Cancer Vaccinations

The different vaccine types and the treatment results of melanoma and prostate cancer vaccines

Figure 1. (RJWestmore Inc., 2009)

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Abstract

Objective – The conventional methods of treating cancer are not always effective enough and in both human and veterinary medicine cancer vaccines could be a significant asset. There are many different vaccine types that have been used, but the most progress has been made with two approved therapeutic vaccines; Provenge® in human prostate cancer and Oncept® in canine melanoma. This literature study focuses on the different vaccine types in general and the treatment results of prostate cancer and melanoma in human and canine patients.

Vaccine types – There are four types of therapeutic cancer vaccines; peptides, genetic (DNA and mRNA), cellular (whole cell or dendritic cell) and recombinant vaccines. In general, all vaccines only cause low grade adverse effects such as irritation around the injection site and each type has its own advantages. Recombinant, whole cell and mRNA vaccines provide the best immune response, whereas genetic and peptide vaccines are the easiest and cheapest to produce. Depending on patient and tumor characteristics, one of the groups can be more appropriate. The most progress in malignant melanoma and prostate cancer has been made with genetic and dendritic vaccines.

Prostate cancer – Prostate cancer, an adenocarcinoma is a spontaneous arising tumor that can be castration-resistant (dogs and late stages in humans) or androgen-dependent (humans). Prostate cancer vaccines have been investigated in humans, while in dogs research is in its early stages and still focuses on inducing prostate cancer. The most investigated human vaccines are Prostvac (recombinant), Sipuleucel-T (Provenge®, dendritic cell) and GVAX (whole cell).

Malignant Melanoma – Malignant melanoma is a common and aggressive tumor of mucosal (dogs) or cutaneous surfaces (dogs and humans). It has the same predilection sites and metastatic properties in both species, and the tumors are chemo and radiation resistant. The most progress in canines has been made with a DNA vaccine (Oncept®), but cellular vaccines have also been investigated. In human research, DNA, dendritic cell and whole cell vaccines have been researched, with comparable results as in prostate cancer.

Discussion and conclusion – All the studies are difficult to compare to each other due to different vaccine types, patient groups, selection criteria, protocols and end points. In prostate cancer GVAX and Sipuleucel-T (Provenge®) are the most promising, in canine melanoma a DNA vaccination even though results in human melanoma research are comparable. Developing vaccines requires a specific and patient-centered approach in which vaccine protocols cannot easily be exchanged between species or cancer types and should not be used as a monotherapy. Further research is therefore not only warranted but also necessary.

Contents

Introduction

Development of cancer
Normal cells can become tumor cells through several alterations. Not every abnormality will lead to cancer, the majority of them will not have any implications. The main alterations to become cancer cells vary from avoiding apoptosis, unlimited reproduction and metastasis, to invading surrounding tissue and self-regulation of growth and angiogenesis. The origin of developing from a normal cell to a tumor cell can result from congenital genetic errors (replication and heredity), or can arise through abnormalities in DNA (smoking, drinking or carcinogens like infectious agents). The alterations usually occur in two classes of genes; the cancer-promoting genes and the tumor suppressor genes, which are both needed for cancer to arise. The promoting genes are activated and lead to growth and division. The suppressor genes are inactivated and are responsible for loss of normal cell function (Aly, 2012).

The functioning of the immune system in relation to cancer
The immune system consists of two parts: the innate immunity and the adaptive immunity. The innate immune system is non-specific and responds quickly. It comprises barriers like the skin and antigen presenting cells (APCs) such as macrophages, dendritic cells (DC) and natural killer cells (NK). APCs can be divided in professional (already presenting MHC II) and nonprofessional (stimulation by T-cells required). The adaptive immune system is the specific part of the immune system and consists of B and T lymphocytes. The T lymphocytes can be divided in helper T cells (CD4 positive, MHC II) and cytotoxic T cells (CD8 positive MHC I) (Beverly et al., 2000). Endogenous antigens are positioned onto MHC I (Major Histocompatibility Complex) after they have been created in the cytoplasm of DCs. MHC II binds exogenous antigens that are processed in endosomes, and the MHC and antigens are presented together on the surface of APCs, to activate cytotoxic T cells (Xu et al., 2011). The B cells produce antibodies that in turn can increase phagocytosis and stimulate the complement system (Bergman, 2007).

The priming (activation of the immune system) usually occurs in the lymph nodes draining the site of the tumor. In the case of a large tumor, it can cause priming on its one. But in the case of a small tumor, it is more likely that inflammation and the subsequent influx of DCs causes a so called danger signal that is part of the adaptive immunity. The dendritic cells, which originate from the bone marrow and spread through the body, are activated by antigens of necrotic or apoptotic tumor cells and migrate to the lymph nodes (Grolleau et al., 2005) (Beverly et al., 2000). Invaders of the immune system contain antigens, which are tumor-specific antigens (TSAs) and tumor-associated antigens (TAAs) in the case of tumor cells. TSAs are antigens that only exist in tumor cells, TAAs are antigens that are present in both tumor cells and normal cells but are expressed in different ways. Even though ideally, TSAs would be used to create cancer therapies, these antigens are so specific that they can only be used for individual patients (Aly, 2012). Therefore TAAs are used, which can be divided into five categories, according to their recognition and expression patterns:

- Viral antigens
- Cancer testes antigens such as MAGE-1 and MAGE-3
- Self-antigens (overexpressed) such as GM2 and GD2 ganglioside
- Melanocyte differentiation antigens such as Melan-A, MART-1, gp100 and tyrosinase
- Antigens from mutated genes such as CDK4 and β-catenin (Campoli et al., 2005) (Nishimura et al., 2005) (Komenaka et al, 2004).

B cells and antibodies can recognize the epitopes (protein fragments) on the antigens and bind them. The recognition of an antigen by a cytotoxic T cell is not that simple. This process requires a minimum of two signals: the first is a recognition signal; the danger signal. It arises after tissue destruction and inflammation. The second signal is a verification signal; the activation of antigen-presenting cells (APCs) that express different molecules on their surface, like CD80 and CD86 (Beverly et al., 2000)

(Pardoll, 1998) (Campoli et al., 2005). A second role of the T cells, and dendritic cells, is the production of cytokines, which stimulate growth and differentiation of the cells, and chemokines that causes migration to the initial site of both these cell types (Beverly et al., 2000).

Mechanisms of tumor evasion

The immune system is not highly stimulated by tumors in general (Pardoll, 1998). Besides that, there are several ways through which the tumor can actively avoid the immune system. First of all the tumor can induce tolerance by deleting T cells or promoting the Th2 response, which lead to B cells producing antibodies (Mosolits et al., 2005). Secondly the tumor can produce immunosuppressive cytokines, like tumor necrosis factor and vascular endothelial growth factor, or can decrease the dendritic cell maturation. Macrophages can be damaged by decreasing the nitric oxide production, thereby affecting their cytotoxic function. The tumor cells can also stimulate regulatory T cells that inhibit the positive CD4/CD8 T cells: the tumor declines the expression of TAAs, thereby preventing recognition by T cells. The tumor can also produce several immunosuppressive substances including prostaglandins, IL-10 and TGF-b or cause the loss of MHC I and MHC I antigens (Bergman, 2007) (Campoli et al., 2005) (Agarwal et al., 2012) (Mosolits et al., 2005). In addition to loss of MHC I there are also several different mechanisms through which the tumor can escape its recognition such as antigen mutation. This recognition is a crucial step for a sufficient cytotoxic T cell response (Eisenlohr et al., 2005) (Agarwal et al., 2012). It can be concluded that the tumor cells that escape the immune system either have mutations that make them unrecognizable or they have been able to prevent the triggering of danger signals (Campoli et al., 2005).

Development of cancer vaccines

Several decades ago, in the 1950's, it was proposed that the immune system could play a role in fighting tumors. According to researchers then, the immune system should be able to recognize the tumor cells and it was noticed that tumors occurred more frequently in case of immunosuppression. In the beginning this association was mainly linked to tumors that involved viruses, like hepatitis B (liver carcinoma), papillomavirus (skin carcinoma) and Epstein-Barr virus (nasopharyngeal carcinoma). In addition, experiments showed that T lymphocytes were essential in this virus-related process. Even though this was not a promising start for the nonviral tumors, the researchers further investigated cancer vaccines and discovered that nonviral tumors also expressed antigens that the immune system could recognize. The main goal of cancer immunotherapy is therefore to boost the immune system against tumors (Beverly et al., 2000). This can take place through improvement of cytotoxic T cell, NK cell, antibody and helper T cell responses and through improving the environment for chemokines, growth factors and cytokines (Kirkwood et al., 2012).

Material and methods

Research questions

Recently there has been much research conducted in the area of cancer vaccines. The conventional methods of treating cancer such as chemotherapy and surgery are not always effective enough and vaccination is expected to be a significant addition. The interest in these vaccines comprises not only veterinary medicine (mainly investigated and used in dogs) but a lot of research is also ongoing in human medicine. Many different types of vaccines have been used; varying from peptides to genetic material with an equal number of different inoculation methods and costimulatory molecules. In 2010 the first human cancer vaccine has been FDA approved for prostate cancer, Provenge®, and the first veterinary vaccine for melanoma, Oncept® by the USDA. Unfortunately many vaccines have not made it to that stage (Kirkwood et al., 2012) (Merial, 2010). Both melanoma and prostate cancer have been investigated in different protocols in numerous trials. The goal of this literature study is therefore to explore and compare the different mechanism of the cancer vaccines, with the emphasis on the treatment results of the most promising vaccines; those against prostate cancer and melanoma. Considering almost all the research has been conducted in dogs, this specific target group was chosen alongside human medicine to focus on in the treatment results. Specific exclusions in patient population regarding age, breed, sex, previous treatments or location and extent of the tumor have not been made.

The research questions are therefore:

- What kind of vaccines are being used in human and veterinary research?
    - Which material forms the basis of the vaccine?
    - What are the advantages/disadvantages of the different types?
    - What is their route of administration?
- What are the different mechanisms of action of cancer vaccines?
- Which cancer vaccines for prostate cancer and melanoma are there at this moment?
    - Which types of vaccine are being used?
    - What are the treatment results of the most advanced vaccines?
        - In which development phase are these vaccines?
        - In which population has it been tested? (Species? metastatic disease? castrate resistant?)
        - What is considered to be a positive result (Immune response, improved overall survival, improved progression free survival, complete healing)?
        - What are the results and can the studies be compared?
        - Which vaccines are the most promising?

Retrieval of literature

The literature search has taken place from February 2012 until November of 2013. The first keywords that were used were ‘cancer vaccines’ in combination with several other terms: ‘human’, ‘dog’, ‘peptide’, ‘dendritic’, ‘prophylactic’, ‘genetic’, ‘DNA’, ‘mRNA’, ‘whole cell’ ‘viral’ and ‘recombinant’. Numerous quality articles were found, including a book describing the different types of vaccines. This book was used as a starting point for the research into the different cancer types. The keywords that were used for this were again ‘cancer vaccinations’, in combination with: ‘melanoma’, ‘prostate’, ‘Oncept’, ‘Provenge’, ‘Prostvac’, ‘GVAX’, ‘Sipuleucel-T’, ‘canine’, ‘veterinary’, ‘human’. The retrieved articles were selected on the basis of:

- Aim of the study; only articles comparing the efficacy of cancer vaccines, no articles regarding gene signature, the exact cellular mechanism of action etcetera
- Patient group; in the second search no articles regarding other species than human/veterinary or other cancer types than melanoma/prostate cancer were selected
- Study design; no combination trials such as vaccines with chemotherapy, selecting of only clinical trials as much as possible

In addition, the references of the articles were viewed and, if useful, retrieved. The same applied for clinical trials which were described. The databases CAB Abstracts, PubMed and Scopus have been used for the literature search as well as the Omega library. For general information regarding clinical trial phases and terminated trials the search engine Google has been used.

Mechanisms of action of cancer vaccines

The purpose of a cancer vaccination is to provoke an immune response. This reaction can be activated trough active and passive immunity. The design of a cancer vaccination is to activate the active immunity, in which the immune system itself is stimulated. The arising of an immune response can take several weeks to months due to the slow development of the adaptive immunity. An ideal cancer vaccine would destroy tumor cells; the primary tumor cells as well as the metastatic ones, differentiate between tumor and normal cells, and would prevent recurrence. The aim is to create a vaccine that can achieve all this and have minimal side effects (Bergman, 2007) (Aly, 2012).
Tumor antigens are regularly low immunogenic and all cancer vaccines therefore contain adjuvants that activate and stimulate the immune system. There are several kinds of immunostimulants: cytokines (GM-CSF, IL-2, IL-15, IL-7, interferons) and chemokines, autologous dendritic cells, biologic and chemical adjuvants, gene therapy/gene transfer vectors and immune modifiers. These adjuvants are used to create a stronger and prolonged immune response (Aly, 2012) (Dasanu et al., 2012) (Schlom, 2012).

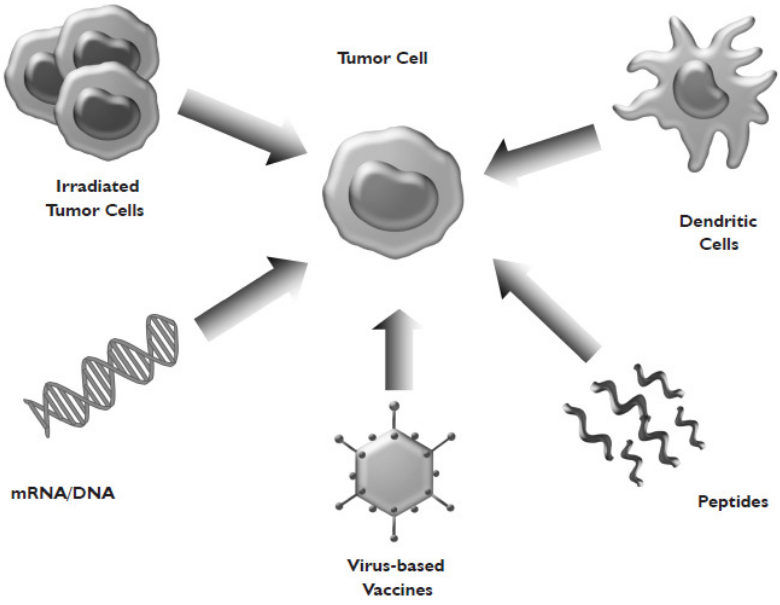

Figure 2. Overview of the different types of vaccines (Winter et al., 2011)

There are several types of vaccines that have been explored up to now, as shown in figure 2. Not only does the basis of the vaccines differ but also the route of administration and the immunostimulants that are being used can vary. The vaccination types can be divided into categories:

- Therapeutic vaccines
    - Peptide
    - Genetic
        - DNA
        - mRNA
    - Cellular
        - Whole cells
        - Dendritic cells
    - Recombinant (virus, bacteria, fungus)
- Prophylactic (Vonka, 2012)

Peptide vaccines
Peptide vaccines are based upon short amino acid portions of tumor proteins (epitopes) (Kirkwood et al., 2012). After administration of the peptide vaccine the cytotoxic T cells recognize the epitopes on the administered antigens. It starts searching and eliminating the epitopes (tumor cells) and producing chemokines and cytokines (Celis, 2002). This type of vaccine has several advantages; i) peptides are easy to modify and are relatively cheap to produce, ii) they can target specific TAAs, thereby avoiding targeting normal cells and iii) peptides can be combined with different immunostimulants (Agarwal et al. 2012) (Berzofsky et al., 2005). Disadvantages of peptides vaccines include i) the fact that they are low immunogenic, ii) their inability to properly stimulate $CD8^+$ T cells, which is of great importance for a strong and prolonged immune response and iii) their limited use because of their specificity (Agarwal et al., 2012) (Kirkwood et al., 2012). To enhance the immune response of these vaccine types the use of immunostimulants is required. These stimulants can imitate danger signals, see to a slow release of the peptides and prevent degradation by proteases (Celis, 2002). Examples include liposomes, to improve the delivery of the vaccines, and CpG-DNA that increases the amount of T helper cells and cytokines (Kwon et al., 2012). The administration can take place in several ways such as intradermal but even intra-lymph node (Berzofsky et al., 2005).

Genetic vaccines
Genetic vaccines consist of DNA or mRNA that contains the coding region of the tumor antigen(s) (proteins). These vaccines use the host to express the selected antigens and induce the T and B cell responses. The genetic material is picked up by the host cells and leads to the production of the antigens. (Aurisicchio et al., 2012). A genetic vaccine comprises a bacterial plasmid, the targeted genetic material (mRNA or DNA) and a viral promoter (Aly, 2012). An important part of the immune response provoked by a genetic vaccine is the activation of DCs. (Aurisicchio et al., 2012). Enhancement of the activation of APCs, including DCs, and the following migration to local lymph nodes is therefore an important part of improving a genetic vaccine (Choo et al., 2005).

DNA
A DNA vaccine consists of inactivated DNA that is not able to replicate anymore. The vaccine contains recombinant DNA that is created through cloning of the coding region of the TAA(s). This DNA is placed into an expression vector; a bacterial plasmid, as illustrated in figure 3. The bacteria grow and magnify the plasmid, after which the plasmid is cleared of bacteria and dissolved in saline (Aly, 2012). A DNA vaccine stimulates both sides of the immune system: the T and B cells. Other advantages of these vaccines are i) easy and cheap production, ii) specificity of producing the tumor antigens and iii) applicable for repeated use (Agarwal et al., 2012) (Choo et al., 2005) (Wolchok et al., 2007). The disadvantages are the limited entry of DNA into living cells (into the nucleus) and the lack of inflammation and therefore the low immunogenicity of DNA vaccines (Agarwal et al., 2012) (Aurisicchio et al., 2012).

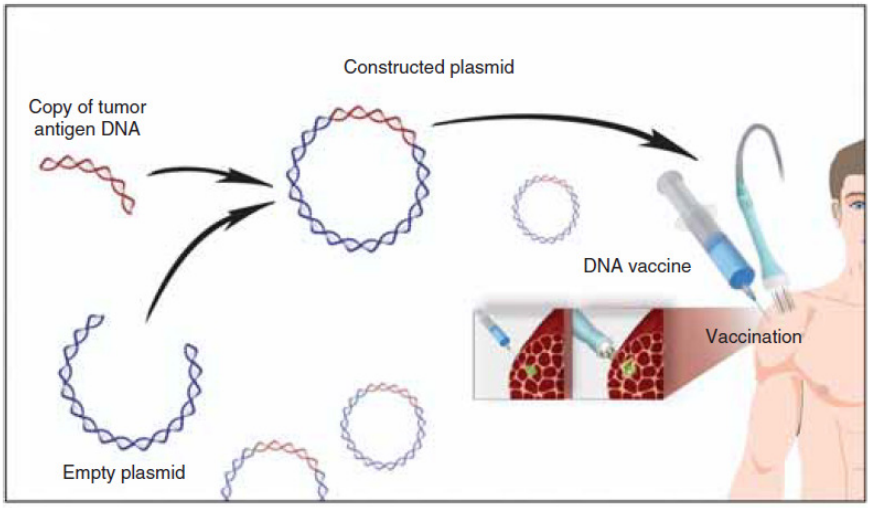

Figure 3. Recombinant DNA placed into an expression vector (Aurisicchio et al., 2012)

The plasmids can simply be directly administered intradermal or intramuscular with additional resources for distribution. The more complex administration routes are into the lymphatic system (intranodal) or through laser, gene gun, tattooing, ultrasound, electro-gene-transfer or DNA electroporation (Aurisicchio et al., 2012).

mRNA

Another genetic approach is the direct vaccination with messenger RNA, the transcription product of DNA, instead of the DNA coding region (Aurisicchio et al., 2012). The advantages of using mRNA are i) the lack of nuclear membrane (it does not need to reach the nucleus, just the cytoplasm) and need to use a promoter, ii) easy to produce in large amounts, iii) the ability to stimulate the innate and adaptive immunity at the same time and iv) the fact that there is no chance of insertional mutagenesis (insertion of bases into DNA). There are several routes of administration for mRNA vaccines varying from injection of mRNA itself, incorporating them in liposomes to gene gun delivery (Agarwal et al., 2012).

Whole cell-based vaccines

Instead of cell extracts like peptides and genetic material, vaccines can also consist of whole tumor cells. The advantage of this type of vaccine is that multiple TAAs are targeted at the same time. Not only does this vaccine targets the known and unknown TAAs, it also passes by the time-consuming testing and selecting of the most immunogenic TAAs (Aly, 2012). The tumor cells, autologous or allogeneic, are removed from the body, biochemically altered and then incorporated in the vaccine (Dasanu et al., 2012). The whole tumor cells can be administered in exosomes, in DCs or as necrotic whole tumor cell lysate: cellular components that are generated through several freeze-thaw cycles or UV ray irradiation. Fusion of DCs with tumor cells or DCs pulsed with whole tumor cell RNA are also possible. The vaccines can activate CD8+ and CD4+ responses through presentation on respectively MHC I and II, and cross presentation of exogenous antigens on MHC I generating a cytotoxic T cell response (Aly, 2012). The parallel presentation of both MHC classes is an advantage because it generates a stronger immune response and memory cells which reduces the chance of tumor escape. Another advantage is the use of the unique TAAs specific to the patient's tumor, in case of autologous tumor cells, that can give a stronger response. The drawback is the intensive and expensive process to select the specific TAAs. The allogeneic tumor cells on the other hand, that only resemble some TAAs, can be produced easily and inexpensive in large amounts of good quality. The administration can take place via different routes such as intradermal, subcutaneous and intranodal (Chiang et al., 2010).

Dendritic cell-based vaccines
In this type of vaccine the DCs can be loaded with TAAs in the form of mRNA, (c)DNA, whole proteins, tumor lysates, irradiated/inactivated tumor cells, apoptotic tumor or (acid-eluted) peptides. An alternative approach is to fuse the DCs with tumor cells, use exosomes or to infect the DCs with viral vectors encoding TAAs (Le et al., 2010) (Aly, 2012). Through the fusion of DCs with tumor cells, the DCs can take the TAAs of the tumor cells in, resulting in stimulation of CD8+, CD4+ and NK responses against the primary tumor and metastases. Another option is using DCs in combination with exosomes. Not only DCs themselves but also the exosomes, which can be produced by DCs or tumor cells, can trigger a T cell response. The DCs can stimulate T cells through direct contact and the exosomes can trigger the response through the MHC-peptide complexes or passively by being incorporated in APCs. The route of administration is of great importance in the development of the immune response: the possible routes are SC, IV, ID or intranodal. The different routes induce a different migration route: DCs that have been administered intravenously migrate to the spleen whereas subcutaneously or intradermal administered DCs gather in lymph nodes (Grolleau et al., 2005) (Xu et al., 2011). In addition to the route of administration the type of DC can also make a difference. Mature DCs induce T cell immunity with a stronger cytotoxic and helper T cell response and can migrate to the lymph nodes to present the TAAs. Immature cells can induce T cell immunity tolerance but are more able to take up exogenous antigens such as proteins, RNA, etc. (Figure 3) (Grolleau et al., 2005) (Aly, 2012).

Recombinant vector vaccines
The goal of recombinant vaccination is to improve the immune response against TAAs through IM or SC injection. The most commonly used vector in recombinant vaccines is the viral one. These vaccines cause a high level of gene expression and use viruses to transport specific TAAs into DCs and other APCs, which in turn activate a strong immune response. Viral vectors can contain large quantities of genetic material and costimulatory molecules like CD80 and IL-2, and are easily produced (Agarwal et al., 2012) (Cawood et al., 2012). Lentiviruses, adenoviruses and adeno-associated viruses, retroviruses and vaccinia viruses have been used as vectors (Kirkwood et al., 2012). The poxviruses (a vaccinia virus) are most commonly used in recombinant vaccines. They are double-stranded DNA viruses that replicate in the cytoplasm of the host cells. The replication results in high neutralizing antibody titers (neutralizes biological effects of antigens) which lead to cell death and cellular debris. This debris contains the TAAs, which can be presented by APCs to cytotoxic and helper T cells and boost the immune response. The resulting antibodies are not only aimed at the tumor cells but also against the (poxviral) vector. A disadvantage of a viral recombinant vaccine is therefore that booster vaccination with the same virus is not useful (antibodies may neutralize the infection) and another virus such as avipox has to be used (Agarwal et al., 2012) (Harrop et al., 2006). The advantages of viral vectors are that they provide potent danger signals and imitate a natural infection (Harrop et al., 2006). In addition, the replication takes places in the cytoplasm of the host cells. There is therefore no risk of mutation because the viral genome will not integrate into the host DNA (Kim et al., 2012).

Prophylaxis
Prophylactic vaccines can be used in different ways: against virus infections such as hepatitis B virus (hepatocellular carcinoma) and human papillomavirus infection (cervical cancer) that cause cancer, or in the prevention of tumors and metastases (Weiner et al., 2010) (Benencia et al., 2011). The HPV vaccine has already been applied to young (not sexually active) girls in the USA and Europe. In Europe the vaccine contains virus-like particles (without internal content) corresponding to the HPV subtypes 16 and 18 and is administered intramuscular (IM) (Kawana et al., 2012). Vaccines have not been used to prevent tumors but have been applied to prevent tumor growth and the growth and spread of metastases. This is more a therapeutic mechanism of action than prophylactic and is therefore discussed further on (Weiner et al., 2010).

Treatment results

As previously mentioned, the Oncept® and Provenge® vaccine were the first to be officially registered. There are several phases which a vaccination should go through before it can be approved for application.

- Phase I: up to 30 patients, determines side effects, dose ranges and efficacy;
- Phase II: up to 100 patients, determines side effects and dose ranges;
- Phase III: comparing a new treatment, dosage or administration method with a standard treatment;
- Phase IV: after the vaccine is approved, determines functioning and long term risks and benefits (Cancer research UK, 2011).

Besides registered vaccines, there has been much research conducted into other approaches of these cancer types by using a different vaccine type or be using different protocols. For prostate cancer and malignant melanoma the most important vaccine trials have been reviewed.

Prostate cancer

Prostate cancer, an adenocarcinoma, is the most prevalent form of cancer and the second leading cause of cancer death in men in Europe. 10 to 20% of the patients develop castration-resistant prostate cancer (CRPC). These patients do no longer benefit from the standard types of treatment such as chemotherapy, castration and androgen deprivation because the plasma testosterone concentrations are already much suppressed. The exact mechanism behind CRPC is unknown but may lay in hypersensitivity of cancer cells to testosterone, androgen receptor mutation, or production of testosterone by the tumor. With CRPC there is a high risk of developing metastases, as prior studies described more than 80% of patients already having metastases at diagnosis, or developing them within two years; metastatic castration-resistant prostate cancer (mCRPC) (Geary et al., 2013) (Yin et al., 2013).

Dogs are the only other mammalian species that can develop spontaneous prostate cancer, even though the incidence is lower (0.2-0.6%) (Fan et al., 2007). Just like humans, dogs develop prostate cancer with age and the tumor metastasizes to the same tissues (bone, lung and lymph nodes). A difference among the two species is the androgen-independence which in dogs in present from the onset but develops in a later stage in humans. Another difference is that prostate cancer frequently forms in castrated dogs as in men it forms only with intact testes. Clinical trials for prostate cancer vaccination in dogs have not been established yet. Scientific research is first focusing on developing a canine model for prostate cancer by inducing prostate tumors (Schmidt et al., 2013) (Keller et al., 2013). This part of the literature study is therefore focused on human prostate cancer.

Development of cancer vaccines has been directed at prostate cancer for several reasons. It is a slow growing tumor type and recurrence is often diagnosed early, with no apparent signs on radiographic images except for an increased serum PSA (Prostate Specific Antigen). In addition, many TAAs for prostate cancer have been identified, which can induce strong immune responses, and the vaccines can be safely combined with other treatments as radiotherapy and anti-androgenics (Schlom et al., 2008). Several different types of prostate cancer vaccinations have been developed and tested in clinical trials over the last couple of years, such as Prostvac, GVAX and Sipuleucel-T vaccine.

Sipuleucel-T

Sipuleucel-T, also known as APC8015, is a dendritic cell vaccine in which the APCs are obtained from the patient's own hematopoietic progenitor cells (leukapheresis). The APCs are loaded with Prostatic Acid Phosphatase (PAP), which is a PSA that is expressed in 95% of prostate cancer. Sipuleucel-T is intravenously administered into the patient on three separate occasions. In the body it activates cytotoxic lymphocytes by binding with their receptors. Due to the PAP epitopes that are presented to the T lymphocytes, the T cells are directed at targeting the tumor cells. The immune response can

also contain other T cells (such as helper T cells), B cells and NK cells (Gulley et al., 2013) (Yin et al., 2013).
After years of clinical trials the FDA has approved a Sipuleucel-T vaccine, Provenge®, in 2010 for human CRPC, and the manufacturer is now striving to get European approval (Fiercepharma, 2013). Several clinical trials are recruiting or active at this time, with focuses on immune monitoring and combination therapies (ClinicalTrials.gov, 2014b).

Table 1. Summary of selected human trials of Sipuleucel-T

Study	*Design*	*Endpoint or Objective*	*No.*	*Eligibility*	*Results*
Burch et al., 2000	Phase I	Safety, immune response	13	mCRPC	Safe and well tolerated, reduction of PSA and PAP levels
Small et al., 2000	Phase I/II	Safety, efficacy, immune tolerance	31, (12/19)	CPRC, non-metastatic	Safe and well tolerated, T cell proliferation response observed
Small et al., 2006	Phase III, placebo controlled	TTP	127, 2:1 control	Asymptomatic mCRPC	No significant difference in TTP, trend towards improved OS
Higano et al., 2009	Phase III, randomized, double-blind, placebo-controlled	TTP	225, 2:1 control	mCRPC	No significant difference in TTP, trend towards improved OS
Kantoff et al., 2010	Phase III, randomized, double-blind, placebo-controlled	OS	512, 2:1 control	Asymptomatic to mild symptomatic mCRPC	OS improved (4.1 months), no trend towards improved TTP

(No: number of patients enrolled, OS: overall survival, PFS: progression-free survival, TTP: time to progression, mCRPC: metastatic castration-resistant prostate cancer)

GVAX
GVAX is a whole cell based vaccine that consists of two irradiated allogeneic prostate cancer cell lines: PC3, a cell line from bone metastasis, and LNCaP, a cell line from lymph node metastasis. The cell lines are administered intradermal (Joniau et al., 2012). The immune system is stimulated by APCs in the body that haven taken up the irradiated tumor cells, and through the presentation of TAAs to T cells (Schlom et al., 2008) (Small et al., 2007). Due to the termination of VITAL-1 and VITAL-2 the research into GVAX has an uncertain future. There is only one clinical trial ongoing and currently recruiting patients, that is investigating if GVAX can be safely combined with cyclophosphamide after removal of the affected prostate (ClinicalTrials.gov, 2013).

Table 2. Summary of selected human trials of GVAX

Study	*Design*	*Endpoint or Objective*	*No.*	*Eligibility*	*Results*
Small et al., 2007	Phase I/II	Safety, TTP, PSA changes, OS	34	mCPRC	Well tolerated, trend towards improved OS, TTP and lower PSA levels
Higano et al., 2008	Phase I/II, three dose groups	Safety, OS, TTP, PSA changes, pharmacokinetics serum GM-CSF	80	Asymptomatic mCRPC	Well tolerated, OS varied with dose but was improved compared to Kaplan-Meier estimates
VITAL-1 (Fiercepharma, 2008) (Joniau et al., 2012)	Phase III, vaccine compared to chemotherapy and prednisone	OS	626	Asymptomatic mCRPC	Terminated on base of futility analysis: less than 30% chance of reaching improved OS

VITAL-2 (Joniau et al., 2012) (Fiercepharma , 2008b)	Phase III, vaccine and chemotherapy compared to chemotherapy and prednisone	OS	408	Symptomatic mCRPC	Terminated due to excess in deaths, OS shorter in vaccine group

(No: number of patients enrolled, OS: overall survival, TTP: time to progression, mCRPC: metastatic castration-resistant prostate cancer)

Prostvac

Prostvac is a viral vector based vaccine that consists of a recombinant fowl pox and vaccinia virus and is administered subcutaneously. It contains PSA and a triad of human T cell co-stimulatory molecules (TRICOM): B7-1 (CD80), InterCellular Adhesion Molecule-1 (ICAM-1/CD54) and Leukocyte Function-Associated antigen 3 (LFA-3/CD58) (Kirkwood et al., 2012). The viruses are used for vaccine delivery and can infect APCs. After the transferring of genetic material into the APCs, the TAAs are expressed by the APC (Madan et al., 2009). At this time Prostvac is in phase III testing for men with asymptomatic to minimally symptomatic mCRPC (ClinicalTrials.gov, 2014b).

Table 3. Summary of selected human trials of Prostvac

Study	*Design*	*Endpoint or Objective*	*No.*	*Eligibility*	*Results*
DiPaola et al., 2006	Phase I	Safety, immunogenicity	10	CRPC, with or without metastasis	Safe, well tolerated, antibodies against vaccinia, no antibodies against PSA
Arlen, et al., 2007	Phase I, different protocols with rV-PSA/TRICOM, rF-PSA/TRICOM and rF-GM-CSF	Safety, immune response, detection of vaccinia after vaccination	15	mCRPC, disease progression (increasing PSA or by bone scan/ CT)	Safe, no detection of live vaccinia virus at other sites
Gulley et al, 2010	Phase II, randomized, with or without adjuvants as GM-CSF	Immune response, TTP and OS	32	mCRPC, chemotherapy-naïve	12/32 patients: decline in serum PSA, improved OS (9.2 months) especially in more indolent disease
Kantoff et al., 2010b	Phase II, randomized, double-blind, placebo controlled	PFS, safety, OS	122	mCRPC	No difference in PFS, improved OS (8.5 months)

(No: number of patients enrolled, OS: overall survival, PFS: progression free survival, mCRPC: metastatic castration-resistant prostate cancer, rV: recombinant vaccinia, rF: recombinant fowlpox, TTP: time to progression)

Other vaccine strategies such as TroVax® have been investigated. This is a vaccinia virus that has been tested in phase II trials alone and in combination with other therapies. In addition, DNA vaccines encoding PAP in phase II trials and other peptide and whole cell vaccines are currently investigated (Agarwal et al., 2012) (ClinicalTrial.gov, 2014b) (Joniau et al., 2012).

Malignant melanoma

Melanoma is a brown to black tumor that can be benign (melanocytoma, often on hairy skin areas) or malignant (malignant melanoma, often on mucosal surfaces) and arises from melanocytes (Bergman, 2007b). It is a common tumor in dogs and is responsible for approximately 7% of all malignant tumors in this species (Kim et al., 2009). It can occur on several places on the body such as the oral cavity; gingiva, lips, tongue and palatum durum (in order of decreasing frequency), around the digits and the eyes (Bergman, 2007b) (Manley et al., 2011). This melanoma is a spontaneous neoplasm that is highly aggressive in dogs and metastasizes frequently to lymph nodes and lungs but also to the brain, heart and spleen (Kim et al., 2009). The different stages that are recognized by the

WHO (World Health Organization) on canine oral and digital melanoma are the following (Manley et al., 2011) (Bergman, 2007b):

Table 4. Canine melanoma staging

Stage	**Oral**			**Digit**		
	Size	*Local lymph node*	*Distant metastasis*	*Size*	*Local lymph node*	*Distant metastasis*
I	≤ 2 cm diameter	No involvement	No involvement	< 2 cm diameter	No involvement	No involvement
II	2-4 cm diameter	No involvement	No involvement	2-5 cm diameter	Histologically negative	No involvement
III	2-4 cm diameter	Histo- or cytologically positive	No involvement	> 5 cm diameter	Histologically positive	No involvement
III	> 4 cm diameter	No involvement	No involvement	Invading fascia/bone	Histologically positive	No involvement
IV	Any size	Possible involvement	Involvement	Invading fascia/bone	Possible involvement	Involvement

The therapy protocol usually consist of surgically removing the neoplastic tissue, followed by radiation in case of incomplete removal or local metastasize. The median survival using this protocol in oral melanoma was however disappointing with a survival between 12-14 and 19 months for stage I, 5-6 months for stage II and three months for stage III (Bergman, 2007b). New (vaccine) therapies are therefore being developed for this specific tumor type.
The dog seems to be a good model for melanoma treatment in humans. Just as in canines, human malignant melanoma has the same predilection sites, the same metastatic properties and occurs spontaneously in immune-competent individuals. Even though mucosal melanoma is a rare finding in humans, cutaneous melanoma has an incidence of 0,02% (U.S. Cancer Statistics Working Group, 2014). In both species the tumors are chemo resistant and radio resistant, making them interesting candidates for vaccine therapy (Bergman et al., 2008). The American Joint Committee on Cancer have recognized the following clinical stages (simplified) in cutaneous melanoma (Balch et al., 2001):

Table 5. Human melanoma staging

Stage	**Cutaneous**		
	Size	*Local lymph node*	*Distant metastasis*
I	≤ 2 mm	No involvement	No involvement
II	2-4 mm	No involvement	No involvement
III	Any size	Involvement	No involvement
IV	Any size	Possible involvement	Involvement

Canine
Since oral melanoma in canines is more often malignant and aggressive than digital melanoma, most vaccines have been tested in these patients. The DNA vaccines have been investigated the most extensive. This has led to the 2010 USDA approval of the Oncept® vaccine for canines and testing in other country's such as the Netherlands. The DNA vaccine is xenogeneic and consists of human tyrosinase, a glycoprotein that is essential in melanin synthesis. After presenting the antigen on DC's,

the tyrosinase is recognized by the body as foreign. The resulting immune response of cytotoxic T cells and antibodies targets not only the human tyrosinase but also the own canine tyrosinase (Bergman, 2007b). This is due to the 85% similarity between both tyrosinase variants (Grosenbaugh et al., 2011). The vaccine is administered four times at two week intervals. The Oncept vaccine is administered through a transdermal injection but other administration routes are possible such as intramuscular of intradermal for the vaccines listed below (Grosenbaugh et al., 2011) (Bergman et al., 2008).

Table 6. Summary of selected studies of DNA vaccines against canine melanoma

Study	*Design*	*Endpoint or Objective*	*No.*	*Eligibility*	*Results*
Bergman et al., 2003	Phase I, huTyr vaccine, three dose groups	Safety, efficacy	9	MM, stage II, III or IV	Safe, trend towards clinical efficacy
Manley et al., 2011	muTyr vaccine	Safety, efficacy, OS	58, retrospective	MM of the digits	Safe, trend towards prolonged OS
Grosenbaugh et al., 2011	huTyr vaccine	Safety, efficacy, OS	111 and 53 historical controls	Stage II or III oral MM, after local surgery	Safe, trend towards prolonged OS (still to be determined)
Ottnod et al., 2013	huTyr vaccine	PFS, DFI, MST	22 and 23 controls, retrospective	Stage I, II or III oral MM after local surgery/radiation	No improvement in PFS, DFI and MST in the vaccine group

(No: number of patients enrolled, huTyr: human tyrosinase, muTyr: murine tyrosinase, OS: overall survival, MM: malignant melanoma, PFS: progression-free survival, DFI: disease-free interval, MST: median survival time)

Although most research in canines has been conducted into DNA vaccinations, a number of other vaccine designs have also been developed. The base of these vaccines is different and more administration routes have been investigated (such as subcutaneously for Gyorffy et al., 2005).

Table 7. Summary of selected trials of other vaccine protocols against canine melanoma

Study	*Design*	*Endpoint or Objective*	*No.*	*Eligibility*	*Results*
Alexander et al., 2006	Phase II, allogeneic whole cell	TTP, OS, DTH response	34	Stage II, III or IV MM	Biological response is related to improved OS, DTH response correlates to clinical response
Gyorffy et al., 2005	DC vaccine with BM from canine MM patients	CTL activity, efficacy	3 and 1 disease-free control	Stage I or II oral MM after local surgery and radiation	Antigen-specific immune response in 1 MM patient and the control

(No: number of patients enrolled, OS: overall survival, MM: malignant melanoma, TTP: time to progression, DTH: delayed-type hypersensitivity, Biological response: at least 50% decrease in tumor volume, BM: bone marrow, CTL: cytotoxic T lymphocyte)

Human

In human medicine the focus in melanoma vaccine research has not been so directed at one specific vaccine protocol as it has been in canine research. A few clinical trials into DNA vaccines have been designed, with both xenogeneic tyrosinase (mouse) as human tyrosinase. But this has not led to approval or phase III testing. Many other different approaches have been developed and tested over the years. The vaccines can be administered in several ways including intramuscular, intradermal and subcutaneous.

Table 8. Summary of selected trials of DNA vaccines against human melanoma

Study	*Design*	*Endpoint or Objective*	*No.*	*Eligibility*	*Results*
Wolchok et al., 2007	Phase I, randomized, muTyr and huTyr, three dose levels	Safety, immunogenicity	18	Stage III or IV MM, some after local surgery	Safe, T cell response in 7 patients, no relation with dose or schedule
Yuan et al., 2013	Phase I, muTyr at three dose levels	Safety, immune response, OS	24	Stage II, III or IV MM after local surgery	Safe, T cell response at highest dose level, OS still to be determined
Yuan et al., 2009	Phase I, randomized, muTyr and huTyr at three dose levels	Safety, immune response, PFS, OS	19	Stage II, III or IV MM	Safe, no association between PFS and immune response, OS still to be determined

(No: number of patients enrolled, MM: malignant melanoma, muTyr: murine tyrosinase, huTyr: human tyrosinase, OS: overalls survival)

Table 9. Summary of selected trials of other vaccines against human melanoma

Study	*Design*	*Endpoint or Objective*	*No.*	*Eligibility*	*Results*
Oshita et al., 2012	Phase II, DC	Efficacy, OS, immunologic response	24 and 37 retrospective controls	Stage III or IV metastatic MM	Safe, improved OS (6.3 months), anti-MAGE-A1 autoantibody positively correlated with OS
Dillman et al., 2012	Phase II, randomized, two groups: DC and autologous tumor cells (TC), both with GM-CSF	OS, DFS, EFS, FFS	18 DC and 24 TC	Stage III or IV metastatic MM	OS still to be determined, DC vaccine associated with longer survival compared to tumor cells
Hsueh et al., 2002	Phase II, allogeneic with three melanoma cell lines, five different adjuvant protocols ('Canvaxin')	OS, immunologic response	150 and 113 controls, retrospective	Stage IV MM, symptomatic and radiographic disease free	Improved OS (19 months), survival is correlated with a DTH immune response to the vaccine
Sondak et al., 2002	Phase III, randomized, allogeneic lysate of melanoma cells	DFS	300 and 300 controls	Stage II after local surgery	DFS not improved in vaccine group

(No: number of patients enrolled, MM: malignant melanoma, DFS: disease-free survival, DC: Dendritic cell, OS: overall survival, EFS: event-free survival (events being death or disease progression), FFS: failure-free survival (failure being, death, disease progression or discontinuing protocol), DTH: delayed-type hypersensitivity)

At this time there are several ongoing trials including phase I and II trials of DC and peptide vaccines, a phase I/II DNA vaccine trial and a phase III allogeneic POL-103A vaccine consisting of multiple melanoma-associated antigens. The vaccines in these trials are often compared to other therapeutic options such as chemotherapy and radiation (ClinicalTrials.gov, 2014).

Discussion and conclusion

There are several remarks that can be made regarding cancer vaccination research in general. Negative remarks include the fact that patient groups are mainly very small and that there is often no control group, blinding or sometimes even randomization. In most cases, the new vaccine is compared with another treatment, such as chemotherapy, surgery, a different vaccine protocol or to historical controls. Especially the latter option results in statistical difficulties due to lack of randomization, changes in the population and changes in standard of care treatment. The lack of control group is however not surprising given the aggressive course and often fatal outcome of cancer, making it not ethical to withhold all treatment. But it also raises the question if vaccination in some cases may not reduce overall survival instead of prolonging it, as was the case for VITAL (GVAX).

The immensity of different types of tumor, vaccines, adjuvants and protocols (doses, route and quantity of administration) makes it difficult to compare the different vaccines and even the endpoint and definition of success differ greatly. Some researches consider T cell reactions or tumor shrinkage to be a success, whereas others only use prolonged overall survival or the time to progression. New criteria, instead of the already existing RECIST (Response Evaluation Criteria in Solid Tumors), need to be drawn up to accurately compare the trials to each other (Kim et al., 2012).

In addition to response criteria, further research is necessary to determine when an immune response can be expected it the first place and how it can be measured. From HIV research it is known that it can take up to one year for an immune response to arise after DNA vaccination (Wolchok et al, 2007). It could very well be that many of the trials try to determine immune responses too soon or in the wrong way. The effect of vaccination may also be underestimated due to the composition of the patient group. These usually consist of patients with severe and even metastatic disease, whereas several researches have suggested that vaccines are more effective in the early stages without large tumor burden (Kim et al., 2012). Cancer vaccination should therefore probably not be seen as a monotherapy, but as part of a larger and long-lasting treatment protocol.

Vaccine types
The four major groups of therapeutic vaccine types (genetic, cellular, peptide and recombinant) have all been studied in many different cancers. In general, all vaccines only cause low grade adverse effects such as irritation around the injection site (Schlom, 2012). Each type has its own advantages; genetic and peptide vaccines are the easiest and cheapest to produce, whereas a whole cell vaccine produces a stronger immune response. Peptide and DNA can also target specific TAA's but have the disadvantage that they are low immunogenic. In contrast to DNA vaccines, mRNA vaccines only need to reach the host cytoplasm (not the nucleus) and they stimulate the innate and adaptive immunity at the same time. Recombinant vaccines have other strengths and weaknesses; it is not possible to booster a recombinant vaccine with the same virus but they do provide potent danger signals. Depending on patient and tumor characteristics, one of these groups can be more appropriate. The most progress in malignant melanoma and prostate cancer has been made with genetic and dendritic vaccines.

Prostate cancer
There are several difficulties in comparing the selected human prostate cancer studies with each other. The inclusion criteria for patients to enter the studies can differ greatly: the Kantoff and Gulley study (Kantoff et al., 2010) (Gulley et al., 2010) required a life expectancy of over six months, compared with three months in other studies. In addition there are differences in metastatic and symptomatic properties and allowing the use of other treatment modalities during the vaccination course and after progression. The trial designs differed greatly with regard to exclusion criteria; in the

Burch and Kantoff studies (Burch et al., 2000) (Kantoff et al., 2010) patients did not need to complete the entire course to be eligible for statistical analysis, just one vaccination was enough. Whereas in other studies such as the Small study (Small et al., 2006) patients needed to complete all three vaccinations. A few studies even offered the patients in the control arm the option to crossover to the treatment arm in case of disease progression (Higano et al., 2009) (Kantoff et al., 2010b). Even though this will most likely influence the distinction in overall survival between the two groups, overall survival was still determined. The best end point for testing vaccines against prostate cancer has not been established yet. Due to the quick progression of disease in some patient groups, median time to progression (sometimes as little as 10-12 weeks) may not be the best option. Other suggestions such as PAP or PSA are also unsuitable due to the lack of antibodies against PSA in some studies (DiPaola et al., 2006) or no significant drop in PSA in 140 out of 147 patients in others (Higano et al., 2009).

The most advanced vaccine in human prostate cancer, Sipuleucel-T dendritic cell vaccine, has been FDA approved. It has shown to be safe, well tolerated and phase III double-blind randomized testing resulted in a 4.1 months OS improvement, even though not all patients had to complete the entire vaccine course. Other frequently used vaccine types are whole cell based such as GVAX, which has had some setbacks over the last couple of years due to futility analysis and excess in deaths. It has recently been revived in a new clinical trial, but all other trials have been terminated. Lastly viral vectors such as Prostvac have been intensively researched and phase II testing resulted in 8.5 months and 9.2 months improvement. Based on OS improvement and safety, Prostvac and Sipuleucel-T therefore seem the most promising. Considering that human mCRPC and canine prostate cancer have the same characteristics, these vaccine approaches might also be appropriate protocols for canine prostate cancer.

Malignant melanoma

There are several difficulties comparing the different vaccine studies into malignant melanoma to each other. The first problem is located in the staging of tumors, due to the lack of distinction in the WHO staging system between a Chihuahua with a 3 cm size tumor and a Great Dane with a tumor the same size. Even though this has different consequences for the individual patients. In addition, there is often no homogeneous patient group. This is due to the different previous treatment modalities, the lack of free surgical margins, the eligibility of multiple stages of the disease such as in the Grosenbaugh study and Manley study (Grosenbaugh et al., 2011) (Manley et al., 2011) or the lack of a good diagnostic work-up for staging (Ottnod et al., 2013). Just as in prostate cancer, not all patients have gone through the complete vaccine dose schedule as shown in the Grosenbaugh study (Grosenbaugh et al., 2011) and often there is non-matched control group. For instance, the Alexander (Alexander et al., 2006) and Grosenbaugh study (Grosenbaugh et al., 2011) compared patients to historical controls, where the Gyorffy study (Gyorffy et al., 2005) compared them to healthy dogs. As well as in prostate cancer, some patients in melanoma research have also been allowed to undergo other treatments during the vaccine trials. In the Dillman study (Dillman et al., 2012) the vaccinations would even be postponed in order to undergo other treatment modalities. In the Manley study, the lack of availability/approval of the vaccine led to postponing the therapy. Patients had to wait up to 24 months before receiving the vaccine. Lastly, the general criticism regarding sample size (three dogs in the Gyorffy study), randomization and blinding of all the studies applies here as well (Gyorffy et al., 2005).

The most advanced vaccine in malignant melanoma is the canine DNA vaccine Oncept®, which has been USDA approved and is applied and investigated at several institutes. It has shown to be safe, well tolerated and prolonging OS (exact improvement still to be determined). Other melanoma vaccines have not made that much progress and have not progressed beyond completing phase II testing.

In human melanoma a variety of different vaccines and protocols have been developed, leading to promising first results. Canvaxin showed a nineteen-month improvement in human phase II testing, but a large scale phase III trial in stage III and IV human malignant melanoma patients showed no survival benefit compared to patients who received the placebo. This and another phase III trial have therefore been terminated (CancerVax, 2006). A small phase II dendritic cell vaccine trial however, has led to a 6.3 month improvement of OS, which is comparable to vaccine results seen in prostate cancer (Oshita et al., 2012). In human malignant melanoma there is therefore no vaccine that has shown great improvement of results in phase III testing, but there are some vaccines that are promising. The results in human melanoma research are therefore comparable to results in other cancer types and that of vaccine trials in other species.

Conclusion

Even though cancer vaccines hold promises for the future, taking all information, researches and reviews into account, there is much more research needed into developing and testing therapeutic cancer vaccines. In the context of follow-up studies it is important to determine the appropriate endpoints, patient groups, vaccine types and admission strategies before the results of researches can be compared and conclusions can be drawn about efficacy. It is suggested that patients without tumor bulk make the best candidates and appropriate staging systems should be created to optimize patient selection. The size of patient groups should be appropriate and be powered to detect significant differences. In early vaccine testing this is often a difference of 50%, which may lead us to underestimate the effect of vaccine therapy (Sondak et al., 2002). In addition, it has been shown that vaccine protocols do not have the similar effect in all species and all cancer types. This indicates that developing vaccines requires a specific and patient-centered approach. Vaccine therapy should not be seen as a monotherapy, but its effect is probably most valuable in combining it with other treatment modalities such as surgery. Overall survival is therefore a difficult endpoint in patients who will receive other treatment modalities prior or after vaccination or are lost to follow-up. Progression free survival might therefore be a better option in cancer types that are slowly progressing. Even though Oncept® and Provenge® have now been available and approved for several years, cancer vaccines still have a long way to go.

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Addendum

Context
This literature study has been made in combination with a practical participation in an ongoing study into a canine melanoma vaccine; Oncept® as part of a multimodal treatment. At the start of my research traineeship a total of twelve patients had been enrolled in the study. Two of the patients had already passed away, the remaining ten were still alive. Each dog had undergone an extensive work-up including fine needle aspiration biopsy of the tumor and local lymph nodes, bloodwork and a full body CT scan. This scan was made to visualize the tumor and the possible metastases and to determine the tumor stage. Subsequently the tumor and affected lymph nodes were surgically removed. The post-operative treatment consisted of a three week radiation protocol in case of local metastasis or incomplete removal and could be combined with the first two sessions of the vaccine protocol. The vaccine protocol and follow-up is listed in the table below;

Table 10. Canine melanoma vaccine protocol and follow-up at Utrecht University

Time	*Action*	*Details*
1 week after surgery	Vaccination	
3 weeks after surgery	Vaccination	
5 weeks after surgery	Vaccination	
7 weeks after surgery	Vaccination	
1 month after last vaccination	Check-up	Physical examination
3 months after last vaccination	Check-up	Physical examination and X-rays
6 months after last vaccination	Check-up	Physical examination and CT scan

Several dogs were not operated at the university hospital in Utrecht, but at their own veterinary clinic. These dogs started the vaccine protocol at the day of the first appointment and the other three vaccinations were administered every fortnight. After six months the dogs could be subjected to another vaccine protocol consisting of four vaccines. This decision was made after assessment of the new CT scan and in consultation between the owners and the specialist.

Contribution to the study
The main tasks during the practical period were providing information to interested owners and patient owners while guiding them through the study. This started by going through all the fine needle biopsy samples that the Universitair Veterinair Diagnostisch Laboratorium (UVDL) at Utrecht University receives and contacting the veterinarians of dogs with melanoma. They provided the information to the interested owners, which could in turn contact me. In this way or initiated by the referring veterinarian, the owners were made aware of the study.

I have guided three owners and their dogs from the start of the study: the family Osterwald with Raya, the Müller family with Peggy and the Schmidt family with Charley. Raya was a 13-year old Newfoundlander with stage 2 oral melanoma, Peggy was an 8-year old Spitz with stage 3 oral melanoma and Charley a 12-year old crossbreed with stage 4 oral melanoma. All patients were referred by the same veterinarian and all had already been treated with laser excision in Germany. I have been present at the appointments of these patients during my traineeship which included the vaccinations, CT scans and control visits. In addition I was present at the appointments of the other ten enrolled patients. The owners of those ten patients, who had already completed the first vaccine protocol, were contacted every couple of months to discuss the disease progress and set new appointments. Lastly I was present at the surgical oncology consultations on Wednesdays where patients with surgically removable melanoma and other tumor types can be referred to. All the information gathered about patients in the study during consultations, telephone contact or e-mail was collected and processed in Excel-sheets for data-analysis.