalized by dendritic cells that digest the vaccine, extract antigen fragments and present these fragments as peptides on their cell surface. The presented peptides are recognized by T helper cells ($CD4^+$ T-cells), a subset of T-cells, that subsequently help IgG antibody production of specific B-cells. These vaccine-induced pathogen-specific B-cells will remain present as memory cells after vaccination so when the pathogen is detected these cells can rapidly scale up antibody

production to prevent infection (1, 9). This antibody based form of immunity is also named humoral immunity (1).

While antibody mediated immunity is very effective in the prevention of viral and bacterial infections in the body fluids, it is not functional against readily infected cells. T-cells, however, are able to detect intracellularly infected cells; the so-called cellular immunity. The recognition of e.g., virus-infected cells is mediated by antigen fragments, peptides derived from the viral pathogen inside the cell, that are presented by MHC molecules (see below) on the surface of infected cells, which can be recognized by the T-cell receptor (TCR) of the specific T-cell (1, 8). Similar to the humoral response the dendritic cells play a major role in the orchestration of the T-cell response. The dendritic cells present antigen fragment from the pathogen, derived from dead infected cells or a vaccine, to cytotoxic $CD8^+$ T-cells (by MHC class I molecules) and to helper $CD4^+$ T-cells (by MHC class II molecules). Additional to antigen presentation, the dendritic cells strongly stimulate T-cell activation and division by expression of co-stimulatory signals (10-13). The induced $CD8^+$ T-cells travel throughout the whole body and upon detection of the foreign antigen fragments they bind and kill the infected cell. The $CD4^+$ T-cells will help induce and shape the $CD8^+$ T-cell response (hence the name T-helper cell) and ensure clearance of the infected cells (1, 8, 11). Since a wide variety of cancer cells present tumor-specific antigen fragments the T-cell arm of the immune system is able to 'see' cancerous cells (8, 14-16). The increased understanding of T-cell mediated tumor cell recognition and subsequent clearance has revolutionized the treatment of cancer and resulted in the introduction of cancer immunotherapy, the collective name of drugs that are able to induce and improve tumor-specific immune responses.

CANCER IMMUNOTHERAPY

Cancer is a collective of diseases in which uncontrolled cell growth invades and/or spreads throughout the body. It is only recently appreciated that the immune system is able to recognize tumors via antigens that can be (over)expressed by the tumor. This concept is strengthened in the past decade by a number of newly developed drugs that aim to improve cancer immunity that have been successfully introduced in the clinic. One of the most widely used immunotherapeutic drugs are immune checkpoint inhibitors, which are antibody-based drugs that block immune signals, which modulate T-cell functioning in cancer patients. Currently, inhibitors for the programmed cell death receptor-1 (PD-1) and cytotoxic T-lymphocyte antigen-4 (CTLA-4) are now part of the clinically approved treatment of a variety of tumors (8, 17-20). Both PD-1 and CTLA-4 play a role in the negative feedback loop of the normal immune system to ensure that T-cell immunity is dampened when an infection is cleared, likely to prevent autoimmune reactions (8, 20, 21). However, it became clear that tumor cells can utilize expression of such co-inhibitory molecules to "defend" themselves against T-cell mediated killing. This mechanism allows tumor cells to escape T-cell immunity when the tumor antigens

are recognized by cancer-specific T-cells. The discovery of these molecules and the subsequent development of blocking antibodies, that can prevent inhibitory signals to- and reactivate cancer-specific T-cells, has shown the power of cancer immunotherapy, which was a real breakthrough in the clinic (20, 22).

Checkpoint inhibitors mainly affect a class of highly tumor-specific T-cells in cancer patients, the so-called neoepitope-specific T-cells (14-16). These antigenic neoepitopes originate from mutations, generally DNA point mutations, which are not necessarily involved in the uncontrolled cell growth but can be present in any gene. The mutations can lead to the expression of tumor-specific proteins with small amino acid changes (the neo-antigens which can potentially be recognized by specific T-cell receptors present in the T-cell repertoire as seen as non-self. Parts of these mutated proteins can be translated into peptides and presented on the tumor cell surface via major histocompatibility complexes (MHC) class I and II, enabling T-cells to recognize these cancer-specific molecular changes even when they are intracellular (14-16, 23). Such intracellular defects cannot be recognized by antibodies produced by the humoral part of the immune response. The checkpoint inhibitors that are able to dampen immunosuppression, by which these T-cells are normally kept non-functional in tumors, revealed the potential of neoepitope specific T-cells. Treatment of tumors with a high number of mutations (e.g., melanoma, lung cancer) with checkpoint blocking therapies resulted in durable clinical benefit and progression-free survival (22, 24).

Despite the clinical success in melanoma and lung cancer, still only limited number of other cancer patients benefits from checkpoint inhibition, likely due to other mechanistic reasons, like low antigenicity, T-cell exhaustion and other ways of immune escape by tumor cells (8, 19, 25-27). Additionally, checkpoint inhibition has led to side effects as autoimmunity since the brakes of the immune system are released throughout the whole body (8, 19, 21, 25, 26). In most cases fortunately these side effects are tolerable and transient, but in some cases severe. The limited number of responders shows the need for improved and more specific immunotherapies that elicit high numbers of functional tumor-specific T-cells which can effectively reach the tumor and metastases. Therapeutic vaccination is a strategy to induce, amplify and diversify tumor-specific immunity, resulting in high numbers of activated T-cells that selectively recognize and kill malignant cells (15, 28-30) and leave healthy cells unharmed. Therefore, therapeutic vaccination has the potential advantage of no expected side effects. The technological and scientific advancements have enabled rapid genome sequencing, cancer mutation mapping and systematic epitope identification, enabling the design of truly personalized cancer vaccines (14-16, 28, 29, 31). For the design of personalized cancer vaccines, multiple antigen formats (e.g., proteins, peptides, antigen-encoding mRNA or DNA) are available (14, 29, 32).

FORMULATION OF SYNTHETIC PEPTIDE-BASED CANCER VACCINES

The research described in this thesis is focused on peptide-based cancer vaccines. Peptides in cancer vaccines are parts of the amino-acid sequence of tumor antigens that contain the tumor epitopes, the 'instructions' for specific activation of the immune system. In earlier research our lab has shown that length of the antigenic peptide is important for effective vaccination. A short peptide which contains only the MHC class I epitope can bind directly to MHC class I molecules on the cell surface, since every nucleated cell express MHC class I. This results in antigen presentation by cells that are, unlike dendritic cells, not able to provide co-stimulation to the T-cell and thereby immune tolerance can be induced rather than tumor immunity (33, 34). Elongation of the peptide circumvents this problem since the peptide becomes processing dependent, meaning that dendritic cells have to engulf and process the peptide before the epitope can be loaded in MHC molecules (10, 34, 35). Research of our lab has shown that that these synthetic long peptides are more effectively processed and presented by dendritic cells than the native protein or the short minimal analogue (36). Peptide elongation can be done by embedding tumor epitopes in flanking sequences of amino acids, which can be the natural sequences of the tumor antigen, different tumor epitopes or man-designed sequences. A main advantage of these long peptides is the full synthetic production, eliminating the need for a cell based production system and offer the possibility to include potency enhancing modifications (34, 35, 37). Also the production time is greatly reduced, since no cell transfection, culturing and complex purification steps are required. In our studies we have made use of processing dependent, long synthetic peptides (SPs) in all conducted studies.

Previous studies have shown that SPs encoding tumor epitopes are able to induce antigen-specific effector T-cells in multiple preclinical and clinical studies (30, 31, 38-40). In order to establish effective tumor immunity the SPs needs to be delivered to, and subsequently activate, dendritic cells. Previous studies in our lab have made use of cationic liposomes; positively charged lipid spheres on a nanometer scale ($1*10^{-9}$ meter) that have an aqueous core. The synthetic lipids DOTAP and DOPC were used to prepare the liposomes and different antigen-containing SPs were loaded in the liposomes. When the liposomal vaccine is combined with a defined adjuvant, a toll-like receptor ligand (TLR-L), a class of immune stimulating small molecules prior to injection, the SP loaded liposomes induced antigen-specific and functional $CD8^+$ as well as $CD4^+$ T-cell responses (41-43). Vaccination with liposomal encapsulated SP containing tumor antigens of the human papilloma virus (HPV)-induced tumor specific T-cells that were able to fully clear established tumors in a HPV tumor-bearing mouse model (41).

A different strategy to activate dendritic cells, to ensure efficient induction of an antigen-specific immune responses, is by direct conjugation of the SP to an immune stimulating

molecule. Previous work of our lab has shown effective induction of tumor specific T-cells after administration of such peptide-based conjugates. These conjugates have been used to efficiently to induce tumor-specific T-cells that were able to clear tumors in multiple tumor-bearing mouse models. In an *ex* vivo setting the conjugates efficiently activated patient-derived tumor-specific T-cells (44-46). Recently, a phase I clinical trial revealed that TLR2-ligand-SP conjugates, containing HPV epitopes, were safe with limited side effects upon delivery in the skin and induced significantly higher T-cell responses in the blood of these patients (Speetjens et al. In preparation) (47).

Combined, both liposomal encapsulation and peptide conjugation offer an option to improve personalized peptide-based cancer vaccines. An important feature of personalized cancer vaccines is that such vaccines will be composed of different SPs, which are based on tumor-specific mutations, to induce immunity against multiple neoepitopes. This thesis, “Formulation of peptide-based cancer vaccines”, describes two strategies applicable for neoepitope-based cancer vaccines.

THESIS AIM AND OUTLINE

The aim of this thesis is to design and optimize prototype vaccines for personalized cancer vaccination. The described research in this thesis was focused on two different strategies of synthetic peptide-based vaccines:

1. DOTAP:DOPC based cationic liposomes loaded with antigenic synthetic peptides

2. antigenic synthetic peptides conjugated to a newly developed TLR-Ligand as an adjuvant

In **Chapter 2** the current status of cationic nanoparticle-based cancer vaccines is reviewed. The application of cationic nanoparticles in cancer vaccines is discussed including their molecular mechanisms of adjuvanticity and biodistribution profiles when administered via different administration routes.

Since personalized peptide-based cancer vaccines will consist of multiple patient specific SPs, with varying physicochemical characteristics, the cationic liposomes should be able to harbor a wide variety of synthetic peptides. In **Chapter 3** the application of cationic liposomes as a flexible vaccine delivery system for physicochemically diverse antigenic peptide sequences is described. A library of physicochemically different SPs, all harboring a model T-cell epitope, were synthesized. Three liposome encapsulation methods were developed to individually encapsulate all different SPs and an improved immunogenicity for encapsulated peptides was shown in vitro.

In **Chapter 4** the intradermal administration of cationic liposomes via a digitally-controlled hollow microneedle injection system was studied. The intradermal route has shown great potential for peptide-based T-cell cancer vaccines and hollow microneedles allow for a more controlled administration.

Chapter 5 describes an ultra-pressure liquid chromatography (UPLC) method that separates the synthetic peptides and both lipids, DOTAP & DOPC, of our liposomal cancer vaccine. The development and validation of quantification methods, according to ICH guidelines, for both peptides and lipids is a requirement for the further clinical translation of the cationic liposomal formulations.

In **chapter 6** a multi-neoepitope vaccine formulated in cationic liposomes is described. In the vaccine MHC class I and class II neoepitopes of a mouse colorectal cancer model, MC-38, were formulated and characterized via the methods developed in chapters 3 and 5. The liposomal multi-neoepitope vaccine efficiently induced and activated neoepitope specific T-cells, and could control outgrowth of MC-38 tumors in mice and induced long-term immunity.

The immunological mechanisms of action and pharmacokinetics of SP loaded cationic liposomes were explored in **chapter 7**. Cationic liposomes loaded with SP resulted in prolonged intracellular antigen storage in dendritic cells and antigen deposition at the site of injection upon intradermal administration. SP loaded cationic liposomes induced the highest frequencies of antigen specific CD8+ T-cells compared to neutral and anionic SP loaded liposomes.

In **chapter 8** a novel human-specific Toll-like receptor 2 ligand mini-UPam, which was directly conjugated to two different SPs comprising human neoepitopes derived from a patient's melanoma, was evaluated. Both an MHC class I and MHC class II neoepitope conjugated to the mini-UPam could effectively activate the cancer patient's T-cells *ex vivo*. This flexible system allows further exploration for clinical translation.

In **chapter 9** the findings of this thesis and their implications for the development of personalized cancer vaccines are discussed.

REFERENCES

1. Pollard AJ, Bijker EM. A guide to vaccinology: from basic principles to new developments. Nat Rev Immunol. 2021;21(2):83-100.
2. Roush SW, Murphy TV, Vaccine-Preventable Disease Table Working Group, the. Historical Comparisons of Morbidity and Mortality for Vaccine-Preventable Diseases in the United States. JAMA. 2007;298(18):2155-63.
3. WHO. WHO Coronavirus (COVID-19) Dashboard 2021 [updated 03-09-2021; cited 2021 06-09-2021]. Available from: https://covid19.who.int/.
4. Riedel S. Edward Jenner and the history of smallpox and vaccination. Proc (Bayl Univ Med Cent). 2005;18(1):21-5.
5. Blass E, Ott PA. Advances in the development of personalized neoantigen-based therapeutic cancer vaccines. Nat Rev Clin Oncol. 2021;18(4):215-29.
6. Smith KA. Louis pasteur, the father of immunology? Frontiers in immunology. 2012;3:68-.
7. Plotkin S. History of vaccination. Proceedings of the National Academy of Sciences. 2014;111(34):12283.
8. Waldman AD, Fritz JM, Lenardo MJ. A guide to cancer immunotherapy: from T cell basic science to clinical practice. Nature Reviews Immunology. 2020.
9. Chaplin DD. Overview of the immune response. Journal of Allergy and Clinical Immunology. 2010;125(2, Supplement 2):S3-S23.
10. Kloetzel PM, Ossendorp F. Proteasome and peptidase function in MHC-class-I-mediated antigen presentation. Curr Opin Immunol. 2004;16(1):76-81.
11. Borst J, Ahrends T, Babala N, Melief CJM, Kastenmuller W. CD4(+) T cell help in cancer immunology and immunotherapy. Nat Rev Immunol. 2018;18(10):635-47.
12. Vyas JM, Van der Veen AG, Ploegh HL. The known unknowns of antigen processing and presentation. Nat Rev Immunol. 2008;8(8):607-18.
13. Sanchez-Paulete AR, Teijeira A, Cueto FJ, Garasa S, Perez-Gracia JL, Sanchez-Arraez A, et al. Antigen cross-presentation and T-cell cross-priming in cancer immunology and immunotherapy. Ann Oncol. 2017;28(suppl_12):xii44-xii55.
14. Sahin U, Türeci Ö. Personalized vaccines for cancer immunotherapy. Science. 2018;359(6382):1355-60.
15. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science. 2015;348(6230):69-74.
16. Saxena M, van der Burg SH, Melief CJM, Bhardwaj N. Therapeutic cancer vaccines. Nat Rev Cancer. 2021;21(6):360-78.
17. Scheetz L, Park KS, Li Q, Lowenstein PR, Castro MG, Schwendeman A, et al. Engineering patient-specific cancer immunotherapies. Nat Biomed Eng. 2019;3(10):768-82.
18. Robert C. A decade of immune-checkpoint inhibitors in cancer therapy. Nature Communications. 2020;11(1).
19. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. New England Journal of Medicine. 2015;372(26):2521-32.
20. Borst J, Busselaar J, Bosma DMT, Ossendorp F. Mechanism of action of PD-1 receptor/ligand targeted cancer immunotherapy. European Journal of Immunology.n/a(n/a).
21. Khan S, Gerber DE. Autoimmunity, checkpoint inhibitor therapy and immune-related adverse events: A review. Seminars in Cancer Biology. 2020;64:93-101.
22. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. Science. 2018;359(6382):1350-5.
23. Alspach E, Lussier DM, Miceli AP, Kizhvatov I, DuPage M, Luoma AM, et al. MHC-II neoantigens shape tumour immunity and response to immunotherapy. Nature. 2019;574(7780):696-701.
24. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer. Science. 2015;348(6230):124-8.

25. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, Activity, and Immune Correlates of Anti–PD-1 Antibody in Cancer. New England Journal of Medicine. 2012;366(26):2443-54.
26. Finn OJ. The dawn of vaccines for cancer prevention. Nat Rev Immunol. 2018;18(3):183-94.
27. Kalbasi A, Ribas A. Tumour-intrinsic resistance to immune checkpoint blockade. Nat Rev Immunol. 2020;20(1):25-39.
28. Wells DK, van Buuren MM, Dang KK, Hubbard-Lucey VM, Sheehan KCF, Campbell KM, et al. Key Parameters of Tumor Epitope Immunogenicity Revealed Through a Consortium Approach Improve Neoantigen Prediction. Cell. 2020;183(3):818-34 e13.
29. Hu Z, Ott PA, Wu CJ. Towards personalized, tumour-specific, therapeutic vaccines for cancer. Nat Rev Immunol. 2018;18(3):168-82.
30. Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. Nature. 2017;547(7662):217-21.
31. Hos BJ, Camps MGM, van den Bulk J, Tondini E, van den Ende TC, Ruano D, et al. Identification of a neo-epitope dominating endogenous CD8 T cell responses to MC-38 colorectal cancer. Oncoimmunology. 2019;9(1):1673125.
32. Heuts J, Jiskoot W, Ossendorp F, van der Maaden K. Cationic Nanoparticle-Based Cancer Vaccines. Pharmaceutics. 2021;13(5).
33. Melief CJ, van Hall T, Arens R, Ossendorp F, van der Burg SH. Therapeutic cancer vaccines. J Clin Invest. 2015;125(9):3401-12.
34. Melief CJ, van der Burg SH. Immunotherapy of established (pre)malignant disease by synthetic long peptide vaccines. Nat Rev Cancer. 2008;8(5):351-60.
35. Stephens AJ, Burgess-Brown NA, Jiang S. Beyond Just Peptide Antigens: The Complex World of Peptide-Based Cancer Vaccines. Frontiers in Immunology. 2021;12.
36. Rosalia RA, Quakkelaar ED, Redeker A, Khan S, Camps M, Drijfhout JW, et al. Dendritic cells process synthetic long peptides better than whole protein, improving antigen presentation and T-cell activation. European Journal of Immunology. 2013;43(10):2554-65.
37. Truex NL, Holden RL, Wang BY, Chen PG, Hanna S, Hu Z, et al. Automated Flow Synthesis of Tumor Neoantigen Peptides for Personalized Immunotherapy. Sci Rep. 2020;10(1):723.
38. Melief CJM, Welters MJP, Vergote I, Kroep JR, Kenter GG, Ottevanger PB, et al. Strong vaccine responses during chemotherapy are associated with prolonged cancer survival. Science Translational Medicine. 2020;12(535):eaaz8235.
39. Kenter GG, Welters MJ, Valentijn AR, Lowik MJ, Berends-van der Meer DM, Vloon AP, et al. Vaccination against HPV-16 oncoproteins for vulvar intraepithelial neoplasia. The New England journal of medicine. 2009;361(19):1838-47.
40. Kenter GG, Welters MJ, Valentijn AR, Lowik MJ, Berends-van der Meer DM, Vloon AP, et al. Phase I immunotherapeutic trial with long peptides spanning the E6 and E7 sequences of high-risk human papillomavirus 16 in end-stage cervical cancer patients shows low toxicity and robust immunogenicity. Clin Cancer Res. 2008;14(1):169-77.
41. Varypataki EM, Benne N, Bouwstra J, Jiskoot W, Ossendorp F. Efficient Eradication of Established Tumors in Mice with Cationic Liposome-Based Synthetic Long-Peptide Vaccines. Cancer Immunol Res. 2017;5(3):222-33.
42. Varypataki EM, Silva AL, Barnier-Quer C, Collin N, Ossendorp F, Jiskoot W. Synthetic long peptide-based vaccine formulations for induction of cell mediated immunity: A comparative study of cationic liposomes and PLGA nanoparticles. J Control Release. 2016;226:98-106.
43. Varypataki EM, van der Maaden K, Bouwstra J, Ossendorp F, Jiskoot W. Cationic liposomes loaded with a synthetic long peptide and poly(I:C): a defined adjuvanted vaccine for induction of antigen-specific T cell cytotoxicity. AAPS J. 2015;17(1):216-26.
44. Zom GG, Welters MJP, Loof NM, Goedemans R, Lougheed S, Valentijn RRPM, et al. TLR2 ligand-synthetic long peptide conjugates effectively stimulate tumor-draining lymph node T cells of cervical cancer patients. 2016;7(41).
45. Zom GG, Welters MJP, Loof NM, Goedemans R, Lougheed S, Valentijn RRPM, et al. TLR2 ligand-synthetic long peptide conjugates effectively stimulate tumor-draining lymph node T cells of cervical cancer patients. Oncotarget. 2016;7(41).

46. Zom GG, Filippov DV, van der Marel GA, Overkleeft HS, Melief CJ, Ossendorp F. Two in one: improving synthetic long peptide vaccines by combining antigen and adjuvant in one molecule. OncoImmunology. 2014;3(7):e947892.

47. Speetjens FM, Welters MJ, Slingerland M, Steenwijk PdVv, Roozen ICFM, Boekestijn S, et al. Phase I trial to determine safety and immunogenicity of amplivant, a synthetic toll-like receptor 2 ligand, conjugated to two HPV16 E6 synthetic long peptides. Journal of Clinical Oncology. 2021;39(15_suppl):2614-.